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# Consumers' behavior in quantitative microbial risk assessment for pathogens in raw milk: Incorporation of the likelihood of consumption as a function of storage time and temperature

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## ABSTRACT

Foodborne disease as a result of raw milk consumption is an increasing concern in Western countries. Quantitative microbial risk assessment models have been used to estimate the risk of illness due to different pathogens in raw milk. In these models, the duration and temperature of storage before consumption have a critical influence in the final outcome of the simulations and are usually described and modeled as independent distributions in the consumer phase module. We hypothesize that this assumption can result in the computation, during simulations, of extreme scenarios that ultimately lead to an overestimation of the risk. In this study, a sensorial analysis was conducted to replicate consumers' behavior. The results of the analysis were used to establish, by means of a logistic model, the relationship between time-temperature combinations and the probability that a serving of raw milk is actually consumed. To assess our hypothesis, 2 recently published quantitative microbial risk assessment models quantifying the risks of listeriosis and salmonellosis related to the consumption of raw milk were implemented. First, the default settings described in the publications were kept; second, the likelihood of consumption as a function of the length and temperature of storage was included. When results were compared, the density of computed extreme scenarios decreased significantly in the modified model; consequently, the probability of illness and the expected number of cases per year also decreased. Reductions of 11.6 and 12.7% in the proportion of computed scenarios in which a contaminated milk serving was consumed were observed for the first and the second study, respectively. Our results confirm that overlooking the time-temperature dependency may yield to an important overestimation of the risk. Furthermore, we provide estimates of this dependency that could easily be implemented in future quantitative microbial risk assessment models of raw milk pathogens.

Key words: raw milk, quantitative microbial risk assessment, consumer behavior, milk spoilage

## INTRODUCTION

Probabilistic modeling is becoming established as one of the main tools to inform risk management decisions with regard to foodborne hazards. Quantitative microbial risk assessment models (QMRA) are increasingly applied to scenarios involving established and emerging food safety hazards as risk analysis becomes standard practice to manage food safety and ensure that regulatory decisions about foods are science based and transparent (FAO, 2006; WHO/FAO, 2010).

One of the most significant examples from the public health perspective in recent years has been the use of QMRA to estimate risks associated with the consumption of unpasteurized milk. Growing interest in raw milk consumption by some groups of consumers and an increasing number of foodborne incidents in which raw milk has been identified as the source have lead agencies such as the UK Food Standards Agency, the European Food Safety Authority, or the US Centers for Disease Control to conduct consultations and issue scientific opinions on the risk posed by milk-borne hazards (CDC, 2014; FSA, 2014; EFSA, 2015).

The public health risk related to consumption of raw milk is a particularly relevant (and debated) topic. Raw milk can contain human pathogens, which can be inactivated by appropriate heat treatment (pasteurization or sterilization). However, the perception of raw milk as a more natural product has led to several consumers opting for raw as opposed to heat-treated milk. In light of this trend, models have been developed in recent

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years to assess probability of exposure or infection by pathogens such as *Salmonella*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Escherichia coli* O157, or *Staphylococcus aureus* as a result of raw milk consumption (Heidinger et al., 2009; Latorre et al., 2011; Giacometti et al., 2012; Giacometti et al., 2015).

Quantitative microbial risk assessment models aimed at assessing the risk from farm to table include a consumer phase module, a stage of the model that occurs at household level, where the food is no longer controlled by professionals and where control of storage conditions or application of sufficient heat treatments cannot be enforced by legislation (Nauta and Christensen, 2011). In QMRA related to unpasteurized or pasteurized (Koutsoumanis et al., 2010) milk, the time and temperature of storage in the consumer phase modules are usually described and modeled as independent distributions. Time and temperature are the most important parameters that regulate microbial growth in milk and are regularly identified in sensitivity analysis as the factors with greatest effect on the model output (Koutsoumanis et al., 2010; Latorre et al., 2011).

