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

K. E. HEIMAN[1]*, V. B. GARALDE[2], M. GRONOSTAJ[3], K. A. JACKSON[1], S. BEAM[4], L. JOSEPH[1], A. SAUPE[5], E. RICOTTA[6], H. WAECHTER[7], A. WELLMAN[8], M. ADAMS-CAMERON[9], G. RAY[10], A. FIELDS[2], Y. CHEN[2], A. DATTA[2], L. BURALL[2], A. SABOL[1], Z. KUCEROVA[1], E. TREES[1], M. METZ[2], P. LEBLANC[2], S. LANCE[2], P. M. GRIFFIN[1], R. V. TAUXE[1] AND B. J. SILK[1]

[1] *Division of Foodborne, Waterborne, and Environmental Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA;* [2] *U.S. Food and Drug Administration, Silver Spring, MD, USA;* [3] *Allegheny County Health Department, Pittsburgh, PA, USA;* [4] *California Department of Food and Agriculture, Sacramento, CA, USA;* [5] *Minnesota Department of Health, St Paul, MN, USA;* [6] *Maryland Department of Health and Mental Hygiene, Baltimore, MD, USA;* [7] *New York City Department of Health and Hygiene, NY, New York, USA;* [8] *Virginia Department of Health, Richmond, VA, USA;* [9] *New Mexico Department of Health, Santa Fe, NM, USA;* [10] *District of Columbia Department of Health, Washington, DC, USA*

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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

* Author for correspondence: K. E. Heiman, 1600 Clifton Rd NE MS A-38, Atlanta, GA, USA. (Email: uwj0@cdc.gov)

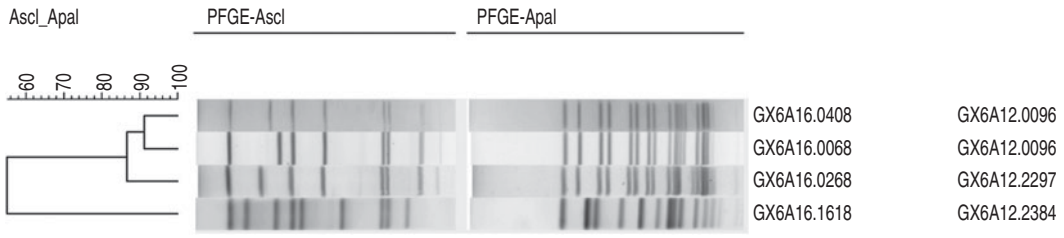

Fig. 1. Dendrogram of the four pulsed-field gel electrophoresis (PFGE) patterns comprising the outbreak strain. The outbreak strain comprised four PFGE pattern combinations: GX6A16·0408/GX6A12·0096 (pattern 1), GX6A16·0268/ GX6A12·2297 (pattern 2), GX6A16·0068/ GX6A12·0096 (pattern 3), and GX6A16·1618/GX6A12·2384 (pattern 4). All four PFGE patterns were indistinguishable by MLVA.

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 pulsotype, (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 (*Asc*I and *Apa*I) 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

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 *queso fresco* 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

Fig. 2. Persons infected with the outbreak-associated strain of *Listeria monocytogenes* by state of residence ($n = 22$), United States, March–October 2012.

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).

