Widespread Listeriosis Outbreak Attributable to Pasteurized Cheese, Which Led to Extensive Cross-Contamination Affecting Cheese Retailers, Quebec, Canada, 2008

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ABSTRACT

A major Listeria monocytogenes outbreak occurred in the province of Quebec, Canada, in 2008, involving a strain of L. monocytogenes (LM P93) characterized by pulsed-field gel electrophoresis (PFGE) and associated with the consumption of pasteurized milk cheese. This report describes the results of the ensuing investigation. All individuals affected with LM P93 across the province were interviewed with a standardized questionnaire. Microbiological and environmental investigations were conducted by the Quebec’s Food Inspection Branch of Ministère de l’Agriculture, des Pêcheries et de l’Alimentation du Québec among retailers and cheese plants involved in the outbreak. Between 8 June and 31 December 2008, 38 confirmed cases of LM P93 were reported to public health authorities, including 16 maternal-neonatal cases (14 pregnant women, and two babies born to asymptomatic mothers). The traceback of many brands of cheese that tested positive for LM P93 collected from retailers identified two cheese plants contaminated by L. monocytogenes strains on 3 and 4 September. PFGE profiles became available for both plants on 8 September, and confirmed that a single plant was associated with the outbreak. Products from these two plants were distributed to more than 300 retailers in the province, leading to extensive cross-contamination of retail stock. L. monocytogenes is ubiquitous, and contamination can occur subsequent to heat treatment, which usually precedes cheese production. Contaminated soft-textured cheese is particularly prone to bacterial growth. Ongoing regulatory and industry efforts are needed to decrease the presence of Listeria in foods, including pasteurized products. Retailers should be instructed about the risk of cross-contamination, even with soft pasteurized cheese and apply methods to avoid it.

Listeria monocytogenes is a ubiquitous pathogen, capable of causing infection in humans and animals (28). Although uncommon in humans, it occurs in sporadic and epidemic forms throughout the world (21, 31). Several large epidemics of human listeriosis have been reported, but no source of the organism was demonstrated until 1981, when an association between epidemic disease and the consumption of contaminated coleslaw was shown (11, 31). A common source of pasteurized milk was found to be associated with epidemic disease in an outbreak in Massachusetts in 1983 (3). After a 1985 L. monocytogenes outbreak in California resulting from the consumption of inadequately pasteurized cheese, listeriosis became a concern for the food industry (6). Since then, L. monocytogenes has been involved in many outbreaks linked to food items such as dairy products, deli meat, fish, and seafood (1, 9, 16, 22, 30–35).

Unpasteurized dairy products can carry a markedly elevated risk, and it is recommended that vulnerable individuals refrain from consuming such products (12, 25, 28). The L. monocytogenes risks associated with products made from pasteurized milk have been reported less frequently (3, 8, 18), but recently, European food safety alerts because of L. monocytogenes–contaminated cheese has been related more frequently to products made from pasteurized or heat-treated milk rather than from raw milk (18).

This report examines a province-wide listeriosis outbreak in the year 2008, linked to a commercial cheese made from pasteurized milk. The outbreak generated extensive cross-contamination among cheese retailers. Consequently, the Quebec’s Food Inspection Branch of Ministère de l’Agriculture, des Pêcheries et de l’Alimentation du Québec (MAPAQ) conducted one of the largest food recall ever carried out in the province.

On 19 August 2008, the Laboratoire de santé publique du Québec (LSPQ) detected by pulsed-field gel electrophoresis (PFGE) a cluster of three human cases of L. monocytogenes. This PFGE profile was designated as LM P93, according to the Quebec nomenclature, and LMACI.0149–LMAAI.0265, according to the PulseNet
Canada database. This profile had not been seen before in the other Canadian provinces and was considered a rare *L. monocytogenes* profile. The LM P93 subtype is 1/2a.

An investigation was then initiated by a team of epidemiologists at the ministère de la Santé et des Services sociaux (MSSS) in Quebec and the MAPAQ’s Food Inspection Branch. All public health authorities in Quebec were asked to interview and promptly report all listeriosis cases and immediately submit the strain to the PFGE analysis done by the LSPQ. Thereafter, cases were classified by the PFGE profile when available.

