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Research Article

Prevalence of *Listeria Monocytogenes* in Public Health Risk Assessment of Zoonotic Illnesses and Influence the Occurance from Frozen Buffalo Meat Exported from India

Ujjal Sen[1*] and Anil Mahadeo Garode[1]

Department of Microbiology, Kalinga University, Raipur, Chattishgarh, India

Abstract

A survey of literature about the effect of freezing and low temperature storage on buffalo meat in frozen condition was performed for *Listeria monocytogenes* detection. The reviewed data point out that freezing is the most convenient method for prevention of foodstuff spoilage by developing its own enzyme which can even kill pathogens. But, here *L. monocytogenes* can withstand a very low temperature in buffalo meat while freezing. It is considered that low temperature can contribute to meat foodstuffs safety because of their effect on parasite too. But, a scientific and practical challenge is the differentiations of frozen-thawed from fresh chilled meat pathogenic bacteria like *Listeria monocytogenes* and other species can survive such an extreme condition. In this literature, it is dealt with the processing and packaging of frozen buffalo meat in India practicing with precise hygienic condition or not and prevalence of *L. monocytogenes* present or absent in different cuts of frozen buffalo meat. Microbiologically 55 samples collected from abattoir and tested for *L. monocytogenes* and 44 observed as positive which can withstand freezing temperature. *L. monocytogenes* important from public risk assessment due to creation of rare disease called liseriosis in humans. The reviewed literature concerning this problems leads to the conclusion of solution that enzymatic methods of differentiation are comparatively the most precise and easiest to perform, at plant or abattoir effectiveness of appropriate cleaning and sanitation, good manufacturing practice, effective temperature control, maintenance of hygienic condition in whole food process area in abattoir absolutely perform by training the food handlers about contamination and prevention measure to restrict the food to contaminate. Even if preservative is used food may be free from *L. monocytogenes.*

Keywords: *Listeria monocytogenes*; Frozen Buffalo Meat; Zoonotic Disease; Public Health

Introduction

Listeria monocytogenes is an important food-borne pathogen having common occurrence in variety of foods [1,2,3,4]. *Listeria monocytogenes* are one of the most human pathogens among the six scecies like *L. monocytogenes*, *L. innocua*, *L. seeligeri*, *L. welshimeri*, *L. ivanovii*, and *L. grayi* respectively and one of the causes of zoonotic illness. It was first described by Murray in 1926 as *Bacterium monocytogenes*, the cause of an infection of laboratory rabbits where it was associated with peripheral blood monocytes as an intracellular pathogen, and it has since been established as both an animal and human pathogen. As a veterinary problem it causes two main forms of disease in sheep and cattle: a meningiencephalitis most common in adult ruminants such as sheep and cattle and a visceral form most common in and young ruminants which attacks organs other than the brain causing still birth, absorption and septicemia. Reported incidence of human listeriosis in England and Wales remained at around 100 cases p.a. until 2001 when numbers increased to more than 200 cases p.a. in 2003 and 2004. In the United States, the Center for Disease Control (CDC) has estimated an annual incidence of around 1700 cases resulting in 450 adult deaths and 100 foetal and postnatal deaths [5]. Overall the disease listeriosis severe and fatal illness with clinical manifestations likes sepsis or meningitis in immunocompromised patients or neonatal babies and flu-like illness or abortion during pregnancy in women [6]. It is reported that low temperature contributes for buffalo meat safety as well from the parasites. Thus, freezing of foodstuffs has a favorable effect because of two primary factors: the low temperature and the low amount of free water. The products are considered as frozen when a certain temperature is reached in depth (Fennema et al. 1973). This temperature should be −18°C or lower in product's depth [7]. This microorganism can survive and grow under conditions found in refrig- erated foods [8]. For example, it has been reported to grow in more than 10% NaCl [9].

These micro-organisms can contaminate raw frozen buffalo meat and its products. The series of outbreaks of the 1980s showed that *L. monocytogenes* causes a very serious invasion and often life-

***Corresponding author:** Ujjal Sen, Department of Microbiology, Kalinga University, Raipur, Chattishgarh, India, E-mail: ujjal_sen@hotmail.com

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threatening disease, constituting an economic burden for both public health services and food industry [10].