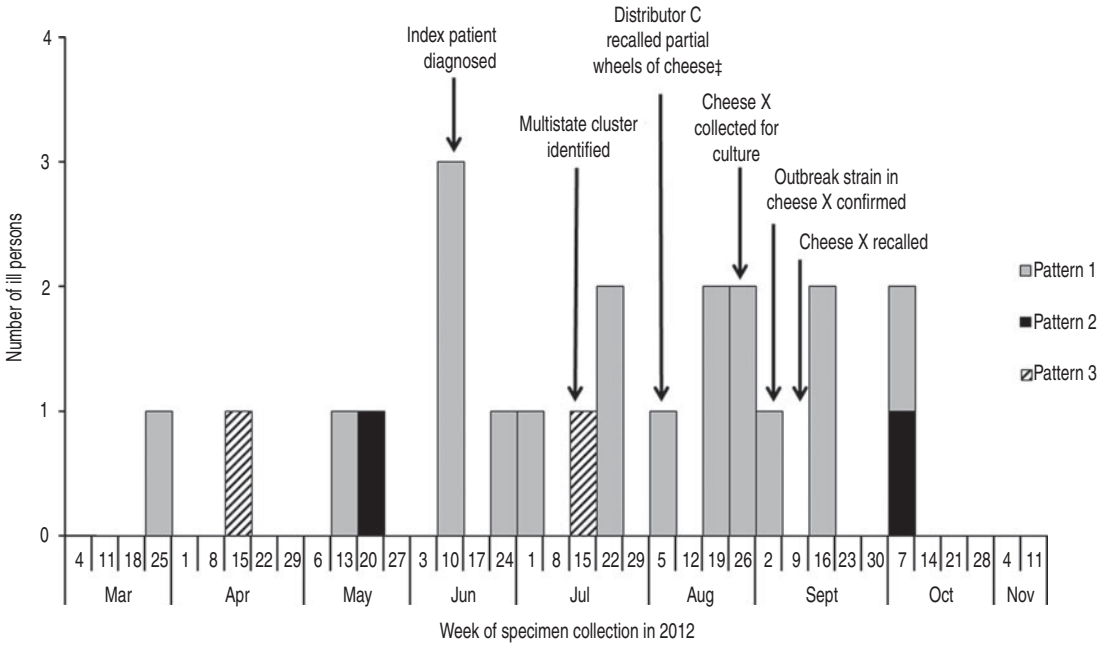

Fig. 3 Persons* infected with the outbreak-associated strain† of *Listeria monocytogenes* ($n = 22$) by week of clinical specimen collection and pulsed-field gel electrophoresis (PFGE) pattern combination, United States, March–October 2012. [* Persons includes mother and her infant, counted as two persons. † 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), which was only isolated from cheese X.] ‡ A blue cheese and a farmstead cheese.

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

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**

Food exposure	Food frequency, *n*/*N* (%)				mOR	(95% CI)	*P* value
	Outbreak-related cases (*N* = 10)		Sporadic cases (*N* = 204)				
Any soft cheese†	9/10	(90)	51/138	(37)	17·3	(2·0–825·7)	0·003
Brie	4/9	(67)	14/134	(10)	5·7	(1·0–31·7)	0·04
Feta	5/9	(56)	22/134	(16)	8·9	(1·4–96·6)	0·02
Blue or Gorgonzola	5/9	(56)	16/134	(12)	4·9	(0·8–29·2)	0·09
Camembert	3/9	(33)	2/133	(2)	26·4	(1·6–1921·7)	0·02
Goat's cheese	2/9	(22)	11/136	(8)	3·9	(0·3–31)	0·33
Mexican-style cheese	1/10	(10)	24/135	(18)	0·4	(0·01–4·0)	0·76
Farmer's cheese	0/10	(0)	2/137	(1)	17·3	(0–145·0‡)	1·0
Other soft cheese	7/10	(70)	20/136	(15)	17·2	(2·9–182·4)	0·0006
Yogurt	8/10	(80)	52/129	(40)	11·9	(1·4–563·4)	0·01
Turkey delicatessen meat	4/10	(40)	52/138	(38)	1·0	(0·2–4·8)	1·0
Pâté	2/10	(20)	5/134	(4)	3·6	(0·2–34·3)	0·43

* 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.

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 ~9000 to ~3 750 000 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.

FDA determined that the Italian exporter shipped Marte Brand Frescolina Ricotta Salata through a single US importer, which distributed it to firms in 30 states. On 10 September 2012, the importer voluntarily recalled a single lot of Marte Brand Frescolina Ricotta Salata. The recall was expanded to include all lots on 14 September 2012 after testing identified presumptive *Listeria* contamination in additional lots. The importer also withdrew from the market all other cheeses purchased from the Italian exporter. On 13 September the exporter was put on import alert, so that no cheeses from this exporter would be allowed into the United States until the exporter provided evidence that the conditions that led to contamination with *L. monocytogenes* were resolved. Further, the exporter was required to provide assurance that future entries would not contain *L. monocytogenes* and to have testing of a minimum of five cheese shipments into the United States not yield *L. monocytogenes*. Additional recalls were issued by stores that had received Marte Brand Frescolina Ricotta Salata, including grocery chain A. Recall audit checks found the recall was effective. Recalls of the other cheeses that yielded the outbreak strain during the investigation also occurred. The outbreak strain was not recovered from environmental samples obtained in the United States.

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

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

* 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.

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) ($P = 0{\cdot}18$).

One patient with a single exposure to Marte Brand Frescolina Ricotta Salata (patient no. 6, Table 3) consumed an estimated 55 g. However, the production date or sell-by date of the cheese was unknown, and because *L. monocytogenes* can grow rapidly in soft cheese over time, we were unable to estimate the dose present in the cheese at the time it was consumed.

