# A STUDY ON THE PREVALENCE OF *Salmonella* spp. IN ICE CREAM MARKETED IN THRISSUR CITY

**K. Radha* and V. Jinitha**
Department of Dairy Science, College of Veterinary and Animal Sciences,
Mannuthy, Kerala Veterinary and Animal Sciences University, Thrissur -680651, Kerala.

**Received:23.2.2016** **Accepted: 27.2.2016**

**ABSTRACT**

A *study was carried out to check the prevalence of Salmonella spp. in ice cream marketed in Thrissur city, Kerala. Altogether 80 ice cream samples belonging to 10 different brands were randomly collected from different retail outlets of the city and were analysed for total viable count, coliform count and for the presence of Salmonella spp. The mean total viable count and coliform count in ice cream samples were* 11.90±0.06 *and* 3.72±0.17 log cfu/g respectively. Thirty *per cent of the ice cream samples exceeded the standards prescribed for coliform count by Food Safety and Standards Act* (2006). *Salmonella spp. was isolated from one of the ice cream samples. The presence of potential pathogen such as Salmonella in ice cream samples reveals the importance of strengthening the quality control measures to be followed during manufacture as well as storage and distribution of ice cream in order to reduce public health risk.*

**Key words:** Ice cream*, Salmonella spp, prevalence of Salmonella in ice cream, Microbiological quality of ice cream*.

## INTRODUCTION

Ice cream is a nutritionally rich frozen dairy product preferred by all age groups in the population. It is prepared by the combination of milk, sweeteners, stabilizers, emulsifiers and flavouring agents. The popularity of ice cream can be attributed to its refreshingly cool and delightfully sweet characteristics besides its nutritive value. The high content of nutrients like lactose, proteins and the neutral pH of ice cream make it as an excellent growth medium for microbes some of which may cause serious disease outbreaks like cholera, typhoid and bacillary dysentery in human beings (Ahmed *et al.,* 2009). The major pathogens encountered in ice cream include *Listeria monocytogenes, Staphylococcus aureus and Salmonella spp*. Even though, pasteurization of the complete ice cream mix will reduce the number of micro-organisms, still it acts as a source of deteriorative and pathogenic microorganisms probably due to post pasteurization contamination. Incorporation of air during the freezing process also facilitates the survival and growth of microorganisms.

Recently, it has been seen that consumption of contaminated ice creams were responsible for many food poisoning outbreaks. Among these, *Salmonella* had been responsible for the largest number of food-poisoning outbreaks worldwide (Scallan *et al.,* 2011).

Several *Salmonella* outbreaks associated with serovars of *S. bongori and S. enterica* subspecies *salamae, arizonae, diarizonae*, *houtenae*, and *indica* has been reported. It is estimated that approximately 11% of the

food borne illness is caused by non-typhoidal *Salmonella* and these infections were the leading cause of hospitalization and death (Scallan *et al.,* 2011). Hence, the present study was designed to determine the bacteriological quality of ice cream marketed in Thrissur city with particular reference to the prevalence of *Salmonella* spp.

## MATERIALS AND METHODS

The ice-cream samples were collected from different retail outlets of Thrissur city. Samples were brought to the laboratory in an ice box. Altogether 80 ice cream samples belonging to 10 different brands were collected from retail outlets over a period of five months. All samples were evaluated for total viable count (TVC) and total coliform count by using plate count agar and violet red bile agar respectively by pour plate method. For the detection of *Salmonella* spp enrichment was done by using tetra thionate broth and then plated on brilliant green agar as per the standard procedure (ISO: 6579, 1993). Isolates were identified by cultural, morphological, biochemical characteristics and confirmed by polymerase chain reaction. A PCR based assay was devised to specifically detect contamination of ice cream with *Salmonella* serovar. This method utilizes specific primers to amplify *hilA* gene, which is conserved across *Salmonella* serovars (Marathe *et al.,* 2012). Agarose gel electrophoresis of the amplified PCR product was carried out along with 50 bp DNA marker in 1X TBE buffer. The data generated from this study were analysed by one way analysis of variance (Snedecor and Cochran, 1994). The bacterial counts of samples were compared with the standards prescribed by Food Safety and Standards Act (FSSA) for ice cream.

