Research Note

Incidence of Listeria monocytogenes in Cheese Produced in Rio de Janeiro, Brazil

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ABSTRACT

The present study evaluated the incidence of Listeria spp. in some Brazilian cheeses obtained from retail stores in Rio de Janeiro. Of 103 samples of various types of cheese examined as recommended in the Listeria isolation protocol of the Health Protection Branch of Canada, 11 (10.68%) were contaminated by Listeria monocytogenes, 13 (12.62%) by Listeria innocua, 6 (5.83%) by Listeria grayi, and 1 (0.97%) by Listeria welshimeri. A higher incidence of L. monocytogenes was observed mainly in the homemade Minas Frescal cheeses (a Brazilian soft white cheese, eaten fresh), 7 of 17 (41.17%), followed by ripened cheeses, 3 of 53 (5.70%), and industrially manufactured Frescal (Minas and Ricotta) cheeses, 1 of 33 (3.03%). Three serotypes (1/2a, 1/2b and 4b) were observed among the strains of L. monocytogenes isolated, all of them being frequently involved in outbreaks of foodborne listeriosis and sporadic cases of the disease all over the world.

Listeria monocytogenes is ubiquitous and is now recognized as an important foodborne pathogen. Incidents of foodborne listeriosis have been reported involving both immunocompromised (30, 36) and immunocompetent (1, 18) persons. Epidemiological evidence has pointed to dairy products as alleged vehicles for some cases of foodborne listeriosis. Many outbreaks have involved pasteurized milk (15), as well as soft Swiss (4) and Mexican-style (24) cheese. Two sporadic cases of listerial meningitis were also linked to consumption of dairy products (1, 2). In Brazil, the first reports concerning Listeria spp. have focused on its occurrence in vegetables (20), sewage (19), soil (22), and humans (21). Later it was also verified in shrimp (23), meat and dairy products (10), including raw and pasteurized milk (7, 11, 27), and in a shrimp processing plant (9). Despite the importance of L. monocytogenes in dairy products, there are few studies as to the incidence of this microorganism in cheese manufactured in Brazil (7, 10, 16, 33), and so far neither clinical cases nor outbreaks of listeriosis associated with the consumption of any food have been described. Therefore, the purpose of this study was to evaluate the incidence of L. monocytogenes and other species of Listeria in different types of cheese manufactured in the city of Rio de Janeiro, Brazil.

MATERIALS AND METHODS

Sampling. A total of 103 samples of different types of cheese were examined, including 32 Gorgonzola, 2 Roquefort, 3 Ricotta, 2 Cheddar, 11 Brie, 6 Camembert and 47 Minas Frescal (17 home-made and 30 manufactured Brazilian white soft cheese, eaten fresh), purchased between March and December 1995 at five different supermarkets and two street markets in the city of Rio de Janeiro, Brazil.

Isolation and identification of Listeria spp. Twenty-five grams of each cheese sample were added to 225 ml of Listeria enrichment broth (LEB; Merck), as recommended by Health Protection Branch of Canada (34) for primary enrichment (Fig. 1). After incubation at 30°C for 24 and 48 h, 0.1 ml of the enrichment broth was transferred into 10 ml of Fraser broth (secondary enrichment) and simultaneously streaked onto plates of modified Oxford agar (MOX; Oxford agar base; Merck) supplemented with moxalactam (15 mg/liter) (Sigma) and colistin sulfate (10 mg/liter), and also onto Palcam agar (Merk) supplemented with polymyxin B (10 mg/liter), acriflavin (5 mg/liter), and ceftazidime (20 mg/liter). The plates were incubated at 35°C for 48 h, Palcam agar plates being incubated under microaerobic conditions (5% CO₂, 80% N₂ and 10% H₂). Blackened Fraser broth was plated onto palcam and MOX agars, following incubation under the same conditions previously described (Fig. 1). Ten typical colonies from each plate were tested as Listeria spp. and L. monocytogenes by microscopic examination and phenotypic analysis, as described elsewhere (29). Briefly, each typical colony was tested for Gram staining and morphology, motility, catalase reaction, ß-hemolysis on sheep blood agar plates, CAMP reaction with Staphylococcus aureus, carbohydrate fermentation (rhamnose, xylose, mannitol, mannose, and glucose) and nitrate reduction (Fig. 1).

Serotyping. Colonies identified as L. monocytogenes were serotyped according to Seeliger and Hohne (31).

RESULTS AND DISCUSSION

Owing to the importance of L. monocytogenes as a foodborne pathogen, documented by various outbreaks and
sporadic cases of human listeriosis all over the world, the importance of carrying out a prospective study on the prevalence of this microorganisms in different types of cheese is widely acknowledged.