DISCUSSION

Molecular surveillance for *L. monocytogenes* detected a complex foodborne disease outbreak associated with

Table 3. *Estimated incubation period for patients who consumed Marte Brand Frescolina Ricotta Salata or only one type of another cut and repackaged cheese (n = 9)*

Patient	Age (years)	Pregnancy-associated?	Clinical syndrome	Outcome	Type of cheese consumed	No. of days consumed	Estimated incubation period (days)*
1	68	No	Meningitis	Survived	l'Édel de Cléron, blue†	⩽13	12
2‡	86	No	Bacteraemia	Died	Ricotta salata§ or cross-contaminated menu item	2	7
3	65	No	Meningitis	Survived	Blue	⩽7	7‖
4	82	No	Bacteraemia	Survived	Brie		26
5	56	No	Bacteraemia	Survived	Ricotta salata§	3	2
6	30	No	Meningitis	Survived	Ricotta salata§	1	3
7	38	Yes	–	Infant died	Ricotta salata§	⩽7	14
8	29	Yes	–	Delivered healthy baby	Ricotta salata§		8
9	29	Yes	–	Delivered healthy baby	Ricotta salata§	2	3

* 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.

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

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' illnesses. 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 investigation'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 several 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 illness and consumption of soft cheeses found early in the investigation could have been a result of confounding (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 listeriosis outbreaks in Europe, North America, and elsewhere [20–25]. In 1985, Mexican-style soft cheese caused 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 relatively high water activity ($a_w > 0{\cdot}94$). *L. monocytogenes* has been isolated from other imported Italian cheeses, including Ricotta Piatta, Talleggio, and unnamed 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 period 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-pregant (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 median incubation period in non-pregnant patients who ate the ricotta salata compared with those who consumed another single cut and repackaged cheese. This suggests that larger numbers of *L. monocytogenes* cells likely contaminated the ricotta salata (i.e. ingestion of more cells decreases the time needed to overwhelm the immune system) [33], compared to cross-contaminated cheeses that may have been inoculated 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 incubation period, although such information was not routinely collected for all patients. For example, a 56-year-old patient known to have a history of leukaemia 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

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 uninoculated 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 $\log_{10}$, 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 $\log_{10}$ 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 cheese-manufacturing 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 repackaging 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.

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DECLARATION OF INTEREST

None.

REFERENCES

1. **Painter J, Slutsker L.** Listeriosis in humans. In: Ryser ET, Marth EH, eds. Listeria, Listeriosis, and Food Safety, 3rd edn. Boca Raton: CRC Press, 2007, pp. 85–110.
2. **Scallan E, *et al.*** Foodborne illness acquired in the United States – major pathogens. *Emerging Infectious Diseases* 2011; **17**: 7–15.
3. **Mook P, O'Brien SJ, Gillespie IA.** Concurrent conditions and human listeriosis, England, 1999–2009. *Emerging Infectious Diseases* 2011; **17**: 38.