India has made rapid strides in frozen meat exports to about 50 countries of the world. Quality meat is produced adopting OIE guidelines and international quality standards. Out of total meat production of more than 6 million tones, the frozen buffalo alone contributes about 1.47 million tones and India is 5th largest exporter of frozen buffalo meat in the world (Agnihotri, 2008). Listeriosis is a relatively rare disease, but fatality rate ranges from 15.0 to 30.0% with the highest hospitalization rates (90.5%) amongst known food-borne pathogens [11]. There is paucity of comprehensive information regarding occurrence of *Listeria* spp. in buffalo meat in India [12].

According to European Food Safety Authority, *Listeria monocytogenes* counts exceeds the level of 100 cfu/g at the end of shelf life was 0.43 % in ready to eat meat products [13]. *L. monocytogene* are also reported from Egypt in different research of minced beef where it survives refrigeration temperature and frozen temperatures [14].

Listeria monocytogenes Morphology

L. monocytogenes is a Gram-positive, facultatively anaerobic, catalase positive, oxidase-negative, non-spore former. The coccoid to rod shaped cells (0.4–0.5 mm to 0.5–2.0 mm) cultured at 20–25 1C possess peritrichous flagella and exhibit a characterisitic tumbling motility [5]. It is occurring singly or in pairs which often angled at point of contact and may resemble diplococcic or diphtheroids. Filamentous form may develop in old cultures, sometime with loss of gram reaction. Non-sporing, non-acidfast, non-capsulate, flagellate and show 'tumbling' motility at 20-25°C not at 35-37°C (Mackie & McCartney).

Materials and Methods

55 various cuts of Frozen Buffalo meat samples investigated in this study were collected everyday from different slaughter houses and transferred in sterile food bags and analyzed on each day when collected.

Preperation of Samples

25 gm frozen buffalo meat samples weighed in sterile petridishes. The sample should homogenize in a blender containing sterile dilution 225mL of 0.1% peptone water under the aseptic condition. Rinse the pre-enrichment bottle for two minutes for thoroughly mixing of sample. The above mentioned method considers as pre-enrichment method and incubated at 30°C for 4 hours [15,16].

Isolation

Place 1mL pre-enrichment to 9mL of Listeria Enrichment Broth at 30°C for 48 hours for selective enrichment. Streak from pre-enrichment after 4 hours directly to the selective media Listeria Oxford Agar Base with moxalactum supplement. Alternatively, after 48 hours from selective enrichment streak on Listeria Oxford Agar Base. Incubate both the plates at 30°C for 24 hours. The plates are observed with grey or black colonies along with grey or black halos appearance on media. Sometime if more bacteria are present they colonized by mushrooming the whole plate with blackening the surface of the plate media. It was also observed that *Listeria monocytogenes* can grow on temperatures like 25°C, 37°C, 35°C etc. [16]. Streak from pre-enrichment to Oxford agar which supplemented with moxalactum and charcoal, whereas charcoal provides dark background on media and *L. monocytogenes* colonies appear as creamish or whitish in color against dark back ground mentioned in figure. Few samples of frozen buffalo meat also tested on serial dilution basis to observe the load of *L. monotogenes* on frozen buffalo meat sample. In this study it observed that *L. monocytogenes* are present in frozen buffalo meat ranges from 10^4 to10^5 cfu/gram in frozen buffalo meat. Below serial dilution picture is given.

Identification and Confirmation of *Listeria monocytogenes* Isolates

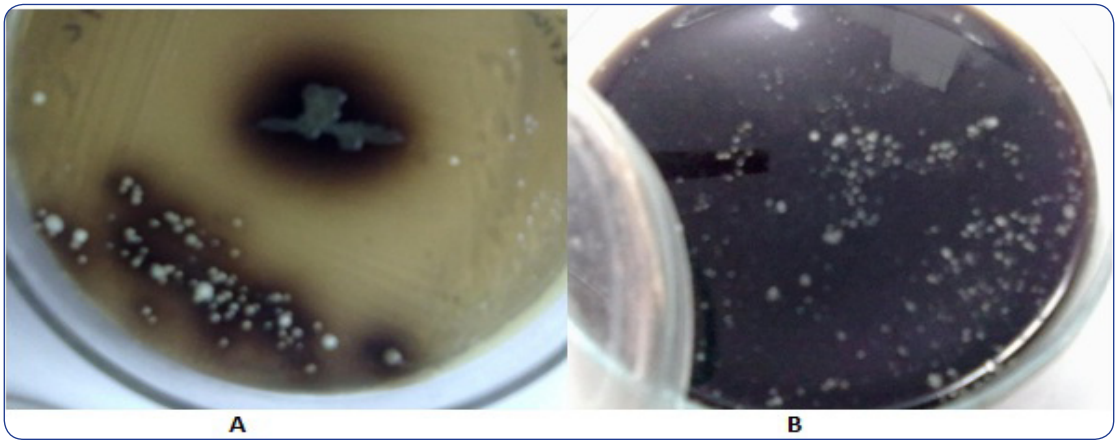
Listeria monocytogenes are identified and confirmed based on certain conventional biochemical reaction Gram Positive Rods with filamentous form occurring singly or in pairs, *Listeria spp.* are catalase positive, Voges-Proskauer Positive, methyl red positive, oxidase negative, urease negative, indole negative, motility is tumbling hence motile, on TSI agar produce H_2S. They can ferment glucose without gas; acid from salicin, aesculin positive. [16,17].

