TITLE: 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

AUTHORS: Crotta, M., Paterlini, F., Rizzi, R. and Guitian, J.

JOURNAL: Journal of Dairy Science

PUBLISHER: American Dairy Science Association

PUBLICATION DATE: February 2016

DOI: 10.3168/jds.2015-10175

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

Matteo Crotta[a*]

Franco Paterlini[b]

Rita Rizzi[a]

Javier Guitian[c]

[a] Department DIVET, Università degli Studi di Milano, Via Celoria 10, 20133 Milan, Italy

Email: matteo.crotta@unimi.it

Email: rita.rizzi@unimi.it

[b] Lombardy and Emilia Romagna Experimental Zooprophylactic Institute, Via Rovelli 53, 24100 Bergamo, Italy

Email: franco.paterlini@izsler.it

[c] Veterinary Epidemiology, Economics and Pubic Health Group. The Royal Veterinary College. Hawkshead Lane. North Mymms. AL9 7TA, Hatfield, UK

Email: jguitian@rvc.ac.uk

[*]Corresponding author. Tel.: +39 0250318056. E-mail address: matteo.crotta@unimi.it (M.Crotta)

ABSTRACT

Foodborne disease as a result of raw milk consumption is an increasing concern in Western countries. Quantitative Microbial Risk Assessment (QMRA) 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 modelled as independent distributions in the Consumer Phase Module (CPM).

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' behaviour. 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, two recently published QMRA models quantifying the risks of listeriosis and salmonellosis related to the consumption of raw milk were implemented. Firstly, the default settings described in the publications were kept, secondly, 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 QMRA models of raw milk pathogens.

Keywords Raw milk; quantitative microbial risk assessment, consumer behaviour, milk spoilage

1. INTRODUCTION

Probabilistic modelling is becoming established as one of the main tools to inform risk management decisions with regard to foodborne hazards. Quantitative Microbial Risk Assessment models (QMRAs) 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, 2007; WHO/FAO, 2010).

One of the most significant examples from the public health perspective in recent years has been the use of QMRAs to estimate risks associated with the consumption of unpasteurized milk. Growing interest on 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 (FSA), the European Food Safety Authority (EFSA) or the US Centres for Disease Control (CDC) to conduct consultations and issue scientific opinions on the risk posed by milk-borne hazards (CDC, 2014; EFSA, 2015; FSA, 2014).

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 a number of consumers opting for raw as opposed to heat-treated milk. In light of this trend, models have been developed in recent years to assess probability of exposure or infection by pathogens such as *Salmonella*, *Listeria monocytogenes*, *Campylobacter jejuni*, *E. coli* O157 or *Staphylococcus aureus* as a result of raw milk consumption (Giacometti et al., 2015; Giacometti et al., 2012; Heidinger et al., 2009; Latorre et al., 2011).

QMRA models aimed at assessing the risk from farm-to-table include a consumer phase module (CPM), 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 & Christensen, 2011). In QMRAs related both to pasteurized or unpasteurized (Koutsoumanis et al., 2010) raw milk, the time and temperature of storage in the CPMs are usually described and modelled 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 modelled as independent probability distributions (most often Triangular or Pert) there will be instances during simulations in which 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 to 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 impact on the final output.

This limitation was already highlighted by Latorre *et al.* (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 (i) model the dependencies between time and temperature in order to express the likelihood for a raw milk serving to be actually consumed for any computed storage time-temperature combination and (ii) 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 five days 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 two recently published QMRAs of raw milk consumption and comparing published results with those of the modified model.