## RESULTS AND DISCUSSION

The results of the study are presented in Table No.1. The mean total viable count (TVC) of ice cream samples was 11.90±0.06 log cfu/g. According to Food Safety and Standards Act (2006), TVC of ice cream should not exceed 2, 50,000/g. In the present study, it was observed that the TVC of nine ice cream brands were within the prescribed limit. The samples belonging to brand "H" with a mean TVC of 12.52±0.03 cfu/g had exceeded the standard prescribed by FSSA (2006). Anuranjini *et al.* (2008) analysed the bacteriological quality of ice creams marketed in Mangalore city. They had reported that the total bacterial count and coliform count have exceeded the standards prescribed by BIS in all the 90 samples tested. Ambili and Beena (2012), found that the TVC of all the industrially produced ice cream samples tested were within the prescribed limit ($1.2x\ 10^2$ to $8.2x10^3$ cfu/g) whereas, the street vended samples showed higher TVC ranging from $5.2x10^5$ to $6.6x10^6$ cfu/g. According to Alam *et al.* (2015), primary sources of microbial contamination in ice cream include water and raw milk whereas secondary sources include flavouring agents, utensils and human handlers. Quality control measures such as use of high quality ingredients, proper pasteurization and maintenance of cold chain will improve the microbiological quality of ice cream.

The mean coliform count of ice cream samples in the present study was 3.72±0.17 log cfu/g (Table No.1). As per FSSA (2006), the coliform count in ice cream should not exceed 100/g. In the present study, 30 percent of samples had exceeded this limit. Anuranjini *et al.* (2008) also reported heavy contamination of ice cream samples with coliforms in Mangalore city. A study conducted by Jadhav and Raut (2014), revealed that all the ice cream samples tested were contaminated with coliform bacteria while 40 percent of ice cream samples were contaminated by the *E. coli*, 33 percent samples were positive for *Salmonella* and 40 percent showed growth of *Staphylococcus aureus.* Coliforms being non spore formers are susceptible to pasteurization. Their presence in ice cream indicates post pasteurization contamination. The other possible reasons for contamination

include use of poor quality water, lack of personal hygiene and improper cleaning and sanitization of utensils used for ice cream manufacture.

*Salmonella* spp. was isolated from one ice cream sample belonging to brand "G" which also had high TVC and coliform count (Table No.1). For the detection of *Salmonella* spp. enrichment was done by tetra thionate broth and then plated on brilliant green agar as per the standard procedure (ISO: 6579, 1993). The colonies of *Salmonella spp.* appeared as white to pinkish red within 18-24 hours of incubation on Brilliant Green Agar. The colonies were identified by primary, secondary tests and further confirmation was done by PCR technique.

**Primary identification tests**

***Gram's staining***

Pink coloured small rod shaped organisms were observed under oil immersion objective of microscope which indicated that the test organism was Gram negative bacilli (Table 2)

***Catalase test***

The test organism was mixed with 3% hydrogen peroxide solution. Active bubbling was observed within few seconds. This result confirmed that the organism was positive for catalase reaction (Table 2).

***Oxidase test***

When the colony of test organism was mixed with Oxidase disc, no colour change was produced on the disc. This indicated that the organism was negative for oxidase reaction (Table 2).

***Motility test***

Spreading growth on the Hugh and Leifsons medium from the line of inoculation indicated the motility of organism (Table 2).

**Secondary identification tests**

***Indole test***

Indole test was done to observe the ability of the organism to produce indole from amino acid tryptophan. After the addition of kovac's reagent a yellow coloured ring indicated that the organism was negative for indole test (Table 2 and Fig.1).

***Methyl red test***

Methyl red test was done to check the production of acid during the fermentation of glucose. Positive methyl red test was indicated by the development of red colour on addition of methyl red reagent (Table 2 and Fig.1).

***Voges-proskauer test***

Negative Voges-proskauer test was indicated by lack of colour change after the addition of Barritt's A and Barritt's B reagents (Table 2 and Fig.1)

***Citrate Utilization Test***

This test was done to check the ability of the organism to utilize citrate as a sole source of carbon. After the utilization the medium became blue in colour which indicated that the medium had become alkaline. The test organism was positive for this test (Table 2 and Fig.1).

***Triple sugar iron test***

Red coloured slant indicated that the test organism did not ferment either lactose or sucrose and black butt was developed due to hydrogen sulphide production. The test organism was positive for this test (Table 2 and Fig.2).

**POLYMERASE CHAIN REACTION (PCR)**

The increasing industrial interest towards rapid methods had led to the development of PCR based molecular methods for the detection

of microbes present in food principally during various stages of commercial production chain. Oligonucleotide primers targeting the *hilA* gene were used in the study for the confirmation of the isolated strains.