All *L. monocytogenes* cases were reported to public health authorities as well as to the MAPAQ. After these notifications, some cheese was collected from the first three LM P93 cases at home or from retailers. Between 22 and 26 August 2008, the MAPAQ confirmed the presence of LM P93 in two raw milk cheeses and one cheese made with pasteurized milk, all collected from different retailers. Both affected raw milk cheeses were produced by the same plant, but under different brands. All three cheeses (raw milk and pasteurized) were made and sold only in the province of Quebec. Isolation of LM P93 from three different brands of cheese collected from three different retailers led public health authorities to suspect broader contamination by one of these products or by another possible source, requiring urgent identification.

The information gathered from LM P93 cases and by the MAPAQ’s food inspectors was used to trace two cheese plants contaminated by *L. monocytogenes*. On 3 September, cheese plant B was first identified as contaminated after *L. monocytogenes* was isolated from an intact wheel of cheese taken from a retailer where an LM P93 case had purchased cheese. Cheese plant A was identified after notification from a sick consumer who had purchased some cheese at the store adjacent to the plant. Environmental and cheese samples collected from the store on 29 August tested positive for *L. monocytogenes* on 4 September 2008. On 8 September 2008, LM P93 was confirmed in soft washed-rind cheese made with pasteurized milk from plant A only. Unrelated *L. monocytogenes* strains were identified in both cheese plants.

This article summarizes the clinical, epidemiological, and laboratory information obtained during this investigation and the reasons that supported the recall in the province of Quebec.

**MATERIALS AND METHODS**

**Case definition.** A case was defined as a person with onset of illness between 8 June and 31 December 2008, in association with isolation of LM 93 from a sterile site, and was a Quebec resident or a person who visited the province within 70 days preceding the onset of symptoms.

Cases were then classified as maternal-neonatal when the strain was isolated from a pregnant woman, a newborn, or a fetus. If the LM P93 strain was isolated from the mother and her baby, it was counted as one case. The pregnancy outcome was classified according to gestational age as determined by the World Health Organization’s *International Statistical Classification of Disease, 10th Revision: preterm delivery (<37 weeks of gestation), term delivery (≥37 weeks of gestation), and fetal death (death of a fetus before expulsion, regardless the gestational age).*

Cases were interviewed for potential risk factors including underlying conditions, medications, and food exposures during the month prior to illness. If a patient died or could not be interviewed, we attempted to identify a surrogate (most often the person’s spouse). Even if the source of contamination was identified early, all subsequent LM P93 cases notified after 5 September were interviewed to determine the level of cross-contamination in this outbreak.

**MAPAQ’s Food Inspection Branch.** Food samples were collected with official MAPAQ’s procedures (5–7, 19, 27). Samples collected were analyzed with standardized methods (MFHPB-30 and MFHPB-07) either at the Direction du laboratoire d’expertise d’analyses alimentaires, MAPAQ’s food analysis laboratory, or at the City of Montreal’s Food Inspection Service Laboratory, depending on the specimen’s origin (26, 37). These methods are Health Canada’s standard reference cultural methods. Samples came from homes of the cases, retailers, cheese plants, and dairy herds. In homes, food items, including various brands of cheese, and environmental samples were collected from the refrigerators of several LM P93 cases. Some food samples were collected at the retailer level when food items were not available for analysis from the home of the cases. More than one retailer might have been identified by LM P93 case. MAPAQ’s food inspectors visited these retailers and collected the same food items (primarily cheese) consumed by the LM P93 cases. Cheese plant A operates a store adjacent to the plant where products are sold directly to the consumer. Cheese and environmental samples were taken directly from the store. Cheese plants A and B were also visited by the MAPAQ’s food inspectors. Noncompliance reports, production and construction records, and staff-kept records of product and environmental sampling for *Listeria* spp. were reviewed. Time and temperature records for pasteurizer operation at cheese plant A were reviewed as well. Samples of all brands of cheese, all ingredients, and the plant environment were collected for culture, and records of deliveries and shipments were reviewed at both plants. Additionally, dairy herds providing milk to cheese plants A and B were visited. Milk samples were collected from the bulk tanks and from each lactating cow, along with water and silage samples.

