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ORIGINAL ARTICLE

Prevalence of *Bacillus cereus* in food products in Poland

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Abstract

Introduction and Objective. *Bacillus cereus* is a foodborne pathogen causing two main types of gastrointestinal diseases: emetic and diarrheal. The aim of this study was to investigate the prevalence of the *Bacillus cereus* group in ready-to-eat (RTE) food products available in retail in Poland.
Materials and method. Samples were collected by Sanitary and Epidemiological Stations within the framework of the national official control and monitoring sampling programme in Poland. In 2016–2020, a total of 45,358 food samples, such as: 'confectionery products and products with cream', as well as 'cereal grains and cereal and flour products', 'milk and milk products', 'sugar and others', 'meat offal and meat products', 'poultry offal and poultry products', 'eggs and egg products', 'fish, seafood and their preserves', 'vegetables' (including legumes), 'coffee, tea, cocoa, fruit, and herbal teas', 'delicatessen and culinary products', and 'foods for particular nutritional uses' were collected.
Results. The presence of the presumptive *B. cereus* group was monitored mainly in two categories of food products: 'confectionery products and products with uncooked cream' and 'confectionery products and products with heat-treated cream'. The number of samples disqualified due to presumptive *B. cereus* was 339 (0.75%).
Conclusions. This study provides useful information regarding the contamination of RTE products with the *B. cereus* group, which may have implications for food safety.

Key words
food safety, *Bacillus cereus*, microbiological contamination

INTRODUCTION

The *Bacillus* species can be grouped into the family *B. cereus* group (presumptive *B. cereus* or *B. cereus sensu lato*) based on their genetic similarity. The *B. cereus* group includes *B. cereus* sensu stricto, *B. anthracis*, *B. thuringiensis*, *B. mycoides*, *B. pseudomycoides*, *B. weihenstephanensis*, *B. cytotoxicus*, *B. toyonensis*. The *B. cereus* group shows numerous similarities, both phenotypically and genetically [1, 2]. *B. cereus* can grow over a wide temperature range from 5 °C-50 °C, with the optimum between 28–35 °C, and pH range from 4.9–9.3, the optimum being 6–7. The *B. cereus* spores can survive in gastric juice. Sporulation is observed after 2–3 days in most microbiological media. The spores of the *B. cereus* group bacteria show heat resistance [3, 4].

The natural reservoir of the *B. cereus* group is primarily decaying organic matter, vegetables, invertebrate digestive tracts, contaminated soil and water [2, 5, 6]. The *B. cereus* group is also isolated from food: rice, confectionery (cakes, puddings), flour dishes (pasta), meat and meat products, sauces, raw and pasteurized milk and dairy products, seafood, spices, and vegetables [7, 8, 9]. The major threat to food safety is mainly related with the production of spores and biofilm, which is particularly difficult to eliminate [10]. The ability of the *B. cereus* group to form heat-resistant spores is particularly dangerous in the food industry, as spores surviving pasteurization may adversely affect the quality of the final product or contribute to secondary food contamination. The decimal reduction time of the *Bacillus* spores at 100°C takes 2.2–5.4 minutes [11].

B. cereus sensu stricto is an etiological agent responsible for several intestinal and extraintestinal diseases, e.g. gastroenteritis, brain abscess, meningitis, endophthalmitis, emesis, fulminant bacteraemia, respiratory tract infection, endocarditis, hepatitis, and bone infection [12, 13, 14]. Both the *B. cereus* cells and spores can survive in the gastrointestinal tract, where the cells produce toxins in amounts sufficient to cause intoxication [15]. *B. cereus* produces various virulence factors, such as cereulide, haemolysins, enterotoxins, pore-forming toxins, proteases and phospholipases [14, 16]. *B. cereus* is responsible for two main types of gastrointestinal diseases: (a) emetic type and (b) diarrheal type.

