Staphylococcus aureus in unripened ewes' lump cheese. Part 2: Exposure assessment at the time of consumption

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Summary

An exposure assessment of *Staphylococcus aureus* in unripened ewes' lump cheese traditionally produced in Slovakia, and consumed cooled usually up to three day from manufacture, is presented in this study. Predictive microbiological models, together with survey data, were combined with probabilistic modelling to simulate the level of *Staph. aureus* in unripened lump ewes' cheese at the time of consumption (model output). The main factors involved in the risk of being exposed to unacceptable levels of *Staph. aureus* were the distribution density of *Staph. aureus* in cheese after first 24 h of fermentation, the initial level of lactic acid bacteria (LAB) culture in raw ewes' milk, the storage at retail/home temperature and the time (model input). The three cases, use by date (UBD), use by date + 1 day (UBD+1) and the worst one (the case of a contaminated udder when the initial counts of *Staph. aureus* after milking may increase up to 4 log CFU·ml^{-1}), were considered. The results of exposure assessment for the cases use by date, use by date + 1 day and the worst one indicated that, at the time of consumption, 0.4%, 0.5% and 11.5% of manufactured cheeses exceeded the level of 10^5 CFU·g^{-1} of *Staph. aureus*, respectively. Taking the higher content of 10^6 CFU·g^{-1} into account as a criterion for unacceptable products, the ratio reached the maximum of 0.1% for the worst case.

Keywords

ewes' lump cheese; *Staphylococcus aureus*; modular process risk model; quantitative risk assessment

The most frequent potential pathogens associated with raw milk products, including ewes' cheeses are *Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes* and pathogenic *Escherichia coli* [1, 2]. Generally, they are able to multiply rapidly in milk or fresh curd, in particular during the initial phase of preparation when natural lactic acid bacteria (LAB) are in lag phase and a sufficient amount of lactic acid has not been produced. Based on prevalence and content of the pathogens mentioned above, only *Staph. aureus* can be considered as ubiquitous in dairy farms producing raw milk cheeses [3, 4]. When reaching high numbers, thermo-stable staphylococcal enterotoxins can be produced and staphylococcal food poisoning (SFP) may occur. After a short time from ingestion (1 h to 7 h), SFP typically results in sudden onset of nausea, violent vomiting, abdominal cramps and sometimes diarrhoea. On the other hand, of course, not all *Staph. aureus* strains can produce staphylococcal enterotoxins [5, 6], and even those that are capable of toxin production, do not performed it usually at conditions prevailing during proper curd fermentation or cheese ripening [7]. Generally it is recognized, that *Staph. aureus* can reach maximal densities within the first 24 h followed by a slow decline during ripening [8, 9]. Therefore, the presence as well as fast growth and metabolism of LAB accompanied with sufficient production of lactic acid or other antimicrobial compounds, and pH decrease during fermentation, are needed [10–12].

The microbiological safety of foods is of fundamental importance to all companies involved in the primary production, processing and distribution of foods. As preventive measures and for the customer protection, the good manufacturing and hygiene practice (GMP/GHP) followed

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by the process control based on Hazard Analysis and Critical Control Points (HACCP) principles should be applied. Microbiological risk assessment (MRA) takes place within a risk management context to aid decision-making on microbial hazard managing, considers nature knowledge and the likelihood of exposure on/to that hazard. The core elements of MRA are the following consecutive steps: (1) hazard identification, (2) exposure assessment, (3) dose-response assessment, (4) risk characterization [13].

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 microbiological risk assessment (QMRA) works with numerical data [14]. The available modelling techniques of QMRA are generally based on some 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 [15]. The main goal of the modelling in microbiological food safety is to evaluate possible presence or growth of undesirable microorganisms in foods by 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 the model representing various pathways or scenarios that can occur as product moves from the farm to consumer. In general, a model should be broken down into smaller components, disaggregated, as much as necessary but not more for an efficient but accurate modelling in relation to the purpose of the assessment [16].

The general framework for quantitative exposure assessment modelling, the Modular Process Risk Model (MPRM) as proposed by Nauta [17], was applied in our study describing a model of *Staph. aureus* exposure in the retail and consumer phase of the food pathway, using the distribution density of *Staph. aureus* in cheese after first 24 h of fermentation (the end of the production phase) as the input. The consumer phase is also taken into account, because it is less controlled than other phases of the food pathway. During transport or at home, consumer storage temperatures may be too high to maintain the chill chain and thus insufficient to prevent the pathogen from growth. The exposure assessment ends at the moment when consumer takes the product from the refrigerator and eats it.