When both, storage time and temperature, are modeled as independent probability distributions (most often Triangular or Pert), in some instances during simulations, values from the tails of the distributions are sampled together yielding scenarios with high bacteria concentration at the time of consumption. An implicit assumption underlying the cited models is that 100%of the computed scenarios will result in milk being consumed, whatever the time-temperature combination is. However, in reality some time-temperature combinations are unlikely to result in milk being consumed as it would be perceived by the consumer as unsuitable (raw milk stored at high temperature for extended periods might be spoiled and thus not actually consumed). Therefore, given that in microbial dose-response models the probability of illness is directly dependent on the number of bacteria ingested per serving (i.e., each bacteria has the same probability to generate infection), the amount of simulated scenarios under extreme conditions may have a significant effect on the final output.

This limitation was already highlighted by Latorre et al. (2011), who noted that some correlation between these variables may exist and that without any restriction, the model cannot take into account that some extreme scenarios may not occur or end with milk not being consumed. However, to our knowledge, this limitation and the effect that this assumption may have on model output have never been formally assessed.

Following these considerations, the objectives of this work were to (1) model the dependencies between time

and temperature to express the likelihood for a raw milk serving to be actually consumed for any computed storage time-temperature combination and (2) assess the extent to which this dependency would affect the output of a QMRA model.

To this end, results of a simplified sensorial analysis on raw milk stored for 5 d at different temperatures were used to estimate the probability that at given time-temperature combinations, the milk is spoiled, recognized as such, and thus not consumed. The potential effect of the estimated time-temperature relationship on model output was than evaluated by its inclusion in 2 recently published QMRA of raw milk consumption and comparison of published results with those of the modified model.

### MATERIALS AND METHODS

#### Raw Milk Sample Collection for Sensorial Analysis

A total of 1.5 L of raw milk was collected from 30 automatic vending machines in Lombardy by the public veterinary services, univocally coded, placed in cold boxes at 5°C  $\pm$  3, and taken to the laboratory within 30 min. Upon arrival, 5 aliquots of 200 mL were obtained from each sample and kept in different isothermal conditions at 3, 5, 8, 12, and 16°C for 5 d (temperatures were chosen to reflect the range of temperatures at which the domestic refrigerators can be expected to operate).

A total of 500 mL from each sample was used to test the samples for pH, SCC, lactic acid bacteria, total mesophilic flora, Enterobacteriaceae, and the major pathogens to ensure operator safety. An instrument with automatic temperature compensation (Hanna instrument HI9321, Hanna Instruments Inc., Woonsocket, RI) was used for pH measurement. The SCC was determined by an Optofluorimetric accredited internal method MP02/063 (Fossomatic, Foss Electric, Hillerød, Denmark). The ISO standards ISO4833-2, ISO21528-2, and ISO16649-2 (ISO, 2001, 2004, 2013) were used for surface plate enumeration of total mesophilic flora, Enterobacteriaceae, and E. coli, and the standards AF-NOR BRD 07/10 and AFNOR BRD 07/06 (AFNOR, 2008, 2009) were used for PCR real-time detection of L. monocytogenes and Salmonella. Enumeration of lactic acid bacteria was performed by the accredited internal method MP01/048 (decimal dilution and plating in MRSA agar plate incubated under microaerophilic condition at  $37 \pm 2^{\circ}$ C for  $72 \pm 2$  h and decimal dilution and plating on M17 agar plate at 37  $\pm$  2°C for 48  $\pm$ 2 h for enumeration of mesophilic lactic flora and lactococci, respectively). The accredited internal method

Table 1. Descriptors used in the sensorial analysis of raw milk samples stored at different time-temperature combinations

Item	Description	Score
Aroma	None	1
	Acid aroma perceived when poured from the bottle	2
	Acid aroma perceived immediately at the opening of the bottle	3
Texture	Milk appears homogeneous when observed through the bottle. When poured from the bottle, milk appears smooth without any visible flake or residual on the bottle surface.	1
	Milk appears homogeneous when observed through the bottle. Small flakes are observed on the surface. Small flakes adhered to the bottle are clearly visible when milk is poured.	2
	Milk in advanced coagulation phase; clear phase separation is observable through the bottle.	3

(MP 09/135) was used to test the samples for the presence of *C. jejuni* by PCR real time (*Campylobacter* Kit, Bio-Rad, Hercules, CA).