Following the opinion of the BIOHAZ Commission, the European Food Safety Authority has released requirements relating to *B. cereus* in foodstuffs [17]. The Commission concluded that one of the most important control measures is temperature control, and establishing a system based on the principles of Hazard Analysis Critical Control Points (HACCP). Dehydrated powdered foods (including powdered infant formulas and powdered dietary foods), in which spores of pathogenic *Bacillus* spp. are often present, may constitute a risk. Adding warm water to dehydrated foods may allow *B. cereus* to grow. In accordance with the opinion of the EFSA, the number of the *B. cereus* spores in powdered infant formulas and powdered dietary foods should be as low as possible during processing, and in addition to good practices designed to reduce the delay between preparation and consumption, a process hygiene criterion should be introduced. Pursuant

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to the above recommendation, Commission Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs (the latest consolidated version) has been implemented [18]. Based on Section 2.2.11 of this Regulation, food testing for presumptive *B. cereus* has been introduced to the process hygiene criteria in the category 'Dried infant formulae and dried dietary foods for special medical purposes intended for infants below six months of age'. Sample testing takes place at the end of the manufacturing process according to the analytical reference EN/ISO 7932 method which is the current version [19]. The expected results are satisfactory when all the values observed are ≤50 cfu/g; acceptable when the maximum one of five values is between 50 and 500 cfu/g, and the rest of the values observed are ≤50 cfu/g; unsatisfactory when one or more of the values observed are >500 cfu/g, or more than one of five values are between 50 and 500 cfu/g [18].

In Poland, based on national guidelines, if the presence of *B. cereus* above 10^5 cfu/g is detected in food products other than those listed in Regulation (EC) No. 2073/2005 or food suspected of causing poisoning in humans, a risk assessment is carried out [18]. The risk assessment should be performed for such foods as: cereal products, herbs and spices, cakes and confectionery, rice and rice dishes, dried mushrooms, sprouts, meat products, fruit and vegetable products.

OBJECTIVE

This study aimed to investigate the occurrence of the *B. cereus* group in ready-to-eat (RTE) products available in retail in Poland and estimate the related risk.

MATERIALS AND METHOD

Monitoring studies. Monitoring studies were conducted in all 16 provinces in Poland over a 5-year period (2016 – 2020) based on plans prepared by the Department of Food Safety of NIPH NIH – NRI. As part of the planned monitoring tested in total from categories: 'confectionery products and products with cream' (43,152 samples), and 'cereal grains and cereal and flour products (1847). Additionally, samples collected from food poisonings or consumer notifications were included, such as: 'milk and dairy products' (106), 'other food products' (81), 'delicatessen and culinary products' (89), 'sugar and others' (40), 'fish, seafood and their preserves' (12), 'poultry offal and poultry products, eggs and egg products' (11), 'foods for particular nutritional uses' (7), 'meat offal and meat products' (5), 'vegetables' (including legumes) (5), and 'coffee, tea, cocoa, fruit and herbal teas' (3). A total of 45,358 food samples were collected from both small and large retail outlets. The samples were tested at the accredited laboratories of the Sanitary and Epidemiological Stations. The analyses were performed in accordance with PN-EN ISO 7932:2005 for the presumptive *B. cereus* group using MYP Agar and the haemolysis test [19]. Briefly, 10 g of food sample was taken in an aseptic manner and homogenized in 90 mL of buffered peptone water. An aliquot of 0.1 mL of the initial suspension and further decimal dilution were transferred to mannitol egg yolk polymyxin agar plates (MYP Agar). After incubation for 24–48 h at 30 °C, typical colonies were counted and then subjected to the haemolysis reaction test. These strains were sent to our laboratory at NIPH NIH – NRI for confirmation and further studies. Samples were disqualified according with the national guideline if *B. cereus* counts above 10^5 cfu/g were detected in food products other than those listed in Commission Regulation (EC) No. 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs (the latest consolidated version).

Statistical analysis. Statistical analysis was performed using Statistica v. 13 (StatSoft, Kraków, Poland). Chi-square tests were performed in the disqualification of tested samples of *B. cereus* between matrices and year of the isolation. The differences were considered significant at $P < 0.05$.

RESULTS

A total of 45,358 samples were collected at the retail level conducted in 2016 – 2020, of which 339 were disqualified according to the adopted criteria from the national guide (Tab. 1).

The overall occurrence of disqualified samples due to *B. cereus* contamination during the 5-year period was 0.75% Statistical analysis revealed no significant relationship between the percentages of disqualified samples in 2016–2020 ($P < 0.05$).