The Camp Test

The most important biochemical test to identify *Listeria spp*. as per BIS in 1994 and FDA. The Christie-Atkins-Munch-Peterson (CAMP) test is useful in confirming species particularly when blood agar stab test results are equivocal. To perform the test, streak a β-hemolytic *Staphylococcus aureus* and a *Rhodococcus equi* culture in parallel and diametrically opposite each other on a sheep blood agar plate. Streak several test cultures parallel to one another, but at right angles to and between the *S. aureus* and *R. equi* streaks. After incubation at 35°C for 24-48 h, examine the plates for hemolysis. *L. monocytogenes* hemolytic reactions are enhanced in the zone influenced by the *S. aureus* streak. The other species remain non-hemolytic. The *L. monocytogenes* reaction is often optimal at 24 h rather than 48 h. To obtain enough *R. equi* to provide a good streak of growth, incubate the slant culture 48 h rather than 24 h. Sheep blood agar plates should be as fresh as possible [16].

Antimicrobial Antibiotic Sensitivity Disc Test

L. monocytogenes is sensitive to penicillin, amoxicillin, gentamycin, chloramphenicol, trimethoprim, cotrimoxazole, erythromycin, vancomycin, rifampicin and tetracyclines. It is resistant to most cephalosporins but appears to be sensitive to imipenem. Penicillin or amoxicillin/ampicillin combined with gentamicin is bactericidal and this is generally the treatment of choice [17]. Here few sample of frozen buffalo meat tested with few antibiotic discs like 10µg of amoxyclave disc, 30µg of Vancomycin disc and 5µg of methicillin disc in oxford agar at 30°C for 24 hours against *Listeria monocytogenes* pathogen. It is observed that amoxyclave produced 2.4cm of clear and non-clear zone of inhibition. Hence, amoxyclave is sensitive, 1.7cm of distinct zone of inhibition observed around Vancomycin disc, Hence Vancomycin is sensitive against *Listeria*



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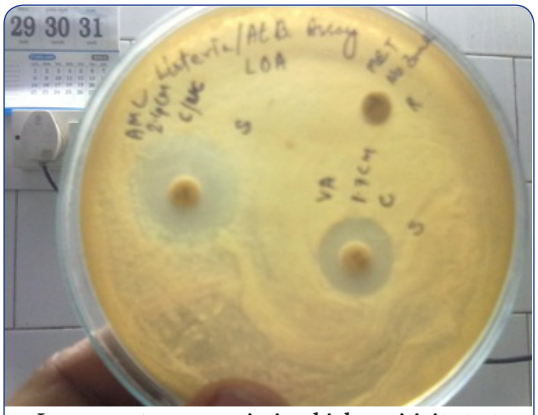
monocytogenes. But 5µg of methicillin disc found to be resistant against Listeria as there is no zone of inhibition.



***L. monocytogenes* antimicrobial sensitivity test**

Listeria Biofilms Formation in the Food Processing Environment

The persistence of *Listeria monocytogenes* in food processing facilities has the ability of these pathogens to live in biofilms. A biofilm may be defined as "a microbially derived sessile community characterized by cells that are irreversibly attached to a substratum or interphase, or to each other, are embedded in a matrix of extracellular polymeric substance that they have produced, and exhibit an altered phenotype". The *Listeria* present in chiller in biofilms are resistant to both heat and sanitizer. Dense clustering of cells and production of extracellular polymers effectively changes the heating menstruum, providing additional heat tolerance. The *Listeria* biofilm consists of many other *Listeria species* growth, pre-existing in biofilms consider to provoke the growth of other pathogenic organisms. Indeed many *Listeria* species can exist within the same environment. Biofilms are more difficult to remove as present as food residues. High fat substrates increase heat resistance to *L. monocytogenes*. Hence, hygienically the slaughter processing environment should be cleaned from fat and food residues first and apply fat cleaning and other sanitizing agent to not to deposit these food residues and fat substances in the food processing environment of slaughter house area. A lot of research scope available to study these biofilms and secure the hygienity of the abattoir [18].