**LSPQ.** PFGE was performed according to the PulseNet standardized protocol, with restriction enzymes Ascl and Apal (2). PFGE profiles were designated with BioNumerics (Applied Maths, Sint-Martens-Latem, Belgium) software and then submitted for comparison to the PulseNet (Canada database). As *L. monocytogenes* strains were prioritized by LSPQ, the laboratory provided evening and weekend PFGE service to facilitate outbreak investigations. As a result, the turnaround time for PFGE analysis for most strains was reduced to 2 days after receipt. PFGE results obtained from clinical, food, and environmental strains were immediately reported to public health authorities MAPAQ and MSSS. Between 8 June and 31 December 2008, LSPQ conducted PFGE analyses of 75 environmental and 218 food strains received as part of outbreak investigations.

**RESULTS**

**Epidemiological investigation.** Between 8 June and 31 December 2008, 38 confirmed cases of LM P93 (Fig. 1) displayed symptoms that met the case definition. They or their surrogates were all interviewed. Among all cases, two came from Ontario, and both visited the province of Quebec in the weeks preceding onset of their symptoms.
In total, 16 (42%) of 38 cases were maternal-neonatal cases, including 14 pregnant women and two neonates born to an asymptomatic mother. The characteristics of the maternal-neonatal and non-maternal-neonatal cases are described in Table 1. Eleven pregnant women delivered prematurely. Three delivered at term: 2 had acquired listeriosis before they were 20 weeks pregnant, had been hospitalized and treated with antibiotics, and had pregnancies with normal outcomes; 1 delivered at term, without symptoms. We observed three fetal deaths among pregnant women who delivered before 26 weeks of pregnancy (median gestational age, 21 weeks; range, 20 to 25 weeks).

Twenty-two adult cases were not related to pregnancy. The median age was older than that of the maternal-neonatal cases. Only one case (28 years old) had no underlying conditions. Two Quebecers, aged 78 and 80, respectively, died. No deaths were observed among the Ontario cases.

Information about cheese consumption was available for 37 LM P93 cases. Interviews revealed that 16 (43.2%) of 37 cases including seven pregnant women had consumed cheese made by cheese plant A, notably a washed-rind cheese made with pasteurized milk and bought exclusively from retail stores. The same proportion (43.2%) of cases had not consumed any cheese from cheese plant A, but had bought cheese from retailers selling products from cheese plant A, including a washed-rind cheese involved in this outbreak. Five (13.5%) of 37 case patients remembered having consumed cheese, but did not recall the cheese brand names or where the cheese had been purchased. Both Ontario cases had consumed cheese from Quebec, and 1 of them had consumed cheese at a hotel (hotel A) located in Quebec City. The MAPAQ's food inspectors visited this hotel and collected cheese-plate samples, which tested positive for LM P93.

MAPAQ's food investigation at the cheese plants A and B, the dairy herds, the retailers, and the homes of

![Epidemic curve of Listeria monocytogenes PFGE profile 93 cases by onset of symptoms, CDC weeks, and provinces of Quebec and Ontario, 2008 (n = 38).](image-url)
the cases. MAPAQ’s food inspectors visited cheese plants A and B, the two dairy herds providing milk to each plant, 74 retailers including the store adjacent to the plant A, one distributor, and 12 homes where food was still available for analysis. In total, 764 cheese samples from numerous brands were collected from different locations and analyzed, along with 1,572 environmental samples. The results are summarized in Table 2.

Cheese plant A makes seven varieties of cheese: six are made with pasteurized milk (two soft washed rind, two spread, and two Cheddar) and one with raw milk. This plant sells cheese directly to customers from a store adjacent to the cheese plant and to 50 retailers in Quebec. Samples collected from the dairy herd providing milk to the plant were all negative. The pasteurization process was adequate. Among 114 wheels of cheese collected at the plant, including soft washed-rind and Cheddar, 17 (14.9%) of 114 tested positive for \( L. \) monocytogenes. Of the sampled cheese, primarily the soft washed-rind cheese from different batches tested positive for LM P93. The whole wheels of cheese were enumerated for \( L. \) monocytogenes, and over \( 10^6 \) CFU/g were found. Microbiological analysis did not distinguish between the interior and the surface. Both soft washed-rind cheeses matured in the same aging room. The same brine solution was being used for both cheeses and might have been used for many days without renewing it. In addition, two samples of a raw milk cheese tested positive with an unrelated \( L. \) monocytogenes strain. This cheese did not mature in the same room as the soft washed-rind cheese.

