Quantitative Risk Assessment of *Bacillus cereus* in Pasteurised Milk Produced in the Slovak Republic

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Abstract

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Quantitative risk assessment of *Bacillus cereus* using data from pasteurised milk produced in Slovakia was performed. Monte Carlo simulations were used for probability calculation of *B. cereus* density at the time of pasteurised milk consumption for several different scenarios. The results of the general case exposure assessment indicated that almost 14% of cartons can contain > 10^4 CFU/ml of *B. cereus* at the time of pasteurised milk consumption. Despite the absence of a generally applicable dose-response relationship that limits a full risk assessment, the probability of intoxication per serving and the estimated number of cases in the population were calculated for the general exposure assessment scenario using an exponential dose-response model based on Slovak data. The mean number of annual cases provided by the risk assessment model for pasteurised milk produced in Slovakia was 0.054/100 000 population. In comparison, the overall reporting rate of the outbreaks in the EU in which *B. cereus* toxins were the causative agent was 0.02/100 000 population in 2010. Our assessment is in accordance with a generally accepted fact that reporting data for alimentary intoxication are underestimated, mostly due to the short duration of the illness.

Keywords: exposure assessment; risk characterisation; Monte Carlo simulations

The microbiological safety of foods is of fundamental importance to all those companies and government organisations involved in the production, processing, distribution, retail, and regulation of foods and drinks. For the customer protection, the good manufacturing and hygiene practice (GMP/GHP) followed by the process control based on the HACCP principles should be applied as preventive measures. Microbiological risk assessment (MRA) has been undertaken since the mid-1990s and is performed within a risk management context to aid decision-making on microbial hazard management, considers nature knowledge and the likelihood of exposure to that hazard. The core elements of an MRA are the following consecutive steps: (1) hazard identification, (2) exposure assessment, (3) dose-response assessment, (4) risk characterisation (Bassett *et al*. 2012).

Whereas the qualitative risk assessment involves the description treatment of information in order to estimate the magnitude of risk and the impact of factors affecting risk, the quantitative risk assessment (QRA) works with numerical data (Fazil 2005). The available modelling techniques of the quantitative risk assessment (QRA) are generally based on a certain form of Monte Carlo simulations which result in frequency distribution of the output of interest providing not only extreme values but also the most likely outcome based on the combinations of input probability values that could occur (Lindqvist *et al.* 2002). The main goal of the modelling in microbial food safety is to evaluate possible presence or growth of undesirable microorganisms in foods using predictive primary and secondary models and their health impact on consumer using dose-response models. The most common approach requires development

or application of a model representing the various pathways or scenarios that can occur as the product moves from farm to consumer.

First, growth models as a function of environmental conditions are applied for prediction of the numbers of bacteria ingested (described in the form of probability distributions) and then they are translated through dose-response models into a risk probability of infection/illness. According to Vose (2008), the possibilities of modelling are nearly endless, but complex models should be simplified yet still adequately representing the reality. It should also be reminded as Zwietering (2009) added that simple models are not always wrong and complex always right.

B. cereus is the most important spore-forming microorganism taken into account in pasteurised milk when considering its quality and safety. It is able to survive the pasteurisation process and may produce enterotoxins that can cause food poisoning, e.g. nausea, diarrhoea, vomiting (Granum *et al.* 1995). The optimal growth temperature of *B. cereus* is 28–35°C, with a minimum growth temperature of 4°C and a maximum of 55°C. Growth can occur in pH ranges from 4.9 to 9.3, and the organism tolerates 7.5% salt concentration (Lampel *et al.* 2012). Generally, foods containing > 10^4 *B. cereus*/g are not considered as safe. Except dehydrated foods, e.g. dried infant or dietary formulae in which presumptive *B. cereus* counts (m < 50 CFU/g or M < 500 CFU/g) are embedded in food legislation (Regulation EC 1441/2007), no regular estimation in other foods, including pasteurised milk, is performed within end-product testing. Under the well-established HACCP system, control of temperature in each step of production, proper cleaning and disinfection of the equipment, rapid cooling after heat treatment (below 4°C), low pH (below 4.5), reduction in a_w (below 0.92), and refrigeration are the major measures to prevent the growth of *B. cereus*, including psychrotrophic strains (Anonymous 2005).