## $\operatorname{logit}(p_i) = \ln[p_i/(1 - p_i)] = \alpha + \beta_1 T^{\circ} + \beta_2 h, \quad [1]$

## Sensorial Analysis

To replicate consumers' behavior, a simplified descriptive sensorial analysis of the milk samples stored at different temperatures was performed. The evaluation was carried out independently by 2 internal panelists experienced with sensory evaluation of milk (Experimental Zooprophylactic Institute of Lombardy and Emilia Romagna, Bergamo, Italy). Descriptors used in the evaluation sessions were selected following consultation with the panelists and based on their experience and the scope of the analysis (Table 1).

Panelists were asked to evaluate all the milk samples every day at the same hour for 5 d. Each raw milk sample required the judgment of 5 subsamples per session (one sample for each temperature); thus, for practical reason, no more than 5 samples per week were processed and a total of 6 wk were necessary to complete the experiment.

All the milk samples were presented in transparent plastic bottles, and panelist were asked to spill the milk into glasses to simulate consumer behavior. As reference, 500 mL of fresh raw milk was also taken to the laboratory every day from the nearest automatic vending machine and presented to the panelists before each evaluation. Samples were presented in random order, and panelists were asked to give their scores independently.

## Data Analysis

Following a conservative approach, the time at which a sample kept at a given temperature was considered spoiled was the moment when at least one descriptor was scored as 3 or both the descriptors were scored as 2 or more.

Results from the panelists were analyzed separately by means of binomial multiple logistic regression with time (h) and temperature  $(T^{\circ})$  as covariates:

$$\operatorname{logit}^{-1}(p_i) = \frac{e^{\alpha + \beta_1 T^\circ + \beta_2 h}}{1 - e^{\alpha + \beta_1 T^\circ + \beta_2 h}},$$
[2]

with  $\text{logit}^{-1}(p_i)$  being the probabilities of the outcome events (i.e., the milk is considered spoiled and not to be drunk by consumers) and  $\alpha$ ,  $\beta_1$ , and  $\beta_2$  being the constant terms representing the unknown parameters. The potential interaction between time and temperature was tested by comparing models with interaction term with those without the interaction term by means of the likelihood ratio test.

The Cohen's kappa statistic for agreement was used to estimate the index of interrater agreement between the 2 panelists.

For inclusion in the QMRA model, the most conservative equation (i.e., the one that implies later detection of spoilage) was chosen. Statistical analysis was performed in R 3.1.2 (R Development Core Team, 2014) using packages lmtest (Hothorn et al., 2009) and irr (Gamer et al., 2012).

#### Implementation of QMRA

To evaluate the effect of including our estimates of association between time-temperature combinations and likelihood of milk being spoiled (and as a result not consumed), the 2 most recently published QMRA related to raw milk and indexed in PubMed were identified and reproduced by using the Excel tool @Risk 6.3 (Palisade Corp., Ithaca, NY). The query "Quantitative Risk Assessment Raw Milk," with the filter "published in the last 5 years," was used, and 9 items were found (search date April 2015). The 2 more recently published studies (from different authors) including a formal QMRA were selected. The more recently published studies were used without further consideration of their specific formulation. Use of the most recently published studies rather than purposively selected QMRA was considered the more transparent and sound approach to illustrate the potential effect and highlight the rel-



Figure 1. Distributions describing the storage time and temperature assumed by Latorre et al. (2011) in quantitative microbial risk assessment related to risk of listeriosis due to raw milk in the United States. (A) In the original model all time-temperature combinations can yield a serving that could be consumed. (B) Inclusion of Equation 2 implies that at any time-temperature combination, the milk has a certain probability  $(p_i)$  to be recognized as spoiled by the consumer and thus not actually consumed.  $T^\circ$  = temperature; h = time;  $\alpha$ ,  $\beta_1$ , and  $\beta_2$  = the constant terms obtained by the multiple regression model.