2 MATERIAL AND METHODS

2.1 Raw milk sample collection for sensorial analysis

A total of 1.5 L of raw milk was collected from 30 automatic vending machines (AVMs) in Lombardy by the public veterinary services, univocally coded, placed in cold boxes at 5°C±3 and taken to the laboratory within 30 min. Upon arrival, five aliquots of 200 mL were obtained from each sample and kept in different isothermal conditions at 3°C, 5°C, 8°C, 12°C, and 16°C for five days (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 were used to test the samples for: pH, somatic cell count (SCC), Lactic Acid Bacteria (LAB) Total Mesophilic Flora (TBC), enterobacteriacee (EB) and the major pathogens to ensure operator’s safety. An instrument with automatic temperature compensation (HANNA instrument HI9321) was used for pH measurement; SCC was determined by an Optofluorimetric accredited internal method MP02/063 (Fossomatic, Foss Electric, Hilleroed, DK); the ISO standards ISO4833-2, ISO21528-2 and ISO16649-2; were used for surface plate enumeration of TBC, EB and *E. coli*, while the standards AFNOR BRD 07/10 and AFNOR BRD 07/06 were used for PCR REAL-TIME detection of *L.monocytogenes* and *Salmonella.* Enumeration of LAB was performed by the accredited internal method MP01/048 (decimal dilution and plating in MRSA agar plate incubated under microaerophilic condition at 37±2°C for 72±2h and decimal dilution and plating on M17 agar plate at 37±2°C for 48±2h for enumeration of *Mesophilic Lactic Flora and Lactococci* respectively. The accredited internal method (MP 09/135) was used to test the samples for the presence of *Campylobacter jejuni by PCR REAL-TIME (Campylobacter Kit (Bio-Rad)).*

2.2 Sensorial analysis

To replicate consumers' behaviour, a simplified descriptive sensorial analysis of the milk samples stored at different temperatures was performed. The evaluation was carried out independently by two internal panellists experienced with sensory evaluation of milk[1]. Descriptors used in the evaluation sessions were selected following consultation with the panellists and based on their experience and the scope of the analysis (Table I).

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

	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**

[1] Experimental Zooprophylactic Institute of Lombardy and Emilia Romagna

Panellists were asked to evaluate all the milk samples every day at the same hour for five days. Each raw milk sample required the judgment of five subsamples per session (one sample for each temperature), thus, for practical reason, no more than five samples/week were processed and a total of six weeks were necessary to complete the experiment.

All the milk samples were presented in transparent plastic bottles and panellist were asked to spill the milk into glasses in order to simulate consumers' behaviour. As reference, a 500mL of fresh raw milk was also taken to the lab every day from the nearest AVM and presented to the panellists prior to each evaluation. Samples were presented in random order and panellists were asked to give their scores independently.

2.3 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 predictor was scored as 3 or both the predictors were scored as 2 or more.

Results from the panellists were analysed separately by means of binomial multiple logistic regression with time (h) and temperature (T°) as covariates:

$$logit(p_i) = ln\left(\frac{p_i}{1-p_i}\right) = \alpha + \beta_1 T^\circ + \beta_2 h \qquad \text{(Eq.1)}$$

$$logit^{-1}(p_i) = \frac{e^{\alpha+\beta_1 T^\circ+\beta_2 h}}{1-e^{\alpha+\beta_1 T^\circ+\beta_2 h}} \qquad \text{(Eq.2)}$$

with $logit^{-1}(p_i)$ being the probabilities of the outcome events (i.e. the milk is considered spoiled and not to be drunk by consumers). 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 two panellists.

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 M, 2012).