The highest rate of disqualification in the 'confectionery and pastry products' category was observed in 2020 (1.22%), while the lowest rate occurred in 2016 (0.46%). In 2017, 2018 and 2019, the contamination rates in this category were similar, with percentages of 0.81%, 0.78%, and 0.73% respectively. However, statistical analysis indicated no significant relationship between the percentages of disqualified samples in the 'confectionery and pastry products' category ($P < 0.05$).

Another category of food products in which the presence of bacteria from the *B. cereus* group exceeded the acceptable limits were 'milk and dairy products'. From a total 106 tests conducted in the 'milk and dairy products' category during 2016–2020, 14 of them were disqualified (13.21%). The highest contamination rate was identified in 14 of 15 samples collected from the market in 2018, resulting in a contamination rate of 93.33%. *B. cereus* was not disqualified among in the tested samples obtain in 2016 (2 samples), 2017 (61 samples), 2019 (8 samples), 2020 (20 samples).

Food monitoring for the "cereal grains and cereal and flour products" category was implemented in 2020. Out of the tested 1842 food samples, three samples were disqualified due to the exceeded limit of *B. cereus,* which accounted for the 0.16% of the tested samples in "cereal grains and cereal and flour products" category.

In 2016, *B. cereus* was responsible for the disqualification of 2 out of 21 samples in the "other food products" category, accounting for 9.52% of the tested samples. However, a total of 79 samples collected in the years 2017 (28 samples), 2018 (31 samples), and 2020 (1 sample) were not disqualified in the 'other food products' category. Similarly, in the 'delicatessen and culinary products' category, the limit for bacteria from the *B. cereus* group was exceeded in 2018, with one out of 11 tested samples showing contamination. However, no disqualifications were observed in the 'delicatessen and culinary products' category for the samples collected in 2016 (24 samples), 2017 (38 samples) and 2020 (16 samples).

Furthermore, no exceedances were found in the samples collected from the following categories: 'meat, offal and meat

Table 1. Result of monitoring programme of *Bacillus cereus* in retail products in Poland, 2016-2020. Samples were disqualified according to the national guidelines if the presence of *B. cereus* above 10^5 cfu/g was detected in food products other than those listed in Commission Regulation (EC) No. 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs

PRODUCT GROUP	2016			2017			2018			2019			2020			Summary		
	Tested samples	Disqualified samples	Percent of disqualified samples (%)	Tested samples	Disqualified samples	Percent of disqualified samples(%)	Tested samples	Disqualified samples	Percent of disqualified samples (%)	Tested samples	Disqualified samples	Percent of disqualified samples (%)	Tested samples	Disqualified samples	Percent of disqualified samples (%)	Total tested samples	Total disqualified samples	Total percent of disqualified samples (%)
Meat, offal and meat products	1	0	0.00	2	0	0.00	-	-	-	2	0	0.00	-	-	-	5	0	0.00
Poultry, offal and poultry products. eggs and egg products	-	-	-	-	-	-	1	0	0.00	10	0	0.00	-	-	-	11	0	0.00
Fish, seafood and their preserves	5	0	0.00	-	-	-	1	0	0.00	6	0	0.00	-	-	-	12	0	0.00
Milk and dairy products	2	0	0.00	61	0	0.00	15	14	93.33	8	0	0.00	20	0	0.00	106	14	13.21
Cereal grains and cereal and flour products	-	-	-	-	-	-	-	-	-	5	0	0.00	1842	3	0.16	1847	3	0.16
Confectionery and pastry products	10190	47	0.46	10512	85	0.81	7790	61	0.78	10820	79	0.73	3840	47	1.22	43152	319	0.74
Sugar and others	-	-	-	-	-	-	-	-	-	-	-	-	40	0	0.00	40	0	0.00
Vegetables (including legumes)	-	-	-	-	-	-	-	-	-	5	0	0.00	-	-		5	0	0.00
Coffee, Tea, Cocoa, fruit and herbal teas	-	-	-	3	0	0.00	-	-	-	-	-	-	-	-		3	0	0.00
Delicatessen and culinary products	24	0	0.00	38	0	0.00	11	1	9.09	-	-	-	16	0	0.00	89	1	1.12
Foods for particular nutritional uses	2	0	0.00	-	-	-	5	0	0.00	-	-	-	-	-		7	0	0.00
Other food products	21	2	9.52	28	0	0.00	31	0	0.00	-	-	-	1	0	0.00	81	2	2.47
Total in the year	10245	49		10644	85		7854	76		10856	79		5759	50		45358	339	

