Multistate outbreak of listeriosis caused by imported cheese and evidence of cross-contamination of other cheeses, USA, 2012

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Received 24 February 2015; Final revision 7 May 2015; Accepted 11 May 2015; first published online 30 June 2015

SUMMARY

Listeria monocytogenes is a foodborne pathogen that can cause bacteraemia, meningitis, and complications during pregnancy. In July 2012, molecular subtyping identified indistinguishable L. monocytogenes isolates from six patients and two samples of different cut and repackaged cheeses. A multistate outbreak investigation was initiated. Initial analyses identified an association between eating soft cheese and outbreak-related illness (odds ratio 17·3, 95% confidence interval 2·0–825·7) but no common brand. Cheese inventory data from locations where patients bought cheese and an additional location where repackaged cheese yielded the outbreak strain were compared to identify cheeses for microbiological sampling. Intact packages of imported ricotta salata yielded the outbreak strain. Fourteen jurisdictions reported 22 cases from March–October 2012, including four deaths and a fetal loss. Six patients ultimately reported eating ricotta salata; another reported eating cheese likely cut with equipment also used for contaminated ricotta salata, and nine more reported eating other cheeses that might also have been cross-contaminated. An FDA import alert and US and international recalls followed. Epidemiology-directed microbiological testing of suspect cheeses helped identify the outbreak source. Cross-contamination of cheese highlights the importance of using validated disinfectant protocols and routine cleaning and sanitizing after cutting each block or wheel.

Key words: Cheese, contamination, Listeria, outbreak.

INTRODUCTION

Listeriosis is a severe foodborne infection caused by Listeria monocytogenes [1]. An estimated 1600 invasive cases occur annually in the United States [2]. Most invasive infections occur in older adults, persons with immunocompromising conditions [3, 4], pregnant women, and newborn infants [5]. Patients typically present with bacteraemia or meningitis, and about 21% die [5]. In pregnant women, listeriosis is
usually a non-specific febrile illness, but it can result in fetal loss, preterm labour, and neonatal sepsis and meningitis.

Listeriosis outbreak investigations frequently implicate processed and ready-to-eat meats, dairy products, and raw produce [6–10]. Soft cheeses have relatively high amounts of moisture, which favor *L. monocytogenes* growth. However, other properties (e.g. pH and salt concentration) and processing factors also are important determinants of growth [11]. Unpasteurized (raw) milk used for soft cheese production can become contaminated with *L. monocytogenes* before cheese-making. Cows, sheep, and goats can shed *L. monocytogenes* in their milk during lactation, and faecal contamination of raw milk can occur during milking [11]. The U.S. Food and Drug Administration (FDA) and Health Canada estimate that the risk of listeriosis per serving of soft-ripened cheeses is 50–160 times higher when made with unpasteurized rather than pasteurized milk [12]. However, when sanitation deficiencies exist soft cheeses are susceptible to environmental contamination with *L. monocytogenes* during and after the cheese-making process, even when milk is pasteurized [13, 14].

On 3 July 2012, the Allegheny County Health Department (ACHD) in Pennsylvania began investigating a case of listeriosis in a patient who had consumed two soft cheeses: a commercially produced, domestic blue cheese made from raw milk and imported l'Édel de Cléron made from pasteurized milk. Culture of non-intact samples of both cheeses collected from the patient’s refrigerator yielded a strain of *L. monocytogenes* with a pulsed-field gel electrophoresis (PFGE) pattern, also known as a pulsortype, (pattern 1, Fig. 1) that was indistinguishable from the pattern of the patient’s isolate. The patient had purchased both cheeses at a grocery chain A store. Accompanied by FDA, ACHD visited this store, collected samples of these cheeses, and isolated *L. monocytogenes* from l'Édel de Cléron cheese that had been cut from a wheel and repackaged. One *L. monocytogenes* isolate from this cheese had PFGE pattern 1; another isolate had a second PFGE pattern (pattern 2, Fig. 1). Grocery chain A recalled l’Édel de Cléron sold at this store.

Following reports of this index case and the isolates with indistinguishable PFGE patterns from cheese samples, we queried the database of PulseNet, the national molecular subtyping network for foodborne disease surveillance [15]. Six additional cases with indistinguishable PFGE patterns (patterns 1 and 2) were identified in residents of five other states. A multistate investigation was conducted to identify the outbreak source and implement control measures.