2.4 Implementation of QMRAs

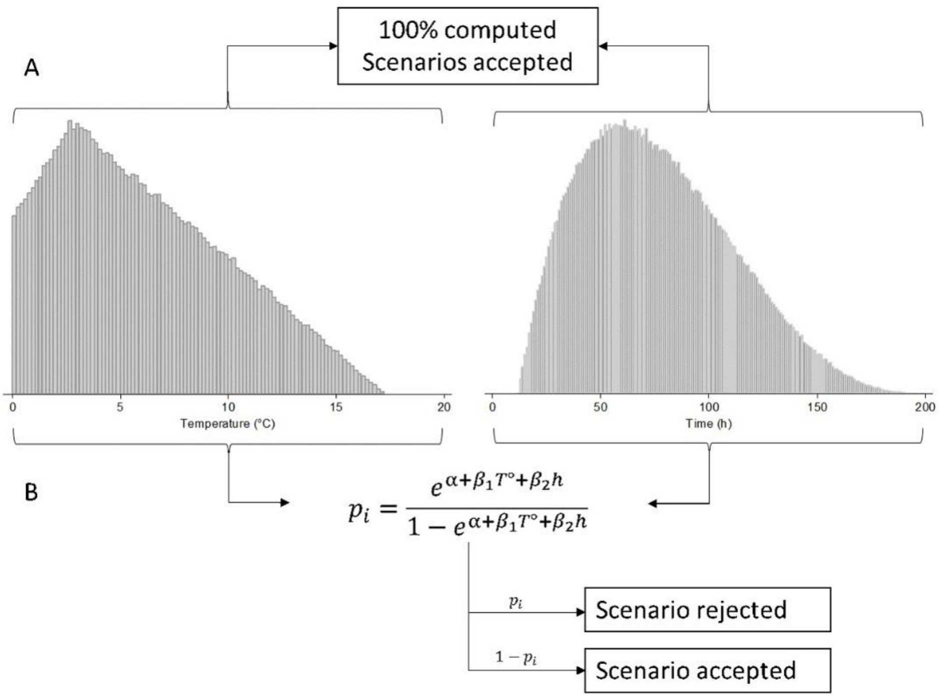
In order to evaluate the effect of including our estimates of association between time-temperature combinations and likelihood of milk being spoiled (and as a result not consumed), the two most recently published QMRAs related to raw milk and indexed in PubMed were identified and reproduced by using the Excel tool @Risk 6.3 (Palisade Corp.). 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 two 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 relevance 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/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 (none heat treatment before consumption and worst storage conditions).

Both models were reproduced as described by the authors, and results (Baseline1, Baseline2) were compared with the ones obtained by the modified models (Model1, Model2) in which the probability that the milk is actually consumed given the sampled values for the time-temperature pair, was considered by including Eq. 2 (Figure1).

Figure 1 Distributions describing the storage time and temperature assumed by Latorre et al. in QMRA related to risk of listeriosis due to raw milk in US. (A) in the original model all time-temperature combinations can yield a serving that could be consumed; (B) inclusion of eq. 2 implies that at any time-temperature combination the milk has a certain probability (pi) to be recognised as spoiled by the consumer and thus not actually consumed.



In the first study, the probability of infection per serving (p_{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:

$$P = 1 - e^{(-rD)} \qquad \text{(Eq.3)}$$

$$p_{ill} = P * prev \qquad \text{(Eq.4)}$$

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_{ill}

is 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 Model1, p_{ill} was estimated as:

$$p_{ill} = P * prev * (1 - p_i) \qquad \text{(Eq.5)}$$

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

In the second QMRA, the beta-Poisson relationship proposed by WHO/FAO (WHO, 2002) was used to calculate p_{ill} for the ingested dose:

$$p_{ill} = 1 - (1 + dose/b)^{-a} \qquad \text{(Eq.6)}$$

where *dose* is the ingested dose (CFU per serving), *a* and *b* are two 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 Model2, p_{ill} was estimated by shifting the sampled dose to 0 according to:

$$Bernouilli(p_i) \qquad \text{(Eq.7)}$$

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

3 RESULTS

3.1 Analytical results

The initial (Time 0) values for: pH, SCC, TBC, LB, and EB are presented in Table II.

Table II Analytical results (mean, standard deviation, minimum and maximum) of microbiological and chemical tests (pH, SCC, TBC, LAB and EB) of raw milk samples collected from automatic vending machines in Lombardy (n=30) for purpose of sensorial analysis; tests carried upon arrival to the laboratory.

Parameter	Unit	MIN	MAX	Mean	Std. dev
pH	-log [H(+)]	6,69	7,7	6,9	0,28
SCC[1]	cells*ml^{-1}	2000	371000	176367	100438
TBC[2]	log CFU/ml	3,38	5,04	4,24	0,48
LAB[3]	log CFU/ml	1,3	4,2	2,88	0,62
EB[4]	log CFU/ml	1	4,3	2,61	0,92

[1]Somatic Cell Count
[2]Total bacteria count
[3]Lactic Acid Bacteria
[4]Enterobacteriaceae

No pathogens were found in any sample and no inhibitory substances were detected. According to regional regulation (Lombardia, 2007), the microbiological and chemical quality of the samples was on average good.