products' (5 samples: one in 2016, 2 in 2017, 2 in 2019); 'poultry, offal and poultry products, eggs and egg products' (11 samples: one in 2018, 10 in 2019); 'fish, seafood and their preserves' (12 samples; 5 in 2016; one in 2018; 6 in 2019; 'sugar and others' (40 in 2020); 'vegetables' (including legumes) (5 in 2019); 'coffee, tea, cocoa, fruit and herbal teas' (2 in 2017); 'foods for particular nutritional uses' (7 samples: 2 in 2016, 5 in 2018).

DISCUSSION

The national monitoring studies on *B. cereus* are the first carried out in Poland on such a large scale. Data on the occurrence of *B. cereus* in these areas are extremely limited. In the current study, the overall disqualified samples amounted to 0.48% in 2016; 0.80% in 2017; 0.97% in 2018; 0.73% in 2019; and 0.87% in 2020. The number of disqualified food samples tested for the presumptive *B. cereus* group in accordance with the PN-EN ISO 7932:2005 standard in Poland can be estimated as very low. The highest number of samples were tested in the category 'confectionery and pastry products', as they were included in the *B. cereus* monitoring plan. There were significantly fewer samples resulting from food poisoning incidents or consumer interventions. However, it was acknowledged that these samples would be valuable for this research.

B. cereus was mainly isolated from dairy-based and flour-based products in Poland. The official monitoring of food samples for the presumptive *B. cereus* group is primarily focused on confectionery products and products with uncooked cream and confectionery products and products with heat-treated cream which are a milk-flour-based product (category: 'confectionery and pastry products').

As mentioned previously, monitoring studies conducted in Poland in this area, due to the relatively high number of samples, are one of the few studies conducted in the field of detecting presumptive *B. cereus* on such a large scale in the world. Food samples from this category were 95% from all tested food samples in the 5-year monitoring period (43,152). The overall exceeding the limit of *B. cereus* in category 'confectionery and pastry products' amounted to 0.74%. Data on the disqualified sample of *B. cereus* in the category 'confectionery and pastry products' are extremely limited. One of the few studies on confectionery products has been carried out in Egypt where a total of 150 milk-based dessert samples, including pudding, rice with milk and custard, were collected from different places in that country [20]. The occurrence of *B. cereus* was 44% in the pudding, 62% in the rice with milk, and 32% in the custard samples. The *B. cereus* count in the samples examined ranged from 10 to $<10^2$ cfu/g. The percentage of contaminated samples was higher than in the current study, but concerned a low level of contamination (10 to $<10^2$ cfu/g). Food preparation using raw milk and the use of powdered milk in production can increase the risk of food poisoning. These factors contribute to the overall risk associated with *B. cereus* contamination In turn, nationwide monitoring for raw co-mingled silo milk intended for pasteurization conducted in the United States demonstrated that a total 18 of 202 (8.9%) samples were positive for presumptive *B. cereus* (positive from <10 – 50 cfu/ml) [21]. A similar level of contamination was reported in a study by Proroga et al. [22]. They analysed samples of 'dairy products' collected from dairies, supermarkets and company canteens in southern Italy and found that 7.2% of 515 tested samples were contamination. In comparison, a higher level of contamination were observed in the 'milk and dairy products' category in Polish studies, where out of a total of 106 tests, 13.21% were disqualified (level of contamination $>10^5$ jtk/g). A by Liu et al. [23] in China demonstrated a high prevalence of *B. cereus* in milk (44%) and milk powder (26.1%). Similarly, an investigation conducted in China from 2011 – 2016 suggested that approximately 27% of pasteurized milk samples were contaminated with *B. cereus* [24]. Consequently, products such as confectionery and pastry products made with unpasteurized milk containing *B. cereus* may pose a health risk.