**METHODS**

**Epidemiological investigation**

We defined a case as an illness in which *L. monocytogenes* with an outbreak-associated PFGE pattern was isolated from a normally sterile site or from a product of conception (i.e. amniotic fluid, placental or fetal tissue) collected from a patient during 1 March 2012 to 31 October 2012. Dates of specimen collection were reported because dates of illness onset were often unavailable. Outbreak-associated patterns were defined by two-enzyme (*Ascl* and *Apal*) PFGE pattern combinations, which public health laboratories determined using the PulseNet protocol [15, 16]. Clinical isolates were forwarded to the U.S. Centers for Disease Control and Prevention (CDC) for serotyping and multiple-locus variable-number tandem repeat analysis (MLVA) to determine genetic relatedness. A pregnancy-associated case was defined as culture-confirmed listeriosis in a pregnant woman or her

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**Fig. 1.** Dendrogram of the four pulsed-field gel electrophoresis (PFGE) patterns comprising the outbreak strain. The outbreak strain comprised four PFGE pattern combinations: GX6A16·0408/GX6A12·0096 (pattern 1), GX6A16·0268/GX6A12·2297 (pattern 2), GX6A16·0068/ GX6A12·0096 (pattern 3), and GX6A16·1618/GX6A12·2384 (pattern 4). All four PFGE patterns were indistinguishable by MLVA.
newborn aged ≤28 days. State public health authorities reported whether deaths were attributed to listeriosis.

Initial patient food histories were collected with the questionnaire used routinely for CDC’s Listeria Initiative (LI) (www.cdc.gov/listeria/surveillance). Since 2004, the LI has collected detailed clinical and epidemiological information from all listeriosis patients (i.e. whether they are a part of a recognized cluster or not), including consumption of higher-risk foods during the month before illness onset. Using the LI database, we conducted an initial case-case analysis, comparing exposure frequencies of 10 outbreak-related patients with those of 204 listeriosis patients who (i) were interviewed during 2008–2012, (ii) were not linked to a recognized cluster or outbreak, and (iii) lived in counties with a grocery chain A store. We assessed exposures to specific soft cheeses and other food items. We created an aggregate variable for ‘any soft cheese,’ which included cheeses named in the LI questionnaire (blue, Gorgonzola, Brie, Camembert, farmer’s, feta, goat’s) and other soft cheeses (to capture types not named in the questionnaire). Mexican-style cheeses like quesos frescos were excluded from this aggregate variable because no patients with outbreak-related illness initially reported consuming them [8, 13, 17]. Matched odds ratios (mORs), 95% confidence intervals (CIs), and two-tailed P values were estimated for each food exposure using exact conditional logistic regression adjusting for the patient’s state of residence. For analyses of exposures, a mother–infant pair was considered as a single exposure, and the mother’s exposures were analysed.

Patients were interviewed (or infants’ mothers) with a supplementary questionnaire to obtain additional information about cheeses consumed (including semi-firm and firm cheeses), packaging and brand information, date and location where cheeses were purchased, and quantity of cheese consumed. Patients were asked to name cheeses they consumed. To investigate the hypothesis that an intact, contaminated cheese could have cross-contaminated other types or brands of cheeses, we defined a cut and repackaged cheese as any cheese cut from a larger wheel or block by a distributor or retailer and then sold as an individually wrapped section.

Product and microbiological investigations

We compiled inventory data voluntarily provided by retail firms in areas where patients purchased cheese during the month preceding illness onset and by firms that distributed cheeses contaminated with the outbreak strain of L. monocytogenes during the investigation period. To identify suspect cheeses to prioritize for microbiological investigation, FDA and CDC investigators analysed commonalities in inventory data. FDA analysed data for 11 firms of interest, including seven grocery chain A stores, three retail stores where patients purchased cheese, and a distributor referred to as distributor C. CDC performed an epidemiological analysis of cheeses cut and repackaged at two locations where the outbreak strain had been cultured from cheese samples (the grocery chain A store in Pennsylvania and distributor C). We identified cheeses that were possible sources of the outbreak strain and prioritized them for testing on the basis of their characteristics (e.g. moisture content, texture) and whether they were made from raw or pasteurized milk.

The California Department of Food and Agriculture (CDFA) collected several wheels of cheese from distributor C for culture at the California Animal Health and Food Safety Laboratory. FDA collected from distributor C intact samples of the suspect cheeses that were prioritized, and cultured these samples using standard methods [18]. Isolates from product samples were subtyped by PFGE, and the patterns were submitted to PulseNet [15, 16]; MLVA was also performed. FDA conducted enumeration studies to estimate counts of L. monocytogenes in intact samples of the implicated cheese using direct plating on RAPID® L. mono and Palcam agar [19].

FDA, CDFA, and ACHD conducted investigations and collected samples of cheese at several locations, including but not limited to one location of grocery chain A, distributor C, and a US cheese importer. FDA conducted focused investigations to ensure the proper scope of national recalls.