To gain information on human exposure assessment, storage tests of pasteurised milk have been applied (Notermans 1997; Nauta *et al.* 2003; Valík *et al.* 2003, 2009). For the present it remains advisable to observe the existing regulations on maximal numbers of *B. cereus* in ready-to-drink milk. Based on the data of the literature sources mentioned above, the real infectious dose may vary from about 1×10^5 to 1×10^8 viable cells. Three factors, initial *B. cereus* count in pasteurised milk, storage temperature, and storage time, are used to consider the exposure assessment of *B. cereus* at the milk consumption time. One of the weak points in the risk assessment of foodborne intoxications is usually a lack of dose-response relationships. The approach of Lindqvist and Westöö (2000) used for calculation of the species specific constant R that helps define the dose-response curve could be used. The aim of our study was to use Slovak data, the approach mentioned above and to run the model through Monte Carlo simulations using the ModelRisk software (www.vosesoftware.com), thus providing *B. cereus* exposure data in pasteurised milk at the time of consumption and finally the probability of illness per serving or estimated number of cases/population.

MATERIAL AND METHODS

Exposure assessment. The MPRM methodology (Lindqvist *et al.* 2002; Nauta *et al.* 2003; Nauta 2005; Mataragas 2010), as an extension of the process risk model, introduced by Cassin *et al.* (1998), was applied in the present food chain model. It simulates the transmission of *B. cereus* by splitting up the food pathway into smaller steps (modules). The following food pathway was recognised by the model description: transport from manufacturer to retail, storage in retail, transport from retail, and storage at home. The main factors involved in the risk of being exposed to unacceptable levels of *B. cereus* at the time of milk consumption were the initial density of *B. cereus* after pasteurisation and storage at retail/domestic temperatures and times. The implementation of the probability distributions of the input model parameters (factors) has to be done. Predictive microbial growth models were used for an exposure assessment study. Final result was found as the probability distribution of *B. cereus* density at the time of pasteurised milk consumption.

Food pathway. The food pathway considered in this study starts after milk pasteurisation process and packing in 1l Tetra Top cartons. They were then handled or stored in four consecutive stages: (1) milk transport from manufacturer to retailer, (2) retail, (3) transport from retail to home, and (4) keeping in domestic refrigerators.

Storage and temperature distribution. The use-by date (UBD) on the aseptically packed 1l Tetra Top cartons after leaving the milk factory was declared at 7 days by the producer. It was assumed that the cartons from the milk manufacturers were stored and transported under strictly controlled conditions until they reach the retailers (1 day at 5°C). In the model, 80% of pasteurised milk cartons were sold in

shops within the first four days, the rest in the last two days before UBD, both with the uniform distribution (Nauta *et al.* 2003). Storage temperature distribution at retail was calculated on the basis of data (Anonymous 1999). The date of purchase (PD) was then the sum of storage time until retail (t_1), storage time at retail (t_2), and time from retail to home (t_3). Transport temperatures and times from retail to home were generally largely unknown. Therefore in this study we applied an analogous approach as did Nauta *et al.* (2003). We assume that the transport time from retail to domestic refrigerator in Slovakia has normal distribution with a mean of 50 min and standard deviation 15 minutes. Assuming that milk was not refrigerated during transport by the consumer, temperature distribution could be approximated by PERT distribution with minimum temperature 4°C, most likely 10°C and maximum 25°C. Despite the fact that the consumer's behaviour was influenced by the UBD on the milk carton, many consumers did not observe the recommended storage times and temperatures in their private household refrigerators, therefore the distribution of storage times was adopted from Notermans *et al.* (1997), presented in Table 3, and the temperature distribution in Slovak private households from Pokrievka (2001), shown in Table 4.

Pasteurised milk, its sampling and determination of the initial B. cereus density. Pasteurised milk was heat-treated in plate heat exchangers at 74°C for 20 s and aseptically packed in 1l Tetra Top cartons. Samples of commercially processed pasteurised milk (1.5% fat) were taken immediately after packaging and were kept at 5.0 ± 0.5°C during their transportation (shorter than 60 min) to the laboratory. Some of the samples were tested for the *B. cereus* presence by using ISO 7932 procedures. The distribution of the initial cell density is generated on the basis of data (Valík *et al.* 2009) and the prevalence of *B. cereus* in pasteurisation milk is 100% ($P_P = 1$).

Growth curves and growth parameters. To model bacterial growth as a function of time and temperature, primary and secondary growth models were applied (Whiting 1995). Whole packages of pasteurised milk were stored in incubators at 5, 7, 9, 11, and 13°C and *B. cereus* plate counts were obtained using 15 ml aliquots taken aseptically from each milk sample at each storage temperature at predetermined time intervals. The growth parameters, lag-time, and growth rate, in relation to the storage temperature (Valík *et al.* 2003), shown in Table 1, were used for the prediction of safety and shelf life defined as the time required for *B. cereus* counts to reach critical density limits (10^4–10^5 CFU/ml) at the time of consumption (Notermans *et al.* 1997).