While several studies on quantitative microbiological risk assessments in raw milk cheese were performed [15, 18–20], no similar effort has been done yet in Slovakia, although several raw cheese varieties are produced from raw ewes' milk in Slovakia [21]. This was the reason why we focused on the exposure to ubiquitous *Staph. aureus* in unripened ewes' lump cheese, traditionally produced in farms in Slovakian mountain areas. Although several studies [12, 22] reported that enterotoxin A was first detected when *Staph. aureus* levels were 6.8 log CFU·g^{-1} and 6.5 log CFU·g^{-1}, respectively, the criterion of 6 log CFU·g^{-1} at the time of consumption is widely accepted as the endpoint for evaluation of acceptability of the product [15, 23].

MATERIALS AND METHODS

Modular process risk model

The MPRM methodology as an extension of the process risk model, introduced by Cassin et al. [24], was applied in the present food chain model. In this model, transmission of hazard is modelled by splitting up the food pathway into smaller steps (modules). The risk model is a stochastic model, analysed by Monte Carlo simulations, and input and output are given in terms of probability distributions reflecting uncertainty or variability [16]. Typically, MPRM starts with a description of the food pathway and the processes that are relevant to assess the risk, not with the collection of available data. This may imply that parameters have to be defined in the process models, values of which cannot be estimated on the basis of scientific data. In that case, food microbiology experts were asked to provide their opinion [19]. Generally, minimum (min), most likely (ml), maximum (max), mean and standard deviation values of the parameter, as assessed by the experts, were implemented as parameters of the applied distributions.

Food pathway

The food pathway considered here starts at the end of a production phase (the first 24 h of fermentation of unripened lump cheese production). It is assumed that lumps of cheese are stored at 7 °C and transported during two hours until they reach the retailers. The *Staph. aureus* growth in co-existence with LAB is practically negligible under these conditions. The following food pathway was recognized by the model description in three consecutive steps: (1) storage in retail, (2) transport from retail to home, and (3) storage at home (keeping the cheese in domestic refrigerators). The main factors involved in the risk of being exposed to unacceptable levels of *Staph. aureus*

(> 6 log CFU·g^{-1}) at the time of cheese consumption were found to be: density of *Staph. aureus* after first 24 h of fermentation, the initial level of LAB culture in raw ewes' milk [4], storage at retail/domestic temperatures, and time. In order to predict the growth of *Staph. aureus* further down the food pathway, implementation of the probability distributions of the input model parameters (factors) have to be done. Information not only from predictive microbial growth models, but also the storage time and storage temperature are required to be involved. Final result of the exposure assessment was formulated as the probability distribution of *Staph. aureus* density at the time of consumption of the unripened ewes' lump cheese.

Storage and temperature distribution

The use-by-date period (UBD) of the unripened ewes' lump cheese fermented for 24 h was declared between 1 to 3 days by the producers (response to a personal request). It was assumed that 70% of the cheese portions were sold in shops within the first day, 25% the next day and the rest on the last one, all with uniform distribution. Storage temperature distribution at retail was calculated on the basis of the previously published data [25]. Both data sets were transferred into distributions via the CumulA function by the ModelRisk software (Vose Software, Gent, Belgium) such as: VoseCumulA (0; 72; {8; 16; 24; 32; 40; 48; 56; 64}; {0.233; 0.466; 0.699; 0.783; 0.866; 0.949; 0.966; 0.982}); VoseCumulA (0; 16.15; {1.15; 2.75; 4.45; 6.15; 7.75; 9.45; 11.15; 12.75; 14.45}; {0.108; 0.275; 0.588; 0.784; 0.873; 0.951; 0.961; 0.971; 0.982}).