Accuracy of the Selective Medium Used

Apart from the Oxford Agar other agar such as PALCAM and DRI agar usefull for the isolation of *L. monocytogenes* from froze buffalo meat respectively. In a study it is observed that DRI agar and oxford agar yielded recovery rate of *L. monocytogenes* 90% and 60% respectively but, in PAKCAM agar 92.2% [19]. But in this experiment only oxford agar is utilized which is FDA recommended Hi-Media product which gives also 90% recovery of *L. monocytogenes* from frozen buffalo meat samples [20]. Among the temperatures utilized for isolation are 30, 35, 25 and 37°C, whereas 30°C is observed to be best temperature to isolate the organism and Oxford agar yielded 90% accuracy rate of recovery of the organism as per this study.

L. Monocytogenes Contamination Associated with Frozen Bovine Meat

It's distribution in the environment and its ability to grow on most non-acid foods offer *Listeria monocytogenes* plenty of opportunity to enter the food chain and multiply. Factors that increase or decrease the risk of listeriosis are associated with consumption of foods contaminated with *L. monocytogenes*. It underlines the fact that *L. monocytogenes* is not only important in public health but also has a socio-economic importance upon the production of food and on food business worldwide, including those involved in international trade. In India, slaughter houses some time not having an improper practice of food hygienity condition by the food handlers which create a path of invation *of L. monocytogenes* entry in frozen bovine meat. Food residues, fats not sweep out properly by the sweepers from floor which create biofilm and the biofilm is more resistant that tolerates heat and promotes the growth of other organisms. Hence from the public point of view *L. monocytogenes* is very important in frozen buffalo meat contamination.

Result

In recent study 55 frozen buffalo meat samples tested whereas 44 samples among them found positive for *Listeria monocytogenes* and 11 samples are negative for *L. monocytogenes*. All the 44 positive samples of *L. monocytogenes* revealed characteristic features of

grey or black colonies along with grey or black halos appearance on Listeria Oxford Agar media which also recommended by FDA BAM for detection of *Listeria monocytogenes*. The organism's exhibit tumbling motility on Oxford agar and during overgrowth or old culture continues on the media which colonizes by mushrooming the whole plate and form total bluish black or grey color on the plate. On preliminary characteristic IMViC pattern *Listeria monocytogenes* produces positive tests as per the species and other biochemical tests mention before in identification and confirmation. Among all these CAMP test is most unique and important for identification of *L.monocytogenes* through their ability to haemolyse the blood cells on blood agar and produce enhanced haemolysis when crossing with *S. aureus* and *Rhodococci equi*. Among few antibiotics used to investigate L. monocytogenes methicillin 5µg resistant strans observed where as amoxyclave and vancomycin yield sensitive to the organism. Hence, risk concern with *L. monocytogenes* solely prevalent in contaminated buffalo meat during processing, handling and post-processing and always risk in public health hazard which will be major problem in food borne cases.

Discussion

In these experiments 55 frozen buffalo meat samples investigated on standard guidance and procedure to isolate only *Listeria monocytogenes,* instead of, other strains of *Listeria spp*. Here 44 samples found positive, it means 90% of positive for *Listeria monocytogenes*. Prevalence of such high rate in frozen buffalo meat exported from India alarming high alert for scientific community to develop rules for export from India. *Listeria monocytogenes* is a major food-borne disease pathogen which always under prevalence of food contamination and creates disease called listeriosis. Listeriosis is a relatively rare disease, but fatality rate ranges from 15.0 to 30.0 percent with the highest hospitalization rates (90.5%) amongst known food-borne pathogens [20]. It is found that all positive samples from frozen buffalo meat, oxford agar may be less sensitive than PALCAM agar for recovery of *L. monocytogenes* as per [19]. But, still as per above result of whole experiments continues with oxford agar yield a good result in different temperatures as mention above for the recovery of *L. monocytogenes*. Good manufacturing practice, appropriate cleaning and sanitation, follow the hygiene program of HACCP, effective temperature control throughout the food production, distribution and storage chain are required for prevention of contamination or inhibition of growth of *L. monocytogenes* to levels exceeding 100cfu/g in foods and may pose a *L. monocytogenes* risk.