Consumptions. The distribution for milk serving sizes was described by a modified triangular distribution (VoseTriangularAlt). This distribution is determined by parameters for the minimum (100 ml), maximum (500 ml), most likely serving sizes (250 ml) and by estimates of the percentages of doses below the min. (5%) and max. serving sizes (95%). The used parameters were estimated on the basis of typical milk doses consumed in Slovakia.

Hazard characterisation. An exponential dose-response model, which relates the probability of illness by consuming a contaminated dose (P), with the number of *B. cereus* in the serving size (N_D), and constant R, specific to each pathogen that expresses the probability of being infected by one microorganism (Vose 2008), is described as follows:

$$P = 1 - e^{-RN_D} \quad (1)$$

We made an assumption that pasteurised milk with a concentration over 10^6 *B. cereus* (high probability of toxin production) causes an illness, and that only members of the high-risk population become ill. In the present work the constant R was estimated according to the approach of Lindqvist and Westöö (2000) based on Slovak data. This number was calculated by assuming that 7.6% (proportion of doses containing toxins, $P_T = 0.076$) of the servings (estimated from the MPRM) have a concentration higher than 10^6 CFU/ml. The total number of pasteurised milk consumers was calculated to be 8.91×10^5, which follows from 5.5×10^6 (the population in Slovakia) × 0.6 (fraction of milk consumers) × 0.27 (fraction of pasteurised milk consumers). This estimation was based on a survey conducted by GFK (TA SR 2012). To quantify the risk consumers of pasteurised milk, the value of 0.33 was used. This was based on the information that one third of the population of developed countries may be affected by food-borne diseases each year (WHO-FAO 2006). Assuming that an average dose size of 250 ml is taken four times per week, then the numbers of portions consumed by these persons are 209 a year. The number of contaminated portions with more than 10^6 CFU/ml consumed by the high-risk population per year is $2.94 \times 10^5 \times 209 \times 0.076 = 4.66 \times 10^6$ portions. The average number of reported illness cases in food caused by *B. cereus* in Slovakia is 0.02 cases/100 000 inhabitants (EFSA and ECDC 2012). Assuming that all these cases are caused by the consumption of pasteurised milk, the probability of illness for high-risk

consumers equals $1.1/4.66 \times 10^6 = 2.36 \times 10^{-7}$ cases per contaminated serving. The ingested dose in these servings was assumed to be 250 ml × 1 ×10^6 CFU/ml, i.e. 2.50×10^8 CFU, which is a conservative assumption by Lindqvist and Westöö (2000) since the level in some of the portions may be higher than 1×10^6 CFU/ml. The constant $R = 9.44 \times 10^{-16}$ was then calculated by rearranging Eq. (1):

$$R = [\ln(1 - P)]/N_D \quad (2)$$

Unfortunately, the generally recognised absence of a relationship between *B. cereus* toxins and dose response does not allow us to use an independent estimate of the constant R and therefore this approach was used.

Risk characterisation. The annual distribution and the mean value of predicted illness cases caused by *B. cereus* in pasteurised milk were estimated based on the number of servings consumed by risk groups in the Slovak Republic per year and the probability of illness per serving, P_{ill}, using the ModelRisk software (www.vosesoftware.com).

Mathematical model. For a quantitative risk assessment study of *B. cereus* present in milk at the time of consumption a Monte Carlo simulation model was constructed.

A simple predictive microbiology model for the *B. cereus* bacterial growth in pasteurised milk (Zwietering *et al.* 1996; Notermans *et al.* 1998, Nauta 2000) was used.

As a primary growth model the exponential growth is assumed:

$$\log(N) = \log(N_0) + kt\log(N) \quad (3)$$

where: N – cell density (CFU/ml) at time t; N_0 – initial cell density (CFU/ml); k – growth rate parameter (log CFU/h); t – storage time (h)

The growth function of Baranyi and Roberts (1994) was applied to fit the growth curves with the data observed (Valík 2009).

The second stage of predictive modelling involved the use of the growth parameters calculated from the primary model in the modified Arrhenius type model (Davey 1989) and the square root model (Ratkowsky *et al.* 1982).

