Imported food risk advice

Bacillus cereus in human milk and human milk products

Context of this risk advice

- Human milk means expressed milk collected from lactating women to be fed to infants that are not the biological infants of the women supplying the milk.
- Human milk products means products derived from human milk that have been specially formulated to meet the specific nutritional needs of infants such as fortifiers and formula.
- The level of risk for this hazard in human milk and human milk products was determined assuming that the most vulnerable category of infants (preterm infants in hospital neonatal intensive care units) would be receiving the products.

Nature of the hazard

Bacillus cereus is a Gram-positive, motile, spore-forming, rod-shaped bacteria that belongs to the *Bacillus* genus. *B. cereus* is commonly found in the environment (e.g. soil) as well as in a variety of foods. The spores produced by *B. cereus* are able to survive harsh environments including normal cooking temperatures (FSANZ 2013). *B. cereus* is one of the agents frequently involved in gastrointestinal diseases as it can produce toxins which cause food poisoning, but it is also a recognised opportunistic human pathogen that can cause severe local and systemic infections among at-risk populations, including neonates (Auger et al. 2009; Decousser et al. 2013; Hilliard et al. 2003).

Transmission

The primary mode of *B. cereus* transmission is via ingestion of contaminated food (Tewari and Abdullah 2015), as such *B. cereus* may be transmitted through human milk. Transmission of *B. cereus* can also result from exposure to environmental reservoirs via spores suspended in the air or present on surfaces and fomites (Glasset et al. 2018; Rigourd et al. 2018). It is often difficult to establish the mode or source of neonatal *B. cereus* infection (Decousser et al. 2013; Girisch et al. 2003; Hilliard et al. 2003; Manickam 2008).

B. cereus has been detected in human milk, including pooled human donor milk before and after pasteurisation (Decousser et al. 2013; Lima et al. 2017; Rigourd et al. 2018; Simmer 2011; Zhang et al. 2015). Between 2006-2011 an Australian human milk bank processed 1,919 batches of donor human milk of which 1.7% showed growth of *Bacillus* spp., including *B. cereus,* after pasteurisation (Simmer 2011). Similarly, in 2003 an American human milk bank processed 303 pooled batches of pasteurised donor milk. After pasteurisation 6% of pooled milk samples showed *Bacillus* spp. growth on routine cultures (Landers and Updegrove 2010). These reports demonstrate that *B. cereus* is an occasional contaminant of pasteurised donor milk. Pasteurised donor milk has not been identified as a source of *B. cereus* infection, however this may be attributed to donor milk being discarded if *B. cereus* is detected post-pasteurisation (as per the processes of the Human Milk Banking Association of North America and other international human milk banks, such as in Australia).

Disease severity

B. cereus generally causes mild gastrointestinal disease. However in vulnerable individuals such as new-borns, *B. cereus* is an opportunistic pathogen and can cause invasive infection. Although it is often difficult to establish the source of *B. cereus*, some literature suggests that under certain circumstances foodborne *B. cereus* could be associated with the development of invasive infections, potentially by translocating from the gastrointestinal tract (Bottone 2010; El Saleeby et al. 2004; Hilliard et al. 2003; Rigourd et al. 2018).

B. cereus is a serious hazard in new-borns as it can cause life threatening illness with or without sequelae. *B. cereus* can cause severe invasive diseases in immunosuppressed individuals such as neonates including septicemia, endophthalmitis[1], pneumonia, endocarditis[2], meningitis[3] and encephalitis[4], with a case fatality rate of approximately 10% (Bottone 2010; Gaur et al. 2001; Glasset et al. 2018; Hilliard et al. 2003; Rigourd et al. 2018).

B. cereus usually causes gastrointestinal disease. There are two main types of intoxication disease syndromes caused by *B. cereus*. The diarrhoeal syndrome is caused by diarrhoeal toxins produced during growth of the bacteria in the small intestine. In the general population, this syndrome is mild and primarily manifested by abdominal cramps, watery diarrhoea and occasional nausea and emesis[5] (Stenfors Arnesen et al. 2008; Tewari and Abdullah 2015). The emetic syndrome, which is more severe and acute than the diarrhoeal syndrome, is an intoxication caused by the *B. cereus* emetic toxin (pre-formed when ingested) and is characterised by nausea and emesis[5] happening shortly after ingestion (Stenfors Arnesen et al. 2008; Tewari and Abdullah 2015). In either syndrome, the illness is usually mild and lasts less than 24 hours (FSANZ 2004; Tewari and Abdullah 2015). Although no specific information on the severity of *B. cereus* gastrointestinal disease in neonates was identified, it is important to note that due to their under-developed immune systems, infants are more susceptible to enteric bacterial pathogens, present severe response to toxins, and have increased mortality (FSANZ 2004).

Infectivity

The infective dose of *B. cereus* in human milk is not known. However, for both types of *B. cereus* foodborne disease, a relatively high number of cells has been found in the implicated foods. For the diarrhoeal disease, 10^5-10^8 cells or spores have been indicated as the infective dose, although doses as low as 10^3 cfu/gram have been found in foods causing illness (Stenfors Arnesen et al. 2008). The number of *B. cereus* cells required to produce sufficient emetic toxin to cause disease has not been determined, but in implicated food levels of 10^3-10^{10} cfu/gram have been found and in most cases at levels $\geq 10^5$ cfu/gram (Stenfors Arnesen et al. 2008). Infants may be susceptible to illness from a lower infectious dose but there is no available data to support this (FSANZ 2004).

