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BACILLUS CEREUS IN RAW, PASTEURIZED AND INFANT FORMULA MILK

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Abstract

Bacillus cereus *is a spore-forming aerobic bacterium that offers a serious public health threat due to its extensive dispersal in the environment, particularly in milk and dairy milk products and its ability to generate toxins. Pasteurization is insufficient for total elimination of* B. cereus *in milk due to low-temperature sterilization for a short period of time, resulting in substantial economic loss and food safety issues. Two types of food poisoning caused by* B. cereus *diarrheal and emetic form. The purpose of this review is to provide information on the incidence of* B. cereus *in raw, pasteurized milk and infant formula milk. Bacillus cereus is a common agent of food poisoning, and its effects human health badly, so should be given special attention. To prevent the contamination of* B. cereus *in food and milk products strict sterilization and storage procedures should be followed.*

Keywords: *Bacillus cereus, Contamination Raw milk, Hygiene, Micro-biota, Proliferation*

INTRODUCTION

Milk is an essential nutrient for everyone, and it has a high water content and slightly acidic pH, making it a perfect environment for microbes to thrive (1). The micro biota in unpasteurized milk is complex, depending on animal health, seasonality, hygiene, species differences, and a range of other factors (2). Sporulating microbes are of main concern among the microorganisms that enter like milk. Despite the improvements in the dairy industry, milk deterioration due to spore contamination continues to be an alarming situation that results in significant financial losses (3). Unfortunately, sporulationg bacteria can widely contaminate milk and milk products. Sporulating bacteria are members of the Firmicutes phylum and are divided into five categories: Erysipelotrichia, Thermo-litho-bacteria, Clostridia, Bacilli, and Negativicutes (4). Among these microbes, *B. cereus* is also a well-known food-borne pathogen. Food borne illness outbreaks associated with milk and milk products have been documented, where raw milk as well as powdered milk, contain 85% enterotoxigenic *B. cereus* (5). The purpose of this review is to shed light on *Bacillus cereus'* importance as a foodborne pathogen in milk and milk products.

THE GENUS *B. CEREUS*

Bacillus is the oldest and the most varied genus of bacteria, belonging to the Bacillaceae family. Ferdinand Cohn discovered this bacterium in 1872. *B. cereus* is a facultative anaerobic, spore-forming, gram-positive rod bacterium with at least 226 species, making it the biggest genus in the Bacillaceae family. In addition, peritrichous flagella is present which aid in movement. This bacterium is also known as *Bacillus cereus* sensu lato (s.l.) and is mostly responsible for human foodborne outbreaks. Liu *et al*. (2015) documented nine closely related species *B. cereus* comprising *B. wiedmannii, B. pseudomycoides, B. mycoides, B. cytotoxicus, B. toyonens, B. weihenstephanensis, B. anthracis, B. thuringiensis,* and *B. cereus* group (6). Moreover, from 2017 onwards, from marine sediments, another nine new species were identified including, *B.*

paramycoides, B. proteolyticus, B. mobilis, B. tropicus, B. paranthracis, B. nitratireducens, B. nitratireducens, B. pacificus and *B. albus* (6, 7).

Members of this genus can survive in a wide range of settings due to their ability to produce endospores, which are resistant to dehydration, heat, and other physical stresses (8). This genus may grow in a wide range of temperatures, with the ideal temperature being 25-37 ºC, where psychotropic species can multiply at low temperature 3-5 ºC and thermophilic species, can proliferate at 75 ºC. Some species may live in extreme alkaline and acidic conditions with pH ranges of 2-10, resulting in a wide spectrum of physiological diversity. Thus Bacillus exist in a wide range of environments, from soil to thermal springs (9).

B. CEREUS CONTAMINATION IN RAW AND PASTEURIZED MILK

B. cereus is predominately present in unpasteurized milk as well as in variety of other dairy products. In addition, dung, hay, soil, and fodder, and contaminated surfaces of the milking utensils, are all main sources of *B. cereus* in milk and milk products. Different strains of *B. cereus* can tolerate industrial pasteurization due to endospore formation, whereas psychrotrophic strains can withstand cooling temperatures and it is a limiting factor for the shelf-life of pasteurised milk and cream stored at temperatures over 6°C . Psychrotrophic *B. cereus* endospores are often found in low concentrations in raw milk (i.e., <1/ml) (10).