The effect of temperature on the lag-phase of *B. cereus* is described by the modified Arrhenius equation:

$$\lambda = a_T + \frac{b_T}{T} \quad (4)$$

where: λ – lag time duration (h); a_T = –45.667 h; b_T = 1035.3 h·°C – equation parameters (Valík *et al.* 2003); T – temperature (°C)

A secondary square-root growth rate model with temperature incorporated was used for the growth rate analysis (Valík *et al.* 2003):

$$\sqrt{k} = b(T - T_{min}) + q \quad (5)$$

where: parameter $b = 0.026°C^{-1}\cdot h^{-0.5}$ is the slope and depends on additional growth conditions and the microorganism involved ($°C^{-1}\cdot h^{-0.5}$); $q = -0.1032\ h^{-0.5}$ is the intercept; T_{min} = 4°C is the theoretical minimum growth temperature (°C) (Blackburn & McClure 2009)

By inserting Eq. (5) into Eq. (3) and applied for considered food pathway phases it follows:

$$\log(N_i) = \log(N_{0i}) + [b^2(T - T_{min})q + q^2]t_i \quad (6)$$

with substituting

$$c_i = [b^2(T - T_{min})^2 + 2b(T - T_{min})q + q^2]t_i \quad (7)$$

where: N_{0i} – initial cell density (CFU/ml); c_i – growth parameter (CFU/ml); t_i – real time for *B. cereus* growth (in days); i = 1, 2, 3, 4 – food pathway phase denoted

Eq. (6) can be rewritten to a simplified form:

$$\log(N_i) = \log(N_{0i}) + c_i \quad (8)$$

To assess the probability of an exposure higher than the critical level (safety criteria vary from 10^4 to 10^5 CFU/ml), the implementation of the probability distributions of the input model parameters has to be done. Frequency distributions of the initial pathogen density in milk, storage temperatures and storage times were performed on the basis of sampling experiments and storage conditions in supermarkets/retail to home transport/households (Notermans *et al.* 1997; Pokrievka 2001; Nauta *et al.* 2003; Valík *et al.* 2003, 2009). Distribution of the parameter c_i, for the consecutive food pathway phases was calculated from model Eq. (7). The ModelRisk software (www.vosesoftware.com) was used not only for probability calculation of *B. cereus* density at the time of pasteurised milk consumption and then the risk factors influencing the output of the model and their threshold values were determined by the application of crude and advanced sensitivity analysis, respectively, but also for probability of illness per serving and annual number of illness cases.

The probability of illness per serving was estimated by the equation:

$$P_{ill} = PP_pP_T \quad (9)$$

where: P – probability of illness from drinking the contaminated dose calculated by Eq. (1); P_p – prevalence of *B. cereus* in milk; P_T – probability of serving sizes containing enterotoxins

The annual number of illness cases, n_C, was then calculated by the following equation:

$$n_C = P_{ill} n_S \quad (10)$$

where: n_S – the number of pasteurised milk servings consumed by risk groups in Slovakia per year

RESULTS AND DISCUSSION

Quantitative risk assessment of *Bacillus cereus* in pasteurised milk is presented in this paper. Monte Carlo simulations were used to calculate the probability of illness per serving and the annual number of illness cases based on the number of exposures Eq. (8) and dose-response relationship Eq. (1). The main factors involved in the risk of being exposed to unacceptable levels of *B. cereus* > 10^4 CFU/ml at the time of milk consumption are the initial cell density, storage temperature and storage time. All pasteurised milk samples were plated on the day of production. Due to its heat-stable spores, *B. cereus* survives the pasteurisation process. The initial counts of *B. cereus* in the samples of pasteurised milk varied from the absence to 1.58 CFU/ml and their distribution density count is shown in Table 1. Data were used for the generation of randomly sampled initial *B. cereus* density distribution and subsequently in Monte Carlo simulations for probability calculations of *B. cereus* density at the time of pasteurised milk consumption.

The above-mentioned approach was applied also to other storage time (Table 2) and storage temperature data (Table 3) in the considered milk pathway: transport form manufacturer to retail, transport from retail to home and storage in domestic refrigerator. The growth parameters, lag-time and growth rate, in relation to the storage temperature were taken from Valík *et al.* (2003) and Valík (2009). To assess the human exposure to *B. cereus* present in pasteurised milk, storage tests, and assumptions were carried out. The cartons from the milk manufacturers were stored and transported under strictly controlled conditions until they reached the retailers (1 day at 5°C). For the retail pathway phase uniform distribution for milk selling in shops was applied in the model: 80% of pasteurised milk cartons are sold within the first four days, the rest in the last two days before UBD (Nauta *et al.* 2003). Storage temperature distribution in retail was calculated on the basis of FDA data (Anonymous 1999) and they are shown in Table 3. For transport time/temperature from retail to home normal distribution (40, 15 min) and PERT distribution (4, 10, 25°C) were proposed. The storage times in domestic refrigerators varied from 1day to 11 days after milk pasteurisation and they are presented in Table 4 (adopted from Notermans 1997). Table 5 shows the temperature distribution in Slovak private households (Pokrievka 2001) varying from 5°C to 11°C.

