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Haumaru Kai Aotearoa

Risk Profile: *Bacillus cereus* in rice and starchy foods

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1 Executive Summary

This Risk Profile considers *Bacillus cereus* in rice and other starchy foods. *B. cereus* is a toxin- and spore-forming pathogen ubiquitous in the environment. Depending on the type(s) of toxin produced, *B. cereus* can cause either diarrhoeal or emetic illness if ingested. *B. cereus* spores are hardy and can survive the cooking process, sporulating into vegetative cells and proliferating if conditions allow. A wide range of environmental conditions support survival and growth of *B. cereus.*

Absence of *B. cereus* colonies by culture does not rule out *B. cereus* as a causative agent in foodborne illness, particularly for the emetic toxin which can be pre-formed in food. The infectious dose for both types of illness is >10^5 cfu/g of food or an estimated concentration of emetic toxin of 8-10 µg/kg body weight.

B. cereus intoxication is regarded almost exclusively as a foodborne illness which can be prevented with storage of cooked foods at appropriate temperatures. This consists of keeping cooked food not eaten immediately above 60 °C, cooling cooked food to below 5 °C within four hours and keeping it at this temperature until it is eaten. These steps minimise the time that foods are at temperatures which support growth of *B. cereus*.

Although the number of outbreaks and linked cases is low in New Zealand, *B. cereus* intoxications are only notifiable in New Zealand if they are part of an outbreak, the person is in a high-risk category or their illness is deemed to be of public health significance. The majority of outbreaks in recent years have been linked to rice-based meals. The generic nature of diarrhoea as a symptom of foodborne illness may mean *B. cereus* is under-represented as a cause of diarrhoeal illness.

2 Introduction

Risk Profiles provide scientific information for risk managers relevant to a food/hazard combination as part of the wider risk management framework[1]. This document provides a Risk Profile considering *Bacillus cereus sensu stricto (s.s.)* in rice and starchy foods, which is associated with *B. cereus* foodborne illness, particularly emetic disease. A Risk Profile on *B. cereus* in rice was previously published in 2004[2].

This Risk Profile focuses on *B. cereus s.s.,* a genomospecies belonging to the '*Bacillus cereus* group' [also known as *B. cereus sensu lato* (*s.l.*)], a group of closely related *Bacillus* species. *Bacillus cereus s.s.* is the most commonly identified species among the *Bacillus cereus* group as causing foodborne illness. The foods considered in this Risk Profile are rice and starchy foods, such as potatoes, pasta, noodles, and flour-based products, which are often implicated as vehicles of *B. cereus s.s.* (referred to in this Risk Profile as *B. cereus*) illness.

The purpose of this Risk Profile is to critically review available information to answer the following Risk Management Questions:

1) How do food products become contaminated with *B. cereus*?
2) What information is available on prevalence of *B. cereus* in New Zealand foods and how does this compare with other countries?
3) How much illness is caused by *B. cereus* in New Zealand?
4) What control measures are the most efficacious?

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[1] https://www.mpi.govt.nz/dmsdocument/23029-new-zealands-food-safety-risk-management-framework Accessed 10 September 2020

[2] https://www.mpi.govt.nz/dmsdocument/35328-Risk-Profile-Bacillus-cereus-in-rice Accessed 18 December 2020

3 Hazard identification: the organism

3.1 *BACILLUS CEREUS*

Bacillus spp. are large (0.6-1.2 µm wide; ~3.5 µm long) Gram-positive (or Gram-variable), spore-forming, aerobic or facultative anaerobic, rods with wide distribution in the environment. The '*Bacillus cereus* group' (also known as *B. cereus s.l.*) includes several closely related *Bacillus* genomospecies, which have been proposed as: *Bacillus pseudomycoides, B. paramycoides, B. mosaicus* (includes the species formerly known as *B. anthracis*), *B. cereus sensu stricto (s.s.)* (includes the species formerly known as *B. thuringiensis*), *B. toyonensis, B. mycoides* (includes the species formerly known as *B. weihenstephanesis*), *B. cytotoxicus*, and *B. luti.* Additions of *"B. bingmayongensis", "B. gaemokensis", "B. manliponensis"* and *"B. clarus"* have also been proposed as novel genomospecies (Carroll et al., 2020). The final taxonomic assignment can include subspecies, serovar, and biovar depending on various genomic, serologic, and phenotypic tests. *B. cereus* s.s. is the most common *Bacillus* genomospecies identified as causing foodborne illness worldwide.

3.2 *B. CEREUS* TOXINS

B. cereus can produce several different toxins which can cause two distinct types of foodborne illness: 1) emetic disease caused by the depsipeptide toxin, cereulide; and 2) diarrhoeal disease caused by a cytotoxin K variant, non-haemolytic or haemolytic enterotoxin complexes (Stenfors Arnesen et al., 2008, Ehling-Schulz et al., 2004, Schoeni & Wong, 2005).

3.3 GROWTH

Strains of *B. cereus* have a growth temperature range of 4-48 °C (optimally 28-35 °C) (FDA, 2012). Toxin can be produced within a narrower temperature range of 10-40 °C (optimally 20-25 °C). The range of pH for growth for vegetative cells is 4.3-9.3 (optimally pH 6-7) and, depending on the solute, the minimum water activity (a_w) range for growth is 0.912-0.950 (Jenson & Moir, 2003). *B. cereus* tolerates NaCl concentrations of 7.5% (FDA, 2012). Although *B. cereus* vegetative cells are facultative anaerobes, emetic toxin production requires oxygen.

3.4 SURVIVAL

B. cereus vegetative cells themselves are not particularly resistant to various environmental stresses. However, *B. cereus* spores (endospores) persist in the environment, particularly in soil and water run-off from soil (Jenson & Moir, 2003). Even at low temperatures, sporulation occurs relatively soon after the cessation of growth (Jenson & Moir, 2003). *B. cereus* spores are hardier than vegetative cells due to their metabolic dormancy and robust physical nature (Jenson & Moir, 2003) and are resistant to acid, heat, UV light, and desiccation (Ehling-Schulz et al., 2015). Spores can survive gastric acidity (pH 1-5.2) (Clavel et al., 2004), cooking at or below 100 °C (van Asselt & Zwietering, 2006) and in cereal with a_w of 0.27-0.28 for almost a year (Jaquette & Beuchat, 1998). The emetic toxins are stable between pH 2-9 and at 121 °C for 80 minutes and 150 °C for 60 minutes (Rajkovic, 2014). Diarrhoeal toxins are stable between pH 4-11 and 45 °C for 30 min (Jenson & Moir, 2003).