Moreover, slow germinating spores germinate in milk at high-heat treatment at 80°C, whereas fast germination spores germinate within 24 hours at 20 - 72°C. Thus slow germination spores are more resistant to heat than quick germinating spores (11). Raw milk contains both types of spores, indicating that the milking procedures are associated with *B. cereus* infection. The study concluded that *B. cereus* endospore concentrations were highest in the summer and early fall, whereas in the winter, hay a dust and in rainy summers soil were key sources of contamination, respectively. Despite the fact that *B. cereus* is the only pathogen in the Bacillus genus that may cause food poisoning several other Bacillus species have been found as the source of bacterial spoilage of milk and milk products including: mesophilic spore-forming bacillus species *B. subtilis, B. licheniformis, B. pumilus*, and is thermotolerant species: *B. licheniform* (12).

DAIRY POWDERED MILK

Microbial growth in powdered dairy ingredients is minimal due to their low water content, while during the manufacturing process they might be adulterated with thermoduric or thermophilic sporulating bacteria (13). Furthermore, vegetative cells as well as endospores of this bacterium may endure pasteurization and form biofilms on the surfaces of stainless steel heat exchangers and evaporators. When fragments of cells or biofilms are sloughed off and enter the processing stream, contaminate the powdered components, affecting product quality and restricting the processing plant's run length (14). Despite the fact that the most thermophilic microbes do not to cause illness under ideal circumstances, latent endospores might propagate, generating acids and enzymes that affect the powder's composition and organoleptic qualities (15) .In extreme situations of spore contamination, concentrations of up to 105/g of milk have been detected, far exceeding standard limits and resulting in lower-value out-of-spec commodities that fail to meet customer expectations (16).

INFANT FORMULA MILK

In the baby formula milk industry, contamination of milk products by *B. cereus* is of major concern. Infants are more vulnerable to foodborne diseases, because of lack of weak normal microflora of gut and their underdeveloped immune systems. Furthermore, infant formula milk frequently comprises raw components from different sources that are nutrient-dense. When these milk products are reconstituted and stored at room temperature for an extended period of time, they create a favorable substrate for *B. cereus* growth and their enterotoxin synthesis (17). As a result, foodborne sickness is caused by high prevalence of *B. cereus* in infant formula milk.

Moreover, *B. cereus* spores have been found in UHT-treated milk and are known to withstand pasteurization (18). According to many research, the effective dose of *B. cereus* taken to induce sickness

symptoms is 105- 106 cells or spores/g. Thus, while considering the hazards of formula milk products, more attention should be paid to newborns and young children whose immune systems are less developed than those of healthy adult people (19).

TOXIGENICITY OF *B. CEREUS* IN MILK AND FORMULA MILK

According to numerous studies, *B. cereus* has been found to generate enterotoxins in fresh and dry milk products and newborn formula milk products. Many studies have found that isolated strain of milk and milk product exhibited cytotoxic activity when cultivated in artificial media in the laboratory, and thus must be considered possibly enterotoxigenic (20). According to Griffiths, inoculating *B. cereus* strains in reconstituted skim milk powder resulted in the production of enterotoxin after incubation at 10- 21°C. Consequently, factors such as aeration, pH, and temperature can effect proliferation and enterotoxin generation. In addition enterotoxin production can be evaded by maintaining the temperature below 4°C and the pH below 5.0 (21). Food intoxication caused by *B. cereus* is now being evaluated whether it is caused by preformed enterotoxin or enterotoxin synthesized in the ileum after consumption of *B. cereus* cells or spores. The amount of bacteria in milk and milk products depends on the type of farm and management and the type of product. It also differs from farm to the next and region to the region. In addition, during the shipping, storage, and disposal of milk and milk products, the bacterial burden may rise.

B. cereus is primarily responsible for two types of illness. The synthesis of heat labile enterotoxins in the host's small intestine during the growth of vegetative cells causes diarrhea. It is known as the "long-incubation" or diarrheal type of the illness. After proliferation period of 8 to 16 hours, this moderate disease causes cramps and diarrhea that last 6 to 12 hours. Diarrhea can be mild or severe, and it can be watery or bloody (22). Another type is emetic disease, also known as brief incubation disease, which is more severe and acute.