Table 1. Initial number of *B. cereus* distribution present in milk after pasteurisation in the respective bin values within 0 to 1

bin_{min}	bin_{max}	*B. cereus* count (CFU/ml)
0	0.015	0.07
0.015	0.031	0.10
0.031	0.046	0.14
0.046	0.046	0.20
0.046	0.077	0.28
0.077	0.092	0.40
0.092	0.185	0.56
0.185	0.631	0.79
0.631	0.923	1.12
0.923	1.000	1.58

Calculated $N_{0,mean}$ = 0.88 CFU/ml

The calculation of *B. cereus* densities at the time of pasteurised milk consumption ($N_4 = N$), based on the calculations with Eq. (3–8), was performed for four cases: deterministic, general, UBD+1 and idealised. Probabilities of *B. cereus* density counts at the time of pasteurised milk consumption for all studied cases are summarised in Table 6.

The first studied deterministic case, where all main input factors were adjusted to their calculated mean values (see the above presented tables), con-

Table 2. Storage time distribution (days after milk pasteurisation) in the respective bin values within 0 to 1 (retail)

bin_{min}	bin_{max}	Storage time (days)
0	0.2	1
0.2	0.4	2
0.4	0.6	3
0.6	0.8	4
0.8	0.9	5
0.9	1.0	6

Calculated t_{mean} = 3.11 days

Table 3. Storage temperature distribution in the respective bin values within 0 to 1 (the retail stage)

bin_{min}	bin_{max}	Storage temperature (°C)
0.000	0.050	0
0.05	0.109	1.15
0.109	0.277	2.75
0.277	0.594	4.45
0.594	0.792	6.15
0.792	0.881	7.75
0.881	0.960	9.45
0.960	0.970	11.15
0.970	0.980	12.75
0.980	0.990	14.45
0.990	1.000	16.15

Calculated T_{mean} = 5.14°C

cerns the simple final number of cell density at the point estimate. For the deterministic estimate, the growth parameters, c_i, and the final number of cell density at the time of milk consumption (N), were calculated in Eq. (5) and Eq. (6), respectively. The final *B. cereus* density count at the time of pasteurised milk consumption equals 3.0×10^2 CFU/ml. This estimate is significantly lower than the critical level (e.g. 10^4 CFU/ml), which implies no health risk caused by *B. cereus* from the consumption of pasteurised milk. However, this is only the average (point) density count of *B. cereus* in 1l cartons after the total mean storage time (9.5 days).

To assess the true exposure to *B. cereus* at the time of pasteurised milk consumption we have to

Table 4. Storage time distribution (days after milk pasteurisation) in the respective bin values within 0 to 1 (domestic refrigerators)

bin_{min}	bin_{max}	Storage time (days)
0	0.022	1
0.022	0.081	2
0.081	0.238	3
0.238	0.396	4
0.396	0.516	5
0.516	0.659	6
0.659	0.813	7
0.813	0.868	8
0.868	0.912	19
0.912	0.963	10
0.963	1	11

Calculated t_{mean} = 5.53 days

Table 5. Storage temperature distribution in the respective bin values within 0 to 1 (the domestic refrigerator stage)

bin_{min}	bin_{max}	Storage temperature (°C)
0.000	0.007	5
0.007	0.051	5.5
0.051	0.154	6
0.154	0.375	6.5
0.375	0.478	7
0.478	0.596	7.5
0.596	0.706	8
0.706	0.787	8.5
0.787	0.882	9
0.882	0.949	9.5
0.949	0.978	10
0.978	0.993	10.5
0.993	1.000	11

Calculated T_{mean} = 7.52°C

implement the probability distributions of the input model parameters. The second general case involves all four consecutive stages with distributions of input parameters mentioned in the above tables and the resulting *B. cereus* probability distribution is shown in Figure 1a.

Calculations of exposure assessment, shown in Table 6, demonstrate that in this general case (Case 2) 13.7% of Tetra Top cartons can contain > 10^4 CFU/ml of *B. cereus* at the time of pasteurised milk consumption while 10.1% > 10^5 CFU/ml. It means that a certain portion of consumed pasteurised milk does not meet the general preventive criterion accepted in most European countries at the time of consumption.

