ISSN: 2278-4632
Vol-10 Issue-6 No. 13 June 2020

PREVALENCE OF *LISTERIA MONOCYTOGENES* AND OTHER *LISTERIA SPP*. IN RAW MILK AND MILK PRODUCTS – A REVIEW

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SUMMARY

The review highlights the chief sources of raw milk contamination with *Listeria monocytogenes*, and a permanent reservoir of the pathogen in the dairy industry. Data on studies on the distribution of *Listeria monocytogenes* in raw milk in different zones and countries were summarised and analysed. The risk from consumption of raw milk and contaminated milk products, the main preventive measures, methods of detection throughout the production chain emphasise the need of such studies.

Key words: *Listeria monocytogenes*, prevalence, raw milk

INTRODUCTION

Listeriosis caused by *Listeria monocytogenes* is an important foodborne infectious disease of humans and animals, prevalent worldwide. In the past few years listeriosis has turn out to be one of the most dangerous foodborne diseases with a high mortality rate of 20-30% (Dmowska and Osek 2010). It is the third main cause of death due to food-borne bacterial pathogens, with the fatality rates exceeding that of *Salmonella* and *Clostridium botulinum* (Ramaswamy *et al* 2007). Apart from emerging foodborne infections, listeriosis is also of leading Veterinary importance because of substantial economic losses in livestock production.

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ISSN: 2278-4632
Vol-10 Issue-6 No. 13 June 2020

Being both mesophilic and psychotropic in nature, it has the tendency to propagate at temperatures as low as 0°C, permitting its multiplication at the refrigeration temperatures and significantly increasing its ability to evade control in human foodstuffs. *Listeria* species are mostly facultative intracellular organisms found in the intracellular state within monocytes and neutrophils (Walsh *et al* 2001), causing Listeriosis.

The genus *Listeria* belongs to the class Bacilli and the order Bacillales. Earlier it contained ten species: *L. fleischmannii*, *L. grayi*, *L. innocua*, *L.ivanovii*, *L. marthi*,*L. monocytogenes*, *L. rocourtiae*, *L. seeligeri*, *L. weihenstephanensis* and *L. welshi meri*. Now seven additional new species, have been reported which includes *L.floridensis, L. aquatica, L. cornellensis, L. riparia, L. grandensis, L. booriae* and *L.newyorkensis*. Out of these seventeen species two are pathogenic *L. monocytogenes* and *L. ivanovii*. *L. ivanovii* is predominantly pathogenic for animals but only *L. monocytogenes* is constantly associated with human and animal illnesses. Hence, *L. monocytogenes* and *L. ivanovii* are considered as pathogenic species of public health (Guillet *et al* 2010). Members of *Listeria* are Gram•positive, non spore•forming, motile, facultatively anaerobe rod•shaped bacterium. expresses a beta hemolysin and exhibits characteristic tumbling\motility (due to peritrichous flagella) when observed with light microscopy.

Listeriosis is characterized by invasive and non-invasive illness and has a tendency to cause severe complications especially in pregnant women, neonates, the elderly, and the immunosuppressed individuals (Vlaemynck *et al* 2000). Specifically in pregnant women it leads to abortion, septicaemia or infections of the central nervous system (Rebagliati *et al* 2009). Moreover it can reach on to the foetus through placenta, and cause miscarriage, still birth or severe diseases in new born babies.

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ISSN: 2278-4632
Vol-10 Issue-6 No. 13 June 2020

Listeriosis affects domestic as well as wild animals, and the most common form of the disease is encephalitis which is sometimes called as 'Circling Disease'. Although the morbidity of listeriosis is comparatively low, the mortality of the systemic disease can be very high, with values in the vicinity of 30% (OIE 2008).

The major cause of infections are vegetables like lettuce, celery and tomatoes (Ho *et al* 1986). Milk and other dairy products have been reported as source of listeriosis in numerous widely publicized incidents. Subsequently *Listeria* has been reported from mastitic milk (Malik *et al* 2002).

