International Journal of Science, Environment and Technology, Vol. 5, No 2, 2016, 638 – 643

ISSN 2278-3687 (O)
2277-663X (P)

PREVALENCE *OF LISTERIA MONOCYTOGENES* IN BOVINE MASTITIC MILK AND DAIRY FARM ENVIRONMENT

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Abstract: Listeria spp. cause abortion, stillbirths, neonatal septicaemia and encephalitis in human beings and domestic animals. The present study was conducted to determine the prevalence of *L. monocytogenes* from bovine mastitic milk and in dairy farm environment. A total of 500 samples which included 100 mastitic milk (clinical and subclinical), 50 soil, 50 dung, 50 fodder, 50 water, 50 handwash and 50 udder wash samples were collected from dairy farms of three different panchayaths namely Thariyode, Porhuthana and Kalpetta municipality of Wayanad district, Kerala. Out of 500 sample*s, L. monocytogenes* was isolated from six (six per cent) mastitic milk, three (three per cent) dung, three (six per cent) udder wash, two (four per cent) milker's hand wash, five (10 per cent) fodder, seven (14per cent) soil and two (four per cent) water samples. A total of 28 (5.60 per cent) samples were positive for *L. monocytogenes.* The observations made during the visit to the farm and the results revealed that milk is getting contaminated with the *L. monocytogenes* mainly from environmental samples rather than the animal being a source of infection.
Keywords: *Listeria monocytogenes*, bovine mastitic milk, dairy farm environment.

Introduction

Listeriosis has been recognized as an emerging foodborne bacterial infection and a nagging public health hazard (Farber and Peterkin, 1991). The genus *Listeria* consists of Gram positive, non-spore forming bacteria (Wong and facultative anaerobic, motile, Freitag, 2004). Listeria has two pathogenic species namely, *Listeria monocytogenes* and *Listeria ivanovii* of these, *L. monocytogenes* is a well-known cause of abortion, encephalitis and septicemia in man and animals. The case fatality rate for this foodborne pathogen, which ranges from 15.0 to 30.0 per cent with the highest hospitalization rates (90.5 per cent) amongst known food-borne pathogens (CDC, 2000).

Ruminant farm animals play a key role in the persistence of *Listeria* spp. in the rural environment via a continuous faecal-oral cycle (Vazquez-Boland *et al.,* 2001) of theses farm animals Cattle play a role in the spread of *Listeria* between animals or people rather than

*Received Mar 16, 2016 * Published April 2, 2016 * www.ijset.net*

small ruminants (Pritchard and Donnelly, 1999). The risk of listeriosis in ruminants increases with poor quality fermented feeds, for example, when dairy cattle are fed with ensilage foods (Donnelly, 2002).

Mastitis is a disease causing heavy economical losses to the dairy industry throughout the world. Listeric mastitis, which is the most stubborn and difficult to treat, results in culling of the infected animals from a herd (Stewart, 1998). It affects one or all the quarters and the organism could be excreted for months posing a potential threat to public health (Hird and Genigeorgis, 1990). Moreover, many of the naturally occurring cases of mastitic cases of listeriosis may go unnoticed or undetected due to lack of suitable techniques employing specific media/antigen(s).

The aim of this study was to determine the prevalence of *L. monocytogenes* in bovine mastitic milk and in dairy farm environment. The epidemiology of Listeriosis plays a major role in prevention of infection by listeria organism.

Materials and Method

Collection of Samples:

The study was under taken for a period of 10 months from June 2014 to March 2015. A total of 500 samples were collected in sterilized sample collection vials which included 100 mastitic milk (clinical and subclinical) based on CMT and changes in the consistency of milk and udder, 50 soil, 50 dung, 50 fodder, 50 water, 50 hand wash and 15 udder wash samples from three different panchayaths namely Thariyode, Porhuthana and Kalpetta municipality of Wayanad district, Kerala. The samples were then carried to the laboratory in an ice box for further processing.

Isolation of *Listeria monocytogenes:*

A standard protocol described by Thomas *et al.* (1991) was followed with slight modifications for the isolation of *L. monocytogenes*. A total of 10g sample (millilitres in case of liquid samples) were transferred into a sterile stomacher bags containing 0.5 per cent sterilized peptone water and homogenized.In this method, a two-step enrichment procedure using UVM I and II broth was followed. In primary enrichment, UVM I was used. Acriflavin (6 mg) and Nalidixic acid (10 mg) are the selective agents used in the UVM I media. One ml of the homogenized suspension was transferred to 9ml of UVM I and incubated at 37^0C for 24 h. The secondary selective enrichment was carried out in UVM II broth, which contained the selective agents at higher concentration (Acriflavin 12.5 mg and Nalidixic acid 10 mg). From the primary enriched UVM I broth culture, 0.1 ml was transferred to 10 ml of UVM II

broth and incubated at37^{0}C for 24 h. Aloopful of the inoculum from the enrichment broth was streaked onto duplicate plates of PALCAM agar medium. The plates were incubated at 37^{0}C for 24 h. At the end of incubation, colonies showing characteristic appearance (grey-green with black centre colonies surround with black halo) on PALCAM agar medium were selected and transferred onto nutrient agar slants and incubated at 37^{0}C overnight. The isolates were stored at refrigeration temperature for characterization. The isolates were subjected to staining, biochemical and molecular test (PCR).