 $p_i$ 

evance and timeliness of our proposal of incorporating time–temperature dependency in future QMRA.

In the first work (Latorre et al., 2011), the risk of listeriosis due to raw milk consumption in the United States was estimated for different scenarios and different susceptible population groups (intermediate age, perinatal or pregnant woman, elderly). The scenario related to raw milk purchased at retail stores was chosen.

In the second (Giacometti et al., 2015), the risk of salmonellosis linked to consumption of raw milk sold in vending machines in Italy was estimated for the best and worst storage conditions. The "worst conditions" scenario was selected (no heat treatment before consumption and worst storage conditions).

Both models were reproduced as described by the authors, and results (baseline 1, baseline 2) were compared with the ones obtained by the modified models (model 1, model 2) in which the probability that the

milk is actually consumed given the sampled values for the time–temperature pair was considered by including Equation 2 (Figure 1).

Scenario rejected

Scenario accepted

In the first study, the probability of infection per serving  $(p_{\rm ill})$  was calculated assuming an exponential dose-response model (WHO/FAO, 2004) and combining multiplicatively the probability of illness given the dose with the assumed overall prevalence of *L. monocytogenes* in raw milk:

$$P = 1 - e^{(-rD)},$$
 [3]

$$p_{\rm ill} = P \times \text{prev},$$
 [4]

where P is the probability of illness, D is the dose per serving (cfu per serving), and r is the parameter describing the probability that one L. monocytogenes cell causes illness (WHO/FAO, 2004). Variable  $p_{\rm ill}$  is

**Table 2.** Analytical results (mean, SD, minimum, and maximum) of microbiological and chemical tests [pH, SCC, total bacteria count (TBC), lactic acid bacteria (LAB), and *Enterobacteriaceae* (EB)] of raw milk samples collected from automatic vending machines in Lombardy (n = 30) for purpose of sensorial analysis<sup>1</sup>

Parameter	Unit	Minimum	Maximum	Mean	SD
pH SCC TBC LAB EB	-log [H(+)] cells/mL log cfu/mL log cfu/mL log cfu/mL	$6.69 \\ 2,000 \\ 3.38 \\ 1.3 \\ 1$	$7.7 \\ 371,000 \\ 5.04 \\ 4.2 \\ 4.3$	$6.9 \\ 176,367 \\ 4.24 \\ 2.88 \\ 2.61$	$\begin{array}{r} 0.28 \\ 100,438 \\ 0.48 \\ 0.62 \\ 0.92 \end{array}$

<sup>1</sup>Tests were carried out upon arrival to the laboratory.

the probability of illness per serving, and prev is the assumed prevalence of L. monocytogenes in raw milk (proportion of raw milk positive servings). Thus, in model 1,  $p_{\rm ill}$  was estimated as

$$p_{\rm ill} = P \times \text{prev} \times (1 - p_i), \qquad [5]$$

where the correction factor  $(1 - p_i)$  expresses the probability that the serving is actually consumed according to time and temperature.

In the second QMRA, the  $\beta$ -Poisson relationship proposed by the World Health Organization/Food and Agriculture Organization of the United Nations (WHO, 2002) was used to calculate  $p_{\rm ill}$  for the ingested dose:

$$p_{\rm ill} = 1 - (1 + \text{dose}/b)^{-a},$$
 [6]

where dose is the ingested dose (cfu per serving) and a and b are 2 coefficients described by triangular distributions with parameters (minimum, most likely, and maximum) 0.0763, 0.1324, 0.2274 and 38.49, 51.45, 57.96, respectively.