3.5 INACTIVATION

Vegetative cells of *B. cereus* do not survive routine cooking temperatures (Desai & Varadaraj, 2010), with *D*-values of 1 min at 60 °C (Byrne et al., 2006). Depending on temperature, vegetative calls can be inactivated in low pH foods like yoghurt (pH 4.5) and fruit juice (pH 3.7). Most food industry sanitisers destroy vegetative *B. cereus* cells on surfaces. Household chlorine-based bleaches (sodium hypochlorite; 100 ppm) show effectiveness against vegetative cells, although not spores (Kwak et al., 2014). Chlorine and chlorine dioxide at concentrations of 100 µg/ml were found to be effective in killing *Bacillus cereus* spores on apple surfaces, with reductions of ≥3.8-4.5 log cfu per apple (Kreske et al., 2006). Experiments have shown *B. cereus* vegetative cells were unable to survive an *in vitro* gastrointestinal passage, even at a high inoculum (7-8 log cfu/ml). Vegetative cells that survived the simulated gastric passage were subsequently inactivated in the simulated duodenum phase by bile (Ceuppens et al., 2012). Depending on the food matrix, *B. cereus* spores can be inactivated in cooked rice at 600 MPa at 60 °C for 15 min, 75 °C for 10 min, 85 °C for 4 min, or 0.1 MPa at 85 °C for 180 min (Daryaei et al., 2013). Domestic cooking requires heating of food to 121 °C for 90 minutes to destroy *B. cereus* emetic toxin. *B. cereus* diarrhoeal toxins are not stable outside the of pH range of 4-11 and can be inactivated by 56 °C for 5 min (Jenson & Moir, 2003).

3.6 IDENTIFICATION

B. cereus cannot always be reliably differentiated from other *B. cereus* group species by biochemical, physiological or morphological characteristics and production of toxin is not specific to any species of *Bacillus* (Jenson & Moir, 2003). This generality extends to analysis by molecular methods too, due to the highly conserved nature of many marker genes (Zheng et al., 2013). Current standard culture methods use Mannitol-egg yolk-polymyxin (MYP) agar and/or chromogenic agars to select for *B. cereus* in foods (ISO, 2020, Tallent et al., 2019). Microscopically, spore staining reveals *B. cereus* spores are ellipsoidal, paracentral or subterminally placed, and do not distend the cell (Logan & De Vos, 2015).

3.6.1 Emetic toxin

During foodborne investigations, absence of *B. cereus* colonies by culture in the food product examined does not rule out *B. cereus* as a causative agent in foodborne illness, particularly the emetic disease because the emetic toxin, cereulide, can be pre-formed in the food (Rajkovic, 2014). Attempts to develop a reliable immunologic test for cereulide have been unsuccessful, as it is not particularly antigenically active, but emetic activity can be detected by a boar spermatozoan motility assay and measured by Liquid Chromatography-Mass Spectrometry (LC-MS) (Jääskeläinen et al., 2003, Häggblom et al., 2002). Mass spectrometry can also distinguish between the 18 variants of cereulide reported (Marxen et al., 2015). Ability to produce the emetic *B. cereus* toxin has been successfully detected by polymerase chain reaction (PCR) assays targeting the cereulide synthetase (*ces*) gene (Fricker et al., 2007), and differentiation between emetic and non-emetic isolates has been achieved with a 99.1% success rate using matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) (Ulrich et al., 2019).

3.6.2 Diarrhoeal toxins

Genes encoding non-haemolytic enterotoxins (Nhe) are ubiquitously found in *B. cereus* group isolates. Their contribution to diarrhoeal *B. cereus* disease is not fully understood (Doll et al., 2013). Haemolytic enterotoxins (Hbl) and cytotoxin K (CytK) are the other major causative toxins produced by diarrhoeal *B. cereus*. Both Nhe and Hbl toxins are composed of three different proteins, while CytK is a single-protein toxin (Stenfors Arnesen et al., 2008). Enterotoxin FM (EntFM) has also been implicated in *B. cereus* virulence. Evidence of *B. cereus* diarrhoeal enterotoxin production or gene carriage can be detected using various biologic, immunologic and molecular assays (e.g., cell culture, western blotting, visual immunoassays, PCR) (Moravek et al., 2006, Guinebretière et al., 2006). It is likely that multiple toxins act together to cause gastroenteritis (Callegan et al., 2003).

3.6.3 Toxin production ability

In terms of the proportion of *B. cereus* group strains that produce toxins, Nhe has detection ranges between 73-100% of strains, Hbl 42-85%, and CytK 28-53% (Tallent et al., 2015). Among their *B. cereus* collection, the gene for cereulide production, *ces*, was detected in 19% of isolates, making it the least common of the toxin genes tested. This differs from an earlier Thai study that found 324 (88.8%) of 365 *B. cereus* isolates from food and soil carried the *cytK* gene and 66.2% carried the *hblCDA* genes (Ngamwongsatit et al., 2008). *entFM* appears to be a gene common to *B. cereus* strains (Ngamwongsatit et al., 2008, Tran et al., 2010).

The mechanisms for triggering emetic toxin production are not well understood, but external physiological signals, like cell respiration, redox potential regulation and growth have been shown to modulate production (Ehling-Schulz et al., 2015). The diarrhoeal syndrome is caused by diarrhoeal toxins produced during vegetative cell growth of the bacteria in the small intestine (Ehling-Schulz et al., 2006). In terms of triggers for toxins, porcine gastric mucin has been shown to greatly enhance enterotoxin production (Jessberger et al., 2019). Human gastric mucin may have a similar role in human pathogenesis. There is evidence to suggest that emetic toxin-producing *B. cereus* do not hydrolyse starch (Pirhonen et al., 2005).