The sensitivity analysis is a very important component of a risk-based decision making. It helps to find out which of the input parameters is driving the final *B. cereus* count uncertainty. Spearman's rank order correlation coefficients of the tornado plot (type of crude sensitivity analysis), which provided a statistical measure of correlation between

Table 6. Probability of *B. cereus* density counts at the time of pasteurised milk consumption

Case	Probability of *B. cereus* counts (%)			
	$N < 10^4$	$N < 10^5$	$N < 10^6$	$N < 10^7$
1	100	–	–	–
2	86.2	89.9	92.4	94.8
3	92.1	94.5	95.7	97.1
4	100	–	–	–

the model inputs and output, showed that variables such as domestic storage time (0.453), retail storage temperature (0.436), initial *B. cereus* count (0.327), and domestic storage temperature (0.193), had the greatest influence on the *B. cereus* count at the time of pasteurised milk consumption (Figure 2a). The remaining inputs of the model had a rank correlation lower than 0.1 (e.g. retail storage time, transport time and temperature from retail to households) and, therefore, they were not considered in further evaluation (Mataragas *et al.* 2010).

In the third case, so called UBD+1, we assume that manufactured milk is consumed within eight days after pasteurisation for all possible combinations of manufacturer /retailer/consumer storage times (1 day)/(1–6 days after pasteurisation)/(1–7 after days after pasteurisation), respectively. The critical *B. cereus* limit (10^4 CFU/ml) should not be exceeded (Notermans 1998). The distribution of *B. cereus* density count at the time of pasteurised milk consumption is depicted in Figure 1b.

Probability distribution of *B. cereus* at the time of pasteurised milk consumption demonstrates that even in the UBD+1 case *B. cereus* density count can be higher than the critical limit (10^4 CFU/ml). Rank correlation values (Figure 2b) for initial *B. cereus* count (N_0) and retail temperature (T_2) drive the output *B. cereus* value uncertainty the most.