In model 2,  $p_{\rm ill}$  was estimated by shifting the sampled dose to 0 according to

Bernoulli
$$(p_i)$$
. [7]

In this way, rejected scenarios are not considered at risk scenarios by the model. For both models, as described by the authors, the numbers of expected cases per year  $(N_{\text{exp}})$  were estimated by multiplying  $p_{\text{ill}}$  by the number of servings per year.

#### RESULTS

### Analytical Results

The initial (time 0) values for pH, SCC, total mesophilic flora, lactic acid bacteria, and *Enterobacteriaceae* are presented in Table 2. No pathogens were found in any sample, and no inhibitory substances were detected. According to regional regulation (Regione Lombardia, 2007), the microbiological and chemical quality of the samples was on average good.

#### Sensorial Analysis Results

Results of the binomial multiple logistic regression analysis are reported in Table 3. Only the results of the models without interaction are presented because the inclusion of an interaction term did not significantly improved the models.

With an overall interrater agreement of 99.44%, the K coefficient for agreement resulted as 0.98, confirming an excellent strength of agreement between the panelists.

As expected, the model predicted that when the storage time, the storage temperature, or both increases, the probability for the milk to spoil and be recognized by the consumer as expired also increases (Figure 2).

Table 3. Coefficients of multiple logistic regression models for the association between the probability of raw milk being recognized as spoiled and the storage time–temperature  $combination^1$ 

Equation	Independent variable	Coefficient	2.5%	97.5%
$A^2$	Constant	-12.273	14.150	10.395
	Time (h)	0.4883	0.403	0.573
	Temperature (°C)	0.0661	0.054	0.078
В	Constant	-13.004	15.025	10.983
	Time (h)	0.5161	0.426	0.606
	Temperature (°C)	0.0718	0.058	0.085

<sup>1</sup>The regression curves were fitted to data from the evaluation of 30 samples of milk stored at different time– temperature combinations by 2 panelists. Results of each panelist (A and B) are reported independently. <sup>2</sup>The equation coefficients were selected to be included in quantitative microbial risk assessment.



Figure 2. Graphical representation of the modeled relationship between storage time and temperature on probability of milk being perceived as spoiled  $(p_i)$ . Color version available online.

#### Implementation of QMRA

After 500,000 simulations of the first study (baseline 1) and according to an assumed prevalence of L. monocytogenes of 2.1%, 10,445 iterations (2.1%) yielded scenarios in which contaminated raw milk servings were ultimately consumed by consumers, for the same study, 9,232 scenarios (1.8%) were predicted when the correction was applied (model 1). An overall reduction of about 11.6% of scenarios ending with consumption of a contaminated serving was observed.

The same approach applied to the second study (baseline 2 vs. model 2) generated a similar difference (12.7%). The effect of this dependency is immediately evident when the densities of the sampled time-temperature pair combinations are compared between baseline 1 and model 1 (Figure 3) and between baseline 2 and model 2 (Figure 4). As expected, the most evident effects are noticed when the extreme time-temperature combinations are computed.

As a consequence, considering that (1) the probability of illness per serving depends on the dose of the pathogen at the time of consumption [equations 3 and 6]; (2) the dose at the time of consumption depends on microbial growth; and (3) microbial growth is regulated by time and temperature, if extreme time, temperature, or both scenarios are unlikely to result in consumption (Figure 2), there is a direct effect of including time– temperature dependency on the number of expected cases  $N_{\text{exp}}$  (Table 4).

