Public Health Significance of *Listeria monocytogenes* in Milk and Milk Products: An Overview

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**ABSTRACT**

Milk and milk products serve as important source of many disease producing microbes including *Listeria monocytogenes*, which is a Gram-positive, motile, psychotropic bacterium, and is the principal cause of listeriosis in humans and in a wide variety of animals including birds. The disease occurs in sporadic as well as in epidemic form, following the ingestion of food contaminated by this organism. In the world, it is becoming an important food-borne bacterial disease, with low incidence but high case fatality rate. *L. monocytogenes* primarily affects older, pregnant women, newborns, and adults with weakened immune systems; and it has been recovered from the soil, dust, water, sewage, decaying vegetation, etc. Raw or inadequately pasteurized milk (or milk contaminated post-pasteurization), soft cheeses, ice cream and other dairy products are important sources of *L. monocytogenes* in humans. The disease has two forms, one febrile gastroenteritis and other invasive systemic disease. The control of *Listeria* in foods relies largely on a HACCP approach and the establishment of effective critical control points in the process. As milk and milk products are important vehicles of *L. monocytogenes* and clear risk factors, it is emphasized that people susceptible for acquiring listeriosis should not consume unpasteurized milk and milk products.

**Keywords**: HACCP, *Listeria monocytogenes*, milk and milk products, pasteurization, public health.

**Introduction**

Milk is supposed to constitute a complex ecosystem for various microorganisms including bacteria. Milk products like cheese, ice cream and curd are widely consumed and market for them has existed in many parts of the world for many generations. Raw milk and other dairy products are consumed by all age groups, including those populations at risk for contracting listeriosis (Pal et al., 2012 a). The disease primarily affects older, pregnant women, newborns, and adults with weakened immune systems. However, rarely, persons without these risk factors can also be affected. Among the different species of the genus *Listeria*, *L. monocytogenes* has been known to cause listeriosis in humans and animals (Schukken et al., 2003 and Pal, 2007).

*L. monocytogenes* emergence as a food borne pathogen dates from 1980, with the occurrence of many outbreaks and sporadic cases of listeriosis associated with the consumption of contaminated foods. The increase in consumption of many types of ready-to-eat foods that are stored for fairly long periods of time, and the fact that many of these foods are consumed without properly reheating or by microwave heating, has given an edge for this pathogen to cause the disease. Any temperature abuse, even for a short time, can accelerate the growth rate. It is quite clear that many of the above conditions have given an advantage to *L. monocytogenes* to become a newly emerging food borne pathogen in many countries (Bibek and Arun, 2008). The present communication delineates the public health significance of *Listeria monocytogenes* in milk and milk products.

**Etiology**

Listeriosis is a serious illness caused by eating food contaminated with the bacterium *Listeria*, which is a Gram-positive, psychotropic, facultative anaerobic, non-sporulating, motile, small rod (Pal, 2007). It displays characteristic tumbling motility that is facilitated by the presence of peritrichous flagella. Motility is temperature dependent, showing high motility at 20-30°C when flagellar expression is maximum. Out of the ten species of genus *Listeria* (*L. monocytogenes*, *L. ivanovii*, *L. seeligeri*, *L.
in particular shedding of tract of animals and the environment, skin of the teats, L. monocytogenes (O'Donnell, 1995). In cows with mastitis, Wales (5.1%) (Siegman-Igra (5.2%); in Ireland, in 1992 (4.9%) and in England and 5.4%), and in 1998 (2.7%); in South Africa, in 1990 raw milk, in different countries; in USA, in 1987 (4.2%),

**Epidemiology**

**Reservoirs and Risk factors**

Listeria species are widespread in nature and live naturally in plants and soil environments. It can grow in a wide range of temperature and pH, and they are relatively resistant to freezing, drying, and high salt concentration (Bhilegaonkar et al., 2001). These adaptabilities enable Listeria to grow in refrigerated raw milk and in low quality silage with a pH>4.5. At high bacterial concentrations, L. monocytogenes can survive minimum HTST pasteurization (Bunning et al., 1988).