Risk mitigation

Controls are required to minimise contamination of human milk with *B. cereus.* The persistent contamination of industrial food processing systems by *B. cereus* is due to its largely heat stable spores which may survive pasteurisation (including Holder pasteurisation, 62.5^{o}C, 30 minutes). Furthermore, the pasteurisation process can cause *B. cereus* spores to germinate (Lima et al. 2017; Simmer 2011). The capacity of *B. cereus* to form biofilms may also contribute to its persistence in hospital environments and food-processing-related conditions given it provides a protected mode of growth on inert surfaces and has been shown to be highly resistant to cleaning procedures (Auger et al. 2009; Bottone 2010; Kwon et al. 2017). In addition, the emetic toxin is extremely resistant to heat (surviving heating at 126°C for 90 minutes) and could remain in the milk even after all viable bacteria have been destroyed by heating (Juffs and Deeth 2007).

The safe production of human milk and milk products is dependent on maintaining a high level of hygiene control during collection, handling, processing, storage and transport to minimise the contamination of milk with *B. cereus*. This is achieved by obtaining and treating donor human milk according to best practice guidelines followed by international donor milk banks, including those in Australia. Milk must be collected hygienically from the donors, with donors instructed about the importance of hand washing, cleaning and sterilising pumps, and the use of appropriate containers. Donor milk should be refrigerated (4^{o}C) immediately after collection and then stored frozen at -20^{o}C (Hartmann et al. 2007; HMBANA 2015; UKAMB 2003).

Human milk products should be produced from milk that has been subjected to Holder pasteurisation or an equivalent thermal treatment during processing to reduce the risk from microbiological contamination. However, if human milk is heavily contaminated with *B. cereus* cells and/or spores or if heat stable bacterial toxins are present, Holder pasteurisation used by international human milk banks may be ineffective (Juffs and Deeth 2007). *B. cereus* spores are able to geminate without preliminary heat treatment; however the rate and proportion of germination are higher when the spores are heat-activated at temperatures in the range of 60-95°C for various times (Juffs and

[1] Infection of the interior of the eye
[2] Infection of the inner lining of the heart
[3] Infection of the membrane covering the brain and spinal cord (the meninges)
[4] Infection of the brain
[5] Vomiting

Deeth 2007; Simmer 2011). Therefore, pre- and post-pasteurisation microbiological criteria are used for human milk as described in international best practice guidelines to ensure the effectiveness of Holder pasteurisation and the microbiological safety of donor human milk (Bharadva et al. 2014; Hartmann et al. 2007; HMBANA 2015; UKAMB 2003). The Human Milk Banking Association of North America and other international human milk banks, such as in Australia, screen human milk for *B. cereus* post-pasteurisation and discard any batches of milk that are positive for this bacterium (Lima et al. 2017; Simmer 2011). Process hygiene criteria are also useful to verify that the hygiene measures in place in the manufacturing facility are working as intended (FSANZ 2018).

Milk banks and manufacturers of human milk products should utilise Good Manufacturing Practices, Good Hygienic Practices and an internationally recognised hazard management tool, such as the hazard analysis and critical control points (HACCP) process to identify, evaluate and control hazards (Codex 2008; Hartmann et al. 2007; HMBANA 2015; PATH 2013). Specifically, facilities and equipment used to process human milk and human milk products should be designed, constructed and laid out to prevent the entry of pathogens into high hygiene areas and to minimize their establishment or growth in harbourage sites, including the prevention of biofilm formation, and designed to facilitate appropriate cleaning (Codex 2008; Marchand et al. 2012).

Pasteurised human milk is stored and transported frozen. Once thawed, human milk should be kept refrigerated (4^{o}C) until use and should be used within 24 hours. The human milk should be discarded after completion of the initial feed. If fortifiers are added to the human milk, the fortified human milk should be kept refrigerated and used within 24 hours. Thawed pasteurised human milk and fortified human milk should not be refrozen (Hartmann et al. 2007; Jones 2011; UKAMB 2003).

Evaluation of uncertainty

Severe and possibly life threatening disease can occur in newborns associated with *B. cereus* infection. However, there is uncertainty around the route of transmission. As human milk banks recommend discarding milk if *B. cereus* is detected post-pasteurisation, there is little evidence of illness. However, in the absence of this practice of screening pasteurised milk, it is unknown how often illness would potentially occur. Therefore, there is uncertainty around human milk as a source of *B. cereus*-associated disease in neonates and the number of cells required to cause infection via this route of transmission. If assumed to be similar to documented cases of contaminated food, the infectivity of *B. cereus* in human milk would be low.

Pooling of human milk from multiple donors is common practice amongst many human milk banks and would dilute the bacterial load from a single donor, however some milk banks only pool milk from individual donors (Haiden and Ziegler 2016). The Australian Red Cross milk bank pasteurises human milk in single donor batches (Australian Red Cross 2018). However, potential environmental contamination of the human milk during collection, processing and/or post-processing may increase the bacterial load of the milk.

Risk characterisation

There is evidence of *B. cereus* being present in human milk, however the epidemiological evidence to link the ingestion of *B. cereus* and the development of *B. cereus*-associated infection in infants is limited. This is thought to be due to *B. cereus* positive human milk being routinely discarded by international human milk banks. In the event of human milk becoming contaminated with *B. cereus* and being fed to a neonate, large doses may be required to cause illness. There is a medium likelihood of exposure to *B. cereus* through human milk and human milk products as Holder pasteurisation does not inactivate spores or pre-formed toxin. Also, inadequate hygiene practices of collection, handling and storage, and/or inadequate processing or post-processing practices, could facilitate its contamination.

B. cereus is a serious hazard in neonates and can cause life threatening disease. In imported human milk and human milk products *B. cereus* presents a potential medium or high risk to public health and safety.

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