The effect of explicitly including in the model the probability of consumption  $(1 - p_i)$  as a function of the storage time and temperature on  $p_{\rm ill}$  and  $N_{\rm exp}$  was evident in model 1 at the 95th percentile, where  $p_{\rm ill}$  was

## Baseline 1

Model 1



Figure 3. Retrospective density plot representing the density of the time-temperature pair combinations behind the computed scenarios characterized by presence of *Listeria monocytogenes* in raw milk servings. In baseline 1 the time-temperature dependency is not modeled; thus, the occurrence of time-temperature combinations only depends on the individual time and temperature distributions. In model 1, each sampled combination generates a specific probability of milk being recognized as spoiled and, ultimately, not consumed. A decrease in the intensity of the extreme scenarios in model 1 with respect to baseline 1 (upper right corner) is evident. Color version available online.

reduced by about 3.5 times for the categories intermediate and perinatal and up to 8 times for the category elderly;  $N_{\rm exp}$  resulted 3.5, 3, and 3.6 times smaller with respect to baseline 1 for the categories intermediate, perinatal, and elderly, respectively.

In model 2 the effect of modeling the time-temperature relationship was evident even on the median values, where a reduction of 1.7 times with respect to results from baseline 2 were observed for both  $p_{\rm ill}$  and  $N_{\rm exp}$ .

## DISCUSSION

Raw milk spoilage is a natural phenomenon, and the time at which it occurs depends on several factors such as the type and initial load of microbial contaminants, pH, enzymes, and time-temperature conditions.

The processes leading to modification of organoleptics properties of milk are time-temperature dependent; therefore, as for the majority of the fresh products, the spoilage occurs more rapidly if the product is not stored at low temperatures. Ignoring spoilage of raw milk in QMRA models and therefore assuming that milk will always be consumed regardless of its organoleptic modifications during storage is not realistic and can have a significant effect on model outputs.

In this study we have demonstrated that overlooking the time–temperature relationship may result in those scenarios in which contaminated raw milk servings are consumed being significantly overestimated (by approximately 11.6 and 12.7% in the case studies we selected).

Coping with all the possible dynamics that might influence raw milk spoilage would require such a level of complexity that analytical solutions might not be possible. An alternative would be the incorporation of a dependency such as the one described in our logistic model. Our equation simplifies the complex dynamics that ultimately determine the spoilage of milk, considering only the relationship between storage time and temperature on likelihood of spoilage (and of consumption) being averted. It provides, for the first time, a concrete and objective basis to explicitly include the logical relationship between storage time-temperature

Model	Probability of illness per serving, median (95th percentile)	Number of expected cases, median (95th percentile)	
Baseline 1 <sup>2</sup>			
Intermediate	$1.4 \times 10^{-13} \ (3.9 \times 10^{-8})$	$4.1 \times 10^{-5} (14)$	
Perinatal	$8.0 \times 10^{-12} (2.3 \times 10^{-6})$	$2.0 \times 10^{-5}$ (6)	
Elderly	$1.3 \times 10^{-12} (8.8 \times 10^{-7})$	$1.0 \times 10^{-4}$ (29)	
Model 1			
Intermediate	$1.3 \times 10^{-13} (1.1 \times 10^{-8})$	$4.5 \times 10^{-5}$ (4)	
Perinatal	$7.4 \times 10^{-12} (6.6 \times 10^{-7})$	$1.9 \times 10^{-5}$ (2)	
Elderly	$1.2 \times 10^{-12} (1.1 \times 10^{-7})$	$9.3 \times 10^{-5}$ (8)	
Baseline $2^3$	$2.6 \times 10^{-4} (1.4 \times 10^{-2})^{\prime}$	28,558 (28,838)	
Model 2	$1.5 \times 10^{-4} (1.0 \times 10^{-2})$	16.243(16.455)	

Table 4. Probability of illness per serving and number of cases per year associated with consumption of raw  $\operatorname{milk}^1$ 

<sup>1</sup>Results from 2 published quantitative microbial risk assessment (QMRA) models with time and temperature as independent distributions (baseline 1, baseline 2) and with inclusion of time–temperature relationship (model 1, model 2). The effect on the shape of the output distributions is mainly shown from the values at 95th percentile.

