Prevalence of *Listeria* spp. in Milk Sold in Different Markets of Mumbai City

R.N. Waghamare*, R.J. Zende, V.S. Waskar, A.M. Paturkar and D.S. Kulkarni

Department of Veterinary Public Health
Bombay Veterinary College, Parel, Mumbai - 400012

(Received 24.06.2012; accepted 10.02.2013)

**ABSTRACT**

Pasteurized and unpasteurized milk samples were procured from different markets in Mumbai city and analyzed for presence of *Listeria* spp. using one step enrichment procedure. The study revealed overall prevalence of *L. monocytogenes* and *Listeria* spp. amongst all the pasteurized milk samples as 0.66% and 3.33%, respectively while 5.88% unpasteurized milk samples showed presence of *L. monocytogenes* and 21.32% samples showed other *Listeria* spp. The study concluded that overall prevalence of *Listeria* spp. and *L. monocytogenes* was found to be 15.03% and 3.14%, respectively. It is therefore necessary to implement strict quality management system during collection, processing and transportation of milk to prevent public health hazard.

**Keywords:** *L. monocytogenes, Listeria* spp., milk, prevalence

**Introduction**

*L. monocytogenes* due to its ability to survive and grow over a wide range of temperature and low pH (Gahan *et al*., 1996 and Kim *et al*., 1998) has emerged as an important milk-borne pathogen which causes potential public health hazard through milk and their products. Over the last two decades *L. monocytogenes* has firmly established as an important food borne pathogen with its widespread outbreaks and sporadic cases linked to a wide variety of contaminated foods of both animal and vegetable origin (Ryser and Marth, 1999). Although, *L. monocytogenes* has been recognized as a human pathogen way back in 1929, but its recognition as a foodborne pathogen in 1981 (Ben Embarek, 1994) is relatively new. The first outbreak of food borne listeriosis was reported in Maritime Province of Canada in 1981, where Coleslaw consumption was mainly associated with illness (Schlench *et al*., 1983), while major outbreak occurred in Massachusetts by drinking of specific brand of pasteurized milk with 29% mortality (Fleming *et al*., 1985).

The incidence of listeriosis seems to be small as compared to other foodborne diseases, however the associated mortality is high at around 30% (Wing and Gregory, 2000). Major outbreaks of listeriosis in the developed countries have been related to the consumption of dairy products such as Swiss soft cheese, Mexican style cheese, chocolate milk and butter. In India too, *Listeria* spp. were isolated from raw milk and ice cream samples (Pednekar *et al*., 1997 and Bhilegaonkar *et al*., 1997). Amongst all *Listeria* spp., *L. monocytogenes* has public health significance as it is pathogenic for animal as well as human beings. *L. monocytogenes* is associated with meningio-encephalitis, septicemia and abortion in humans. Pregnant women, newborns and immunocompromised individuals were particularly susceptible to infections, however, in some instances, apparently healthy individuals developed clinical disease after ingestion of food contaminated with this pathogen (Schlench *et al*., 1983). However,
the risk due to listeriosis from contaminated milk in various regions is not evaluated in detail as annual incidence rate in India is significantly low. It seems that there is a lack of enough data on survival of *L. monocytogenes* in dairy milk samples. Thus the present study was carried out to generate data on prevalence of *Listeria* spp. in pasteurized and unpasteurized milk sold different markets in and around Mumbai city.

**Materials and Methods**

**Samples procurement**

The total of 286 milk samples comprised of unpasteurized and pasteurized milk were procured from different retail outlets and farms (organized and unorganized sectors) situated in and around Mumbai city. All the raw unpasteurized milk samples from retail outlets (*n* = 80) and farm bulk tanks (*n* = 56) were collected aseptically in UV sterilized polyethylene bags, and pasteurized milk samples (*n* = 150) of different brands of organized sector available in packaged form were also collected. Samples were immediately brought to laboratory on ice in an insulated container and stored at 4°C until processed.

**Enrichment and isolation of *Listeria* spp.**

All the pasteurized and unpasteurized milk samples were subjected to isolation of *Listeria* spp. as per method described by EN-ISO 11290 –1 (ISO, 1996). 25 ml of milk sample was inoculated in 225 ml of Demi Fraser’s broth and incubated at 30°C for 18-24 hours. Loopful of inoculum from the broth was streaked directly on Polymyxin- Acriflavine- Lithium chloride- Ceftazidime- Aesculin- Mannitol (PALCAM) and *L. monocytogenes* Differential Agar (LMD) separately and incubated at 30°C for 48 hours at 37°C for 24 hours, respectively.