There are several documented studies which have found milk from infected animal i.e. raw milk (Rahimi *et al* 2010) or mastitic milk or milk available in the retail market contaminated with *L. monocytogenes* (Fretz *et al* 2010). Dairy products such as cheese and ice cream which are produced from unpasteurised milk (Brooks *et al* 2012) are often contaminated with this pathogen. Besides milk, meat and meat products have also been found to be contaminated with *L. monocytogenes* (Schwartz *et al* 1988).

There are thirteen known serotypes of *L. monocytogenes*, but more than 90% of human isolates belong to only three serotypes: 1/2a, 1/2b, and 4b. Indeed, it has been observed that these three serotypes are held responsible for 98% of recognized human listeriosis cases, whereas serotypes 4a and 4c are rarely associated with the disease (Wiedmann *et al* 1996) In humans the occurrence of listeriosis is as low as 1%, but with high percentage of fatal outcome (30%). Moreover, the presence of this bacteria in intestinal tract of 5 to 10% of healthy humans do not as such exhibit any apparent symptoms of the disease (Todar 2009).

In India, *L. monocytogenes* has been isolated from humans (Kaur *et al* 2007), clinical animal cases (Shakuntala *et al* 2006) and foods (Jallewar *et al* 2007).

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ISSN: 2278-4632
Vol-10 Issue-6 No. 13 June 2020

In the United States of America, around 2500 cases of listeriosis occur each year with 20-30% mortality due to contaminated foods irrespective of antimicrobial treatment (Mead *et al* 1998). Thus, it specifies that the prevalence of *L. monocytogenes* in foods poses a significant danger.

The incidence of listeriosis in European Union in 2007 was reported to be 0.3 cases in every 100,000 population (Farber *et al* 1991). Therefore, the European council directive 92/46/EC suggested a `Zero tolerance policy for *L. monocytogenes* in soft cheese which has also been approved by other countries (Anon 1992).

The pathogenicity of *L. monocytogenes* is determined by the presence of several virulence factors, including the hemolysin (listeriolysin O) responsible for bacterial vacuole evasion and cell-to-cell spread (Gedde *et al* 2000), phospholipases mainly *plcA* and *plcB* present in *L. monocytogenes* and *L. ivanovii* and the third *smcL*, is specific to *L. ivanovii* , a protein (*ActA*) several internalins, Metalloprotease, *Clp* proteases and ATPases, *PrfA* and others (Kathariou, 2002).

SOURCES OF CONTAMINATION WITH *LISTERIA* SPP

L. monocytogenes infections have gained a great deal of attention not due to increased report of clinical disease, exhibited by meningo-encephalitis, septicaemia and abortion, in people and animals, but also due to its association as a food-borne pathogen (Low and Donachie 1997). *L. monocytogenes* has been causative agent of major food-borne epidemics in which dairy products including cheese, raw milk and pasteurized milk have been incriminated as the contaminated foods. Reports of listeriosis from human beings in India are scanty, may be due to failure of identifying isolates, use of improper isolation technique or lack of awareness.

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ISSN: 2278-4632
Vol-10 Issue-6 No. 13 June 2020

Listeria spp, including *L. monocytogenes* are ubiquitous microorganism that survive at different places in farm environment and serve as a source of contamination (Sunitha *et al* 2016, Fieseler *et al* 2014, Farber and Peterkin 1991). Fieseler *et al* (2014) isolated *Listeria* spp from the walls, floors and drains of the farms. Sunitha *et al* (2016) also reported *L. monocytogenes* from the dairy farm environment, animal faeces, udder wash, milker's hand and water samples. In some studies it has been found to be excreted intermitantly in the faeces of apparently healthy animals at farm, more in animals that are kept indoor and in cooler parts of the year such as December (Husu 1990). Excretion of these organisms in the faeces and their widespread presence in the farm environment make milk samples highly prone to contamination from these organisms. Contaminated dairy equipments and hands of milkers can also add to the contamination of raw milk with *Listeria* spp (Tahoun *et al* 2017, Sunitha *et al* 2016).