The date of purchase (PD) was then the sum of storage time at retail (t_1), time from retail to home (t_2) and storage time at home (t_3). Transport temperatures and times from retail to home were generally largely unknown. Therefore the analogous approach as [19] was applied in this study. We assumed that transport time from retail to domestic refrigerator in Slovakia has a normal distribution with a mean of 2 h and standard deviation of 0.5 h. Assuming that no refrigeration was applied during the transport by the consumer, temperature distribution could be approximated by the Pert distribution with a minimum temperature of 10 °C, most likely 18 °C and a maximum of 25 °C . Despite the fact that consumer's behaviour was influenced by UBD labelling on the cheese package, many consumers did not adhere to the recommended storage times and temperatures in their private household refrigerators. Assumption that 70% of the unripened lump cheese packages after purchase were consumed within the first day, 20% within the second day, 5% within the next day (UBD) and the rest within the day after UBD (UBD+1), all stored for uniformly distributed storage times in domestic refrigerators, was adopted from personal assessment. The temperature distribution in private households in Slovakia was calculated on the basis of previously published data [26]. Transformation of the last two mentioned data sets into distributions was performed through the CumulA function for the UBD+1 case as follows: VoseCumulA (0; 96; {8; 16; 24; 32; 40; 48; 56; 64; 72; 80; 88}; {0,233; 0.466; 0.699; 0.766; 0.833; 0.899; 0.916; 0.932; 0.949; 0.966; 0.982}); VoseCumulA (5; 11; {5.5; 6; 6.5; 7; 7.5; 8; 8.5; 9; 9.5; 10; 10.5}; {0.051; 0.153; 0.372; 0.474; 0.591; 0.701; 0.781; 0.876; 0.941; 0.971; 0.985}).

Exposure assessment

For the exposure assessment study of *Staph. aureus* present in the unripened ewes' lump cheese at the time of consumption, the Monte Carlo simulation model was constructed for three consecutive steps in the food pathway: (1) storage in retail, (2) transport from retail to home, and (3) storage at home. The exposure model combined the food pathway characteristics with the basic models for growth and the pathogen cells density distributions at the end of a production food pathway [27]. These were used as a starting point for predictions of *Staph. aureus* counts at the moment when consumer takes the product from the refrigerator.

The following exponential growth model was assumed:

$$\log(N_j) = \log(N_{j0}) + k_j t_j \tag{1}$$

where N_j is the cells density (in colony forming units per gram) at time t, N_{j0} is the cells density at the beginning (in colony forming units per gram), k_j is the growth parameter (in logarithm of colony forming units per gram) and t_j is the time (in hours) for the *Staph. aureus* growth for the given food pathway denoted by a subscript $j = 1, 2, 3$.

By substituting $k_j t_j$ as c_j

$$c_j = k_j t_j = \frac{\mu_{\max,j}}{\ln 10} t_j \tag{2}$$

and inserting Eq. (2) into the Eq. (1), Eq. (1) can be re-written to a simplified form:

$$\log(N_j) = \log(N_{j0}) + c_j \tag{3}$$

Dependencies of the specific growth rate as well as the duration of pH lag phase on temperature and initial content of LAB culture, resulting from the linear regression analysis, are expressed by relationships adopted from [4]:

$$\sqrt{\mu_{\max}} = -0.2111 + 0.0487T - 0.0541\ \log(N_{\mathrm{LAB},0}) \quad (4)$$

$$\ln(t_{\mathrm{L,pH}}) = 6.494 - 0.129T - 0.23\log(N_{\mathrm{LAB},0}) \quad (5)$$

where $N_{LAB,0}$ is the initial LAB culture density and $t_{L,pH}$ is the duration of pH lag phase.

The logNormal distribution (ModelRisk) was applied to the initial LAB density distribution in ewes' milk by parameters for minimum (2.6 log CFU·ml^{-1}), maximum (5 log CFU·ml^{-1}) and mean count (3.5 log CFU·ml^{-1}) with the standard deviation of 0.4 log CFU·ml^{-1}.

Risk characterization

Since cheese consumption was not considered the present study, due to the absence of dose-response models, the study does not provide a complete risk assessment. Instead, the final pathogen density was used as an approximation of the potential enterotoxin production that may cause staphylococcal food poisoning. The assessment endpoint selected for evaluation of an unsatisfactory product was the probability that unripened ewes' lump cheese contained at least 6 log CFU·g^{-1} of *Staph. aureus* at the time of consumption. This endpoint was termed as P_{uc}, the probability of unsatisfactory cheese [15].

A spreadsheet model was developed in Microsoft Excel (Microsoft, Redmond, Washington, USA) and simulated using the ModelRisk software. Each simulation consisted of 100 000 iterations (cheese packages) and the P_{uc} above the threshold value (6 log CFU·g^{-1}) was predicted.