**Identification of *Listeria* spp.**

The greenish-yellow glistening, iridescent and pointed colonies of about 0.5 mm diameter surrounded by diffuse black zone of aesculin hydrolysis on PALCAM agar and light blue turquoise colonies showing a halo formation on LMD agar were considered as presumptive of *Listeria* spp. The colonies showing precipitating zone due to phosphatidylinositol specific phospholipase C (PI-PLC) activity were presumed as *L. monocytogenes*.

Morphologically typical colonies were verified by Grams staining, catalase reaction, oxidase, tumbling motility at 20–25°C, MR-VP reactions, PI-PLC activity, CAMP test with *S. aureus* and *R. equi* (McKellar, R.C. 1994), nitrate reduction and haemolysis on sheep blood agar. Isolates were also subjected for sugar fermentation tests (rhamnose, xylose, mannitol and α-methyl D-mannopyranoside) for further characterization. (Cowan and Steel, 1993)

**Results and Discussion**

**Prevalence of *Listeria* spp. in pasteurized milk samples collected from organized sector**

A total of 150 pasteurized milk samples, comprised of 30, each from five different brands renamed as brand I, II, III, IV and V were analyzed. Amongst all 6 (4.00%) milk samples were positive for *Listeria* spp. of which 1 (0.66%), 1 (0.66%) and 4 (2.66%) milk samples showed presence of *L. monocytogenes*, *L. seeligeri* and *L. welshimeri*, respectively. Thus, overall prevalence of *L. monocytogenes* and *Listeria* spp. amongst all the pasteurized milk samples was found to be 0.66% and 3.33%, respectively (Table 1).

However, among all the brands tested, six samples of brand III showed presence of *Listeria* spp., where as remaining brands were negative for the *Listeria* spp. It indicates ineffective pasteurization of milk of brand III or post pasteurization contamination.

Frye et al. (2005) analyzed 5,519 pasteurized milk samples and reported very low (0.018%) prevalence of *L. monocytogenes* as compared to the present study. Moura et al. (1993) reported 0.9% prevalence rate in pasteurized milk samples for the *Listeria* spp. while none of the samples was positive for *L. monocytogenes*. Morobe et al. (2009) observed 5.3% prevalence rate in raw milk. However, Ahrabi et al. (1998) reported slightly higher incidence of *Listeria* spp. in pasteurized milk (5.00%) as compared to present study (4.00%).

The pasteurization temperature destroys *Listeria* spp. present in milk, therefore it is expected that pasteurized milk samples were devoid of the
Prevalence of *Listeria* spp. in milk

Table 1. Prevalence of *Listeria* spp. in pasteurized milk samples from organized sector

<table>
<thead>
<tr>
<th><em>Listeria</em> spp. isolated</th>
<th>Number of samples positive for <em>Listeria</em> spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Brand I (n=30)</td>
</tr>
<tr>
<td><em>L. monocytogenes</em></td>
<td>—</td>
</tr>
<tr>
<td><em>L. ivanovii</em></td>
<td>—</td>
</tr>
<tr>
<td><em>L. seeligeri</em></td>
<td>—</td>
</tr>
<tr>
<td><em>L. innocua</em></td>
<td>—</td>
</tr>
<tr>
<td><em>L. murrayi</em></td>
<td>—</td>
</tr>
<tr>
<td><em>L. welshimeri</em></td>
<td>—</td>
</tr>
<tr>
<td><strong>Total of <em>Listeria</em> spp.</strong></td>
<td>—</td>
</tr>
</tbody>
</table>

Figures in parentheses indicate percent prevalence.

Table 2. Prevalence of *Listeria* spp. in unpasteurized milk samples collected from unorganized sector