3.2 Sensorial analysis results

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

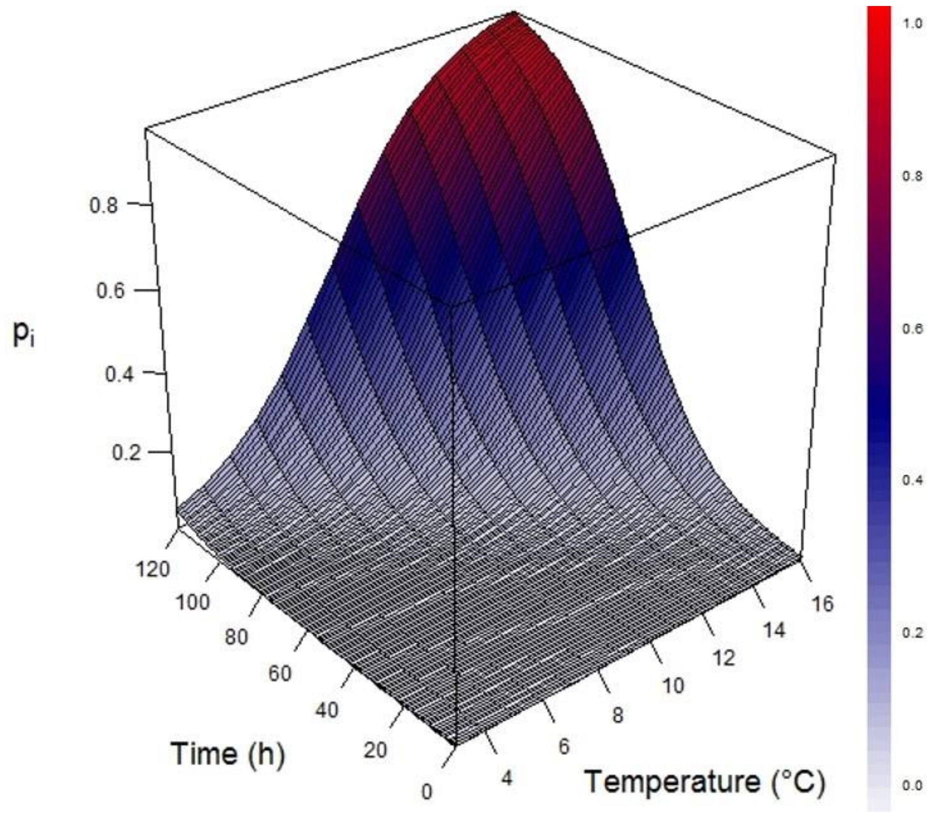
*Table III Coefficients of multiple logistic regression models for the association between the probability of raw milk being recognised as spoiled and the storage time-temperature combination. The regression curves were fitted to data from the evaluation of 30 samples of milk stored at different time-temperature combinations by two panellists. Results of each panellist (A and B) are reported independently. * indicates the equation coefficients selected to be included in QMRAs.*

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

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

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

Figure 2 Graphical representation of the modelled relationship between storage time and temperature on probability of milk being perceived as spoiled (pi)



Implementation of QMRAs

After 500,000 simulation of the first study (Baseline1) 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 are ultimately drank 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 (Baseline2 Vs Model2), 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 Baseline1 and Model1 (Figure 3) and between Baseline2 and Model2 (Figure 4). As expected, the most evident effects are noticed when the extreme time-temperature combinations are computed.

Figure 3 Retrospective density plot representing the density of the time-temperature pair combinations behind the computed scenarios characterized by presence of L.monocytogenes in raw milk servings. In Baseline1 the time-temperature dependency is not modelled, thus, the occurrence of Time-Temperature combinations only depends on the individual Time and Temperature distributions; In Model1, 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 the Model1 with respect to Baseline1 (upper right corner) is evident.