Cheese exposures and incubation periods

We investigated a small exposed cohort that included a patient with listeriosis and others who had consumed the implicated cheese during a family meal. Mean serving size was calculated by dividing the total amount of cheese served by the number of persons who consumed it.

Using dates of consumption and illness onset, incubation periods were calculated for patients who reported consuming either the implicated cheese or only one type of another cut and repackaged cheese. When consumption dates were unknown, purchase dates were used instead. Median incubation periods
were compared across cheese types using the Kruskal–Wallis test.

RESULTS

In total, 22 outbreak-related cases of listeriosis were reported in patients in 13 states and the District of Columbia (Fig. 2). Twenty (91%) patients were hospitalized. Of the adults, three died following infection, and one death was attributed to listeriosis. Nine illnesses were pregnancy-associated; *L. monocytogenes* was isolated from maternal blood or placental tissue (six mothers) and from infant blood (three infants, including an infant whose mother’s blood also yielded *L. monocytogenes*). One fetal loss was reported, and one newborn infant died. Of 13 patients with non-pregnancy-associated listeriosis, ages ranged from 30 to 87 years (median 77 years); seven (54%) were female. One of 14 patients self-identified as Hispanic.

Clinical specimens were collected from 28 March 2012 to 6 October 2012 (Fig. 3). Four PFGE pattern combinations of *L. monocytogenes* serotype 1/2a that were indistinguishable by MLVA were identified from a combination of clinical and food isolates (Fig. 1).

Infection with *L. monocytogenes* PFGE pattern 1 caused 18 of the illnesses. *L. monocytogenes* of pattern 1 was isolated from five cheese samples and had been isolated in 2003 from samples of a frozen imitation seafood item. Pattern 2 was new to the PulseNet database; isolates with this pattern caused two illnesses and were isolated from two cheeses. Pattern 3 had a one-band difference from pattern 1. *L. monocytogenes* of pattern 3, which had last been identified in clinical specimens in 2002, caused two illnesses. This pattern was not identified in cheese. Pattern 4, which was also a new PFGE pattern, was isolated only from intact samples of the implicated cheese.

Epidemiological investigation

On 23 August 2012, a case-case analysis of the initial 10 cases found that patients with outbreak-related listeriosis were more likely than patients with sporadic listeriosis to report having consumed Brie (mOR 5.7, 95% CI 1.0–31.7), feta (mOR 8.9, 95% CI 1.4–96.6), Camembert (mOR 26.4, 95% CI 1.6–1921.7), other soft cheeses (mOR 17.2, 95% CI 2.9–182.4), and any soft cheese (mOR 17.3, 95% CI 2.0–825.7) (Table 1).
Although nine out of 10 patients reported consuming any soft cheese, no single type of cheese was initially reported by more than five patients. Eight patients reported consuming yogurt (mOR 11·9, 95% CI 1·4–563·4), but examination of brand information revealed no common source.

Supplementary questionnaire data were initially available for nine of the 10 patients. All six who provided packaging information reported purchasing cheeses that were cut and repackaged, including ricotta salata (n = 2 patients), blue (n = 2), Brie (n = 2), mozzarella (n = 1), Camembert (n = 1), Monterey Jack (n = 1), and l’Édel de Cléron (n = 1). Because eating cut and repackaged cheese was common, and no two patients ate the same cheese sold under the same brand name, we investigated whether a single, intact cheese (cheese X) may have cross-contaminated others at retail stores and possibly distributors where it was cut and repackaged near these other cheeses.

Product and microbiological investigations

On 10 August 2012, after distributor C had notified CDFA of routine testing that identified the presence of Listeria in the facility, CDFA collected partial wheels of domestically produced blue and farmstead cheeses that had been cut and repackaged at distributor C; FDA isolated L. monocytogenes with PFGE pattern 1 from these two cheeses. However, cultures of intact samples of the blue and farmstead cheeses did not yield L. monocytogenes, and no patients had reported consuming either cheese. Distributor C had cut and repackaged these and several others but did not ship cut and repackaged cheeses to grocery chain A.

Analysis of inventory data revealed no brands or manufacturers in common among all 11 firms definitely or possibly linked to cases. Twenty-four cheeses that could be cheese X were identified by analyses of inventory data; 17 were cut and repackaged, including Italian-imported Marte Brand Frescolina Ricotta Salata, which appeared on inventories at both distributor C and the grocery chain A store where the index patient purchased cheese. Nine cheeses had characteristics that would likely favour L. monocytogenes growth; six were available at distributor C and collected by FDA for culture, including Marte Brand Frescolina Ricotta Salata.