Among environmental samples collected in multiple locations, 12 (4.9%) of 246 tested positive for \( L. \) monocytogenes, and 5 of 12 tested positive for LM P93. Five LM P93–positive samples were collected at different locations in the plant.

Cheese plant B makes eight varieties of cheese. All of them are made with raw milk (six hard and two semihard). This cheese is distributed to 300 retailers in the province of Quebec only. Among wheels of cheese collected from hard and semi-hard, 10 (7.0%) of 142 tested positive for \( L. \) monocytogenes, but none of them were related to the outbreak strain. Among environmental samples, only 1 of 129 collected from a floor drain tested positive for an unrelated strain of \( L. \) monocytogenes.

\( L. \) monocytogenes was found in the facilities of 29 (39.2%) of 74 retailers in 11 regions of Quebec, with the isolates typed as P93 in 22 (75.9%) of the 29 stores. For a retailer to be considered as contaminated with \( L. \) monocytogenes, at least one sample taken from the environment or from cheese tested positive for \( L. \) monocytogenes. In total, 66 (13.8%) of 479 of the cheese sampled were contaminated by \( L. \) monocytogenes. LM P93 was identified for 58 (87.9%) of 66, including 24 (85.7%) of 28 washed-rind cheeses made by the cheese plant A and collected at the retail stores, and 34 (7.5%) of 451 cheeses identified from 25 additional varieties of cheese. These additional 25 cheeses were made with pasteurized or unpasteurized milk and were produced by others plants. This findings showed extensive cross-contamination at the retailer level, and suggested that the initial source of the contamination was upstream.

Some packaged cheese of various brands positive for LM P93 taken from retailers was enumerated for \( L. \) monocytogenes. A level of 50 to 300 bacteria CFU/g was found in these samples. This level was lower than the level observed on the surface of the washed-rind cheese taken directly from cheese plant, A which was over \( 10^6 \) CFU/g. Among environmental samples collected at retailers on numerous surfaces (knives, cutting boards, cheese plates, packers, counters, refrigerator handles, etc.), 41 (4.1%) of 997 tested positive for \( L. \) monocytogenes, and among them, 29 (70.7%) of the 41 tested positive for LM P93, suggesting that the practices used at the retailer level supported potential for environmental contamination.

Seventeen unrelated \( L. \) monocytogenes strains taken from retailers and cheese plants A and B were identified during this investigation. No human cases were knowingly associated with these profiles.

All cheese collected from the homes of the cases had been opened. Among the samples, 5 (17.2%) of 29 tested positive for LM P93. Other food items were also collected, including deli meat, hot dogs, and processed vegetables. No \( L. \) monocytogenes strains were found in those samples.

MAPAQ’s intervention. Prior to the identification of cheese plants A and B and after the identification of contaminated cheese collected from retailers, numerous recalls were issued. The identification of \( L. \) monocytogenes strains in both plants on 3 and 4 September led to plant shutdowns and a review of their operations by the MAPAQ. A risk assessment done by the MAPAQ resulted in a voluntary recall on 5 September of all products made by these two cheese plants, and the elimination of all products that might have been cross-contaminated by either plant. As a result, 364 retailers who had received products from either plant had to dispose of their stock. Moreover, retailers were required to dispose of all cheese that had been opened on site in order to eliminate products that might have been cross-contaminated (including cheese made by other companies). Only intact wheels of cheese made by other companies were preserved.

There were three reasons for this important decision: First, the presence of \( L. \) monocytogenes in ready-to-eat foods with a shelf life of over 10 days is unacceptable in Quebec and in Canada (15, 29). This explains why products of both cheese plants A and B were included in the recall on 5 September, even though the outbreak strain was later found only in cheese plant A. Second, public health concerns related to increasing and as-yet uncontrolled threat as the number of clinical cases continued to rise. Third, analysis of all cheese on the market in the face of apparent extensive cross-contamination among retailers was simply unfeasible.