The DNA extracted from the *Salmonella* positive ice cream sample was subjected to polymerase chain reaction using hilA gene which is conserved across *Salmonella* spp. Agarose gel electrophoresis of the amplified PCR product was carried out along with 50 bp DNA marker in 1X TBE buffer. It revealed an amplified product size of 216 bp, when viewed under gel documentation system (Fig. 3).Thus the isolates were confirmed as genus *Salmonella.*

The presence of *Salmonella* in ice cream was reported by several earlier researchers. Yaman *et al.* (2006) had evaluated the microbiological quality of ice cream samples marketed in retail markets of Turkey and reported that 6.8 percent of ice cream samples tested positive for *Salmonella* spp. Subramanian and Vashistha (2007), reported the presence of *Salmonella* spp. in 44.4 percent of the ice cream samples tested in Udaipur city, Uttar Pradesh.

Jadhav and Raut (2014) also reported that 33 percent of ice cream samples were positive for *Salmonella* in their study conducted at Kolhapur city. According to a study conducted by Mathews *et al*. (2013), consumption of contaminated ice cream had been the cause for several food poisoning outbreaks. Chatli *et al.* (2014) conducted a study on the microbial quality of different milk products marketed in Ludhiana city. *Salmonella* spp. was found in 12.5 per cent of ice cream samples, 14.3 per cent each of rasogolla and paneer samples.

As per FSSA (2006) standards, *Salmonella* should be absent in 25 grams of the sample. In the present study, out of the 80 ice cream samples tested, only one sample showed positive result. Even though, the presence of *Salmonella* spp. was observed in only one sample in this study, it should be viewed seriously due to its public health significance. The findings highlight the importance of raw ingredient quality and hygienic practices to be followed during processing, stages of packaging, marketing and equipment hygiene.

Although, pasteurization is effective in destroying most of the pathogenic bacteria in ice cream, the additives like fruits, nuts, colouring and flavouring agents added after pasteurization process could act as a major source of contamination. The presence of *Salmonella* spp. in ice cream indicates that the quality control measures followed during processing are not adequate. Raw material quality, processing quality and hygiene at all levels till consumption are crucial to assure the safety of the product.

**Conclusion**

The results of the present study shows that TVC of 10 percent of ice cream samples and Coliform count of 30 percent of samples tested have exceeded the standards prescribed by FSSA (2006). The percentage of prevalence of *Salmonella* spp. in ice cream was 1.25. The microbiological quality of ice cream during retail marketing mainly depends upon post production handling of the product, in addition to the sanitary conditions adopted during frozen storage. The present study revealed that much attention is required for attaining desirable quality in the final product. The occurrence of *Salmonella* spp. in ice cream sample, suggests that protective measures are inadequate and stringent quality control measures are required to improve the microbiological quality of ice cream.

Good manufacturing practices (GMP) especially at pre and post pasteurization steps, prompt adherence to cold chain and automatic machines to minimize handling will be effective in assuring the quality of the ice cream.