 $^{2}$ QMRA model of listeriosis due to consumption of raw milk purchased from retail stores in the United States (Latorre et al., 2011).

<sup>3</sup>QMRA model of salmonellosis related to the consumption of raw milk in Italy (worst scenario; Giacometti et al., 2015).

combinations and likelihood of milk being consumed, that is, "As the storage conditions became extreme the likelihood of raw milk being perceived as spoiled increases."

For practical reasons, it will always be difficult to gather accurate information about storage conditions at the household level or about consumer behavior; however, the proposed approach will mitigate the effect of too conservative assumed distributions. In fact, with the incorporation of the proposed equation, if very conservative storage time or temperature distributions are used (i.e., more extreme values are allowed), when high



Figure 4. Retrospective violin density plot representing the density of the time-temperature (temperature was fixed to 12°C in this study) pair combinations behind the computed scenarios characterized by presence of *Salmonella* in a raw milk serving. In baseline 2, the time-temperature dependency is not modelled. In model 2, each sampled combination generates a specific probability of milk being recognized as spoiled and, ultimately, not consumed. A decrease in the intensity of extreme scenarios can be observed in model 2 with respect to baseline 2 approaching the apexes of the violins.

values are sampled, the predicted likelihood of milk being perceived as spoiled will be high (Figure 2) and the amount of rejected scenarios will increase consequently, mitigating the effect of conservative distributions. Conversely, if this dependency is ignored, the effect of too conservative distributions might lead to alarming but poorly representative risk estimates. With the inclusion of this equation, QMRA for hazards in raw milk would be more realistic and their outputs would not be inflated by ignoring the correlation between storage conditions that favor microbial growth and likelihood of milk being perceived as deteriorated and thus not consumed.

The probabilistic modeling of exposure to hazards present in raw milk should explicitly include this relationship, and in the absence of more extensive empirical data on the relationship between storage conditions and perception of spoilage in milk from other sensorial evaluations, it is reasonable for future studies to make use of the estimates provided in this study.

Considering that the main objective of probabilistic risk modeling in food safety is to represent what happens in the real world to provide science-based information to decision makers, our equation improves the current level of understanding, making it closer to reality by excluding consumption scenarios that would not occur in practice. Inclusion of the logistic equation presented in this study would be a simple, transparent, and sound approach and an improvement with respect to previously used QMRA of raw milk.

In many European countries raw milk can be sold at the farm directly to the consumer (EFSA, 2015), and according to the European legislation EU Regulation 852/2004, 853/2004 (EU, 2004a, 2004b), direct sale of milk is regulated by the national law of the member states and, in some cases, additional regulations at the subnational level. Although some differences may exist in national or subnational regulations, farms allowed to sell raw milk for human consumption are asked to comply with strict criteria and operate with high quality standards. Consequently, a substantial homogeneity in the microbiological and biochemical quality of raw milk for human consumption from different regions with similar regulations might be assumed, making the results presented in this paper more directly applicable to future QMRA models aimed to assess the risk for human health related to consumption of raw milk in different European countries.

However, if the raw milk characteristics, hygienic practices, or regulations are likely to be significantly different or subjected to high variability, the coefficients estimated in this study might not be appropriate (e.g., milk produced in systems and geographic regions where the initial bacterial count can be expected to be considerably higher). Furthermore, considering that the equation is aimed to predict consumer behavior through a sensorial evaluation, the social context of the country where the QMRA is to be implemented plays a critical role. In fact, the perception of suitability might be different because of several traditional and social factors; therefore, even the parameters used to score the organoleptic characteristics should be revised accordingly.

Besides raw milk, our approach can be applied to other food products for which the storage conditions at the household level are critical: raw meat and fish, eggs, vegetables, soft cheese, and fresh products in general, which all deteriorate quickly if not conserved properly.

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