Therefore strict hygiene of the farm and during milking is necessary to prevent contamination. In another study, Rawool *et al* (2007) also detected *L. monocytogenes* from subclinical mastitic milk samples, indicating that even if the farm hygiene is good direct excretion of these organism in milk samples also act as another source of milk contamination.

PREVALENCE OF *LISTERIA SPP* IN MILK AND MILK PRODUCTS

The pathogen *L. monocytogenes* is an extensively adaptable environmental bacterium, able to exist in animals, humans and environment with powerful regulated virulence factors. It has emerged as an important foodborne pathogen during last decade causing several outbreaks and sporadic cases of foodborne listeriosis in human beings.

In Sweden Loncarevic *et al* (1995) analysed the prevalence of *L. monocytogenes* to be 6% in the cheese samples. Similarly in another study conducted by Mahmoodi *et al* (2010) a total of 360 raw milk and dairy product samples including white cheese, yoghurt and Iranian

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ISSN: 2278-4632
Vol-10 Issue-6 No. 13 June 2020

yoghurt drink, in Southern Iran revealed the presence of *L. monocytogenes* in raw milk and white cheese samples to be 1.7 and 3.3 %, respectively. The prevalence of *Listeria* spp. in fresh cream, ice cream, butter milk cheese and kareish cheese was 15%, 20%, 12.5% and 12.5% respectively, according to Elshinaway *et al* (2016) . In his study the most prevalent species was *L. grayii* (5.41%), followed by *L. monocytogenes* (3.33%) and *L. welshimeri* (1.25%).

Based on the documented scientific reports we can say that prevalence or contamination of *Listeria* spp, and especially *L. monocytogenes* varies in different studies done in different regions. The studies conducted in Mysore city, Karnataka wherein authors reported 0.76% prevalence of *Listeria* in milk and none of the sample was positive for *L. monocytogenes* (Shantha and Gopal, 2014). Whereas in another study from, Mohali Punjab documented zero prevalence of *Listeria* spp. in the milk samples (Agarwal *et al*., 2013). Khan *et al*., (2012) also specified the presence of *L. monocytogenes* from two samples out of total 250 raw milk samples and milk products in Bareilly. In another study from Odissa by Sarangi *et al*., (2009), three samples revealed the presence of *L. monocytogenes* (2.01%) out of the total 137 raw milk samples examined. Nayak *et al.*, (2015) screened a total of 200 milk samples and milk products, of these 18 (9%) were found positive for the *Listeria* spp. whereas *L. monocytogenes* was isolated from the three milk samples only with the prevalence 1.5%.

On the contrary some of the studies stated higher prevalence of *Listeria*. Kalorey *et al*., (2008) conducted a large survey of central India and reported 5.1% prevalence of *L. monocytogenes* from 2060 raw milk samples. Similarly, Soni *et al*., (2013) reported 5.8% prevalence of *L. monocytogenes* in raw cow milk samples collected from Varanasi, Uttar Pradesh. Another study carried out in Tamil Nadu by Marry and Shrinithivihahshini (2017) reported 52.7% prevalence of *L. monocytogenes*.

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ISSN: 2278-4632
Vol-10 Issue-6 No. 13 June 2020

Tiwari *et al* 2018, author of this review article investigated a total of 1018 retail milk samples and 250 mastitic milk samples for isolation and molecular characterization of *Listeria* spp from different districts of Punjab. The isolates were phenotypically and genotypically characterised by biochemical tests, in-vitro pathogenicity assay followed by detection of genus specific gene and different virulence-associated genes *viz. hlyA, actA, iapA, plcA* and *prfA* using PCR along with multiplex PCR for geno-serotyping of *L. monocytogenes*. A total of seven samples were found positive for *Listeria* spp by biochemical and molecular tests thereby resulting in an overall *Listeria* spp. prevalence of (0.68%) in retail milk samples. These seven *Listeria* isolates belonged to *L. seeligeri* (2 isolates) and *L. grayi* (5 isolates). Taking into consideration the districts, then Ludhiana, Patiala, Tarantaran and Pathankot yielded 3%, 1.66%, 3.33%, and 3.33% prevalence of *Listeria* spp respectively. None of the mastitic milk sample was positive for *Listeria* spp. Retail milk samples in study meet the food safety guidelines of zero tolerance of *L. monocytogenes*, but the presence of other non-pathogenic *Listeria* spp require further scaling of hygiene measures during production, processing and retailing.