**Figure.1. IMVIC test results of *Salmonella* spp.**

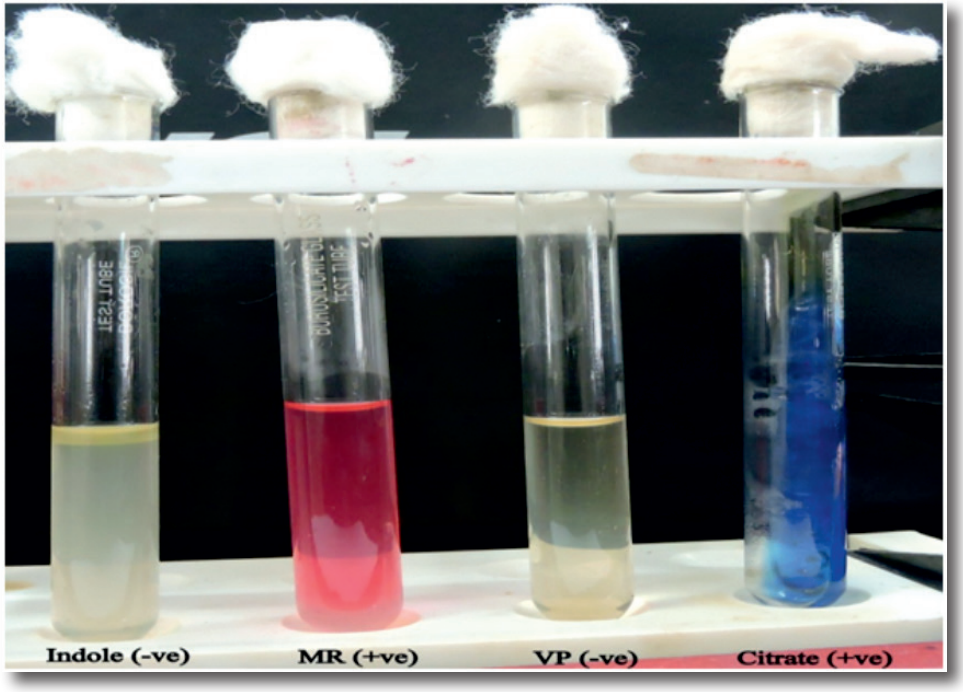
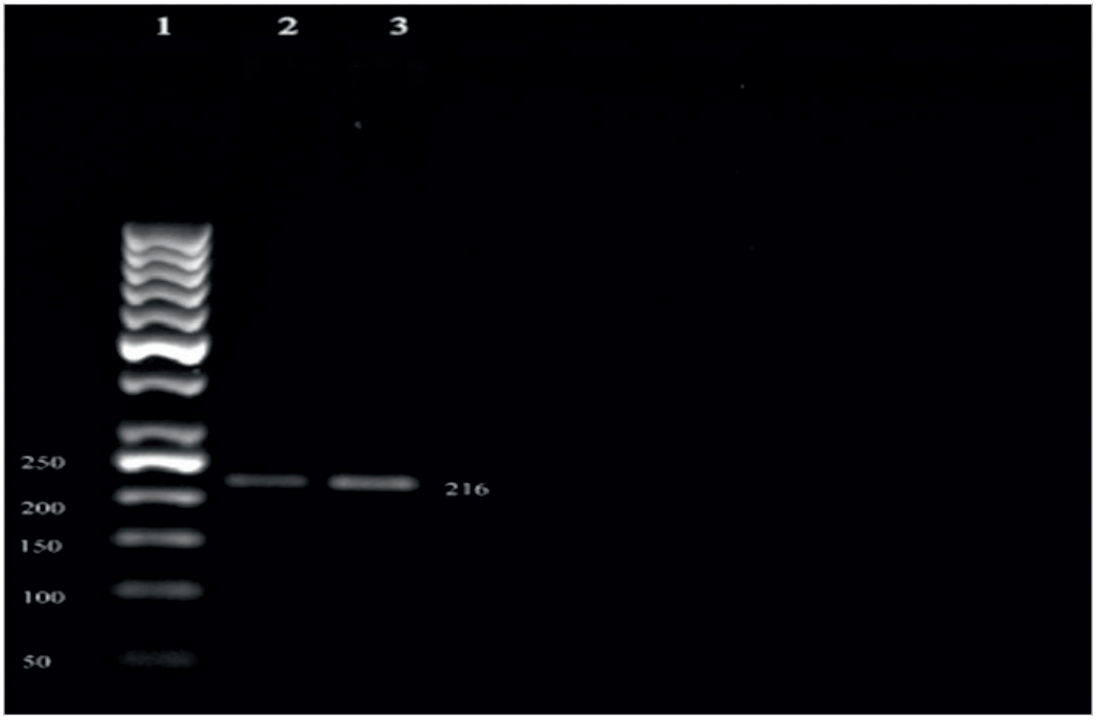



**Figure.2. TSI test result of *Salmonella* spp**

**Figure 2.Agarose gel electrophoresis of PCR amplified *Salmonella* gene showing 216 bp fragment**




Line 1- 50 bp DNA marker; Line 2- 216 bp fragment (Positive control); Line 3- 216 bp fragment (Test organism)

**Table-1. Bacteriological quality of market samples of ice cream**

| Brand name | Total viable count ($log_{10}$cfu/g) | Coliform count ($log_{10}$cfu/g) | *Salmonella* spp. |
|---|---|---|---|
| A | 11.67±0.60 | 4.80±0.15 | − |
| B | 12.15±0.37 | 2.07±0.63 | − |
| C | 10.57±0.09 | 3.90±0.30 | − |
| D | 11.62±0.06 | 3.90±0.30 | − |
| E | 12.42±0.05 | 3.07±0.50 | − |
| F | 12.33±0.05 | 4.20±0.20 | − |
| G | 12.41±0.05 | 5.10±0.10 | + |
| H | 12.52±0.03 | 5.20±0.10 | − |
| I | 11.24±0.09 | 2.80±0.70 | − |
| J | 12.08±0.05 | 2.30±0.70 | − |

**Table-2. Primary and secondary tests for the identification of *Salmonella* spp**

| Sl. No | Biochemical reactions | Results |
|---|---|---|
| 1 | Gram's staining | Gram negative rods |
| 2 | Catalase test | Positive |
| 3 | Oxidase test | Negative |
| 4 | Motility test | Motile |
| 5 | Indole test | Negative |
| 6 | Methyl red test | Positive |
| 7 | Voges-proskauer test | Negative |
| 8 | Citrate utilization test | Positive |
| 9 | TSI | Positive |

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