L. monocytogenes has been recovered from dust, soil, water, sewage, decaying vegetation, at least 42 species of wild and domestic mammals, and 17 avian species, crustaceans, pond trout, ticks, and flies. Among food sources milk and milk products, and uncooked vegetables, fish and shellfish, ready-to-eat meat products, ground beef, and poultry have all been found to contain the organism (Gellin and Broom, 2001). In addition, a human reservoir is suggested by isolation of the organism from human feces at rates ranging from 6% to 16% of the population at any given time (Lamont and Postlethwaite, 2001)

**Modes of Transmission**

The most common route of infection of humans is consumption of foods contaminated by L. monocytogenes (Pal, 2007). Inadequately pasteurized milk (or milk contaminated post-pasteurization), soft cheeses, ice cream and other dairy products also are important sources of L. monocytogenes (MacDonald et al., 2005; Pal et al., 2012a). Milk and milk products are considered as risk foodstuffs for L. monocytogenes (Pal et al., 2012b). Pregnant women can transmit the infection to their unborn fetuses in utero (through hematogenous spread) or during birth (Siegman-Igra et al., 2002).

**Prevalence**

L. monocytogenes has been reported several times from raw milk, in different countries; in USA, in 1987 (4.2%), in 1992 (4.1%), in 1997 (4.6%); in Canada, in 1988 (1.3%, and 5.4%), and in 1998 (2.7%); in South Africa, in 1990 (5.2%); in Ireland, in 1992 (4.9%) and in England and Wales (5.1%) (Siegman-Igra et al., 2002). The source of L. monocytogenes in raw milk is mostly the gastrointestinal tract of animals and the environment, skin of the teats, in particular shedding of Listeria into milk due to mastitis (O’Donnell, 1995). In cows with mastitis, L. monocytogenes may be shed at 10,000-20,000 cells per ml of milk, with the appearance of the milk being normal and there being no inflammation of the affected quarter. So that raw milk collection has the potential of delivering L.monocytogenes to the cheese making facility (Bunning et al., 1988).

Studies conducted in different countries have demonstrated the presence of L. monocytogenes and other Listeria species in milk (Table 1). Most reports concern raw milk but there are few reports for pasteurized milk.

**Population at risk**

Healthy adults and children occasionally get infected with L. monocytogenes, but they rarely become seriously ill. The body’s defense against L. monocytogenes is called “cell-mediated immunity” because it depends on our cells, especially lymphocytes called “T-cells.” Therefore, individuals whose cell-mediated immunity is suppressed are more susceptible to the devastating effects of listeriosis. Pregnant women naturally have a depressed cell-mediated immune system. In addition, the systems of fetuses and newborns are very immature and are extremely susceptible to these types of infections (Richard et al., 2008).

However, in most African countries, there are a few reports on Listeria and listeriosis, when compared to the Europe and USA. This could be associated with lack of awareness of laboratory technicians or lack of diagnostic facilities and limited resources together with the presence of other disease epidemics that claim more priority than listeriosis in developing countries including Ethiopia. However, nowadays there are some reports on prevalence of L. monocytogenes in different samples. For example, in Ethiopia (Addis Ababa) study conducted in 2004 showed that over all prevalence of 32.6% Listeria species out of the total 316 examined samples with high prevalence of L. monocytogenes in ice cream (19.6%) (Molla et al., 2004). Also, of the samples examined (391) in Addis Ababa in 2010, 102 (26.1%) were found to be positive for Listeria. L. monocytogenes was detected in 5.4% of the samples analyzed. It was isolated mainly from raw milk (13%) (Gebrelsadik et al., 2010).