3.6.4 Phenotyping

B. cereus cultures are usually strongly β-haemolytic, produce a lecithinase precipitation zone on MYP and Bacara agars, are Voges-Proskauer (VP) positive, and do not ferment mannitol, although these traits will not distinguish between other *B. cereus* group species (Tallent et al., 2019). Pathogenic and innocuous strains of *B. cereus* have been shown to be distinguishable using a combination of phenotypic characteristics, specifically, motility, epithelial cell adhesion, cytotoxicity, biofilm formation, polymyxin B resistance, and virulence in an insect model (Kamar et al., 2013).

3.6.5 Multilocus sequence typing

A multilocus sequence typing (MLST) scheme based around seven housekeeping genes (*glpF, gmk, ilvD, pta, pur, pycA*, and *tpi*) was devised to separate *Bacillus* spp. lineages (Priest et al., 2004). This scheme is utilised for *B. cereus* isolate data uploaded to the global, open-access database, PubMLST.org (Jolley et al., 2018).

3.6.6 Whole genome sequencing

Whole genome sequencing (WGS) has been suggested as a useful pre-screening tool to detect *B. cereus* toxin genes (*ces*, *nheABC, hblCDA*, *cytK,* and *entFM*) in foods, although further testing would be required to confirm toxin production had occurred (Nguyen & Tallent, 2019). WGS has been successful in detecting toxin genes in otherwise PCR-negative isolates (Miller et al., 2018), perhaps due to better sensitivity of the assay.

4 Hazard identification: the foods

Dairy products are outside the scope of this Risk Profile [see previously published Risk Profile on *B. cereus* in dairy products (Cressey et al., 2016)].

Starchy foods, such as rice, pasta, noodles, potatoes, beans, and bread, are most often implicated with *B. cereus* emetic disease (McDowell et al., 2020). Starchy foods contain starch, a dietary energy source composed of two glucose polysaccharides; amylose and amylopectin (Martens et al., 2018). Foods often implicated in the diarrhoeal syndrome include meat products, fish, poultry, and vegetables products (Webb et al., 2019).

4.1 RAW FOODS

Reflecting that *B. cereus* is ubiquitous in the environment, it is often detected in raw rice grains (Lee et al., 1995, Ankolekar et al., 2009, Bilung et al., 2016). It has been suggested that *B. cereus* migrates into the rice seed as it grows and subsists in the hardened rice seed in spore form (Okunishi et al., 2005). This latter Japanese study reported *B. cereu*s was the only bacterial species isolated in rice beyond the early, softer stages of growth.
B. cereus has been found in the inner tissues of wheat cultivars, including during the heading stage of growth (Comby et al., 2016), indicating flour contamination is likely from the wheat source, although it has also not been observed to survive baking temperatures in bread (Rosenkvist & Hansen, 1995). Presumptive *B. cereus* has been isolated from dried pasta (Akineden et al., 2015) and pasta products (te Giffel et al., 1996) to varying degrees. Potatoes, like other vascular plants, are not sterile - containing microorganisms in the water channels and extracellular spaces (Hoornstra et al., 2013). *Bacillus* spp. have been isolated from dried potato flakes (Heini et al., 2018). Starch has been reported to promote growth of *B. cereus* and the production of emetic toxins (Yu et al., 2020).

4.2 COOKED AND PROCESSED FOODS

As well as dried products, cooked potato has been shown to harbour *B. cereus* (Carlin et al., 2000). Various other starchy cooked food matrices have been shown to support growth of *B. cereus* at room temperature (Blakey & Priest, 1980, Nester & Woodburn, 1982). Cooling of cooked beans, pasta, and rice from 54.5 to 7.2 °C over extended periods of time was observed to allow steady growth of *B. cereus* after spore germination, with an approximately 1.0 log cfu/g increase over 6 h to an approximately 4.0 log cfu/g increase after 21 h (Juneja et al., 2018).

Elevated levels of *B. cereus* were the reason for a recent food recall of fermented bean paste in New Zealand (MPI, 2020). Recent (2019-2020) starchy based food recalls for *B. cereus* contamination internationally include maize and cassava flour of Ghanaian origin in Belgium[3], Paella (with seafood) in Luxembourg[4], bean-based vegetarian burger patties in Canada[5], and hummus in USA[6]

5 Hazard characterisation: the illness

Non-foodborne infections caused by the opportunistic *B. cereus* will not be covered in this Risk Profile. They are comprehensively described by Bottone (2010).

5.1 *B. CEREUS* ILLNESS

Foodborne *B. cereus*-associated illness has two distinct forms; emetic and diarrhoeal, both are usually self-limiting. Symptoms tend to be mild and self-limiting, with recovery usually within 12-24 h without long-term effects. Mortality is rare (FDA, 2012).

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[3] http://www.favv-afsca.fgov.be/rappelsdeproduits/2018/2018-09-21.asp Accessed 27 August 2020

[4] https://securite-alimentaire.public.lu/fr/actualites/alertes/2020/Aout/paella-fruits-de-mer-brasserie-saveurs-maison-bacillus-cereus.html Accessed 24 August 2020

[5] https://www.mapaq.gouv.qc.ca/fr/Consommation/rappelsaliments/2020/08/Pages/4173.aspx Accessed 24 August 2020

[6] https://commissaries.com/our-agency/newsroom/news-releases/hope-foods-organic-hummus-pulled-commissary-shelves Accessed 27 August 2020

5.1.1 Symptoms

The main symptoms of the emetic disease are nausea and vomiting with an onset of 0.5-5 h after ingestion and a duration of 6-24 h. Diarrhoea is uncommon. The main symptoms of the diarrhoeal disease are profuse watery diarrhoea, abdominal cramps, and occasionally nausea, 8-16 h after ingestion with a duration of 12-24 h. Vomiting is rare.