CONCLUSSIONS

The virulent nature of listeriosis has particularly attracted the attention of public and food safety professionals. Therefore rapid diagnosis for the presence of *Listeria* is the need of the day to prevent entry of *Listeria* in the food chain, thereby protecting humans from acquiring this zoonotic infection.

India ranks first among the world's milk producing nations since 1998 and has the largest bovine population in the world. Milk production in India during the period 1950-51 to 2017-18, has increased from 17 million tonnes to 176.4 million tonnes. Owing to the rapid growth of dairy processing industry in India, the possibility of such epidemics cannot be ruled out completely in the country. Hence methods for detection of dairy foodborne *L.monocytogenes* are

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ISSN: 2278-4632
Vol-10 Issue-6 No. 13 June 2020

required to be implemented throughout the production, transport, processing, dispatch and retailing of milk and milk products for protecting the health of human beings and maintaining the standard hygienic levels.

Due to the advancement of Several molecular genotyping techniques such as DNA restriction endonuclease analysis, ribotyping, multilocus enzyme electrophoresis, PFGE for molecular epidemiological studies diagnosis of diseases has become much easier, also the expansion of PCR-based serotyping procedures have provided further benefits for the identification and grouping of *L. monocytogenes.* Prevalence data of *L. monoytogenes* and the opinion of almost all cited authors highlight the risk of raw milk and contaminated milk products consumption thereby emphasising the need for improvement in isolation as well as identification procedures of Listeria

REFRENCES

Anon. 1992a. Council directive 92/46/EC laying down the health rules for the production and placing on the market of raw milk, heat-treated milk and milk based products. *Official Journal of the European Communities* **268**: 1-32

Agarwal A, Awasthi V, Dua A, Ganguly S, Garg V and Marwaha S S. 2013. Microbiological profile of milk: impact of household practices. *Indian Journal of Public Health* **56**(1): 88-94.

Brooks J C, Martinez B, Stratton J, Bianchini A, Krokstrom R and Hutkins R. 2012. Survey of raw milk cheeses for microbiological quality and prevalence of foodborne pathogens. *Food Microbiology* **31**(2): 154-58.

Dmowska K and Osek J. 2010. Molecular aspects of *Listeria monocytogenes* pathogenicity. *Medycyna Weterynaryjna* **66**(4): 236-41.

 Copyright © 2020 Authors

ISSN: 2278-4632
Vol-10 Issue-6 No. 13 June 2020

Elshinaway S H, Meshref A M, Zeinhom M M and Hafez D A. 2017. Incidence of *Listeria* species in some dairy products inbeni-suef governorate. *Assiut Veterinary Medical Journal* **63**: 5-13.

Farber J M and Peterkin P I. 1991. *Listeria monocytogenes,* a foodborne pathogen. *Microbiological Reviews* **55**: 476-511.

Fieseler L, Doyscher D, Loessner MJ, Schuppler M. 2014. Acanthamoeba release compounds which promote growth of *Listeria monocytogenes* and other bacteria. *Applied Microbiology and Biotechnology* **98**: 3091-97.

Fretz R, Pichler J, Sagel U, Much P, Ruppitsch W, Pietzka A T, Stöger A, Huhulescu S, Heuberger S, Appl G and Werber D. 2010. Update: Multinational listeriosis outbreak due to 'Quargel', a sour milk curd cheese, caused by two different *L. monocytogenes* serotype 1/2a strains, 2009-2010. *European Surveillance* **32**: 19543.

Gedde M M, Higgins D E, Tilney L G and Portnoy D A. 2000. Role of listeriolysin O in cell-to-cell spread of *Listeria monocytogenes*. *Infection and Immunity* **68**(2): 999-1003.