RESULTS AND DISCUSSION

For the potential risk evaluation, predictive microbiological models together with survey data were combined with probabilistic modelling in order to simulate the level of *Staph. aureus* in unripened lump ewes' cheese at the time of consumption. Three cases, namely, UBD, UBD+1 and the worst case were considered. The initial *Staph. aureus* density distribution in ewes' milk was described by a LogNormal distribution (Vose-LogNormal) that is determined by parameters for minimum, mean and maximum (1.0 log CFU·ml^{-1}, 2.9 log CFU·ml^{-1}, 3.5 log CFU·ml^{-1}), respectively. For the worst case, maximum was increased to 4 log CFU·ml^{-1} [5, 23]. The parameters used for UBD and UBD+1 cases were estimated on the basis of typical counts in properly drawn milk in the farms in Slovakian mountain areas [4]. All three cases calculated the *Staph. aureus* probability distribution under the presumption that the entire present LAB culture had a potential to inhibit the growth of the pathogen, representing the possible effect of starter LAB cultures on improvement of the product safety.

The effectiveness of LAB culture is related to the rate at which it can produce lactic acid, in particular during the first six hours of fermentation. Here, the phenomenon of pH lag phase is important. Its duration depends not only on the initial LAB density but on temperature as well. The higher the incubation temperature, the more intensive the metabolism of LAB and the sooner pH decrease will occur. The relation between the duration of pH lag phase, temperature and initial counts of LAB is expressed by Eq. 5. When the duration of pH lag phase is smaller than the time passing since milking, growth of *Staph.aureus* will cease and its population will drop down. This period is influenced by the amount of LAB Fresco culture, which should be higher than 10^5 CFU·ml^{-1} at a specific temperature [4]. Because *Staph. aureus* can grow only during the pH lag phase, the growth parameter *c* equals zero in Eq. 3.

The simulated probability that *Staph. aureus* numbers reach above the threshold value of 5 log CFU·g^{-1}, was 0.4% for UBD case, 0.5% for UBD+1 case and 11.5% for the worst case. Almost all of the simulated maximum *Staph.aureus* numbers at the time of consumption were below the value of 6 log CFU·g^{-1}, which is the unacceptability threshold. There was only a low probability

Tab. 1. Probabilities of *Staph. aureus* counts at the time of consumption.

Case	Probability of *Staph. aureus* counts [%]			P_{uc} [%]
	$N \le 10^4$ CFU·g^{-1}	$N \le 10^5$ CFU·g^{-1}	$N \le 10^6$ CFU·g^{-1}	
UBD	19.8	99.6	0	0
UBD+1	18.3	99.5	0	0
Worst case	1.0	88.5	99.9	0.1

UBD – use by date, UBD+1 – use by date + 1 day, *N* – cells density, P_{uc} – percentage of unacceptable cheese portions at the time of consumption.

of 0.1%, in the worst case, that *Staph. aureus* numbers would reach density higher than 10^6 CFU·g^{-1} (Tab. 1).

These data support the approach that it is necessary to manage the cheesemaking process in a way that pH around 5 is reached as soon as possible. This can be controlled by the addition of LAB cultures prior to fermentation of ewes' milk. A low pH is also known to reduce the production of staphylococcal enterotoxins by the strains [28].

The assessment endpoint, termed as P_{uc}, the probability of unripened ewes' lump cheese to contain at least 6 logCFU·g^{-1} of *Staph. aureus* at the time of consumption, was selected as the threshold of unacceptabililty of the product [15, 23]. At this level of contamination, staphylococcal enterotoxins are not necessarily produced, as not all strains are capable to produce staphylococcal enterotoxins [5, 6]. Summarized results (Tab. 1) indicate that only a small portion of the cheeses could contain *Staph. aureus* at an unsatisfactory level of 5 log CFU·g^{-1} at the time of consumption, e.g. on the 3rd or 4th day. However, as staphylococcal food poisoning due to cheese consumption has not been wider reported to the authorities, it is possible that the potential risk predicted for the worst case overestimates the real risk [15]. If LAB starters were used, *Staph. aureus* numbers in cheese would remain below 10^5 CFU·g^{-1} and the cheese could be kept at refrigeration temperatures for 4 days in all cases.