B. cereus generates one emetic toxin (ETE) and three other enterotoxins. Cytotoxin K, Hemolysin BL (Hbl), and Non-haemolytic enterotoxin (Nhe), are three pore-forming enterotoxins that cause diarrhoeal type of food poisoning On the other hand the emetic form of disease is caused by cerulide, a heat- and pH-stable peptide toxin that is pre-formed when eaten, resulting in a rapid onset of the illness (23).

OUTBREAKS OF *B .CEREUS* IN MILK AND MILK PRODUCTS

Among wide range of food-borne pathogens, *B. cereus* has been identified as the cause of food-borne outbreaks in the vast majority of instances. Since 1916, it has been recognized that *B. cereus* is mainly cause contamination in raw milk and milk product. A case study was conducted in china, where the diarrheal illness was reported at a rate of 730 per 1,000 individuals (24). In addition, endospores of *B. cereus* can survive after pasteurization. In dairy industry, psychrothrophic strains of *B. cereus* are known to impair keeping quality of pasteurized milk and develop a high protease activity (25). Smykal and Rokoszewska (1976) examined milk, dried milk and bakery products over a seven-year period (1964–1971) and found high frequencies of *B. cereus*. On the other hand, the results of another investigation showed that the prevalence of B. cereus in milk samples was 8.1% in pasteurized samples (26).

Saleh-Lakha *et al*. (2017) identified *B. cereus* spp. 18.7%, in commercial milk products8.3 % in boiled milk, 5.1 % in pasteurized cream and 15.3% in formula milk samples. A study conducted in Egypt, the findings of this study revealed that *B .cereus* was found in market milk, condensed milk and in milk powder with 52%, 10%, 8 %, respectively (27).

A research stated high prevalence of *B. cereus* was 35%, 48% 14% and 9% in raw milk, ice cream, Cheddar cheese and pasteurized milk, respectively (28). A factory in Hradec Kralove, Czechoslovakia species of *B. cereus* was identified in numerous infant,s' formula milk products (29). Another study conducted in Brazil, high occurrence of *B. cereus* was found in dairy cream, pasteurized milk and full-fat dried milk with 13.3 %, 66.6 % and 80.0 % respectively (30). Lesley *et al.* (2017) detected *B. cereus* 30% in UHT milk samples (31).

In dried milk, Reyes *et al*. (2007) identified 45.9% prevalence of *B. cereus* (32), Salem *et al*. (2015) identified *B. cereus* in pasteurized milk, UHT milk, and white cheese, with 40%, 20% and 30% respectively

(33). A study conducted in Pakistan, the results revealed that the incidence of *B. cereus* was 20% in raw milk, 50% in pasteurized milk and 10% packed milk of different companies (34). Additionally, Mohamed *et al*. (2016) identified *B. cereus* in raw milk and rice pudding with 60% and 55%, respectively (35). A research works conducted in Quetta, Pakistan the finding of this study showed that the prevalence of *B. cereus* was 10% in raw milk samples (36).

PREVENTION AND CONTROL OF BACILLUS FOOD POISONING

Endospores of *B. cereus* are heat resistant, Vegetative cells of *B. cereus* can be destroyed by pasteurization; however endospores cannot easily eliminate. If cooked food is not consumed right away, it must be cooled quickly and reheated properly. Temperature below 8 °C (or better 4–6 °C to avoid *B. cereus* development) must be maintained for long term storage. Foods with a low pH (pH 4.3) are thought to be safe from the development of food-poisoning Bacillus spp. The contaminated and infected cows' udder is the most common source of *B. cereus* contamination in milk. It' is vital to wash and wipe the udder and teats to prevent raw milk contamination by *B. cereus.* To control *B. cereus*, it is necessary to track the incidence of endospores from the farmer to the packaging. Apart from that, poor sanitation and/or food handling practices are the most common causes of food poisoning. Consequently, it is necessary to teach food handlers about their duties for food protection, give awareness about hygiene strategies and fundamental food handling techniques.

CONCLUSION

The bacterium *B. cereus* has been identified as the world's third most common cause of food poisoning. Because of their resistant endospores, Bacillus and related genera have long been a major concern for food producers. The endospore might be present in raw, pasteurized and various infant formula milks and have ability to endure temperature below 7 °C and above 45 °C. Approaches to limit spore germination and stop the growth of vegetative cells in milk and dried milk products may be the best strategy to efficiently avert and manage the incidence of this pathogenic bacterium. Furthermore *B. cereus* prevalence in milk and milk products should be investigated.

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