5.1.2 Infective dose

The infective dose for the emetic syndrome is estimated to be 10^5-10^8 cells/g (Granum & Lund, 1997) or an estimated concentration of cereulide of 8-10 µg/kg body weight (Agata et al., 1995, Shinagawa et al., 1995, Jääskeläinen et al., 2003). The infective dose for the diarrhoeal syndrome is estimated at 10^5-10^7 cells ingested (Granum & Lund, 1997). Although fatalities are rare, both syndromes have been linked to deaths associated with consumption of contaminated foods.

5.1.3 Susceptibility and treatment

All people are considered susceptible to *B. cereus* food illness (FDA, 2012) but the elderly, immunocompromised, and patients with lower stomach acidity may be more vulnerable, particularly to the diarrhoeal syndrome (Clavel et al., 2004, Stenfors Arnesen et al., 2008). *B. cereus* foodborne illness may be treated by rehydration or other supportive therapies if diarrhoea or vomiting were severe. Antimicrobial treatments are not indicated in *B. cereus* intoxication cases but may be effective for patients with invasive (non-foodborne) disease (American Academy of Pediatrics, 2018).

5.1.4 Global burden

The World Health Organization (WHO) Foodborne Disease Burden Epidemiology Reference Group estimated that *B. cereus*, globally, was responsible for 256,775 intoxications [95% uncertainty interval (UI) 43,875-807,547] in 2010 (WHO, 2015). *B. cereus* foodborne illness is likely to be under-reported as both types of illness are often mild and last less than 24 h (European Food Safety Authority, 2005). Unlike other bacterial intoxications, *B. cereus* was determined to have a very low fatality rate [0 (95% UI 0-0)] with Disability Adjusted Life Years (DALYs) estimated at 45 (95% UI 7-171). It was noted that *B. cereus* intoxication data were scarce and only available from seven low-mortality countries, including New Zealand.

5.2 NEW ZEALAND

In New Zealand, *B. cereus* intoxication is classified under the umbrella term ‘acute gastroenteritis’, which includes other toxin-related illnesses such as those caused by *Staphylococcus aureus* and *Clostridium botulinum*. *B. cereus* illness is notifiable as acute gastroenteritis if there is a suspected common source, the person is in a high-risk category (e.g., food handler, early childhood service worker) or it is deemed an infectious gastroenteritis of public health significance (Ministry of Health, 2017). *B. cereus* illness is likely to be under-reported in New Zealand (as elsewhere) due to the illness categorisation, the usually mild nature of illness, and short duration of symptoms (European Food Safety Authority, 2005).

5.2.1 Outbreaks

Figure 1 and Table 1 show the number of *B. cereus* outbreaks and associated cases in New Zealand each year from 2007 to 2019.

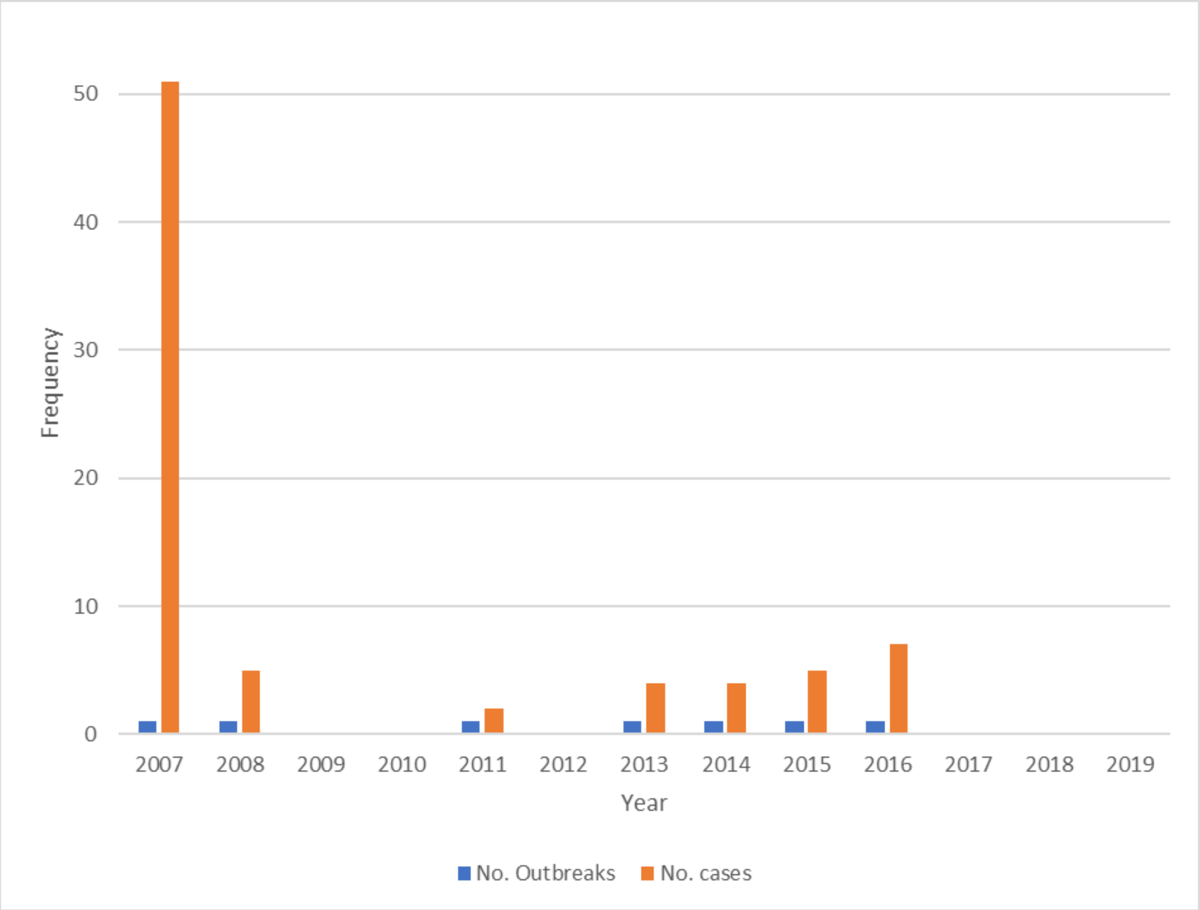

Figure 1: Number of *B. cereus* outbreaks and number of linked cases in New Zealand. 2007-2019.