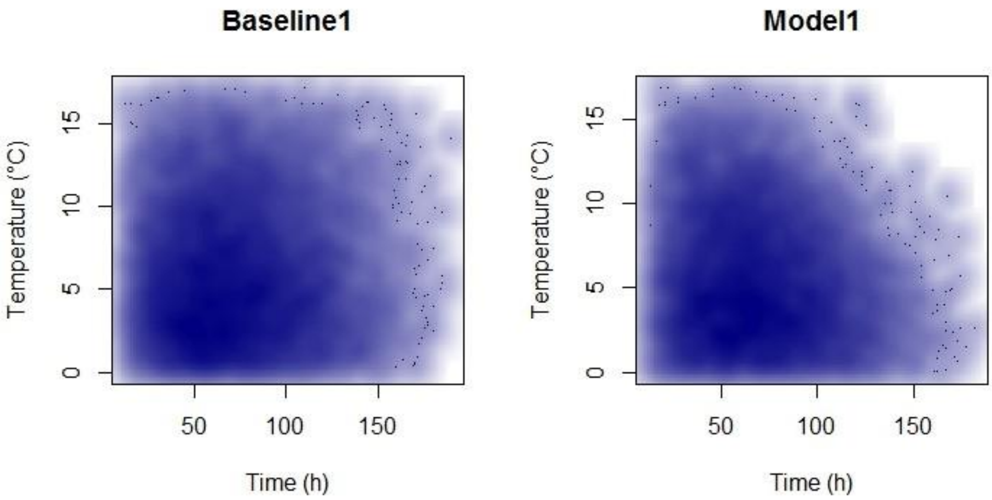
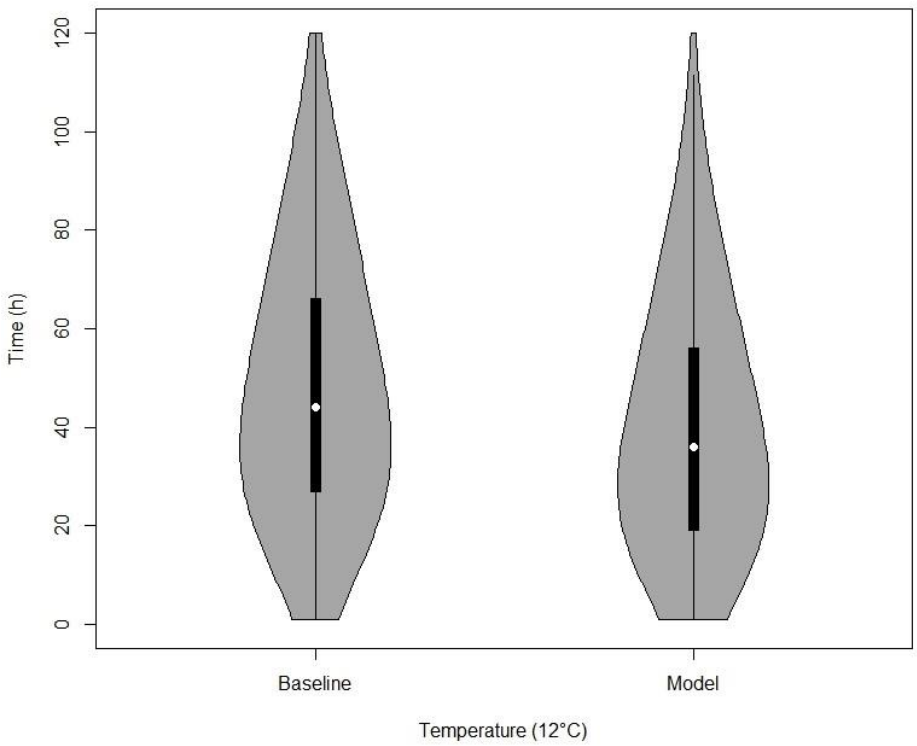


Figure 4 Retrospective Violin density plot representing the density of the Time-Temperature (T° was fixed to 12°C in this study) pair combinations behind the computed scenarios characterized by presence of Salmonella in a raw milk serving. A decrease in the intensity of extreme scenarios can be observed in Model2 with respect to the Baseline2 approaching the violins' apex.



As a consequence, considering that: (i) the probability of illness per serving depends on the dose of the pathogen at the time of consumption (Eq.3, 6); (ii) the dose at the time of consumption depends on microbial growth and (iii) microbial growth is regulated by time and temperature; if extreme time and/or temperature scenarios are unlikely to result in consumption, (Fig.2) there is a direct effect of including Time-Temperature dependency on the number of expected cases N_{exp} (Table IV).