DISCUSSION

The initial contamination of numerous brands of cheese found at the retailer level also involved a rare \( L. \) monocytogenes strain (LM P93); both factors led public health authorities to investigate upstream. The traceback information obtained by the MAPAQ’s food inspectors
<table>
<thead>
<tr>
<th>Collection site</th>
<th>No. of samples</th>
<th>LM$^+$, no. (%)</th>
<th>LM P93$^+$ among LM$^+$, no. (%)</th>
<th>Other PFGE profiles among LM$^+$, no. (%)</th>
<th>No. of samples</th>
<th>LM$^+$, no. (%)</th>
<th>LM P93$^+$ among LM$^+$, no. (%)</th>
<th>Other PFGE profiles among LM$^+$, no. (%)</th>
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<tbody>
<tr>
<td>Cheese plant A</td>
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<td>Dairy herds providing milk to cheese plant A</td>
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<tr>
<td>Lactating cow</td>
<td>114</td>
<td>17 (14.9)</td>
<td>15 (88.2)</td>
<td>2 (12.8)</td>
<td>246</td>
<td>12 (4.9)</td>
<td>5 (41.7)</td>
<td>7 (58.3)</td>
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<td>Bulk tank</td>
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<td></td>
<td>111</td>
<td>0</td>
<td>0</td>
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<td>Cheese plant B</td>
<td>142</td>
<td>10 (7.0)</td>
<td>0</td>
<td>10 (100)</td>
<td>129</td>
<td>1 (0.8)</td>
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<td>1 (100)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>63</td>
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<tr>
<td>Lactating cows</td>
<td></td>
<td></td>
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<td>2</td>
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<td>Bulk tanks</td>
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<td>1</td>
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<tr>
<td>Retailers (n = 74)</td>
<td>479</td>
<td>66 (13.8)</td>
<td>58 (87.9)</td>
<td>8 (12.1)</td>
<td>997</td>
<td>41 (4.1)</td>
<td>29 (70.7)</td>
<td>12 (29.3)</td>
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<tr>
<td>Distributor (n = 1)</td>
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<tr>
<td>Homes of the cases (n = 12)</td>
<td>29</td>
<td>5 (17.2)</td>
<td>5 (100)</td>
<td>0</td>
<td>8</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>Total</td>
<td>764</td>
<td>98 (12.8)</td>
<td>78 (79.6)</td>
<td>20 (20.4)</td>
<td>1,572</td>
<td>54 (4.0)</td>
<td>34 (62.5)</td>
<td>20 (37.5)</td>
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</table>
identified cheese plant A as the likely source of the contamination. Only cheese plant A’s soft washed-rind cheese made with pasteurized milk were identified as the likely source of this outbreak. This cheese was distributed to many retail stores in the province of Quebec. Some practices applied at the retail stores enabled the cross-contamination of other cheese during the cutting or packaging process.

This was the largest listeriosis outbreak in Quebec linked to a contaminated commercial cheese made from pasteurized milk. The majority of cases with available specific epidemiological or microbiological information were linked to the cheese plant A. The outbreak was widely dispersed and lasted several weeks, reflecting the long incubation period and the long shelf life of listeriosis.

Where could the initiating source of the contamination have come from? None of the cows providing milk to the cheese plant A or farm environmental samples were positive for L. monocytogenes. The pasteurization process at cheese plant A was adequate. L. monocytogenes is primarily an environmental pathogen. The primary hypothesis is that the contamination occurred during the postpasteurization process, and the brine solution used for both cheeses might be the most likely source of the contamination, explaining the extensive contamination of both soft washed-rind cheese at plant A and the contamination found at the retailer level. During the aging process, both cheeses were being washed frequently with the same brine solution. The solution might have been used for many days without renewing it. Knowing that the cheese plant’s environment was contaminated by LM P93, L. monocytogenes could have contaminated the solution via the environment. Using a possibly contaminated brine solution for many days might have spread LM P93 to many wheels of cheese. This is similar to a situation in British Columbia, where a ripened pasteurized milk cheese was contaminated by ripening solution sprayed in the aging room in 2002 (24). Unfortunately, no brine solution samples were collected from cheese plant A for microbiological analysis during the initial MAPAQ’s intervention on 5 September.