Table 1: Reported *B. cereus* outbreaks, the number of people reported ill, the implicated food and premise type in New Zealand, 2007-2016.

Year	**No. of cases (deaths)**	**Premise type**	**Implicated food**	**Reference**
2007	51 (0)	Catering	Daal	(Williman et al., 2008)
2008	5 (0)	Cafe	Curry with rice	(Williman et al., 2009)
2011	2 (0)	Home	Fish	(Lim et al., 2012)
2013	4 (0)	Restaurant/café/bakery	Chicken/rice dish	(Horn et al., 2014)
2014	4 (0)	Restaurant/café/bakery	Unknown	(Horn et al., 2015)
2015	5 (0)	Community event	Sushi (chicken/rice)	(Lopez et al., 2016)
2016	7 (0)	Restaurant/café/bakery	Dosai (rice pancake)	(Pattis et al., 2017)

Few New Zealand studies have been published on *B. cereus* prevalence in relevant food stuffs. This may be due to the relatively low numbers of reported illnesses linked to the pathogen each year. Since a single outbreak involving 51 people in 2007 linked to a daal, which was also linked to *C. perfringens*, there have been 27 cases linked to 6 outbreaks. Although not always related to an outbreak, 12 hospitalisations due to *B. cereus* have been reported since 2007. Four of the six outbreaks were linked to rice-based dishes (Williman et al., 2008, 2009; Lim et al., 2010, 2011, 2012; Lopez et al., 2013, 2016; Horn et al., 2014, 2015; Pattis et al., 2017, 2018, 2019, 2020).

In New Zealand, of the notified foodborne illnesses, only outbreaks of toxic shellfish poisoning (n=3) and *Listeria* monocytogenes (n=2) number fewer than *B. cereus* intoxication (n=6) cumulatively between 2008 and 2019 (annual foodborne illness reports). Even after the *B. cereus* outbreak in 2007 involving 51 people, *B. cereus* accounted for <2% of foodborne illness outbreaks and ~10% of foodborne illness cases.

5.3 INTERNATIONAL

5.3.1 Outbreaks

Several countries have reported *B. cereus* to be in the top three most common bacterial pathogens causing foodborne outbreaks, including Hungary, the Netherlands, Norway, and Taiwan (Tewari et al., 2015). In France between 2006 and 2012, *B. cereus* was the second or third most frequently confirmed cause of foodborne outbreaks (Glasset et al., 2016).

Table 2 shows selected international outbreaks of *B. cereus* intoxication linked to specific starchy foods, detailing the food premise type and illness symptoms for outbreak cases.

Table 2: Selected *B. cereus* outbreaks in various countries related to rice and starchy foods, 1971-2016.

Country	Year	No. of cases (deaths)	Site	Implicated food	*B. cereus* count in food (cfu/g)	Initial symptoms	Reference
Austria	2013	14 (0)	Hotel	Mashed potatoes	2-3 x 10^5	V, D	(Schmid et al., 2016)
Belgium	2003	5 (1)	Home	Pasta Salad	10^8 (+ emetic toxin)	V, severe cramps	(Dierick et al., 2005)
Belgium	2008	1 (1)	Home	Pasta	10^7 (+ emetic toxin)	V, D	(Naranjo et al., 2011)
Belgium	2011	8 (0)	Restaurant	Rice-based dish	10^5-10^7 (+ emetic toxin)	V, D	(Delbrassinne et al., 2012)
France	2008	1 (1)	Home	Pasta	Not tested	AB, V	(Saleh et al., 2012)
France	2008	28 (Unk)	Care	Mashed and boiled potatoes	9.2 x 10^5	V, D	(Glasset et al., 2016)
	2010	27 (Unk)	Care	Paella	2.8 x 10^4	D	
	2010	20 (Unk)	Care	Pasta and rice salad	9.6 x 10^7	V, D	
	2012	60 (Unk)	School canteen	Pasta	5.8 x 10^4	V, D	
Germany	2006	17 (0)	Day care centre	Rice (with vegetables)	10^4 (+ emetic toxin)	V	(Fricker et al., 2007)
Germany	2007	46 (0)	Kindy excursion	Rice pudding	Not tested	V, AB	(Kamga Wambo et al., 2011)
Germany	2007	1 (0)	Restaurant	Cooked pasta	3.8 x 10^5	V	(Messelhäusser et al., 2014)
	2009	1 (0)	Home	Cooked potatoes	Isolated	V	
	2010	1 (0)	Catering	Chickpea curry with potatoes and rice	2.8 x 10^4 in rice (+ emetic toxin in chickpeas and rice)	V	
	2010	1 (0)	Restaurant	Cooked pasta (with oysters)	Isolated	V	
	2011	2 (0)	Restaurant	Cooked potatoes (with pork)	1.0 x 10^2	V	
	2011	Several children (0)	Nursery school	Cooked pasta (with tomato sauce)	6.8 x 10^6	V	
Italy	2012	12 (0)	Restaurant	Rice	Isolated	V, D, AB, N	(Martinelli et al., 2013)
Japan	1974	50 (1)	Unspecified	Noodles	1.6 x 10^8 (raw noodle); 6 x 10^7 (cooked noodle)	V	(Takabe & Oya, 1976)
Japan	2008	3 (1)	Home	Fried rice	Isolated (+ emetic toxin)	V, lethargy	(Shiota et al., 2010)

Country	Year	No. of cases (deaths)	Site	Implicated food	*B. cereus* count in food (cfu/g)	Initial symptoms	Reference
Korea	2005	24 (0)	Cafeteria	Rice/fried rice	Not tested	V, H, AB	(Kim et al., 2010)
Malaysia	Unspecified	114 (0)	School hostel	Fried noodles	2.3 x 10^6	AB, N, V, F, D	(Rampal et al., 1984)
Switzerland	Unspecified	2 (1)	Home	Pasta (with pesto)	Isolated	AB, D, V	(Mahler et al., 1997)
United Kingdom (England)	1971	13 (0)	Restaurant	Fried rice	10^6-10^8	V, D	(Mortimer & McCann, 1974)
United Kingdom (England)	2012	200 (0)	Catering	Shepherd's pie	10^4 (in pie); 10^6 haricot beans	V, N, D, AB, F	(Nicholls et al., 2016)
United States	1993	14 (0)	Day care catering	Fried rice (with chicken)	10^6	N, AB, D	(Centers for Disease Control and Prevention, 1994)
United States	2016	179 (0)	Restaurant	Refried beans	Isolated	N, V, AB	(Carroll et al., 2019)