4. **Goulet V, *et al.*** Incidence of listeriosis and related mortality among groups at risk of acquiring listeriosis. *Clinical Infectious Diseases* 2012; **54**: 652–660.
5. **Centers for Disease Control and Prevention (CDC).** Vital signs: *Listeria* illnesses, deaths, and outbreaks – United States, 2009–2011. *Morbidity and Mortality Weekly Report* 2013; **62**: 448–452.
6. **Mead P, *et al.*** Nationwide outbreak of listeriosis due to contaminated meat. *Epidemiology and Infection* 2006; **134**: 744–751.
7. **Olsen SJ, *et al.*** Multistate outbreak of *Listeria monocytogenes* infection linked to delicatessen turkey meat. *Clinical Infectious Diseases* 2005; **40**: 962–967.
8. **Linnan MJ, *et al.*** Epidemic listeriosis associated with Mexican-style cheese. *New England Journal of Medicine* 1988; **319**: 823–828.
9. **McCollum JT, *et al.*** Multistate outbreak of listeriosis associated with cantaloupe. *New England Journal of Medicine* 2013; **369**: 944–953.
10. **Gaul LK, *et al.*** Hospital-acquired listeriosis outbreak caused by contaminated diced celery – Texas, 2010. *Clinical Infectious Diseases* 2013; **56**: 20–26.
11. **Ryser ET, Marth EH.** *Listeria, Listeriosis, and Food Safety*. Boca Raton: CRC Press, 2007, pp. 405–503.
12. **US Food and Drug Administration, Health Canada.** Quantitative assessment of the risk of listeriosis from soft-ripened cheese consumption in the United States and Canada: Draft report (http://www.fda.gov/downloads/food/foodscienceresearch/ucm338617.pdf), 2012.
13. **Jackson K, *et al.*** Multistate outbreak of *Listeria monocytogenes* associated with Mexican-style cheese made from pasteurized milk among pregnant, Hispanic women. *Journal of Food Protection* 2011; **74**: 949–953.
14. **Choi MJ, *et al.*** Notes from the field: Multistate outbreak of listeriosis linked to soft-ripened cheese – United States, 2013. *Morbidity and Mortality Weekly Report* 2014; **63**: 294–295.
15. **Graves LM, *et al.*** Microbiological aspects of the investigation that traced the 1998 outbreak of listeriosis in the United States to contaminated hot dogs and establishment of molecular subtyping-based surveillance for *Listeria monocytogenes* in the PulseNet network. *Journal of Clinical Microbiology* 2005; **43**: 2350–2355.
16. **Halpin JL, *et al.*** Re-evaluation, optimization, and multilaboratory validation of the PulseNet-standardized pulsed-field gel electrophoresis protocol for *Listeria monocytogenes*. *Foodborne Pathogens and Disease* 2010; **7**: 293–298.
17. **MacDonald PD, *et al.*** Outbreak of listeriosis among Mexican immigrants as a result of consumption of illicitly produced Mexican-style cheese. *Clinical Infectious Diseases* 2005; **40**: 677–682.
18. **US Food and Drug Administration.** Compliance Program for Domestic and Imported Cheese and Cheese Products. 1998 (http://www.fda.gov/Food/ComplianceEnforcement/FoodCompliancePrograms/ucm071496.htm). Accessed 20 August 2014.
19. **Hitchins AD, Jinneman K.** Detection and enumeration of *Listeria monocytogenes* in Foods. In: *Bacteriological Analytical Manual*, 8th edn, Revision A, 1998. Chapter 10 (http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm071400.htm#). Accessed 20 August 2014.
20. **Cartwright EJ, *et al.*** Listeriosis outbreaks and associated food vehicles, United States, 1998–2008. *Emerging Infectious Diseases* 2013; **19**: 1–9; quiz 184.
21. **Bille J, *et al.*** Outbreak of human listeriosis associated with tomme cheese in northwest Switzerland, 2005. *Eurosurveillance* 2006; **11**: 91–3.
22. **McLauchlin J, Greenwood MH, Pini PN.** The occurrence of *Listeria monocytogenes* in cheese from a manufacturer associated with a case of listeriosis. *International Journal of Food Microbiology* 1990; **10**: 255–262.
23. **Goulet V, *et al.*** Listeriosis from consumption of raw-milk cheese. *Lancet* 1995; **345**: 1581.
24. **Koch J, *et al.*** Large listeriosis outbreak linked to cheese made from pasteurized milk, Germany, 2006–2007. *Foodborne Pathogens and Disease* 2010; **7**: 1581–1584.
25. **Fretz R, *et al.*** Listeriosis outbreak caused by acid curd cheese 'Quargel', Austria and Germany 2009. *Eurosurveillance* 2010; **15**: 2–3.
26. **Gaulin C, Ramsay D, Bekal S.** Widespread listeriosis outbreak attributable to pasteurized cheese, which led to extensive cross-contamination affecting cheese retailers, Quebec, Canada, 2008. *Journal of Food Protection* 2012; **75**: 71–78.
27. **Spanu C, *et al.*** *Listeria monocytogenes* growth potential in Ricotta salata cheese. *International Dairy Journal* 2012; **24**: 120–122.
28. **Timbo BB, Keys C, Klontz K.** Characterization of *Listeria monocytogenes* recovered from imported cheese contributed to the National PulseNet Database by the US Food and Drug Administration from 2001 to 2008. *Journal of Food Protection* 2010; **73**: 1511–1514.
29. **Genigeorgis C, *et al.*** Growth and survival of *Listeria monocytogenes* in market cheeses stored at 4° to 30° C. *Journal of Food Protection* 1991; **34**: 662–668.
30. **Papageorgiou DK, Marth EH.** Fate of *Listeria monocytogenes* during the manufacture and ripening of blue cheese. *Journal of Food Protection* 1989; **52**: 459–465.
31. **Goulet V, *et al.*** What is the incubation period for listeriosis? *BMC Infectious Diseases* 2013; **13**: 11.
32. **Johnsen BO, *et al.*** A large outbreak of *Listeria monocytogenes* infection with short incubation period in a tertiary care hospital. *Journal of Infection* 2010; **61**: 465–470.
33. **Buchanan RL, Smith JL, Long W.** Microbial risk assessment: dose-response relations and risk characterization. *International Journal of Food Microbiology* 2000; **58**: 159–172.
34. **Vorst KL, Todd EC, Ryser ET.** Transfer of *Listeria monocytogenes* during slicing of turkey breast, bologna, and salami with simulated kitchen knives. *Journal of Food Protection* 2006; **69**: 2939–2946.
35. **Hoelzer K, *et al.*** Estimation of *Listeria monocytogenes* transfer coefficients and efficacy of bacterial removal through cleaning and sanitation. *International Journal of Food Microbiology* 2012; **157**: 267–277.