The risk to cheese safety might come from postpasteurization environmental contamination from the cheese making and/or aging process (17, 25). Contaminated soft-textured cheese is particularly prone to bacterial growth (4, 16, 18). If the contamination occurred during storage, the bacteria would have experienced good growing conditions. Indeed, soft cheese might be an especially effective vehicle for Listeria, a halotolerant organism, because is commonly treated with brine in the production process, which leads to high sodium chloride concentration, which could inhibit competing organisms. As well, soft cheese sold as fresh cheese (i.e., Mexican cheese) never acquires a strongly acidic pH that might discourage bacterial growth (16, 20, 22).

Many other nonoutbreak L. monocytogenes strains were found in both the plant environment and its products, demonstrating the likelihood of ongoing contamination of products within the plant. These nonoutbreak strains were never associated with human illness. It is well known than more than 95% of human L. monocytogenes cases are caused by only three subtypes: 1/2a, 1/2b, and 4b (13, 23, 33). As previously noted, the LM P93 subtype is 1/2a.

According to MAPAQ and Health Canada’s policies, the presence of L. monocytogenes in ready-to-eat foods with a shelf life of over 10 days is unacceptable in Quebec and in Canada (15, 29). As soon as L. monocytogenes is confirmed in products matching these criteria, a recall is initiated. The PFGE profile is used as a complement of information, and it does not influence the recall decision. The recall and elimination of all products made by both plants and the elimination of all products that might be exposed to contaminated products was thus necessary.

Because even soft cheese made with pasteurized milk has potential risk to be contaminated by L. monocytogenes, all the practices employed by retailers when cutting and packaging cheese for individual sale were reviewed with merchants.

In this outbreak, the mortality rate (5%) was low; this could be attributed to the high proportion (41.2%) of affected adults who were young, pregnant women in good health prior to their illnesses (10, 14, 23). The percentage of pregnant women affected by LM P93 was higher than observed by the Centers for Diseases Control and Prevention (36). Two previous major L. monocytogenes outbreaks involving a significant percentage (over 30%) of pregnant women have been reported in the past: one was associated with the consumption of a Mexican cheese produced from inadequately pasteurized milk (20), and the other was associated with the consumption of rillettes in France (14).

During the 2008 Quebec LM P93 outbreak, 44% of cases consumed contaminated soft washed-rind cheese made by the cheese plant A. The fact that this cheese was made with pasteurized milk might have created a feeling of safety in a vulnerable population that included pregnant women. However, the cross-contamination by LM P93 to other cheese including pasteurized milk, raw milk, and imported from many brands and made by multiples companies was so extensive that it became almost impossible to identify which products were or were not safe for the public before 5 September.

The epidemic curve showed a rapid decline in the number of LM P93 cases after the extensive recall among 364 retailers on 5 September 2008. The median incubation period of listeriosis is about 3 weeks. This recall by itself cannot explain the rapid decrease in the number of LM P93 cases immediately after it was carried out. Three weeks before the massive recall on 5 September, numerous preliminary recalls affecting some retailers were issued, and a number of joint press conferences were held by MAPAQ and public health authorities. Those warnings might have affected public confidence with respect to the consumption of cheese produced in Quebec. The recall on 5 September and the elimination of all cheese that might have been in contact with contaminated products likely played a role in the mid- and long-term outcome. Some cases were reported after the massive recall. Knowing that listeriosis has an incubation period that might be as long as 70 days, some cases were expected even after the recall on 5 September.
The proactive role of the MAPAQ in identifying food items at greater risk for contamination by L. monocytogenes and in collecting cheese samples at the beginning of this investigation contributed significantly to controlling the outbreak. Early detection of L. monocytogenes clusters by PFGE analysis under a provincial active surveillance program plays an important role in public health and in (i) detecting outbreaks, (ii) assisting epidemiological investigations to distinguish between outbreaks and sporadic cases, and (iii) identifying the source of outbreaks.

Because the pathogen is ubiquitous, contamination might occur subsequent to heat treatment, which usually precedes cheese production. Contaminated soft-textured cheese is particularly prone to bacterial growth. Ongoing regulatory and industry efforts are needed to decrease Listeria in foods, including pasteurized products. Retailers should be instructed about the risk of cross-contamination and apply methods to avoid it.

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