**Outbreaks associated with milk and milk products**

Milk and milk products are one of the most frequently implicated commodities in the listeriosis outbreaks across the world. The first report of listeriosis from milk was form USA in 1983 (Fleming et al., 1985). Various milk products namely cheese (Azadian et al., 1989; Ries et al., 1990; Gilot et al., 1997; Schoder, D. et al., 2003), curd/yoghurt (Greenwood et al., m 1991), ice-cream (Ryser, 1999), butter (Lyytikainen et al., 1999; Ryser, 1999), chocolate milk (Dalton et al., 1997) etc. have been implicated in the listeriosis cases.
Table 1. Prevalence of \textit{L. monocytogenes} in raw milk samples overseas

<table>
<thead>
<tr>
<th>Country/region</th>
<th>Sample type</th>
<th>No. of samples tested</th>
<th>No.(%) positive for \textit{L. monocytogenes}</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Japan</td>
<td>Raw, farm bulk tank</td>
<td>120</td>
<td>1 (0.8)</td>
<td>Takai et al. (1990)</td>
</tr>
<tr>
<td>India</td>
<td>Raw Milk</td>
<td>180</td>
<td>7 (3.8) summe</td>
<td>Bhilegaonkar et al. (1997)</td>
</tr>
<tr>
<td></td>
<td>Raw cow milk</td>
<td></td>
<td>5.8%</td>
<td>Soni and Dubey (2013)</td>
</tr>
<tr>
<td>Europe</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>France</td>
<td>Raw, bulk tanks</td>
<td>1459</td>
<td>2.4%</td>
<td>Meyer-Broseta et al. (2002)</td>
</tr>
<tr>
<td>Scotland</td>
<td>Raw, bulk tanks</td>
<td>180</td>
<td>0 (0) autumn</td>
<td>Fenlon and Wilson (1989)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>180</td>
<td>2 (1.0) winter &lt;1 cell/ml</td>
<td></td>
</tr>
<tr>
<td>Turkey</td>
<td>Raw</td>
<td>211</td>
<td>2 (0.9)</td>
<td>Uraz and Yücel (1999)</td>
</tr>
<tr>
<td></td>
<td>Pasteurized</td>
<td>1413</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>North America</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canada</td>
<td>Raw, from bulk tanks</td>
<td>455</td>
<td>6 (1.3)</td>
<td>Farber et al. (1998)</td>
</tr>
<tr>
<td>Ontario</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>USA</td>
<td>Raw</td>
<td>124</td>
<td>15 (12.0)</td>
<td>Fleming et al. (1985)</td>
</tr>
<tr>
<td></td>
<td>Raw</td>
<td>121</td>
<td>15 (12.0)</td>
<td>Hayes et al. (2001)</td>
</tr>
<tr>
<td></td>
<td>Raw</td>
<td>650</td>
<td>27 (4.2)</td>
<td>Lovett et al. (1987)</td>
</tr>
<tr>
<td>Tennessee</td>
<td>Raw, bulk farm tanks</td>
<td>292</td>
<td>12 (4.1)</td>
<td>Rohrbach et al. (1992)</td>
</tr>
</tbody>
</table>

Clinical spectrum

Febrile gastroenteritis

The exact mechanism of gastroenteritis is not known; however, epidemiological study suggests that this form is mostly associated with healthy individuals and the infectious dose is in the range of $10^8$-$10^{10}$ cells of \textit{L. monocytogenes} (Bibek and Arun, 2008). Incubation period of this type ranges from 9-48 h after ingestion of contaminated item. Watery diarrhea (blood rarely present in the stool), fever, chills, nausea and vomiting are the main presenting feature (Lorber, 2005).

Invasive systemic disease

This form of disease is associated with immunologically challenged populations. These groups include pregnant women, unborn fetuses, infants, elderly people with reduced immunity due to diseases, and people taking special medications, such as steroids and chemotherapeutic agents to treat cancer. The infective dose in these people is considered to be about 100-1000 cells of \textit{L. monocytogenes} (Bibek and Arun, 2008).

Persistent infection allows the bacterium to infect central nervous system. It crosses blood brain barrier causing inflammation of meninges and brain stem. In pregnant women, it can pass through the placental barrier infecting the fetus. Abortion and stillbirth follows. The incubation period for invasive disease is about 2-6 weeks before the symptoms are visible (Bibek and Arun, 2008). Fever, myalgias, nausea, diarrhea, mild influenza-like illness and CNS signs are the main presenting feature (Lorber, 2005).