The base line survey of different cuts of frozen buffalo meat samples was conducted the test microbiologically for *L. monocytogenes* and 44 samples encountered to be positive. This risk of contamination at level considered to be public health risk and designed to examine general or overall exposure to worldwide consuming and sold by different international traders to different countries can be susceptible to *L. monocytogenes* in frozen buffalo meat exported from India. Prevalence of *L. monocytogenes* in different food categories like vacuum packed frozen cuts of buffalo meat packed with heat treatment and cold treatment of frozen buffalo meat at -38 to -42°C in both blast and plate freezers designed to inhibit these organisms. But in such an extreme condition too *L. monocytogenes* survive and has a tendency to lead a disease like listeriosis. Processed foods (with or without heat treatment) prone to contamination during processing or further handling and with a prolonged storage time under refrigeration can be high-risk foods as regards *L. monocytogenes*.

There are reports available and exhibit that Ryu et al (1992) [21] observed *Listeria spp* were isolated from 43 (56.6%) out of 76 samples of meat products and *L. monocytogenes* occurred in 26 (34%) of the samples. Similarly, Kamat and Nair (1993) [22] showed the presence of L. *ivanovii, L. seeligeri* and *L. welshimeri* in meat samples, however, they were not able to detect incidence of *L. monocytogenes* in any of the samples tested. Retail minced beef in Japan was reported to have a 12.2% con- tamination rates with *L. monocytogenes* [23] Hence, these are certain reports originally exhibit clear picture of isolation of other species of *Listeria* intead of *L. monocytogenes.*

The overall discrepancies of the results of prevalence of *L. monocytogenes* in frozen buffalo meat noticed with great care and examined thoroughly the fact that particularly standard FDA, BAM procedure for isolation were used to study the above organism. Only one or two colonies examined microscopically, morphologically, biochemically to identify and confirm these severe pathogenic food borne organism. *L. monocytogenes* high fatality rate and observation of presence in frozen buffalo meat in high level alarming for public health concern in worldwide should awaken the scientific community allow for more research on the same. The influence of growth rates and possible underestimation of *L. monocytogenes* needs to be further investigated. There are multiple approaches to prevention of contamination, but one of the most effective is amelioration of the critical societal determinants, that is educational activities to alert and inform those concerned about food safety.

Conclusion

Luppi et al. (1988) [24], conducted a bacteriological study in Italy following which identified 13 strains of Listeria (nine of *L. monocytogenes* and four of *L. innocua*) in 113 samples of meat (11.5%) and four strains (two of *L. monocytogenes* and two of *L. innocua*) in 75 samples frozen meat (5.3%). In England, MacGowan et al. (1994) [25], following measurements on samples of meat from different animals, isolated *L. monocytogenes* in these proportions: in chickens' products (21/32 or 65.6%), beef (9/26 or 34.6%), lamb (8/20 or 40%), pig (9/32 or 28.1%) and sausages (8/23 or 34.7%). After revising above studies with reports and experimental facts revealed in this research paper, it is clear that prevalence of above *L. monocytogenes* organism are common and pathogenic form of listeriosis disease emerge in any kind of food in any condition whereas frozen buffalo meat is very susceptible for *L. monocytogenes* contamination. It happens due to improper

handling by the food handlers during pre and post processing. Sanitary condition should be satisfactory, due improper sanitation like use of sanitary agents for toilets, floor and walls, tables and other equipments, hand sanitation and more or less air sanitation play pivotal role at abattoir. As per FDA, BAM use of its method for isolation as one and only species here as *L. monocytogenes* plays a major role as simple diagnostic isolation of the organism for frozen buffalo meat as food borne pathogen. The aim of this experiment is to notice survival of *L. monocytogenes* in extreme low temperature as extremophiles and their isolation. It is remember that only *L. monocytogenes* is a highly pathogenic organism carry out its toxin food contamination and human health risk. But it is also consider that other species and strains of *Listeria* can lead to the disease but may be not create fatally rate as like *L. monocytogenes.* Hence, after consideration of overall reports and facts with these experiment of simple diagnostic lead it become clear and conclude that *Listeria monocytogenes* creates disease listeriosis with other clinical forms, highly fatal and risky if human consumption of frozen buffalo meat occurred and at last and final is great economic loss.

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