Independently, CDFA collected intact wheels of Marte Brand Frescolina Ricotta Salata for culture...
after a review of cutting records at distributor C showed it was the only common cheese at cutting stations used for the blue and farmstead cheeses that yielded the outbreak strain. On 27 August 2012, samples of intact Marte Brand Frescolina Ricotta Salata were collected for microbiological analyses. Those analyses yielded *L. monocytogenes*, and on 8 September 2012, PFGE results identifying the outbreak strain (pattern 1) were confirmed, establishing Marte Brand Frescolina Ricotta Salata as the hypothesized cheese X. Additional cultures from intact wheels of Marte Brand Frescolina Ricotta Salata collected by FDA at distributor C, store B, and the US importer yielded *L. monocytogenes* with PFGE patterns 1, 2, and 4 (Table 2).

Health and regulatory officials in Colorado and New York City later isolated the outbreak strain from leftover samples of Marte Brand Frescolina Ricotta Salata from a patient’s refrigerator (patterns 1 and 2) and a restaurant (pattern 1) (Table 2).

FDA isolated *L. monocytogenes* from all 10 samples of intact Marte Brand Frescolina Ricotta Salata from store B, with counts ranging from 3900 to 750 000 colony-forming units (c.f.u./g) (median 47 700 c.f.u./g). These cheeses were sampled within 7 days of their sell-by date.

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**Table 1. Case-case comparison of cheeses and selected other food exposure frequencies in outbreak-related cases and sporadic cases of listeriosis reported to the Listeria Initiative**

<table>
<thead>
<tr>
<th>Food exposure</th>
<th>Outbreak-related cases (N = 10)</th>
<th>Sporadic cases (N = 204)</th>
<th>mOR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any soft cheese†</td>
<td>9/10 (90)</td>
<td>51/138 (37)</td>
<td>17.3 (2.0–825.7)</td>
<td>0.003</td>
</tr>
<tr>
<td>Brie</td>
<td>4/9 (67)</td>
<td>14/134 (10)</td>
<td>5.7 (1.0–31.7)</td>
<td>0.04</td>
</tr>
<tr>
<td>Feta</td>
<td>5/9 (56)</td>
<td>22/134 (16)</td>
<td>8.9 (1.4–96.6)</td>
<td>0.02</td>
</tr>
<tr>
<td>Blue or Gorgonzola</td>
<td>5/9 (56)</td>
<td>16/134 (12)</td>
<td>4.9 (0.8–29.2)</td>
<td>0.09</td>
</tr>
<tr>
<td>Camembert</td>
<td>3/9 (33)</td>
<td>2/133 (2)</td>
<td>26.4 (1.6–1921.7)</td>
<td>0.02</td>
</tr>
<tr>
<td>Goat’s cheese</td>
<td>2/9 (22)</td>
<td>11/136 (8)</td>
<td>3.9 (0.3–31)</td>
<td>0.33</td>
</tr>
<tr>
<td>Mexican-style cheese</td>
<td>1/10 (10)</td>
<td>24/135 (18)</td>
<td>0.4 (0.01–4.0)</td>
<td>0.76</td>
</tr>
<tr>
<td>Farmer’s cheese</td>
<td>0/10 (0)</td>
<td>2/137 (1)</td>
<td>17.3 (0.145–0.7)</td>
<td>1.0</td>
</tr>
<tr>
<td>Other soft cheese</td>
<td>7/10 (70)</td>
<td>20/136 (15)</td>
<td>17.2 (2.9–182.4)</td>
<td>0.0006</td>
</tr>
<tr>
<td>Yogurt</td>
<td>8/10 (80)</td>
<td>52/129 (40)</td>
<td>11.9 (1.4–563.4)</td>
<td>0.01</td>
</tr>
<tr>
<td>Turkey delicatessen meat</td>
<td>4/10 (40)</td>
<td>52/138 (38)</td>
<td>1.0 (0.2–4.8)</td>
<td>1.0</td>
</tr>
<tr>
<td>Pâté</td>
<td>2/10 (20)</td>
<td>5/134 (4)</td>
<td>3.6 (0.2–34.3)</td>
<td>0.43</td>
</tr>
</tbody>
</table>

* Sporadic cases (i.e. cases not associated with any recognized cluster or outbreak) reported to the Listeria Initiative from 2008 to 2012 in patients residing in counties with a grocery chain A store were included (see text). Matched odds ratios (mORs), 95% confidence intervals (CIs), and two-tailed *P* values were estimated using exact conditional logistic regression adjusting for patient state of residence.
† Any soft cheese was an aggregate variable, which included exposure to the following cheeses: blue or Gorgonzola, Brie, Camembert, farmer’s, feta, goat’s, and other soft cheese. Mexican-style cheese (*queso fresco*) was excluded.
‡ Median unbiased estimate.
In October 2012, Italian authorities issued an international market withdrawal (equivalent to a US recall) of Marte Brand Frescolina Ricotta Salata and four other types of ricotta cheeses from the Italian exporter. The Italian authorities conducted investigations at the Italian exporter and cheese-making plants to identify possible environmental sources of contamination of the ricotta salata.

