Prevalence, Types, and Geographical Distribution of *Listeria monocytogenes* from a Survey of Retail Queso Fresco and Associated Cheese Processing Plants and Dairy Farms in Sonora, Mexico†

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ABSTRACT

In the first part of this study, samples were collected from farms, cheese processing plants (CPPs), and retail markets located in various geographical areas of Sonora, Mexico, over a 12-month period during the summer of 2004 and winter of 2005. Four (all Queso Fresco [QF] from retail markets) of 349 total samples tested positive for *Listeria monocytogenes* (*Lm*). Of these four positive samples, three were collected in the northern region and one in the southern region of Sonora. Additionally, two were collected during the winter months, and two were collected during the summer months. For the second part of the study, a total of 39 samples from a farm, a CPP, and retail markets were collected and processed according to a combination of the Norma Oficial Mexicana NOM-143-SSA1-1995.10 method (NOM) and the U.S. Food and Drug Administration (FDA) Bacteriological Analytical Manual method, and 27 samples from these same locations were collected and processed according to the U.S. Department of Agriculture Food Safety and Inspection Service method (USDA-FSIS). The NOM-FDA method recovered the pathogen from 6 (15%) of 39 samples (one cheese and five product contact surfaces), while the USDA-FSIS method recovered the pathogen from 5 (18.5%) of 27 samples (all product contact surfaces). In addition, the 40 isolates recovered from the 15 total samples that tested positive for *Lm* grouped into five distinct pulsotypes that were ca. 60% related, as determined by pulsed-field gel electrophoresis analysis. The results of this study confirmed a 3.4% prevalence of *Lm* in QF collected from retail markets located in Sonora and no appreciable difference in the effectiveness of either the NOM-FDA or USDA-FSIS method to recover the pathogen from cheese or environmental samples.

In the United States, the association of *Listeria monocytogenes* (*Lm*) with soft Latin-style cheese has been well documented, with outbreaks reported in 1985 in California, 2000 in North Carolina, and 2003 in Texas (2, 13, 17). Despite an ongoing debate as to the definition and standard of identity for these types of cheese, there is little debate that they provide a favorable environment for the growth and survival of *Lm* because of their relatively high moisture and low acid content. Fresh cheese or "Queso Fresco" (QF) made with raw milk by artisanal techniques is very popular in the Mexican culture. In the state of Sonora located in northwestern Mexico, the average consumption of this cheese variety is 4.6 kg per person per year (25). Typically, this variety of cheese is made by small, unregulated, noncommercial processors and is sold both from door to door and in small local markets, where the cheese may or may not be stored at adequate refrigeration temperatures. Depending on the study and method used, the prevalence of *Lm* recovered from fresh cheese samples collected in the state of Sonora, ca. 2000 and 2001, ranged from about 3 to 10% (8, 15). Although the exact route of contamination of this type of cheese with *Lm* is not known, several sources have been proposed, including the use of contaminated raw milk and contaminated processing surfaces, workers, water, and air (5).

Several methods have been developed for the recovery and characterization of *Lm* from food and environmental samples. The U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) Microbiological Laboratory Guidebook (23) and the U.S. Food and Drug Administration (FDA) Bacteriological Analytical Manual (24) are routinely used by laboratories throughout the United States and around the world to recover *Lm* from foods. In addition, in Mexico, a commonly used method for recovering *Lm* from food sources is the Norma Oficial Mexicana NOM-143-SSA1-1995.10 method (NOM) (21). Isolates are frequently confirmed as *Lm* via biochemical tests and by PCR utilizing primers to the *inLA, inLB, iap,* or hlyA virulence-related genes (6, 13, 19, 26). In addition, molecular
typing methods, such as pulsed-field gel electrophoresis (PFGE) and ribotyping, are routinely used to characterize and determine the source of bacterial contamination in food processing plants and in epidemiological analyses of foodborne listeriosis (1, 12, 18).

One objective of this study was to isolate and characterize \textit{Lm} obtained from retail QF samples and from environmental and raw ingredient samples collected from farms and cheese processors located in three geographical regions of Sonora, Mexico. A second objective of this study was to determine if either the USDA-FSIS method or a combination of the NOM-FDA methods was more effective in recovering \textit{Ln} from cheese and environmental samples.