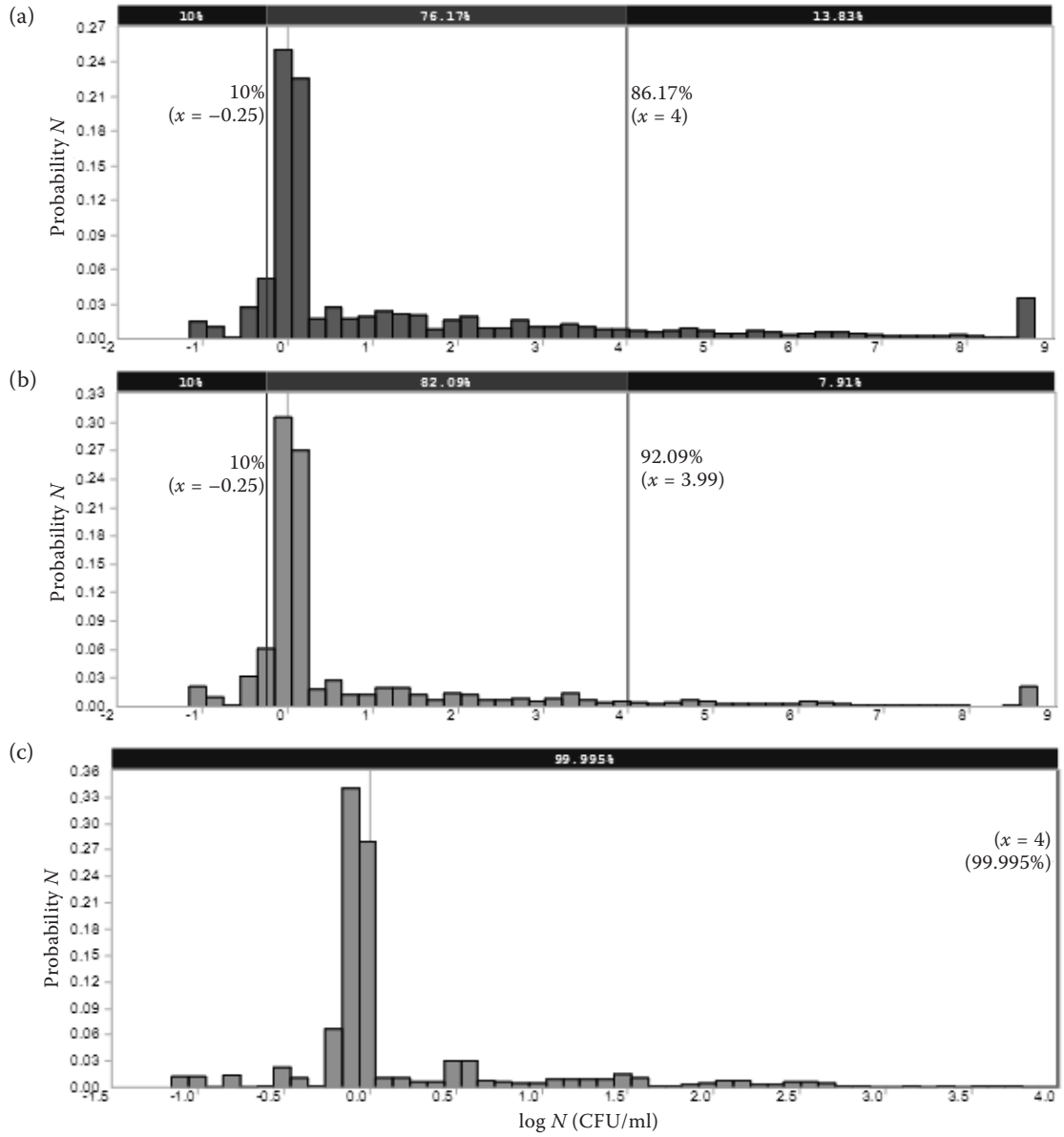

Figure 1. The probability distribution of *B. cereus* at the time of pasteurised milk consumption (a) general case, (b) UBD+1 case, and (c) idealised case

Household storage time (t_4) is below the great limit of influence (less than 0.1). It appears that not only higher temperatures during the retail phase but also the *B. cereus* level at the end of pasteurisation processing may induce growth above the critical limit.

Based on results of the last two cases the idealised case is performed when we assume that the initial *B. cereus* density count in pasteurised milk is ranging from the absence to 1.0 CFU/ml, retail/household storage temperatures varying only from 4°C to 7°C and 5°C to 8°C, respectively, manufactured milk is consumed within eight days after pasteurisation for all possible combinations of manufacturer/retailer/consumer storage times (UBD+1 case). The distribution of *B. cereus* density count at the time of milk consumption is presented in Figure 1c and practically all values of *B. cereus* density count are below the critical limit (10^4 CFU/ml).

Spearman's rank order correlation coefficients of the tornado plot depicted in Figure 2c show that variables such as initial *B. cereus* count (0.597) and retail storage temperature (0.258) have the greatest influence on the *B. cereus* count at the time of pasteurised milk consumption and drive the final *B. cereus* count uncertainty the most.

To carry out a proper quantitative risk assessment study of *B. cereus* in pasteurised milk it is necessary to evaluate the human exposure to this pathogen. In the absence of a dose-response relationship for calculation of infection/illness probability it is not possible to estimate the consequences of being exposed to *B. cereus* (a risk probability of infection/illness) via milk consumption. In this context it remains advisable to observe the existing regulations on maximal numbers of *B. cereus* present in pasteurised milk. It is the shared responsibility of producers (initial count), retailers and consumers (storage time and temperature), especially in the summer season. It requires that producers should keep the initial count of *B. cereus* after pasteurisation as low as possible. The recommended value is lower than 1 CFU/ml, and the critical *B. cereus* limit (10^4 CFU/ml) should not be exceeded within 7 days at 7°C (Notermans 1998) and the idealised case confirmed this. In our study, the initial count of *B. cereus* varied around this recommended value and that is a task for manufacturers to decrease the initial *B. cereus* values below this recommended level. Retailers, who are generally interested in a rapid turnover of products in their shops, should strictly keep storage temperatures below 7°C (our retail temperature threshold value for the general case equals 6.8°C) and the UBD. A more limited expiration date for fresh pasteurised milk could help this process. A part of consumers do not need to observe the recommended storage times and temperatures in their private household refrigerators. In such a case a higher exposure to high numbers of *B. cereus* may result in higher probability of generally underestimated intoxication caused by *B. cereus*.