Guillet C, Join-Lambert O, Monnier A, Leclereq A, Mechai F, Bruneel M F, Bielecka M K, Scortiti M, Disson O, Berche P, Boland V J, Lortholary O and Lecuit M. 2010. Human Listeriosis caused by *Listeria ivanovii. Emerging Infectious Diseases* **16**: 136-38.

Ho J L, Shands K N, Friedland G, Eckind P and Fraser D W. 1986. An outbreak of type 4b *Listeria monocytogenes* infection involving patients from eight Boston hospitals. *Achieves of Internal Medicine* **146**(3): 520-24.

 Copyright © 2020 Authors

ISSN: 2278-4632
Vol-10 Issue-6 No. 13 June 2020

Husu J R. 1990. Epidemiological studies on the occurrence of *Listeria monocytogenes* in the feces of dairy cattle. *Journal of Veterinary Medicine, Series B* **37**(1-10): 276-82.

Jallewar P K, Kalorey D R, Kurkure N V, Pande V V and Barbuddhe S B. 2007. Genotypic characterization of *Listeria* spp. isolated from fresh water fish. *International Journal of Food Microbiology* **114**: 120-23.

Kaur S, Malik S V S, Vaidya V M and Barbuddhe S B. 2007. *Listeria monocytogenes* in spontaneous abortions in humans and its detection by multiplex PCR. *Journal of Applied Microbiology* **103**(5): 1889-96.

Kalorey D R, Warke S R, Kurkure N V, Rawool D B and Barbuddhe S B. 2008 *Listeria* species in bovine raw milk : A large survey of Central India. *Food Control* **19**: 109-12.

Kathariou, S. (2002). *Listeria monocytogenes* virulence and pathogenicity, a food safety perspective. *Journal of food protection, 65(11)*, 1811-29

Khan J A, Rathore R S, Khan S and Ahmad I. 2012. *In vitro* detection of pathogenic *Listeria monocytogenes* from food sources by conventional, molecular and cell culture method. *Brazilian Journal of Microbiology* **44**(3): 751-58.

Loncarevic S, Danielsson-Tham M L and Tham W. 1995. Occurrence of *Listeria monocytogenes* in soft and semi soft cheeses in retail outlets in Sweden. *International Journal of Food Microbiology* **26**(2): 245-50.

Low J C and Donachie W. 1997. A review of *Listeria monocytogenes* and listeriosis. *The Veterinary Journal* **153**: 9-29.

 Copyright © 2020 Authors

ISSN: 2278-4632
Vol-10 Issue-6 No. 13 June 2020

Malik S V S, Barbuddhe S B and Chaudhari S P. 2002. Listeric infection in human and animals in Indian subcontinent. *Tropical animal Health and Production* **34**: 359-81.

Mary M S and Shrinithivihahshini N D. 2017. Pervasiveness of *Listeria monocytogenes* in milk and dairy products. *Journal of Food: Microbiology, Safety and Hygiene* **2**: 125.

Mead P S, Slutsker L, Dietz V, McCaig L F, Bresee J S, Shapiro C, Griffin P M and Tauxe R V. 1999. Food-related illness and death in the United States. *Emerging Infectious Diseases* **5**: 607-25.

Mahmoodi M M. 2010. Occurrence of *Listeria monocytogenes* in raw milk and dairy products in Noorabad, Iran. *Journal of Animal and Veterinary Advances* **9**(1): 16-19.

Nayak D N, Savalia C V, Kalyani I H, Kumar R and Kshirsagar D P. 2015. Isolation, identification, and characterization of *Listeria* spp. from various animal origin foods. *Veterinary World* **8**(6): 695.

OIE. 2008. *OIE Terrestrial Manual*. *Listeria monocytogenes* Chapter 2.9.7: pp. 1238-554.

Rahimi E, Ameri M and Momtaz H. 2010. Prevalence and antimicrobial resistance of *Listeria* species isolated from milk and dairy products in Iran. *Food Control* **21**(11): 1448-52.

Ramaswamy V, Cresence V, Rejitha J S, Lekshmi M U, Dharsana K S, Prasad S P and Vijila H M. 2007. *Listeria*: review of epidemiology and pathogenesis. *Journal of Microbiology and Immunology and Infection* **40**(1): 4-13.