**MATERIALS AND METHODS**

Survey of retail QF and associated CPPs and farms for \textit{Lm}. A total of 349 samples were obtained from farms, cheese processing plants (CPPs), and QF from retail markets located in the cities of Agua Prieta, Cananea, Santa Ana, and Magdalena in the northern region, the cities of Empalme and Hermosillo in the central region, and the cities of Huatabampo, Obregon, and Navojoa in the southern region of Sonora over a 12-month period during the summer of 2004 and winter of 2005 (Table 1). Samples from farms located in these same three regions included udder and fecal samples collected from milking cows, raw milk from individual cows and the bulk tank, and the environment (silage, air, and trough water). Samples from CPPs included surface swabs of milk tanks, curd utensils, curd tanks, floors, aprons, drains, walls, and curd containers, as well as environmental samples of the air, workers’ hands, cheesecloth, ice, and water used in the cheese making process. Samples obtained from retail markets included QF only. In the southern region, a total of 54 cheese samples were collected from 54 retail markets (13 samples in winter and 41 samples in summer), a total of 63 samples were obtained from two different CPPs (five markets, five environmental and 10 surface samples collected from a single CPP, and 20 environmental samples obtained from one farm). Samples collected from CPPs included five different markets, five environmental and 10 surface samples collected from one CPP, and 7 environmental samples collected from one farm. The same markets, CPP, and farm were used for each of the two methods tested.

For each of the two recovery methods, up to five presumptive \textit{Lm} colonies were selected from their respective agar plates and confirmed by biochemical characterization by means of the various methods specified by the FDA *Bacteriological Analytical Manual* method and by PCR amplification of the \textit{hly}A gene prod-

**TABLE 1. Recovery of \textit{L. monocytogenes} from farm, CPP, and QF from retail markets in Sonora, Mexico\textsuperscript{a}**

<table>
<thead>
<tr>
<th>Region of Sonora:</th>
<th>North</th>
<th>Winter</th>
<th>Winter</th>
<th>South</th>
<th>Winter</th>
<th>Summer</th>
<th>Part A</th>
<th>Part B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow</td>
<td></td>
<td></td>
<td></td>
<td>Milk</td>
<td></td>
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<tr>
<td></td>
<td>0/9</td>
<td>0/9</td>
<td>0/25</td>
<td>0/22</td>
<td>0/25</td>
<td>0/17</td>
<td>0/10</td>
<td>0/3</td>
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<tr>
<td></td>
<td>0/3</td>
<td>0/3</td>
<td>0/7</td>
<td>0/15</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/1</td>
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<td></td>
<td>0/3</td>
<td>0/3</td>
<td>0/5</td>
<td>0/13</td>
<td>0/5</td>
<td>0/4</td>
<td>0/4</td>
<td>0/3</td>
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<tr>
<td>CPP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food contact surfaces</td>
<td>0/2</td>
<td>0/2</td>
<td>0/4</td>
<td>0/4</td>
<td>0/0</td>
<td>0/0</td>
<td>5/8 (10)</td>
<td>5/10 (12)</td>
</tr>
<tr>
<td>Environment</td>
<td>0/2</td>
<td>0/1</td>
<td>0/6</td>
<td>0/8</td>
<td>0/1</td>
<td>0/0</td>
<td>5/8 (10)</td>
<td>5/10 (12)</td>
</tr>
<tr>
<td>Retail markets</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheese</td>
<td>2/22 (5)</td>
<td>1/31 (4)</td>
<td>0/11</td>
<td>0/21</td>
<td>0/13</td>
<td>1/41 (3)</td>
<td>1/5 (6)</td>
<td>0/5</td>
</tr>
<tr>
<td>Total</td>
<td>2/41 (5)</td>
<td>1/49 (4)</td>
<td>0/58</td>
<td>0/83</td>
<td>0/50</td>
<td>1/68 (3)</td>
<td>6/39 (16)</td>
<td>5/27 (12)</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Positive samples/total samples (number of isolates retained). CPP, cheese processing plant.
uct. *Lm* ATCC 7644 was used as a positive control for both methods.

**Statistical analyses.** The prevalence of the pathogen based on geographical area, season, and recovery method was analyzed by the proportions test, chi-square methods, or both by the NCSS (Number Cruncher Statistical Systems) statistical analysis and graphical software program (version 6.0, NCSS, Kaysville, Utah).