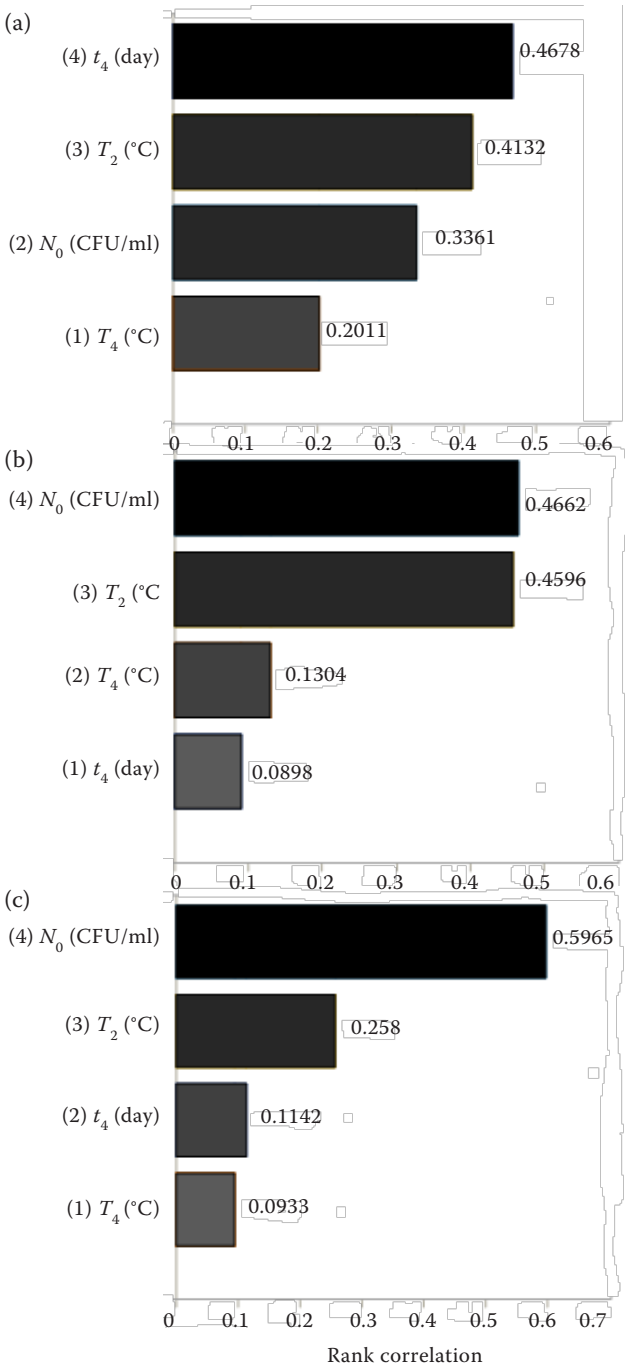

Figure 2. Crude sensitivity analysis with correlation coefficients displaying the most important factors contributing to the *B. cereus* value at the time of pasteurised milk consumption (a) general case, (b) UBD+1 case, and (c) idealised case

The development of a quantitative risk assessment model, although simplified and partially incomplete, can be a helpful tool to evaluate the relationship between the risk and the factors which may be used to reduce such a risk (Whiting & Buchanan 1997; Cassin *et al.* 1998). Despite the absence of a generally applicable dose-response relationship for *B. cereus* in food the probability of illness per serving and the estimated number of cases in the population were calculated for the general exposure assessment scenario using an exponential dose-response model based on Slovak data. The annual number of pasteurised milk servings consumed by risk groups was calculated on the basis of the total consumption and the distribution of serving sizes. This information, together with the probability of illness per serving, was used to predict the number of illnesses per year in Slovakia caused by the presence of *B. cereus* toxins in pasteurised milk. The exponential dose-response model predicted the mean value 0.054/100 000 cases per year (range 0 to 0.884/100 000 cases). This value is in reasonable agreement with the number of reported illnesses in the Slovak Republic (0.02/100 000 cases). The maximum range predicted many more illnesses, overestimating the risk. However, it should be kept in mind that the extent of underreporting illnesses caused by *B. cereus* toxins in milk is unknown and the reported cases all represent cases with serious symptoms. Spearman's correlation coefficients of a crude sensitivity analysis indicated that P_{ill} was the most sensitive to the *B. cereus* distribution at the time of consumption (0.925), and to a lesser extent to the distribution of serving sizes (0.275). The other parameters in the dose-response relationship were not included in the sensitivity analysis, since they were considered as constants, not variables.

CONCLUSION

QRA may give valuable information regarding the presence and development of a microbiological hazard in a food product. The most important parameter determining the risk was the concentration of *B. cereus* at the time of consumption. This study illustrates how the manufacturer, retail and consumer phase of the milk pathway (the contribution of transport from retail to household phase to the final *B. cereus* count shows to be practically negligible) can be modelled by linking predictive models and data. Despite the fact that due to some assumptions and uncertainties the quantitative predictions of *B. cereus* level at the time of pasteurised milk consumption should be handled with reservation they provide a useful tool for quantitative risk assessment and consecutive decisions. It follows from the general case that the easiest way and strategy to decrease the level of *B. cereus* exposure to consumers in pasteurised milk should mainly be aimed at domestic refrigerators where temperature/time abuse is the most frequent and then retailers to maintain storage temperature below 7°C. However, the UBD+1 and idealised cases demonstrate also the increasing role of the initial *B. cereus* density count after milk pasteurisation to keep the final *B. cereus* density count under the critical level (10^4 CFU/ml), which implies no health risk of pasteurised milk consumption. It means that producers, retailers, consumers and regulatory authorities have a certain responsibility for the safety of food products at the time of consumption.

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