Screening of *Bacillus cereus* presence in minced meat and meat products originating from Serbian retail facilities

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Abstract. The goal of this study was to investigate the prevalence and numbers of *B. cereus* in minced meat and meat products. Eighty-seven meat or meat product samples were collected from different retail facilities in Serbia. Meat samples were subjected to microbiological analyses using the conventional cultural method for counting and isolation of *B. cereus*, confirmed by microscopic and biochemical tests. Eighty-seven samples included raw minced meat (7), semi-processed meat (60), processed meat (7) and meat products (13). The prevalence of *B. cereus* in the collected samples was 34.5 % (30/87). This pathogen was not isolated from raw minced meats, unlike semi-processed meat products, where it had a prevalence of 40 % (24 samples). Among processed meats, 28.6 % (2) were positive, while in meat products, the *B. cereus* prevalence was 30.8 % (4). We hope these results will provide better understanding of *B. cereus* isolated from food of animal origin in Serbia and results indicate meat and meat products might play an important role in dissemination of this pathogen through the food chain.

1. Introduction

All over the world, a major cause of mortality is reported to be foodborne diseases, which are a serious threat to public health. Rapid economic development, mass food production, population growth, increasing trans-border movement of people, animals and animal products, etc. play significant roles in the increasing number of outbreaks throughout the world. The main problem is that the greatest number of cases of foodborne disease goes unnoticed because symptoms are often mild, with associated diarrhoea and vomiting, and as such, they remain undiagnosed.

*Bacillus cereus* was identified as a causative agent of foodborne illness in the 1950s. This member of the family Bacillaceae is a gram-positive, rod shaped, aerobic, motile and β-haemolytic bacterium. The vegetative cells are typically 3.0-5.0 μm by 1.0-1.2 μm in size. It is capable of forming ellipsoid-shaped spores in a central or paracentral position without swelling the sporangium. In addition, this bacterium can grow in a broad pH range of 4.9-9.3, with the minimum pH 4.35 for growth in meat [1][2]. It is frequently isolated from soil, but also from food production environments, foods and intestinal tracts of insects and mammals [3][4].

Foodborne outbreaks of *B. cereus* have been reported by many countries, as in Hungary, where it has been found to be the third most common cause of food poisoning cases. Furthermore, the presence of *B. cereus* as a meat contaminant was reported by some investigators, not only in raw meat but also in meat products [5][6]. Consequently, *B. cereus* is also considered as an important pathogen in heat-treated foods [7]. In addition, *B. cereus* has been isolated from the stools of healthy adults and children (43 %) in various concentrations [8].

This pathogen causes two distinct types of food poisoning – diarrhoea and emesis – caused by two different types of toxins [9]. The first form of illness is characterised by abdominal pain and diarrhoea, which occurs between 8 and 16 h after ingestion of the contaminated food. The second form is characterised by nausea and vomiting, which happens within 30 min to 5 h after eating contaminated food [10]. The diarrheal form of disease is associated with three types of enterotoxins: three-
component non-haemolytic enterotoxin, three-component enterotoxin hemolysin BL and the single-component enterotoxin cytotoxin K [11]. Diarrheal toxins are produced by the bacteria during their growth in the small intestine. Unlike the diarrheal form, emetic syndrome is caused by emetic toxin that is synthesized during the bacterium’s growth phase in the food [12]. The infective dose of *B. cereus* in emetic food poisoning is $10^4$ to $10^{11}$ cfu/g or ml, while in diarrheal food poisoning, it is between $10^5$ and $10^{10}$ cfu/g [13], [14].

According to the report by EFSA BIOHAZ Panel’s Working Group, almost all strains of *B. cereus* are resistant to β-lactam antibiotics due to the presence of beta-lactamase genes [15]. Bearing in mind these facts, the goal of this study was to isolate and enumerate *B. cereus* from minced meat and meat products of various animal species from retail facilities in Serbia and to get preliminary results which would be a guide for additional research in this field.

## 2. Materials and methods

### 2.1. Samples

Samples of meat and meat products were collected between January and March 2019 from various retail facilities in different locations in Serbia. Eighty-seven samples (Table 1) including 7 minced meat samples of different species (beef, pork and chicken), 60 semi-processed meat samples (burgers, raw sausages, marinated meats and čevapi), 7 processed meat products (ready meals) and 13 meat products (raw fermented and boiled sausages and pates) were examined for the presence of *B. cereus*.

All samples were collected into sterile containers, stored in an insulated icebox, and transferred in the shortest possible time to the laboratory.

### 2.2. Microbiological method of isolation and identification

Isolation and identification of *B. cereus* in our samples were performed following the SRPS ISO 7932:2009. *B. cereus* selective differential Mannitol Egg-Yolk Polymyxin (MYP) agar base (Park Scientific Ltd., UK) was used to isolate presumptive colonies. MYP plates were surface plated by spreading of 0.1 mL of appropriate serial dilutions of tissue homogenate and then incubated at 30°C for 24-48 h. MYP plates were checked for the presence of pink colour colonies surrounded by a precipitation zone of the same colour, which indicates that lecithinase is produced. Those plates which contained a countable range of 15-150 colonies were counted. The control strain in this study was *B. cereus* ATCC 11778 (Microbiologics Inc., St Cloud, MN, USA).

### 2.3. Confirmation of Bacillus cereus

Presumptive colonies were confirmed using morphological and biochemical tests. The gram stain procedure was used for microscopic examination, where *B. cereus* appeared as large gram-positive bacilli in both short and long chains. Furthermore, biochemical reactions typical for *B. cereus* were utilised for further confirmation (lecithinase, oxidase, β-haemolysis test, motility, sugar fermentation: arabinose, mannitol and xylose) [16].