Cheese exposures and incubation periods

The 22 cases represented 21 possible exposures to cut and repackaged cheeses prior to listeriosis onset; two cases, which occurred in a pregnant women and her newborn infant, were considered a single exposure. Of these 21 patients, six reported consuming ricotta salata, including four pregnant women. Four of these patients had records verifying purchase of Marte Brand Frescolina Ricotta Salata. One person reported consuming ricotta salata after the recall. All six patients purchased it from retail stores. Another patient (Table 3, patient 2) dined at a restaurant where Marte Brand Frescolina Ricotta Salata was served in several menu items. However, we could not determine whether this patient ate this cheese, a cross-contaminated cheese, or both. Of the remaining 14 patients, three ate at least one type of cut and repackaged cheese, six ate various others of unknown packaging, and one ate cheese sliced at a delicatessen. Mothers of two infected newborns did not report consuming any soft cheese, but they were only interviewed using the initial LI questionnaire, not the supplementary questionnaire. Two patients could not be reached.

Using data for nine patients for whom supplementary questionnaire data were initially available, we estimated the overall median incubation period was 7 (range 2–26) days (Table 3). Of the patients who ate ricotta salata, the three pregnant patients had a longer median incubation period (8 days) than the two non-pregnant patients (2·5 days). The incubation period was shorter for the five patients (three pregnant and two non-pregnant) who ate ricotta salata (median 4, range 2–14 days), than for the three non-pregnant patients who consumed cut and repackaged cheese but not ricotta salata (median 12, range 7–26 days) ($P = 0·18$).

In October 2012, Italian authorities issued an international market withdrawal (equivalent to a US recall) of Marte Brand Frescolina Ricotta Salata and four other types of ricotta cheeses from the Italian exporter. The Italian authorities conducted investigations at the Italian exporter and cheese-making plants to identify possible environmental sources of contamination of the ricotta salata.

**Table 2. Cheese characteristics, product sample locations, and pulsed-field gel electrophoresis (PFGE) patterns of Marte Brand Frescolina Ricotta Salata and other cross-contaminated cheeses yielding the outbreak strain of Listeria monocytogenes**

<table>
<thead>
<tr>
<th>Cheese type</th>
<th>Pasteurized milk*</th>
<th>Cheese texture</th>
<th>Packaging</th>
<th>Location</th>
<th>PFGE patterns†</th>
</tr>
</thead>
<tbody>
<tr>
<td>l’Édel de Cléron</td>
<td>Yes</td>
<td>Soft</td>
<td>Cut and repackaged</td>
<td>Index patient refrigerator</td>
<td>1</td>
</tr>
<tr>
<td>l’Édel de Cléron</td>
<td>Yes</td>
<td>Soft</td>
<td>Cut and repackaged</td>
<td>Grocery chain A</td>
<td>1, 2</td>
</tr>
<tr>
<td>Raw milk blue cheese</td>
<td>No</td>
<td>Soft</td>
<td>Cut and repackaged</td>
<td>Index patient refrigerator</td>
<td>1</td>
</tr>
<tr>
<td>Blue cheese</td>
<td>Yes</td>
<td>Soft</td>
<td>Partial wheel</td>
<td>Distributor C</td>
<td>1</td>
</tr>
<tr>
<td>Farmstead cheese</td>
<td>Yes</td>
<td>Semi-hard</td>
<td>Partial wheel</td>
<td>Distributor C</td>
<td>1</td>
</tr>
<tr>
<td>Ricotta salata‡</td>
<td>Yes</td>
<td>Soft</td>
<td>Intact wheel</td>
<td>Distributor C</td>
<td>1</td>
</tr>
<tr>
<td>Ricotta salata‡</td>
<td>Yes</td>
<td>Soft</td>
<td>Cut and repackaged</td>
<td>Additional patient’s refrigerator</td>
<td>1, 2</td>
</tr>
<tr>
<td>Ricotta salata‡</td>
<td>Yes</td>
<td>Soft</td>
<td>Intact wheels and partial wheel</td>
<td>Restaurant</td>
<td>1</td>
</tr>
<tr>
<td>Ricotta salata‡</td>
<td>Yes</td>
<td>Soft</td>
<td>Intact wheels</td>
<td>Store B</td>
<td>1, 2</td>
</tr>
<tr>
<td>Ricotta salata‡</td>
<td>Yes</td>
<td>Soft</td>
<td>Intact wheels</td>
<td>Importer</td>
<td>4</td>
</tr>
</tbody>
</table>