V = vomiting, D = diarrhoea, N = nausea, AB = abdominal cramps, F = fever, H = headache
Unk = unknown

6 Exposure

Essentially all *B. cereus* intoxications are deemed to be foodborne (WHO, 2015), although at least one expert panel has suggested as much as 10% may come from other sources including travel - a proportion of which may also be foodborne (Havelaar et al., 2008). *B. cereus* was not included in a 2013 expect elicitation to estimate foodborne proportions of enteric infections in New Zealand (Cressey & Lake, 2013).

6.1 RICE AND STARCHY FOOD CONSUMPTION

The 2016 New Zealand Total Diet Study analysed foods based on a simulated typical diet of New Zealanders. The addition of composite foods (i.e., rice dishes and noodle dishes) to the 2016 survey substantially increased the amount of rice and noodle-based food included in the simulated diets compared to the 2009 and 2004-2005 surveys. The 2016 survey also specifically included Pacific Island ethnicity adult cohorts for the first time. In terms of starchy foods, the simulated diet for Pacific Islander ethnicity had more rice and rice dishes, instant noodles and noodle dishes, and, more taro, but less potato products (excluding crisps) than the total adult population estimates. Potatoes feature highly in the diets of all age groups, including the 11-14 year-old, 3-6 year-old, and 1-3 year-old categories. Young adult males (19-24 years) are separated out as higher energy consumers. Adult females simulated diets included a mean daily consumption of rice and rice dishes, potatoes (excluding crisps), instant noodles and noodle dishes, and kumara and taro was 54 g, 73 g, 45 g, and 9 g respectively. The mean daily consumption for adult males of those categories was 68 g, 122 g, 70 g, and 11 g, respectively (MPI, 2018). This will likely vary for each ethnic group in New Zealand.

Table 3 shows international prevalence study results for *B. cereus* in a selection of raw starchy foods. The ubiquity of *B. cereus* in the environment is reflected by the presence of *B. cereus* in a variety of raw starchy foods in various countries, at concentrations as high as >10^5 cfu/g (dried pasta sampled in Germany). *B. cereus* was found in cooked starchy foods at levels as high as >10^5 in two samples of fried rice in Canada. Table 4 lists prevalence studies of *B. cereus* in a selection of cooked starchy foods with the New Zealand study highlighted.

Table 3: Selected studies of *B. cereus* prevalence in uncooked starchy foods in various countries, 1971-2018.

Country	**Study year(s)**	**Food type**	***B. cereus* prevalence n/n (%)**	***B. cereus* count (cfu/g or MPN/g where stated)**	**Reference**
Argentina	Unknown	Flour	0/29 (0.0)	n/a	(Iurlina et al., 2006)
Australia	2006-2007	Grain	2/50 (4.0)	log 2.1 - 2.2	(Eglezos et al., 2010)
		Flour	0/350 (0.0)		
Germany $	2010	Pasta (dried)	Unknown 4.6	10^4-10^5 (2.3%) >10^5 (2.3%)	(Akineden et al., 2015)
Hong Kong	Unknown	Rice (raw)	11/16 (68.8)	<5 x 10^2 (n=9) 3 x 10^2-2 x 10^5 (n=2)*	(Lee et al., 1995)
India	Unknown	Rice	6/6 (100)	2 x 10^1-6 x 10^2	(Kamat et al., 1989)
		Rice products	3/7 (42.9)	2 x 10^1-4 x 10^1	
		Pulses	4/10 (40.0)	2 x 10^2-5 x 10^2	
Malaysia #	2009-2010	Breakfast cereals	3/19 (15.8)	Not specified	(Lesley et al., 2013)
		Other cereals	1/7 (14.3)	Not specified	
Netherlands	Unknown	Flour	5/9 (55.6)	10^3	(te Giffel et al., 1996)
		Pasta products	4/8 (50.0)	10^4	
Poland	2007-2017	Breakfast cereals	8/43 (18.6)	log 1.2 ± 0.54 mean	(Berthold-Pluta et al., 2019)
		Pasta	20/54 (37.0)	log 1.5 ± 0.54 mean	
		Rice	13/48 (27.1)	log 1.3 ± 0.54 mean	
Poland	2006-2007	Barley	17/20 (85.0)	<10 MPN/g (n=5) 10-100 MPN/g (n=1) >100 MPN/g (n=11)	(Daczkowska-Kozon et al., 2009)
		Buckwheat	12/14 (85.7)	<10 MPN/g (n=2) 10-100 MPN/g (n=3) >100 MPN/g (n=7)	
		Wheat	14/14 (100)	<10 MPN/g (n=1) 10-100 MPN/g (n=2) >100 MPN/g (n=11)	
Switzerland	2018	Flour	0/89 (0.0)	Non-*Bacillus cereus* isolated in 75 (84.3%) samples	(Kindle et al., 2019)
Taiwan	1992-1995	Cereal	Unknown 5.1	Not specified	(Fang et al., 1999)
		Flour	Unknown 0.0	Not specified	
Tunisia #	2014-2015	Cereal products	28/34 (82.4)	<10^3 (n=16) 10^3-10^4 (n=9) >10^4 (n=3)	(Gdoura-Ben Amor et al., 2018)
Turkey	2006	Wheat flour	6/142 (4.2)	10^2-10^3	(Aydin et al., 2009)
United Kingdom (England)	1971-1976	Rice (uncooked)	98/108 (90.7)	Not specified	(Gilbert & Parry, 1977)

$= Presumptive *B. cereus* reported
*= overlap of ranges reported
#= *B. cereus* group

Table 4: Selected studies of *B. cereus* prevalence in cooked starchy foods in various countries, 1971-2018.