Diagnostic techniques

Conventional cultural method

The two-stage enrichment method for detection of \textit{L. monocytogenes} with isolation on PALCAM agar and Oxford agar (ISO, 1996) are widely used.

The CAMP reaction is useful for identifying \textit{Listeria} species. This test uses horse blood agar and streaks of hemolytic \textit{Staphylococcus aureus} and \textit{Rodococcus equi} in combination with \textit{Listeria} isolates. \textit{L. monocytogenes} and \textit{L. seeligeri} hemolytic reactions are enhanced in the zone influenced by the \textit{S. aureus} streak, while the other species remain non-hemolytic in this zone. In contrast, the hemolytic reaction of \textit{L. ivanovii} is enhanced in the zone influenced by \textit{R. equi} (Hitchins, 2002). For confirmed and specification, different standard biochemical tests can be used. The biochemical confirmatory tests can be done by picking pure colonies and transferring into the following biochemical media and broths. These are motility test medium (motility), blood agar (haemolysis), mannotol, rhamnose, galactose, xylose, Hippurate hydrolysis and xylose broths for carbohydrate fermentation testing (James et al., 2005).

Molecular methods

As molecular methods are accurate, sensitive and
specific, they are increasingly used in Identification of L. monocytogenes form foods. Various molecular methods used are DNA hybridization, polymerase chain reaction and real time PCR (RT PCR). Among these, PCR and real time PCR are now established methods for identification of Listeria monocytogenes from other non virulent Listeria spp. from foods. In the the first reported PCR for identification of L. monocytogenes, the hly sequence published by Mengaud et al. (1988) was used. The real-time PCR is a very sensitive and quantitative method for detection of pathogen and thus has emerged as most important tool for L. monocytogenes detection and quantitution in foods (Hein et al., 2001; Hough et al., 2002).

**Prevention and control**

The control of Listeria in foods relies largely on a HACCP approach and the establishment of effective critical control points in the process. The careful design and layout of processing equipment in conjunction with the implementation of regular, thorough cleaning regimes of the processing environment can significantly reduce the level of Listeria contamination in many processed foods. However, because of its ubiquitous nature it is virtually impossible to totally eliminate the pathogen from many food products. Vulnerable individuals, especially pregnant women, the elderly and the immunosuppressed are advised to avoid consuming unpasteurized dairy products to reduce the risk from listeriosis (Pal, 2007 and Richard et al., 2008).

Early detection of a listeriosis outbreak and efficient intervention are important in preventing the epidemic from continuing. Typing of food isolates and comparison with clinical isolates may also lead authorities to contaminated food processing plants. However, in addition to typing results, epidemiological evidence is needed for the incrimination of a food or a food processing plant (Lukinmaa et al., 2003).

Standards/legislation for the pasteurization of ice cream/frozen desserts adapted in various countries has an importance in reducing of listeriosis. These heat processes are more severe than HTST pasteurization because ingredients such as sugars, fat, emulsifiers and stabilizers in these products protect L. monocytogenes from heat, resulting in an increase in D-value (Richard et al., 2008).

**Conclusions**

L. monocytogenes has gained recognition as a global human pathogen because of the increasing incidence, diagnosis of infections, and also, it is widespread in nature and lives naturally in plants and soil environments and has potential to introduce food plant. It can grow in a wide range of temperature and pH. Milk and milk products are important vehicles of L. monocytogenes, regularly causing listeriosis outbreaks in different countries of the world. The consumption of raw milk or products made of raw milk has caused several listeriosis outbreaks resulting in several hundred cases. Raw milk and raw milk products are therefore, clear risk factors and people that are susceptible for acquiring listeriosis should not consume such products. Good manufacturing and hygiene practices, particularly maintaining hygiene of processing machines, are the keys in preventing L. monocytogenes contamination. It is also equally important to notice that products, which may be subjected to post processing contamination, should be properly reheated before consumption by highly immunocompromised persons in order to eliminate possible contamination. A food safety management system based on the principles HACCP with regular reviews should be developed and implemented in dairy plant.

**References**