*Cheeses made from pasteurized milk.
†Outbreak strain comprised four PFGE patterns: GX6A16-0408/GX6A12-0096 (pattern 1), GX6A16-0268/GX6A12-2297 (pattern 2), GX6A16-0068/GX6A12-0096 (pattern 3), and GX6A16-1618/GX6A12-2384 (pattern 4).
‡Italian-imported Marte Brand Frescolina Ricotta Salata.

Molecular surveillance for *L. monocytogenes* detected a complex foodborne disease outbreak associated with...
Marte Brand Frescolina Ricotta Salata, a soft, salty cheese made from pasteurized sheep’s milk and imported from Italy. Examination of cheese-cutting records and analysis of epidemiological and product inventory data directed microbiological testing of cheese, which ultimately implicated Marte Brand Frescolina Ricotta Salata as the source. All four PFGE patterns of *L. monocytogenes* cultured from this cheese or from patients’ specimens were either extremely rare or had not been identified in the United States before this outbreak. MLVA analysis confirmed these PFGE patterns were closely related, comprising a single outbreak strain. Seven cases were linked directly to the ricotta salata, including a mother–infant pair. Another patient probably ate ricotta salata or a cross-contaminated cheese. Many of the remaining 14 patients were probably exposed to *L. monocytogenes* in cheeses cross-contaminated by Marte Brand Frescolina Ricotta Salata.

We hypothesized early in the investigation that an intact, contaminated cheese (cheese X) could cross-contaminate multiple types or brands of cheeses during cutting and repackaging at retail and distribution locations. This hypothesis was critical to the investigation and directed efforts that eventually led to identifying cheese X as Marte Brand Frescolina Ricotta Salata. Three pieces of information reinforced and focused this hypothesis: (i) the outbreak strain was cultured from samples of cut and repackaged cheeses collected at distributor C and grocery chain A; (ii) distributor C did not ship cut and repackaged cheeses to grocery chain A; and (iii) grocery chain A received only intact wheels of cheese from its distributors. Taken together, these facts meant that cheese X would have to have been cut and repackaged at both locations. This proved true; at distributor C, the process of cutting and repackaging Marte Brand Frescolina Ricotta Salata likely cross-contaminated the two partial wheels of cheese (blue and farmstead) that yielded the outbreak strain. Moreover, cutting records at distributor C revealed that Marte Brand Frescolina Ricotta Salata was the only cheese cut using the

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Pregnancy-associated?</th>
<th>Clinical syndrome</th>
<th>Outcome</th>
<th>Type of cheese consumed</th>
<th>No. of days consumed</th>
<th>Estimated incubation period (days)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>68</td>
<td>No</td>
<td>Meningitis</td>
<td>Survived</td>
<td>l’Édel de Cléron, blue†</td>
<td>≤13</td>
<td>12</td>
</tr>
<tr>
<td>2‡</td>
<td>86</td>
<td>No</td>
<td>Bacteraemia</td>
<td>Died</td>
<td>Ricotta salata§ or cross-contaminated menu item</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>65</td>
<td>No</td>
<td>Meningitis</td>
<td>Survived</td>
<td>Blue</td>
<td>≤7</td>
<td>7‡</td>
</tr>
<tr>
<td>4</td>
<td>82</td>
<td>No</td>
<td>Bacteraemia</td>
<td>Survived</td>
<td>Brie</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>56</td>
<td>No</td>
<td>Bacteraemia</td>
<td>Survived</td>
<td>Ricotta salata§</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>No</td>
<td>Meningitis</td>
<td>Survived</td>
<td>Ricotta salata§</td>
<td>≤7</td>
<td>14</td>
</tr>
<tr>
<td>7</td>
<td>38</td>
<td>Yes</td>
<td>–</td>
<td>Infant died</td>
<td>Ricotta salata§</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>29</td>
<td>Yes</td>
<td>–</td>
<td>Delivered healthy baby</td>
<td>Ricotta salata§</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td>29</td>
<td>Yes</td>
<td>–</td>
<td>Delivered healthy baby</td>
<td>Ricotta salata§</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