Country	Study year(s)	Food type	*B. cereus* prevalence n/n (%)	*B. cereus* count (cfu/g or MPN/g where stated)	Reference
Canada	Unknown	Rice (fried)	20/61 (32.8)	10^1-10^3 (n=13) 10^3-10^5 (n=5) >10^5 (n=2)	(Schiemann, 1978)
		Rice (boiled/steamed)	2/20 (10.0)	10^1-10^3 (n=1); 10^3-10^5 (n=1)	
		Noodles	0/3 (0.0)	n/a	
Canada	2013-2018	Hummus dip/ Salad (potato/pasta)	2/3534 (0.06)	<10^3-10^4	(Canadian Food Inspection Agency, 2019)
China	2011-2016	Rice/Noodles	59/119 (49.6)	<3 MPN/g (n = 10) 3-1100 MPN/g (n = 39) >1100 MPN/g (n=10)	(Yu et al., 2020)
Colombia	2016-2017	Rice (cooked)	14/29 (48.3)	Not specified	(Sanchez Chica et al., 2020)
		Potato puree	2/17 (11.8)	Not specified	
		Pasta	0/5 (0.0)	n/a	
India	Unknown	Rice (boiled)	4/4 (100)	1.5 x 10^3-4 x 10^4	(Kamat et al., 1989)
India	Unknown	Rice (fried with chicken)	53/94 (56.4)	log 2.3 - 4.5	(Sudershan et al., 2012)
		Noodles (with chicken)	56/94 (59.6)	log 2.0 - 4.5	
		Noodle (plain)	62/94 (66.0)	log 2.0 - 4.5	
		Rice (plain)	48/94 (51.1)	log 2.0 - 4.5	
Malaysia	2013-2014	Rice (cooked)	35/70 (50.0)	Not specified	(Jawad & Mutalib, 2016)
Morocco #	2008-2010	Salad (with rice)	9/93 (9.7)	Not specified	(Merzougui et al., 2014)
Netherlands	Unknown	Chinese meals	15/18 (83.3)	10^3-10^5	(te Giffel et al., 1996)
New Zealand	2004	Potato flakes	8/50 (16.0)	<360	(Turner et al., 2006)
		Mashed potato	2/44 (4.5)	1.2 x 10^2-1.0 x 10^3	
United Kingdom (England)	1971-1976	Rice (boiled)	26/252 (10.3)	Not specified	(Gilbert & Parry, 1977)
		Rice (fried)	48/204 (23.5)	Not specified	

#= *B. cereus* group

7 Assessments

A title and abstract screen of the Google Scholar database (https://scholar-google-com) was performed using the terms "Risk Assessment" and "Bacillus cereus". This recovered various types of assessments that have been performed to evaluate aspects of *B. cereus* exposure, hazard, and risk, from raw to cooked products.

7.1 QUANTITATIVE MICROBIAL RISK ASSESSMENT – RICE

A quantitative microbial risk assessment using a Monte Carlo simulation was performed for *B. cereus* emetic disease. The scenario analysed was in which rice, contaminated with *B. cereus*, was prepared and stored under conditions found in Chinese-style restaurants in the United States. The six variables considered were:

1. Distribution of *B. cereus* in a lot of raw rice
2. Concentration of *B. cereus* in raw rice
3. Growth of *B. cereus* after cooking
4. Dose-response
5. Proportion of emetic *B. cereus* strains
6. Rate of attack of emetic illness

The authors concluded that the risk of illness was highly correlated with temperature abuse of cooked rice (i.e., storage at 20 or 30 °C) (McElroy et al., 1999).

7.2 QUANTITATIVE MICROBIOLOGICAL EXPOSURE ASSESSMENT – PASTEURISED RICE CAKES

Also using a Monte Carlo simulation, a quantitative microbiological exposure assessment was performed using computational fluid dynamics to determine the risk of *B. cereus* in pasteurised rice cakes. Differences in packaging method and acidification process to extend shelf-life were considered along with time and temperature of heat transfer during the pasteurisation process and storage. It was found that oval-shaped packaging of rice cakes had a higher final contamination level of *B. cereus* than slab-shaped packaging. Acidification significantly inhibited the growth of *B. cereus* and decreased the thermal resistance of *B. cereus*. The authors suggested a combination of acidification and low temperature pasteurisation could improve the safety of the product (Park & Yoon, 2019).

7.3 EXPOSURE ASSESSMENT – RICE ROLLS

An exposure assessment of takeaway cold Korean rice rolls accounted for four steps: 1) the initial contamination level in the store, 2) storage conditions and time before sale, 3) time and temperature of transport and home storage, and 4) final levels of *B. cereus* at time of consumption. Contamination levels had a median of 1.39 log cfu/g with a minimum of -3.63 log cfu/g and a maximum of 7.31 log cfu/g (stated to be the 5% and 95% percentile, respectively). The authors noted the difficulty in accounting for human behaviour and product treatment after purchase (Bahk et al., 2007).

7.4 RISK ASSESSMENT – TOXINS

A risk assessment of foodborne *B. cereus* and toxins in food concludes that not all *B. cereus* strains will cause illness in humans; consumption of *B. cereus* at a concentration in food <1 x 10^4 cfu/g should not be considered hazardous; and cereulide in food (but not necessarily other pre-formed toxins), due to the thermal stability, should be considered hazardous (Notermans & Batt, 1998).

7.5 HAZARD ASSESSMENT – RICE

A hazard assessment of boiled and fried rice in six restaurants evaluated the amount of *B. cereus* at various stages of cooking and storage (Bryan et al., 1981). All 16 batches of raw rice tested were positive for *B. cereus*. Boiled and fried rice cooled at room temperature and fried rice cooled in deeper layers had the highest cfu/g counts. The authors identified four specific preventive measures.

1. Boil small quantities of rice on several occasions
2. Keep boiled and fried rice above 55 °C (preferably 60 °C)
3. When cooling rice, do so in layers no deeper than 9 cm for no longer than 1 h at room temperature, then refrigerate
4. Ensure an internal temperature of at least 74 °C for fried rice

These steps minimise the time that boiled or fried rice is at temperatures which support growth of *B. cereus*.