Molecular confirmation of *Listeria monocytogenes:*

The PCR confirmation *L. monocytogenes* isolates as per the protocol described by Mcclain and Lee (1998).

PCR Technique:

The primer pair consisting of primer *hly*A1 5'- GCAGTTGCAAGCGCTTGGAGTGAA-3' and *hly*A2 5'- GCAACGTATCCTCCAGAGTGATCG-3' was used for the amplification of a 432 bp region of the hlyAgene. PCR was performed in a 25µl reaction mixture with a PCR buffer containing 200 µm concentration of each deoxynucleoside triphosphate (dNTP), 10 mMTris-Hcl (pH 8.3), 1.5 mM MgCl2, 1 unit of Taq polymerase (Promega), 0.25 µM concentration of each primer and 2.5 µl of DNA template. DNA amplification was carried out for 34 cycles in 25 µl of reaction mixture as follows: denaturation at 94°C for 50 seconds, annealing at 60°C for 50 seconds, extension at 72°C for 90 seconds, with final extension at 72°C for 5 minutes.

The amplified DNA was analyzed by agarose gel electrophoresis on a 1.5 % agarose (GeNei™, Mumbai) gel prepared in 1X Trisacetate EDTA buffer (40mM Tris, 20mM acetic acid and 1mM EDTA) stained with ethidium bromide (0.5µg/ml). A 100 bpDNA ladder (Bangalore Genei, India) was used as a reference marker. Tris-acetate EDTA (0.5) was used as the running buffer and the gel was viewed using UV transillumination at a wavelength of 254 nm.

Results:

A total of 28 (5.60 per cent) samples were positive for *L. monocytogenes.* Out of 500 sample*s, L. monocytogenes* was isolated from six (six per cent) mastitic milk, three (three per cent) dung, three (six per cent) udder wash, two (four per cent) milker's hand wash, five (10 per cent) fodder, seven (14per cent) soil and two (four per cent) water samples

The observations made during the visit to the farm and the results revealed that milk is getting contaminated with the *L. monocytogenes* mainly from environmental samples rather

than the animal being a source of infection. Presence of *L. monocytogenes* in soil in the immediate surroundings of the farm is a point for further spread of the organism in the farm including milk. The fodder being fed to the animal has been found to be harbouring the organism after being unloaded onto the contaminated soil. This assumes significance in farms where workers perform multiple tasks from without proper hand washing protocols in between tasks. It was observed in the present study that the same worker handles the cleaning of the farm and the surrounding, handles the fodder, cleans the animal, cleans the milking utensils and also milks the animal without proper hand washing protocols in between. Disinfection of the soil in the immediate surroundings of the farm by spraying bleaching powder will help in preventing the ingress of *L. monocytogenes* into the food chain.

Discussion

In the present study, *L. monocytogenes* was isolated from six per cent mastitic milk samples from all the three regions. The percent isolation of *L. monocytogenes* is more than 3.52 per cent reported by Yadav *et al*. (2010) in mastitic milk samples in Gujarat. The present findings were in agreement with 6.1 per cent as recorded by Vilar *et al.* (2007) in Spain. A total of three per cent dung samples were found positive for *L. monocytogenes* these findings were lower than 9.3 per cent reported by Vilar *et al*. (2007) in Spain. *L. monocytogenes* was detected in three per cent udder wash samples. Mohammed *et al.* (2009) reported the presence of *L. monocytogenes* in 19 per cent udder wash samples in Pakistan. A total of 14.00 per cent of soil samples were positive for *L. monocytogenes.* The present findings were higher than 0.37 per cent of soil samples as reported by Kumar *et al.* (2014) in kerala. *L. monocytogenes* was isolated from 10.00 per cent fodder samples and four per cent of water samples. The present findings were higher than Atil *et al.* (2011) who isolated *L. monocytogenes* from 0.80 per cent of water samples in Turkey and Kumar *et al*. (2014) in Kerala reported that none of the water samples were positive for *L. monocytogenes.*

Conclusion

The present study revealed a significantly high prevalence of *L. monocytogenes* in mastitic milk and dairy farm environment. The observations made during the visit to the farm and the results revealed that milk is getting contaminated with the *L. monocytogenes* mainly from environmental samples rather than the animal being a source of infection. Disinfection of the soil in the immediate surroundings of the farm by spraying bleaching powder will help in preventing the ingress of *L. monocytogenes* into the food chain. Good management practices

have to be implemented in the farm to maintain the health of the animal and to prevent the contamination.

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