The incidence of Listeria spp. in different types of cheese is shown in Table 1. Of the 103 cheese samples analyzed, 11 (10.68%) were contaminated by L. monocytogenes, 13 (12.62%) by L. innocua, 6 (5.83%) by L. grayi, and 1 (0.97%) by L. welshimeri. Similar rates have been reported for dairy products by other researchers (3, 10, 11, 28). However, lower rates, from 1.6 to 3.0% have been also described (5, 8, 13, 26).

Among the different types of cheese analyzed, homemade Minas Frescal showed the highest frequency of L. monocytogenes, which was in 7 of 17 (41.17%), samples, and L. innocua, in 3 of 17 (17.65%) (Table 1). These high frequencies are due to Minas Frescal usually being made from raw milk under poor hygienic conditions and not being subjected to federal health inspection. Recently, a similar frequency of L. monocytogenes (41%) was observed in a homemade cheese sold in Spain. This cheese showed a low pH, high water activity, and moisture levels that could have favored the survival of Listeria monocytogenes (25). Lower incidences have been reported in Morocco: 18% for Listeria spp. and 4% for L. monocytogenes in homemade Iben Frescal cheese samples (12). Previous research conducted in Brazil by Destro et al. (10) detected L. monocytogenes in 10% of manufactured Minas Frescal cheese, while Cass-arotti et al. (7) failed to detect Listeria spp. in 20 samples of the same type of cheese. The discrepancies between the results of these authors were due to different procedures (method and isolation media) being used and to differences in the source of their samples, which should also be considered.

Despite the lower rate of L. monocytogenes, found in 1 of 33 (3.03%) manufactured Minas Frescal and Ricotta samples, the incidence of Listeria spp., in 9 of 33 (27.27%), was relevant. Thus, the possibility exists for this bacterium to survive and grow in this kind of product, representing a potential risk to consumers. Improper pasteurization, postprocessing contamination, and/or poor hygiene in the processing plant must be responsible for the high frequency of Listeria spp. in this type of cheese. Proper processing and hygienic conditions could minimize this problem (14, 35). Another problem to be considered is the cold storage of Minas Frescal cheese. This type of cheese has a shelf life of 10 days. Despite the low incidence of L. monocytogenes in Brazilian raw milk (27), infective doses can be reached during the cold storage of the cheese.

Ripened cheeses showed a prevalence of L. monocytogenes (5.67%) and L. innocua (13.21%) similar to that of other reports (6, 17, 32). Two factors may have contributed to this rate: lactic bacteria used as starter cultures did not totally inhibit L. monocytogenes, and a rapid rise in pH during processing and ripening steps allowed the growth of the microorganisms (14). This result indicates that if L. monocytogenes is present in the raw milk, even in reduced numbers, it will be concentrated during milk coagulation and will grow during maturation of the cheese, leading to a final product containing high levels of L. monocytogenes (14).

Species of Listeria other than L. monocytogenes were isolated from many of the samples analyzed. It is important to point out that L. innocua was the most frequent species isolated, and thus its presence can very well be considered an indication of L. monocytogenes contamination.

Of 313 strains of Listeria spp. isolated, 207 were identified as L. monocytogenes. Of this total, 59.90, 27.54, and 12.56% belonged to serotypes 1/2a, 1/2b, and 4b, respectively. The latter was found in homemade and manufactured Minas Frescal cheese samples, while serotype 1/2a was found in Gorgonzola and homemade Minas Frescal samples. Serotype 1/2b was found only in ripened cheeses (Gorgonzola and Roquefort). Two serotypes (4b and 1/2a) were observed simultaneously in one homemade Minas Frescal sample. These serotypes have been isolated from the

### TABLE 1. Incidence of Listeria species in some types of Brazilian cheese

<table>
<thead>
<tr>
<th>Types of cheese</th>
<th>No. samples examined</th>
<th>No. (%) of samples positive for Listeria species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>L. monocytogenes</td>
</tr>
<tr>
<td>Homemade Minas Frescal</td>
<td>17</td>
<td>7 (41.17)</td>
</tr>
<tr>
<td>Manufactured Minas Frescal and Ricotta</td>
<td>33</td>
<td>1 (3.03)</td>
</tr>
<tr>
<td>Ripened (Gorgonzola, Brie and Roquefort)</td>
<td>53</td>
<td>3 (5.67)</td>
</tr>
<tr>
<td>Total</td>
<td>103</td>
<td>11 (10.68)</td>
</tr>
</tbody>
</table>

* Dash: no positive samples found.
majority of listeriosis cases in humans. Their distribution varies according to each geographical region. Farber and Peterkin (14) reported that serotype 4b was found in most of Europe and serotypes 1/2a, 1/2b, and 4b in the United States and Canada. In Brazil the three serotypes 4b, 1/2a, and 1/2b also predominate (21). Thus, the isolation of these serotypes in the samples analyzed in this study may indicate the probability of the occurrence of foodborne listeriosis in Brazil. However, no cheese-associated outbreaks of listeriosis have been described. More attention must be given to epidemiological investigations of listeriosis in Brazil.

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REFERENCES