8 Control measures

Bacterial contamination can be mitigated using a variety of hygiene measures from food production, preparation, cooking and storage.

8.1 COOKED FOODS

Food Standards Australia New Zealand (FSANZ) updated their guidelines in 2018 for microbiological quality for food, including ready-to-eat foods, with limits for aerobic plate counts (APC), indicator bacteria and individual pathogen numbers. For *B. cereus*, $<10^2$ cfu/g is considered satisfactory, 10^2-$<10^3$ cfu/g marginal, 10^3-$\leq 10^5$ cfu/g unsatisfactory, and $\geq 10^5$ cfu/g potentially hazardous (Food Standards Australia New Zealand, 2018). Beyond good hygiene practices (GHP) in food production, the European Union (EU) appears to only have limits for presumptive *B. cereus* in 'dried infant formulae and dried dietary foods for special medical purposes intended for infants below six months of age' (European Union (EU), 2005). These limits are for one sample in a batch of five allowed to fall between but not exceed 50-500 cfu/g.

B. cereus associated with emetic toxin production are mesophilic with optimal growth temperatures of 30 to 40 °C. To control growth and toxin production, cooked foods should be either:

- cooled rapidly and stored at 5 °C or below
- held warm at 60 °C or above
- displayed and handled according to the 2-h/4-h rule[7]

8.2 SUSHI

Sushi rice is designated a specialist food within MPI Food Control Plans (FCPs). FCPs detail steps a business making or selling higher-risk foods needs to take to make safe food. Cooked sushi rice must be cooled from 60 °C to 21 °C within 2 h and to 5 °C within another 4 h. Sushi rice must be at pH 4.6 or lower. To do this a vinegar solution must be added to the rice as soon as it is cooked. An exception to this is for brown rice, which cannot be acidified because the hard surface of the grain limits acid penetration of the rice. Sushi made with brown rice has a shorter shelf life.

8.3 SURFACES

Although the generally recommended bacteriocidal aqueous ethanol at 70% is effective against vegetative *Bacillus* spp. cells, 90% ethanol was found to be more effective against *Bacillus* spp. spores in liquid culture (Thomas, 2012). Hand washing and use of 2% chlorhexidine are effective in a clinical setting (American Academy of Pediatrics, 2018).

8.4 BACTERIOCIN AND PHAGE TREATMENTS

B. cereus growth and enterotoxin production was shown to be inhibited by a bacteriocin, enterocin AS-48, in rice-based foods (Grande et al., 2006). The extent to which enterocin AS-48 was a successful additive was dependent on pH and temperature. A strain of *Bacillus amyloliquefaciens* isolated from fermented soybean paste was shown to have strong antimicrobial activity against *B. cereus* and reduce enterotoxin production. The authors suggested it could be used as an additive for biological control in fermented soybean products and other foods (Eom & Choi, 2016). Several families of bacteriophage have phage

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[7] FSANZ. Food held between 5 °C and 60 °C for 1) less than 2 hours can be used, sold or put back in the refrigerator to use later; 2) 2-4 hours can still be used or sold, but can't be put back in the fridge; 3) 4 hours or more must be thrown away. https://www.foodstandards.gov.au/foodsafety/standards/Pages/2-hour-4-hour-rule.aspx

strains that have been shown to be active against the *B. cereus* group (Gillis & Mahillon, 2014). Application of high concentration of phage of the *Myoviridae* family has proven effective in reducing the concentration of *B. cereus* by >6 log cfu/ml within 24 h in mashed potato (Lee et al., 2011). The high concentrations of various active agents required to be effective in food matrices has proven to be one of the barriers, in practical terms, to wider applications.

9 Conclusions

In terms of answers to Risk Management Questions for this Risk Assessment of *B. cereus* in rice and starchy foods:

9.1 SOURCE OF FOOD CONTAMINATION

B. cereus is widely distributed in the environment and raw foods, particularly plant-based foods, can be naturally contaminated early in growth cycles. Subsequent cross-contamination during food preparation and unsuitable storage temperatures can enable *B. cereus* to proliferate and/or produce toxins.

9.2 DATA GAPS

There are limited data available from New Zealand on *B. cereus* prevalence in starchy foods and no recent data. A single study looking at *B. cereus* in mashed potato and potato flakes was published in 2006. Too little information is available about the prevalence of *B. cereus* in rice and starchy foods in New Zealand to compare to international studies. Even less is known about the prevalence of *B. cereus* in foods associated with *B. cereus* diarrhoeal toxin in New Zealand.

9.3 ILLNESS IN NEW ZEALAND

B. cereus intoxications are only notifiable in New Zealand if they are part of an outbreak, the person is in a high-risk category or their illness is deemed to be of public health significance. Illness caused by *B. cereus* is likely to be under-reported in New Zealand, as it is in other countries, due to limited notifiability, the usually mild nature and short duration of symptoms. *B. cereus* causes a small proportion of foodborne outbreaks each year in New Zealand, the majority of which, in recent years, have been linked to rice-based meals.

9.4 RISK MANAGEMENT OPTIONS

B. cereus intoxication is regarded as an exclusively foodborne illness which can be prevented with appropriate storage temperature of cooked foods. This consists of keeping hot food above 60 °C, cooling cooked food to below 5 °C within four hours and keeping it at this temperature until it is eaten. Although the general public may not be aware of these guidelines, food businesses should be, through their Food Control Plans and proper training of staff.

Emphasis remains on foods linked to *B. cereus* emetic illness, with no information on food stuffs associated with *B. cereus* diarrhoeal illness, such as meat products, fish, poultry, soups, sauces and stews, and vegetables, in New Zealand. The generic nature of diarrhoea as a symptom of foodborne illness may mean *B. cereus* is under-represented as a cause. Studies on the prevalence of *B. cereus,* particularly in retail cooked rice and starchy food products in New Zealand, would determine if *B. cereus* was present at unacceptable levels. Analysis of *B. cereus* isolates virulence potential would help determine if any *B. cereus* isolated were innocuous or potentially pathogenic.

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