* The first date of consumption was used for patients reporting they may have consumed cheese on multiple days.
† The index patient was included in analysis (even though multiple cut and repackaged cheeses were reportedly consumed) because both the l’Édel de Cléron and blue cheese, which were contaminated with the outbreak strain, were bought on the same day.
‡ Patient 2 dined at a restaurant on 21 and 22 March. Records show Marte Brand Frescolina Ricotta Salata was delivered to the restaurant 20 March and used in several menu items. Whether the patient consumed ricotta salata or a cross-contaminated menu item is unknown.
§ Italian-imported Marte Brand Frescolina Ricotta Salata.
|| An estimate of 7 days between purchase and illness onset was used for one patient who reported purchasing a cut and repackaged cheese the week before illness onset.
same cutters as the blue and farmstead cheeses. At
grocery chain A, Marte Brand Frescolina Ricotta
Salata likely cross-contaminated the two cheeses
bought by the index patient (another blue cheese
and l’Édel de Cléron).

There are several reasons to think cross-
contamination of other cut and repackaged cheeses
occurred and caused illnesses. First, the index patient
consumed two cut and repackaged cheeses from which
the outbreak strain was isolated but did not report
consuming ricotta salata. The fact that the outbreak
strain was also isolated from wedges of l’Édel de
Cléron at the store where the patient purchased the
cheese (and where Marte Brand Frescolina Ricotta
Salata was cut and repackaged) make it likely that
this cross-contaminated cheese was the source of this
patient’s illness. Yet, culturing of intact wheels did
not yield the outbreak strain, making it unlikely that
l’Édel de Cléron was the source of other patients’
ilnesses. Second, when asked to list cheeses consumed
in the past month, eight patients did not report eating
ricotta salata. However, patients were not specifically
asked if they had consumed ricotta salata. Although
some may not have remembered eating ricotta salata,
all eight reported eating other cheeses; three reported
other cut and repackaged cheeses. Third, the same
rare outbreak strain was isolated from open samples,
but not intact wheels, of four other cheeses cut and
repackaged at distributor C and the grocery chain A
location where Marte Brand Frescolina Ricotta
Salata was cut and repackaged, suggesting cross-
contamination from the ricotta salata. By the investi-
gation’s end, the outbreak strain had been isolated
from five types of cheeses in six places. These results
also provide evidence of cross-contamination in sev-
eral geographically distinct locations and in both retail
and distribution settings.

We suspected that cheese X would support the
growth of *L. monocytogenes* well. The statistically
significant associations between outbreak-related ill-
ness and consumption of soft cheeses found early in
the investigation could have been a result of con-
founding (i.e. people who buy Brie and other soft
cheeses may also buy cheese X) or a true association
with illness (other soft cheeses may have been cross-
contaminated by cheese X). Soft cheeses have been
implicated for decades as the source of numerous lisi-
eriosis outbreaks in Europe, North America, and
elsewhere [20–25]. In 1985, Mexican-style soft cheese
causėd the second largest listeriosis outbreak in US
history (142 illnesses) [8]. During a 2008 outbreak of
listeriosis in Quebec, extensive cross-contamination
at retail led to a province-wide recall of many cheeses
[18]. As in our investigation, a minority (43%) of
patients in the Quebec outbreak recalled eating an
implicated brand of cheese [26]. Although this is the
first known outbreak linked to ricotta salata, one
study found ricotta salata supported growth of *L.
monocytogenes* at refrigeration temperatures, despite
it being a relatively salty cheese [27]. Although ricotta
salata may be perceived as ‘hard’ by touch, it has rela-
tively high water activity (*a_w* > 0.94). *L. monocyo-
togenes* has been isolated from other imported Italian
cheeses, including Ricotta Piatta, Talleggio, and un-
named others [28]. While two different blue cheeses
became cross-contaminated in this outbreak, blue
cheese has not been shown to support the growth of
*L. monocytogenes* when surface-inoculated [29],
although it has been shown to survive in blue cheese
for at least 120 days [30].

Our investigation adds useful data for comparison
with other published estimates of the incubation per-
iod of listeriosis. Overall, our median estimate of 8
days was consistent with a French review of 37
patients with outbreak-related listeriosis [31]; however,
our specific estimates for three pregnant (8 days) and for
six non-pregnant (7 days) patients were significantly
shorter and longer, respectively, compared to larger
datasets from other outbreak investigations [8, 31, 32].
In an outbreak linked to Camembert cheese [32], high
doses of contamination (up to 360 million c.f.u. of
*L. monocytogenes* per portion) may have contributed
to patients’ relatively short incubation periods (3–4
days). Similarly, we found a significantly shorter me-
dian incubation period in non-pregnant patients who
ate the ricotta salata compared with those who con-
sumed another single cut and repackaged cheese.
This suggests that larger numbers of *L. monocytogenes*
cells likely contaminated the ricotta salata (i.e. inges-
tion of more cells decreases the time needed to over-
whelm the immune system) [33], compared to cross-
contaminated cheeses that may have been inocu-
lated with a small number of bacteria, on a medium
that may or may not have supported the growth of
*L. monocytogenes*. The presence of certain medical
conditions also may have played a role in the incuba-
tion period, although such information was not rou-
tinely collected for all patients. For example, a
56-year-old patient known to have a history of leukae-
mia had the shortest incubation period (2 days) of
those we estimated (Table 3). Further investigation
into the clinical history of a 30-year-old patient with
Listeriosis outbreak and contaminated cheese