Table IV Probability of illness per serving and number of cases per year associated with consumption of raw milk. Results from two published QMRAs with time and temperature as independent distributions (Baseline1, Baseline2) and with inclusion of time-temperature relationship (Model1, Model2). The effect on the shape of the output distributions is mainly shown from the values at 95th percentile.

Model	Probability of illness per serving Median (95th %ile)	Number of expected cases Median; (95th %ile)
Baseline1[1]		
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)
Model1		
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)
Baseline2[2]	2.6×10^{-4} ($1,4 \times 10^{-2}$)	28558 (28838)
Model2	$1,5 \times 10^{-4}$ ($1,0 \times 10^{-}2$)	16243 (16455)

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_{ill} and N_{exp} was evident in Model1 at 95th percentile where: p_{ill} was reduced by about 3.5 times for the categories 'intermediate' and 'perinatal' and up to 8 times for the category 'elderly'; N_{exp} resulted 3.5, 3 and 3.6 times smaller with respect to Baseline1 for the categories 'Intermediate', 'Perinatal' and 'Elderly' respectively.

In Model2 the effect of modelling the time-temperature relationship was evident even on the median values were a reduction of 1.7 times with respect to results from Baseline2 were observed for both p_{ill} and N_{exp}.

4 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 raw milk spoilage is a biological phenomenon that occurs in a few days if the product is not conserved properly. 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 impact 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's spoilage, would require such 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 adverted). It provides, for the first time, a concrete and objective basis to explicitly include the logical relationship between storage time-temperature 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 household level or about consumers’ behaviour; 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 and/or temperature distributions are used (i.e. more extreme values are allowed), when high 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, QMRAs for hazards in raw milk would be more realistic and their outputs would not be inflated by ignoring the correlation between storage conditions that favour microbial growth and likelihood of milk being perceived as deteriorated and thus not consumed.

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

Considering that the main objective of probabilistic risk modelling in food safety is to represent what happens in the real world in order 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 QMRAs of raw milk.

In many European countries raw milk can be sold at the farm directly to the consumer (EFSA, 2015) and in accordance to the current regime of hygiene rules adopted by the European Union in 2004, the so-called 'Hygiene Package' (Regulation, 2004a, 2004b), direct sale of milk is regulated by the national law of the member states and, in some cases, additional regulations at subnational level. Although some differences may exist in national or sub-national 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 consumers' behaviour 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 due to a number of 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 household level are critical: raw meat and fish, eggs, vegetables, soft cheese, and fresh products in general which are all subjected to a fast deterioration if not conserved properly.

REFERENCES

CDC. (2014). The Ongoing Public Health Hazard of Consuming Raw Milk, from http://www.cdc.gov/foodsafety/pdfs/raw-milk-letter-to-states-2014-508c.pdf

EFSA. (2015). Scientific opinion on the public health risks related to the consumption of raw drinking milk. *EFSA Journal, 13*(1).

FAO. (2007). Food safety risk analysis: A guide for national food safety authorities *FAO food and nutrition paper* (Vol. 87). Rome.

FSA. (2014). Impact Assessment on the review of the controls governing the sale and marketing of unpasteurised, or raw drinking milk and raw cream (RDM) in England, Wales and Northern Ireland, from http://www.food.gov.uk/sites/default/files/multimedia/pdfs/consultation/rawmilk-pack-england.pdf

Gamer M, L. J., Fellows I, Singh P. (2012). Various Coefficients of Interrater Reliability and Agreemen (Version 0.84). Retrieved from http://www.r-project.org

Giacometti, F., Bonilauri, P., Albonetti, S., Amatiste, S., Arrigoni, N., Bianchi, M., Bertasi, B., Bilei, S., Bolzoni, G., & Cascone, G. (2015). Quantitative Risk Assessment of Human Salmonellosis and Listeriosis Related to the Consumption of Raw Milk in Italy. *J Food Prot, 78*(1), 13-21.