The probability distribution and the crude sensitivity analysis are shown only for UBD case and depicted in Fig. 1 and Fig. 2. The final levels of the pathogen at the moment when the consumer takes the product from the refrigerator depend on the interplay between input model parameters (factors). Their effects were determined by performing sensitivity analysis, which is an important component of risk-based decision making. It helps to find out which of the input parameters is driving the final pathogen count uncertainty. The Spearman's rank order correlation coefficients of the tornado plot (type of crude sensitivity analysis), which provided a statistical measure of correlation between the model inputs and the output, showed that variables such as *Staph. aureus* density after 24 h of fermentation (0.928), initial LAB culture in raw ewes' milk (–0.153) connected with the period of no pH decrease, domestic storage time (0.107) and temperature, transport temperature (–0.061) and retail storage temperature (0.057) had the greatest influence on *Staph. cereus* counts at the time of unripened ewes' cheese consumption. The remaining inputs of the model had a rank correlation lower than 0.05 (retail storage time and trans-

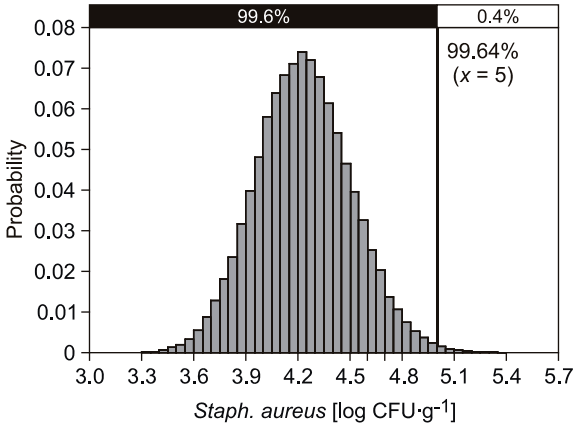

Fig. 1. Probability distribution of *Staph. aureus* in ewes' cheese at the time of consumption (UBD case).

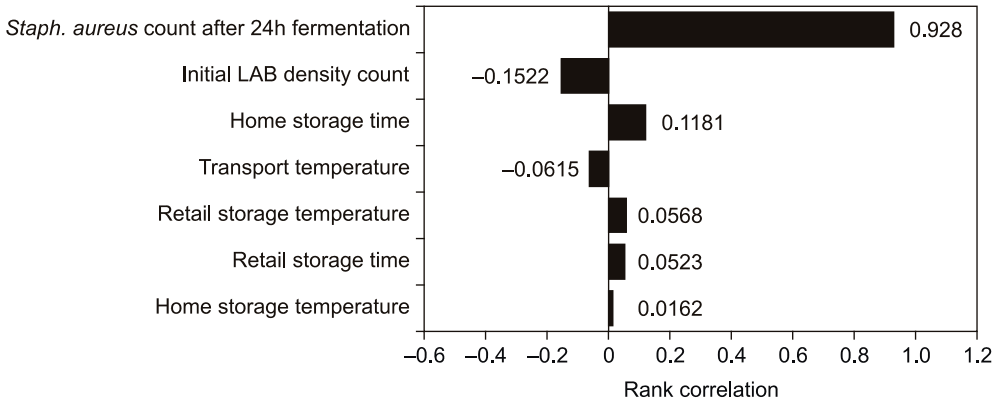

Fig. 2. Crude sensitivity analysis with correlation coefficients for individual factors contributing to *Staph. aureus* levels at the time of cheese consumption (UBD case).

port time from retail to households) and, therefore, they did not need to be further considered [20]. The crude sensitivity analysis revealed that the most important factors contributing to *Staph. aureus* numbers in unripened ewes' lump cheese at the time of consumption were: *Staph. aureus* counts after 24 h of fermentation and the initial LAB numbers in raw ewes' milk that determined the duration of pH lag phase. Simulations also showed that the consumer phase (transport from retail to home and subsequent storage at home), despite the broad distribution of temperatures and times (in particular in domestic refrigerators) had less significant effect than expected. This was due to a more acidic environment (lower pH) during this consumer pathway phase. This influence would be even greater if a starter LAB culture would be added to raw ewes' milk prior to fermentation.

CONCLUSION

The study illustrates that retail and consumer phase of the food pathway can be modelled by linking currently available predictive models and data. Due to a lack of data, some assumptions and parameter estimates had to be used, which were based on estimates of experienced experts. Nonetheless, the results provided an interesting information based on the present knowledge in the fields of food microbiology and mathematical modelling. *Staph. aureus* density after first 24 h of cheese fermentation was found to be a good predictor for the level of contamination at the moment when cheese is removed from the refrigerator and consumed (up to 4 days). Addition of LAB prior to milk fermentation facilitated keeping *Staph. aureus* numbers at the acceptable level until UBD. However, this effect should be verified also for other pathogens, e.g. *Listeria monocytogenes* or *Salmonella enterica* that may contaminate the ewes' lump cheese.

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