a 3-day incubation period did not identify any pre-existing conditions.

Transfer of *L. monocytogenes* from ricotta salata to cut surfaces of other cheeses (via shared cutting utensils and surfaces) may have led to a lower level of initial contamination of the cut cheese and a smaller dose. This would be consistent with the results of a study that repeatedly transferred *L. monocytogenes* from inoculated to unoinoculated delicatessen meats via stainless-steel kitchen knives, demonstrating decreasing levels of contamination on the recipient meats [34]. However, another study suggests that transfer of *L. monocytogenes* may be affected by characteristics of the contaminated food (i.e. moisture and fat content) and by food contact surfaces [35].

The FDA Food Code specifies when food contact surfaces in retail and food establishments are expected to be cleaned and sanitized. The FDA Food Code defines sanitization as ‘the application of cumulative heat or chemicals on cleaned food-contact surfaces that, when evaluated for efficacy, is sufficient to yield a reduction of 5 log10, which is equal to a 99.999% reduction, of representative disease microorganisms of public health importance’ (parts 1–2). Studies that reviewed efficacy of methods for sanitizing mechanical slicers used for delicatessen meats and salmon show variation in log10 reductions of *L. monocytogenes* and also show protein residues can reduce the effectiveness of sanitizers [34, 35]. However, we are not aware of studies examining efficacy of such methods for cheese cutting boards, knives, or wire cutters. Validation of protocols for these tools may be warranted. It is important that retail and food service establishments have procedures in place that ensure compliance with state and local regulations modelled after the FDA Food Code provisions that focus on the prevention of cross-contamination (see part 3–3) and the proper cleaning and sanitizing of food contact surfaces (see parts 4–6 and 4–7). FDA works closely with state, local, and tribal agencies and the industry to improve compliance and promote best practices in these areas. Evaluating avenues for cross-contamination in cheesemaking facilities is also an important part of what FDA investigators look at during inspections, as control of cross-contamination is part of FDA’s Good Manufacturing Practice (GMP) requirements. FDA is continuing routine surveillance sampling of soft cheeses.

Many cheeses are susceptible to bacterial contamination during manufacture, ripening, storage, and during cutting and repackaging for retail distribution and sale. Our report documents a listeriosis outbreak associated with an imported, soft cheese made from pasteurized milk [13, 14, 26, 32]; this demonstrates the risks posed by environmental contamination of soft cheeses made under unsanitary conditions. Because cross-contamination of cheese through cutting and re-packaging can occur, it is important to use validated disinfectant protocols routinely, and to clean and sanitize wire cutters, cutting boards, knives, and utensils after cutting each block or wheel of cheese. Cross-contamination is a vital consideration when investigating an outbreak with suspected links to cheese, and even more so if, during the investigation, a single type or brand is not identified early.

ACKNOWLEDGEMENTS

We dedicate this work to the memory of our esteemed colleague and friend Dr William Keene, who provided technical assistance and his characteristic insight during this investigation. The authors thank the following individuals for assisting this investigation: Anna Blackstock, Karen Blickenstaff, Stacey Bosch, Laura Burnworth, Mary Cartagena, Shaun Cosgrove, Roberta Hammond, Emily Harvey, Capt. Thomas Hill, Manjiri Joshi, Doug Karas, Christine Keys, Akiko Kimura, Seth Levine, Kathryn MacDonald, Kim Machesky, Michelle Malavet, Marika Mohr, Thai-An Nguyen, Kenneth Nieves, Marguerite Pappiaonou, Don Prater, Roshan Reporter, John Sheehan, Cheryl Tarr, Jennifer Thomas, Ian Williams, Cecilia Wolyniak, Katie Wymore, FDA Northeast Region Emergency Response Group, FDA ORA’s Central Region Emergency Response Group, and FDA ORA’s Pacific Region Emergency Response Group.

DECLARATION OF INTEREST

None.

REFERENCES