Data from Luxembourg and Portugal from 2021 show that 2.6% of the analysed food samples (165 batches and 1,130 single samples) classified as 'other processed food products and prepared dishes or sauces and dressings' contained *B. cereus* [25]. In 2019, Slovenia submitted data regarding the detection of *B. cereus* in 5.5% of 200 food samples [26]. Based on the data concerning 'other food products' it can be inferred that the incidence of the *B. cereus* group in Poland is significantly lower compared to results obtained by other researchers. Out of 41 samples, only 2 were disqualified due to contamination with the *B. cereus* group.

A study conducted in Switzerland shows that, out of a total number of 89 flour or dough products that were tested at the retail level during 2 months of 2018, 75 samples were positive [27]. As part of a study conducted in Tunisia, a total of 687 food samples were collected from various food categories during the period from April 2014 – 2015. Among these food samples, 27.8% were found positive in terms of the *B. cereus* group. The level of contamination with the *B. cereus* bacteria in food products was found to be $<10^3$ cfu/g or ml in 77.5% of the samples tested. Higher counts of $>10^4$ cfu/g or ml were found in 6.8% of samples, including 'fresh-cut vegetables', 'cooked foods', 'cereals' and 'pastry products'. The contamination of the food samples was as follows: cereals – 67.6%; pastry products – 46.2%; cooked food – 40.8%; cooked poultry meat – 32.7%; seafood products – 32.3%; spices – 28.8%; canned products – 16.7%; raw poultry meat – 9.4%; fresh-cut vegetables – 5.0%; and dairy products – 4.8% [28]. Therefore, analyzing the available data on various food categories, it can be concluded that exceeding the limit of the *B. cereus* group in Poland is much lower than in other countries.

Notifications of *B. cereus* in food are deposited in the Rapid Alert System for Food and Feed (RASFF). The RASFF notification in the period 2020–2022 showed that the most frequent alerts concerned 'herbs and spices' (8 notifications), followed by 'dietetic foods, food supplements and fortified foods' (6 notifications) and 'prepared dishes and snacks' (6 notifications). In most cases, the 'herbs and spices' category referred to ginger. In 2021, *B. cereus* was detected in 9 products: organic barley grass powder, food supplement, turmeric, ground cinnamon, Italian Herbs, ground ginger, potato gnocchi, curry powder, and salad. In 2022, on the other hand, the bacteria were detected in prepared dishes, ready-to-eat salad, ground ginger, fresh spinach, humus, tofu, ginger powder, food supplements, and sesame seeds.

Despite the very low of disqualified samples of *B. cereus* in the monitoring studies performed, it should be remembered that these bacteria are one of the factors of food-borne outbreaks. In our research entitled 'Extended panel of laboratory testing in outlets of food-transmitted diseases in which an etiological factor was not determined as part of routine laboratory tests in 2020 in Poland', *B. cereus* was identified as an etiological factor of the food-borne outbreak. In 2021, 15 strong-evidence *B. cereus* food-borne outbreaks and 72 weak-evidence outbreaks with a total of 679 human cases (one death) and 9 hospitalizations were reported in European countries. The main sources of foodborne outbreaks were: 'pig meat and products thereof' (1 outbreak); 'fish and fish products' (1); 'crustaceans, shellfish, mollusks and products thereof' (1); 'vegetables and juices, and other products thereof' (2); 'cereal products, including rice and seeds/pulses' (nuts, almonds) (4); and mixed foods (6) [25].

CONCLUSIONS

The analysed ready-to-eat (RTE) food samples originating from retail stores in Poland were found to be contaminated with the *B. cereus* group. The overall percentage of disqualified samples in the 5-year period (2016–2020) was 0.75%. The number of disqualified food samples related to the *B. cereus* group in the monitoring study can be estimated as very low. However, with the occurrence of outbreaks related to poisoning with *B. cereus*, and outbreaks of unknown etiology, it is reasonable to continue monitoring studies on the *B. cereus* in food and expanding the scope of monitoring to include products that are most frequently associated with outbreaks.

It seems justified to test isolates obtained in monitoring studies. Extending the scope of monitoring to include the characteristics of the *B. cereus* strains will allow for a better assessment of the risks associated with the consumption of food contaminated with *B. cereus* and its metabolites, and will also enable a retrospective search for the sources of food infections.

Conflict of Interest

The authors report that they do not have any financial or personal connections with other persons or organizations that might negatively affect the contents of this publication and/or claim authors rights to this publication.

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