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th><em>Listeria</em> spp. isolated</th>
<th>Retail outlets (n=80)</th>
<th>Bulk tank milk (n=56)</th>
<th>Total (n=136)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>L. monocytogenes</em></td>
<td>5 (6.25)</td>
<td>3 (5.30)</td>
<td>8 (5.88)</td>
</tr>
<tr>
<td>2</td>
<td><em>L. ivanovii</em></td>
<td>1 (1.25)</td>
<td>—</td>
<td>1 (0.73)</td>
</tr>
<tr>
<td>3</td>
<td><em>L. seeligeri</em></td>
<td>9 (11.25)</td>
<td>4 (7.10)</td>
<td>13 (9.56)</td>
</tr>
<tr>
<td>4</td>
<td><em>L. innocua</em></td>
<td>2 (2.50)</td>
<td>1 (1.70)</td>
<td>3 (2.20)</td>
</tr>
<tr>
<td>5</td>
<td><em>L. murrayi</em></td>
<td>3 (3.75)</td>
<td>—</td>
<td>3 (2.20)</td>
</tr>
<tr>
<td>6</td>
<td><em>L. welshimeri</em></td>
<td>6 (7.50)</td>
<td>3 (5.30)</td>
<td>9 (6.60)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>26 (32.50)</td>
<td>11 (19.50)</td>
<td>37 (27.20)</td>
</tr>
</tbody>
</table>

Figures in parentheses indicate percent prevalence.

pathogens. In light of this the occurrence of listerial organisms in pasteurized milk samples could be attributed to improper pasteurization time-temperature combination and the post pasteurization contamination of the milk.

**Prevalence of *Listeria* spp. in unpasteurised milk samples collected from unorganized sector**

Out of 80 loose raw milk samples sold in retail outlets, 26 (32.50%) samples were found to be positive for *Listeria* spp., of which 5 (6.25%), 9 (11.25%), 6 (7.50%), 2 (2.50%), 1 (1.25%) and 3 (3.75%) samples showed presence of *L. monocytogenes, L. seeligeri, L. welshimeri, L. innocua, L. ivanovii and L. murrayi*, respectively (Table 2). Therefore, amongst all the *Listeria* spp. higher prevalence was observed for *L. seeligeri* followed by *L. welshimeri, L. monocytogenes, L. murrayi, L. innocua* and *L. ivanovii*. Carlos et al. (2001) reported 23% raw milk samples positive for *Listeria* spp. with prevalence rate as high rate of 13% for *L. monocytogenes*. Similarly, Deniz et al. (2004)
reported higher prevalence (37.90%) of Listeria spp. in raw milk as compared to present study (26.25%). The present observations are also in agreement with those of Ince et al. (1993) who reported 28% prevalence rate for Listeria spp.

From Table 2 it is evident that out of 56 raw bulk tank milk samples from four different farms, 11 (19.5%) samples were positive for Listeria spp. Out of 11, 3 samples (5.3%) were positive for L. monocytogenes. while remaining 14.2% were positive for other Listeria spp. of which 4 (7.1%), 1 (1.7%) and 3 (5.3%) samples showed presence of L. seeligeri, L. innocua, and L. welshimeri, respectively (Table -2). Similarly Parihar et al. (2007) observed prevalence of L. innocua from 1.6%, L. seeligeri from 3.3%, L. welshimeri from 1.6% and L. monocytogenes from 17.9% bulk milk samples which is similar with the other Listeria spp. except L. monocytogenes findings of present study. In the similar studies Muraoka et al. (2003) and Desmasures et al. (1997) observed prevalence of L. monocytogenes in farm raw milk as 4.9 - 7%. Lower prevalence of L. monocytogenes in bulk tank milk was reported by Adesiyun et al. (1996). Analysis of raw milk samples from 943 farms by Yoshida et al. (1998) also revealed 0.3% milk samples positive for L. monocytogenes.

Out of 136 unpasteurized milk samples analyzed from unorganized sector, 8 (5.88%) samples showed presence of L. monocytogenes and 29 (21.32%) samples revealed other Listeria spp. (Table 2). The higher incidence of Listeria spp. and L. monocytogenes observed in the present study could be due to poor sanitary conditions at the retail outlets, fluctuations in the storage temperature, use of unhygienic equipments, handling of milk by the unhygienic personnel or due to milk from animals with listeriosis or sub-clinical mastitis. Presence of L. monocytogenes in unpasteurized milk may pose great public health hazard.

The study concluded that milk samples from unorganized sector (unpasteurized milk) showed more prevalence of Listeria spp. as compared to organized sector (pasteurised milk). Therefore the control of Listeria spp. in milk samples from organized and unorganized sectors requires, strict implementation of quality management systems like HACCP and GMP at all the stages of production, processing, packaging, storage and distribution is advocated and also implementation of food regulations to improve the existing local retail shop is essential.

References
Ince, F.K. 1993. The incidence of Listeria monocytogenes in white cheeses produced in Elazig city and the investigation of
liveliness period of _Listeria monocytogenes_ in white cheeses produced in the lab. Doctoral thesis Firat University. The institute of science. 1993, Elazig.