## 3. Results and discussion

Eighty-seven retail meat samples (Table 1) from various locations in Serbia were tested for the presence of *B. cereus* using MYP medium. On MYP medium, 30 samples of meat products produced pink coloured colonies surrounded by precipitation zones, and which were presumed as *B. cereus* (Figure 1). Presumptive positive *B. cereus* colonies were sent on for further confirmation.
Figure 1. (A and B) Colonies of *Bacillus cereus* grown on Mannitol Egg Yolk Polymyxin agar plate (pink colonies surrounded by a zone of precipitation [lecithinase positive], after overnight incubation at 30°C).

Every one of the 30 presumptive meat products produced colonies positive for sugar fermentation (arabinose, mannitol and xylose) characteristic of *B. cereus*. Oxidase and motility were also positive and β-haemolysis was produced by all of them (Figure 2). Furthermore, microscopic examination also confirmed positive results in the 30 presumptive meat products, since the bulky gram-positive bacilli in both short and long chains were typical of *B. cereus* (Figure 3).

Figure 2. *B. cereus* on sheep blood agar gives rise to β-haemolysis.
In general, the prevalence of \textit{B. cereus} in the collected samples was 34.5\% (Table 1). The pathogen was not isolated from minced meat, unlike semi-processed products where the prevalence of \textit{B. cereus} was 40\% (24 samples). Numbers of \textit{B. cereus} in positive samples ranged between $2\times10^1$ cfu/g and $4.9\times10^6$ cfu/g. Among 7 processed meat samples, 28.6\% (2) were positive, with levels ranging from $1.7\times10^3$ cfu/g to $2.0\times10^5$ cfu/g. Furthermore, in meat products, the \textit{B. cereus} prevalence was 30.8\% (4), with levels ranging from $2\times10^2$ cfu/g to $8.2\times10^4$ cfu/g.

| Sample category     | Number of tested samples | Number of positive samples | Prevalence (%) |
|---------------------|--------------------------|----------------------------|----------------|
| Minced meat         | 7                        | 0                          | /              |
| Semi-processed meat | 60                       | 24                         | 40             |
| Processed meat      | 7                        | 2                          | 28.6           |
| Meat products       | 13                       | 4                          | 30.8           |
| Total samples       | 87                       | 30                         | 34.5           |

The results obtained in this study concurred with the results of Eldaghayes et al., who reported a prevalence of 29\% [17]. A similar prevalence (27.8\%) was obtained by Tewari et al. [18]. The prevalence in meat and meat products consumed in Turkey was 22.4\%, which is also close to our results [19]. Mudasir Bashir et al. [1] reported a prevalence of 29.33\% in their research in India. On
the other hand, Smykal and Rokoszewska conducted research over a 7-year period (1964-1971), where the prevalence of \textit{B. cereus} was 13.3 \% in meat and meat products [20]. The different (higher) prevalence in our study could be explained by poor hygiene and cross-contamination during production in our meat products. Our meat products could initially have been contaminated with larger numbers of microorganisms, meaning they were more prone to carry-over of these organisms during processing. However, it has been proved that the prevalence of \textit{B. cereus} is often much higher in raw or undercooked products compared with cooked ones, because the absence of heating process limits reduction of microbial load [21]. Still, this study failed to detect \textit{B. cereus} in the raw minced meats sampled. The reasons may be the low bacterial loads as well as the relatively small number of minced meats sampled. The largest number of tested samples belonged to the group of semi-processed meat products, and in this group, the \textit{B. cereus} prevalence was also the highest. The main reason might be the fact that these are raw meats, but during the manufacturing processes, additional manipulation occurs. All these subsequent operations could lead to additional contamination. Furthermore, in this group of meat products, spices are also added, and spices are one of the main recognised sources of \textit{B. cereus}. Still, the prevalence of \textit{B. cereus} in semi-processed meat products was lower than reported previously [22]. The prevalences of \textit{B. cereus} in cooked meats (30.8 \%) and processed meats (28.6\%) was lower than in semi-processed meats (40.0 \%), which was anticipated since these former products are thermally treated [21]. Nevertheless, our prevalences in cooked meats were higher than results recorded by Eglezos et al. for cooked sausage rolls [23]. The \textit{B. cereus} prevalence in our meat products was lower than that found by Smith et al. [24]. However, the relatively high prevalence of \textit{B. cereus} in our meat products indicates there might be problems during production processes. These products are ready-to-eat, so they could be a high risk to consumers [25].

4. Conclusion
The presence of \textit{B. cereus} in meat products is an important aspect of food safety for consumers, especially for immunocompromised people. Of course, our research is just one piece of the puzzle and if we want to complete the whole picture, we must continue with extensive and detailed research. Nevertheless, we hope these results will provide a better understanding of the presence of \textit{B. cereus} isolated from food of animal origin in Serbia. We believe the results indicate meat and meat products might play an important role in spreading \textit{B. cereus} through the food chain. Generally, the high prevalence of \textit{B. cereus} in meat products is associated with poor hygiene in abattoirs, contaminated additives and cross-contamination during preparation and storage. These findings surely demand strict implementation of good hygienic practices (GHP) and hazard analysis and critical control point (HACCP) procedures at all stages of the food chain, to decrease the prevalence and number of \textit{B. cereus} and prevent its further dissemination.

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