Giacometti, F., Serraino, A., Bonilauri, P., Ostanello, F., Daminelli, P., Finazzi, G., Losio, M., Marchetti, G., Liuzzo, G., Zanoni, R., & Rosmini, R. (2012). Quantitative risk assessment of verocytotoxin-producing Escherichia coli O157 and Campylobacter jejuni related to consumption of raw milk in a province in Northern Italy. *J Food Prot, 75*(11), 2031-2038. doi: 10.4315/0362-028x.jfp-12-163

Heidinger, J., Winter, C., & Cullor, J. (2009). Quantitative microbial risk assessment for Staphylococcus aureus and Staphylococcus enterotoxin A in raw milk. *J Food Prot, 72*(8), 1641-1653.

Hothorn, T., Zeileis, A., Millo, G., & Mitchell, D. (2009). Package" lmtest": Testing linear regression models. *Retrieved June, 13*, 2010.

Koutsoumanis, K., Pavlis, A., Nychas, G., & Xanthiakos, K. (2010). Probabilistic model for Listeria monocytogenes growth during distribution, retail storage, and domestic storage of pasteurized milk. *Appl Environ Microb, 76*(7), 2181-2191.

Latorre, A. A., Pradhan, A. K., Van Kessel, J. A. S., Karns, J. S., Boor, K. J., Rice, D. H., Mangione, K. J., Gröhn, Y. T., & Schukken, Y. H. (2011). Quantitative risk assessment of listeriosis due to consumption of raw milk. *J Food Prot, 74*(8), 1268-1281.

Ledenbach, L. H., & Marshall, R. T. (2010). Microbiological spoilage of dairy products *Compendium of the microbiological spoilage of foods and beverages* (pp. 41-67): Springer.

Lombardia, R. (2007). Vendita diretta al consumatore di latte crudo vaccino, ovi-caprini e bufalini nell'azienda agricola di produzione – Modifiche e integrazioni alle Circolari n.39/SAN del 17 novembre 2004 e n.20/SAN del 24 maggio 2005" - Precisazioni., from http://www.sanita.regione.lombardia.it/cs/Satellite?c=Page&childpagename=DG_Sanita%2FDGLayout&cid=1213480043116&packedargs=stype%3Dlist&pagename=DG_SANWrapper&numr=621

Ma, Y., Ryan, C., Barbano, D. M., Galton, D. M., Rudan, M. A., & Boor, K. J. (2000). Effects of Somatic Cell Count on Quality and Shelf-Life of Pasteurized Fluid Milk1. *J Dairy Sci, 83*(2), 264-274. doi: http://dx.doi.org/10.3168/jds.S0022-0302(00)74873-9

Nauta, M., & Christensen, B. (2011). The impact of consumer phase models in microbial risk analysis. *Risk Anal, 31*(2), 255-265.

R Development Core Team. (2014). R: A language and environment for statistical computing. (Version 3.1.2). Vienna, Austria.: R Foundation for Statistical Computing, .

Regulation, E. (2004a). No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the Hygiene of Foodstuffs. *Offic. J. L Counc. Eur. Communities, 18*.

Regulation, E. (2004b). No 853/2004 of the European Parliament and of the Council of April 29, 2004 laying down specific hygiene rules for food of animal origin. *Offic. J. L Counc. Eur. Communities, 139*, 55.

Santos, M. V., Ma, Y., & Barbano, D. M. (2003). Effect of Somatic Cell Count on Proteolysis and Lipolysis in Pasteurized Fluid Milk During Shelf-Life Storage1. *J Dairy Sci, 86*(8), 2491-2503. doi: http://dx.doi.org/10.3168/jds.S0022-0302(03)73843-0

Sørhaug, T., & Stepaniak, L. (1997). Psychrotrophs and their enzymes in milk and dairy products: Quality aspects. *Trends Food Sci Tech, 8*(2), 35-41. doi: http://dx.doi.org/10.1016/S0924-2244(97)01006-6

WHO. (2002). *Risk assessments for Salmonella in eggs and broiler chickens* (Vol. 1): World Health Organization.

WHO/FAO. (2004). Risk assessment of Listeria monocytogenes in ready-to-eat foods. *Microbiological risk assessment series* Nineteen. from http://whqlibdoc.who.int/publications/2004/9241562625.pdf

WHO/FAO. (2010). *Codex Alimentarius Commission: procedural manual* (Nineteen ed.). Rome.