

## DETECTION OF *BACILLUS CEREUS* IN PASTEURIZED MILK SOLD IN LOCAL MARKET OF HISAR, HARYANA

DEVAN ARORA, DAVINDER SINGH, P.K. KAPOOR, Y. SINGH\*, V.J. JADHAV and ASHWANI KUMAR

Department of Veterinary Public Health and Epidemiology, College of Veterinary Sciences  
Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar- 125 004, India

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### SUMMARY

The present investigation was undertaken to assess the occurrence of *Bacillus cereus* in pasteurized milk of various brands marketed in Hisar city of Haryana. A total of sixty samples of pasteurized milk consisting fifteen each of four different brands were collected randomly from various retail stores ensuring that the 'best before' date was not exceeded. Isolation of *B. cereus* was attempted using polymyxin pyruvate egg yolk mannitol bromothymol blue agar and confirmed by standard procedures of staining and biochemical tests. Twenty two samples (36.67%) were found positive for *B. cereus*. The isolation frequency did not differ much amongst various brands. The results of this study revealed that significant proportion of pasteurized milk samples were positive for *B. cereus* which is of public health concern. The study emphasizes the need for production of safe, clean and wholesome milk under hygienic conditions following sanitary milking, adequate equipment sanitization and proper hygiene of the employees during all stages of production.

**Key words:** *Bacillus cereus*, pasteurized milk, food borne pathogen, PEMBA

*Bacillus cereus* is a Gram positive spore forming bacterium widely distributed in the soil and water. It is considered as an important enterotoxigenic food borne pathogen causing diarrhoeal and emetic food poisoning (Johnson, 1984; Kramer and Gilbert, 1989). Apart from gastroenteritis, *B. cereus* has a role in causing variety of non-gastro intestinal tract infections such as meningitis, endophthalmitis, endocarditis, periodontitis, osteomyelitis, wound infections and septicaemia in humans (Schoeni *et al.*, 2005). The presence of *B. cereus* in pasteurized milk affects its shelf life and poses other defects such as off flavors, sweet curdling and bitty cream. This organism is also responsible for spoilage of different food products (Meer *et al.*, 1991). Public health significance of its presence in pasteurized milk is due to its heat resistance and potential pathogenic character.

The importance of *B. cereus* in the dairy industry has been recognized since 1938, when the occurrence of bitty cream was recorded. However, *B. cereus* food poisoning was first described in 1950 following consumption of contaminated vanilla sauce (Granum and Lund, 1997). Since 1950, increasing awareness and recognition of *B. cereus* associated illness has resulted in a substantial increase in the number of reports of this type of food poisoning. In India, presence of this

organism has been reported in milk (Garg *et al.*, 1977; Chopra *et al.*, 1980) and other foods of animal origin (Bachil and Jaiswal, 1988).

The objective of this study was to investigate the occurrence of *B. cereus* in pasteurized milk of various brands marketed in Hisar city of Haryana. A total of sixty pasteurized milk samples, fifteen each of four different brands, were collected aseptically from local market ensuring that the 'best before' date did not exceed. The process of sample collection extended over a summer period of three months from April to June, 2013. The samples were brought to the laboratory on ice and processed within two hours of collection. Isolation of *B. cereus* was attempted by both enrichment with brain heart infusion broth as well as by direct inoculation on polymyxin pyruvate egg yolk mannitol bromothymol blue agar (PEMBA, Hi-Media), a selective *cum* differential medium recommended for isolation of *B. cereus* (Tewari *et al.*, 2013).

One ml milk sample was inoculated in nine ml of brain-heart infusion (BHI) broth (1:10) and processed for heat shock at 70° C for 15 min before incubation at 37° C for 24 h. 0.1 ml inoculum from enriched broth or neat milk sample was streaked on PEMBA medium plates and incubated at 37° C for 24 h. Colonies having fimbriate peacock blue colour of 3-5 mm size surrounded

\*Corresponding author: y.singhjadon@yahoo.com

by blue zone against green back ground were considered as presumptive *B. cereus*. These colonies were purified by growing thrice in BHI broth and streaking on PEMBA. The purified cultures were stored in maintenance media till their identification. Presumptive isolates of *B. cereus* were identified by procedures described by Cowan and Steels (1974) and MacFaddin (1976). These isolates were further subjected to different biochemical tests using Hi-Bacillus identification kit (Hi-Media).

Out of 60 milk samples, 22 (36.66%) samples were found to have characteristic fimbriate peacock blue coloured colonies surrounded by a blue zone against green background on PEMBA that were considered as presumptive *B. cereus*. Based on cultural (Gram positive, spore forming and motile rods) and biochemical (positive for catalase, nitrate, Voges–Proskauer and citrate, negative for oxidase, indole, urease and methyl red and producing acid from glucose and sucrose but not from lactose) characters, the presumptive isolates were characterized as *B. cereus*. Occurrence of *B. cereus* in pasteurized milk due to presence of their heat-resistant spores in the raw milk or by milk recontaminations through inadequately cleaned and sanitized surfaces have been described by Salustiano *et al.* (2009). There was not much variation amongst various brands with respect to isolation of this pathogen.

Higher incidence of *B. cereus* (37%) in raw milk had been reported by Martin *et al.* (1962). Matta and Punj (1999) found *B. cereus* as predominant organism in pasteurized milk and isolated 59 (32.2%) different *Bacillus* strains. Similarly, Reis *et al.* (2013) reported a higher prevalence rate (24.23%; 63 out of 260) of *B. cereus* in pasteurized milk and powdered milk sold in commercial establishments in Brazil. However, Banyko *et al.* (2009) reported 15.5% raw milk and pasteurized milk samples to be contaminated with *B. cereus*.

This study revealed the presence of *B. cereus* in pasteurized milk samples supporting the evidence of unsanitary conditions either during milk production at dairy farm or post-pasteurization contamination. Under the impression of milk being pasteurized is safe, public may consume it without heating but reports of milk borne illnesses may be correlated with such practices. This study emphasizes the need for production of safe, clean and wholesome milk under hygienic conditions following good milking as well as manufacturing practices during all stages of production.

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