**Molecular characterization.** Confirmed *Lm* isolates were further analyzed by PFGE with the *AscI* restriction endonuclease (New England Biolabs Inc., Ipswich, Mass.) in accordance with the standardized Centers for Disease Control and Prevention PulseNet protocol (11) essentially as described by Gilbreth et al. (10). Pattern images were compared by version 4.0 of the Applied Maths BioNumerics (Sint-Martens-Latem, Belgium) software package. Pattern clustering was performed by algorithms within BioNumerics, specifically the UPGMA (unweighted pair group method using arithmetic averages) and the Dice correlation coefficient with a position tolerance of 1.1%. Isolates were considered indistinguishable when the number and location of the bands were identical (7). At least one isolate representing each pulsotype was further characterized by automated ribotyping with *EcoRI* and following the manufacturer's guidelines (RiboPrinter Microbial Characterization System, DuPont, Qualicon, Wilmington, Del.).

**RESULTS**

In part A of this study, all of the environmental and surface samples obtained from farms and CPPs located in the northern, central, or southern regions of Sonora, Mexico, were negative for the presence of *Lm* by the NOM-FDA method (Table 1). However, 4 (2.9%) of 139 QF samples obtained from retail markets in these same three regions tested positive for *Lm*. Of these four positive cheese samples, three were collected from markets in the northern region, and one was from the southern region of Sonora. Additionally, two were collected during the winter months, and two were collected during the summer months. A total of 12 isolates were retained from these four positive samples.

Results for part B of this study comparing the efficiency of two recovery methods are also shown in Table 1. The results for the NOM-FDA method showed that none of the 20 environmental samples that were collected from the farm located in the city of Santa Ana, which is in the northern region of Sonora, tested positive for *Lm*. However, by this method, the pathogen was recovered from one (20%) of five of the retail QF samples and from five (63%) of eight of the CPP surface samples (utensils, curd tank, and container). The NOM-FDA method recovered the pathogen from 6 (15%) of 39 of the total samples collected. In comparison, by the USDA-FSIS method, it was not possible to recover *Lm* from the seven farm samples, the five QF samples, or the five environmental samples from the CPP; however, it was possible to recover the pathogen from 5 (50%) of 10 of the surface samples (utensils, milk tank, floor, and cheese mold) from the CPP. In total, the USDA-FSIS method recovered the pathogen from 5 (19%) of 27 of the total samples collected. For part B of the study, a total of 28 isolates were retained from the 11 *Lm*-positive samples testing positive by the NOM-FDA and USDA-FSIS methods.

Regardless of which method was used, there was no apparent difference in the recovery of *Lm* related to seasonality or geography.

**Molecular characterization of retained isolates.** A total of 40 isolates from the 15 positive samples were analyzed by PFGE. Analyses of the banding patterns revealed five distinct *AscI* pulsotypes (Fig. 1). The overall similarity among the five pulsotypes was about 60%. Pulsotypes 1, 2, 3, and 4 were distributed among 39 of the 40 isolates, whereas pulsotype 5 was displayed by only one isolate. A representative isolate from each of the five pulsotypes was further characterized by ribotyping: pulsotypes 1, 2, 3, and 4 displayed the DUP-1038 ribotype, whereas the pulsotype 5 isolate displayed the DUP-1042 ribotype (Fig. 1).

**DISCUSSION**

In the state of Sonora, located in northwestern Mexico, where making cheese with raw milk is a common practice, *Lm* was isolated from 5 (3.4%) of the 149 total QF samples tested from retail markets throughout this study. Other studies conducted in North, Central, and South America reported a prevalence of *Lm* in Latin-style cheese ranging from 2 to 41% (9, 20, 22). Several factors could have contributed to the differences reported in the prevalence of the pathogen in these studies, including the type of transport and plating media, the number of samples analyzed, the size of sample, the strain-to-strain variation, and the time from sample collection to testing.

In part B of this study, samples collected from retail markets, CPPs, and farms during the winter in northern Sonora were analyzed by the NOM-FDA and USDA-FSIS methods to determine which, if either, of these methods was more effective at recovering *Lm*. The results of this part of the study showed that there was essentially no difference between the USDA-FSIS method and the NOM-FDA method for the recovery of *Lm* from the type of samples tested. It is also significant that *Lm* was not recovered from any of the farm samples but was recovered from CPP (10 [55%] of 18) samples and QF (1 [10%] of 10) samples obtained from retail markets.