Rawool D B, Malik S V S, Shakuntala I, Sahare A M and Barbuddhe S B. 2007. Detection of multiple virulence-associated genes in *Listeria* monocyotgenes isolated from bovine mastitis cases. *International Journal of Food Microbiology* **113**(2): 201-07.

 Copyright © 2020 Authors

ISSN: 2278-4632
Vol-10 Issue-6 No. 13 June 2020

Rebagliati V, Philippi R, Rossi M and Troncoso A. 2009. Prevention of foodborne listeriosis. *Indian Journal of Pathology and Microbiology* **52**: 145-49.

Sarangi L N, Panda H K, Priyadarshini A, Sahoo S, Palai T K, Ranabijuli S, Senapati S and Mohanty D N. 2009. Prevalence of *Listeria* species in milk sample of cattle of Odisha. *Immunology and Infectious Diseases* **2**: 135-35.

Schwartz B, Broome C, Brown G, Hightower A, Ciesielski C, Gaventa S and Listeriosis Study Group. 1988. Association of sporadic listeriosis with consumption of uncooked hot dogs and undercooked chicken. *The Lancet* **332**(8614): 779-82.

Shakuntala I, Malik S V S, Barbuddhe S B and Rawool D B. 2006. Isolation of *Listeria monocytogenes* from buffaloes with reproductive disorders and its confirmation by polymerase chain reaction. *Veterinary Microbiology* **117**: 229-34.

Shantha S and Gopal S. 2014. Prevalence of *Listeria* species in environment and milk samples. *Advances in Animal and Veterinary Sciences* **2**(5): 1-4.

Soni D K, Singh R K, Singh D V and Dubey S K. 2013. Characterization of *Listeria monocytogenes* isolated from Ganges water, human clinical and milk samples at Varanasi, India. *Infection, Genetics and Evolution* **14**: 83-91.

Sunitha R, Raghunath R R, Vindo V K, Sunil B, Prejit, Asha K, Samitha and Vergheese J. 2016. Prevalence of *Listeria monocytogenes* in bovine mastitic milk and dairy farm environment. *International Journal of Science, Environment* **5**(2): 638-43.

Tahoun A B, Elez R M A, Abdelfatah E N, Elsohaby I, El-Gedawy A A and Elmoslemany A *M.* 2017. *Listeria monocytogenes* in raw milk, milking equipment and dairy workers:

 Copyright © 2020 Authors

ISSN: 2278-4632
Vol-10 Issue-6 No. 13 June 2020

molecular characterization and antimicrobial resistance patterns. *Journal of Global Antimicrobial Resistance* **10**: 264-70.

Todar, K. (2009) *Listeria monocytogenes*. u: Todar/s online textbook of bacteriology, htt://textbookof bacteriology.net/*Listeria*.html

Tiwari R. 2018. 'Prevalence and molecular characterisation of *Listeria* spp in retail and mastitic milk in Punjab .' M.V.Sc. Thesis, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana

Tiwari R, Saini S R, Kaur S,and Aulakh, R.S. 2018. Prevalence and Molecular Characterisation of Listeria spp. in Retail and Mastitic Milk of Punjab, India.Int.J.Curr.Microbiol.App.Sci. 7(10): 2484-2495.

Vlaemynck G, Lafarge V and Scotter S. 2000. Improvement of the detection of *Listeria monocytogenes* by the application of ALOA, a diagnostic, chromogenic isolation medium. *Journal of Applied Microbiology* **88**: 430-41.

Walsh D, Duffy G, Sheridan J J, Blair I S and McDowell D A. 2001. Antibiotic resistance among *Listeria* including *Listeria monocytogenes* in retail foods. *Journal of Applied Microbiology* **90**(4): 517-22.

Wiedmann M, Bruce J L, Knorr R, Bodis M, Cole E M, McDowell C I, McDonough P L and Batt C A. 1996. Ribotype diversity of *Listeria monocytogenes* strains associated with outbreaks of listeriosis in ruminants. *Journal of Clinical Microbiology* **34**(5): 1086-90.

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