Numerous investigators have also reported finding *Lm* in environmental and cheese samples collected from processing plants and from cheese obtained at retail. Among others, Wagner et al. (27) reported a prevalence of 27.6% (50 of 182 samples) for the pathogen in environmental samples, food contact surfaces, and cheese samples collected from an Austrian CPP, and Kabuki et al. (16) reported an incidence of *Lm* of 11% (27 of 246 samples) and 6.3% (7 of 111 samples) in environmental and fresh cheese samples, respectively, collected in three Latin-style fresh CPPs located in the United States. Even though, in the present study, we were unable to recover *Lm* from samples collected at two farms, other studies have reported on their ability to recover the pathogen from farm environmental samples. As an example, Borucki et al. (3) reported an incidence of *Lm* at 43% (91 of 211 samples) on dairy farms. Additionally, other studies have demonstrated that the pathogen can be recovered from cheese samples that were pur-
FIGURE 1. Sources and subtypes of L. monocytogenes. MFS # refers to the laboratory designation for each strain. Strain # refers to the random number assigned to each sample tested (e.g., "173"), followed by a designation for each isolate recovered from each positive sample (e.g., if three isolates were retained from sample 173, the isolates would be designated 173-1, 173-2, and 173-3). Source, season, and region refer to whether the isolate was recovered from Queso Fresco ("cheese") or a food contact surface ("surface"), during the summer or winter, or from the northern or southern regions of Sonora, respectively. Pulsotype refers to each of the five distinct Ascl banding patterns generated by PFGE analyses, and ribotyping refers to either of two ribotype patterns generated by the DuPont RiboPrinter System with EcoRI.
chased from retail markets. For example, Brito et al. (4) recovered \textit{Lm} from 1 of 10 brands of a Latin-style cheese, that being "brand F" Minas Frescal cheese, and from 6 of 10 samples of brand F cheese collected from retail markets located in southeast Brazil. In general, with the possible exception of the negative farm samples, our results are essentially in agreement with other studies that reported the association of \textit{Lm} with environmental samples from CPPs and retail cheese samples.

The technique of PFGE delineated the 40 isolates from the 15 positive samples into five pulsotypes (Fig. 1). In general, multiple isolates (strains 173-2, 173-3, and 173-4; pulsotype 1) obtained from a single positive sample displayed an indistinguishable pulsotype. However, for one cheese sample, some isolates, namely strains 131-2 and 131-1, displayed pulsotypes 4 and 5 and ribotypes DUP-1038 and DUP-1042, respectively, which could indicate that there were multiple sources of contamination. Regardless, these results show the importance of analyzing multiple isolates from a single sample, because there may be more than one type of \textit{Lm} per sample and, thus, there may be multiple sources of contamination. The isolates obtained from surface samples grouped into pulsotypes 3, 4, and 5, whereas the QF isolates displayed all five pulsotypes. All samples collected during summer grouped mainly within pulsotypes 1 and 4. Relative to geographical distribution, strains displaying pulsotype I were isolated from the southern region of Sonora, whereas strains displaying pulsotypes 2, 3, 4, and 5 were isolated from the northern region. It also is interesting that strains 238-2, 238-4, 238-5, and 238-6 were isolated from cheese during the summer and displayed pulsotype 4, while the other pulsotype 4 strains, with the exception of strain 131-2, were isolated during the winter and were recovered from surface samples. Thus, we observed that some strains recovered from different samples or from different sites or seasons displayed the same pulsotype, suggesting the predominance and persistence of select strain types. Other studies have reported similar findings. For example, in a study conducted to determine the prevalence and distribution of \textit{Lm} in a smoked fish plant, similar profiles were observed for strains isolated from the final product and from the processing environment (brine injector needles and the slicing machines) (1). In another study conducted at a shrimp processing plant, Destro et al. (7) isolated \textit{Lm} displaying the same molecular profile from the plastic boxes used to transport the product and product utensils, as well as from the frozen product. In a comprehensive 3-year survey for \textit{Lm} in a CPP, the pathogen was recovered from drains, floors, and equipment samples; the isolates displayed at least four distinct ribotype patterns (14). Collectively, these studies show that finished products and environmental samples, as well as food contact surfaces both in processing plants and at retail establishments, can be sources for \textit{Lm}. Thus, it is important to improve cleaning and sanitation processes to lessen the prevalence and levels of \textit{Lm} within a CPP and at retail. Our results indicate that from an economic, public health, and production viewpoint, it is also very important to detect potential sources of \textit{Lm} at various points along the production chain and concomitantly take the proper measures to better manage the threat of listeriosis. Additional resources should be directed to prevent the contamination and dissemination of this bacterium within CPPs and at the retail level.

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