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Running title: modelling of *Escherichia coli* concentration in raw milk

**Abstract:**

Climate change is one of the threats to the dairy supply chain as it may affect the microbiological quality of raw milk. In this context, a probabilistic model was developed to quantify the concentration of *Escherichia coli* in raw milk and explore what may happen to France under climate change conditions. It included four modules: initial contamination, packaging, retailing, and consumer refrigeration.

The model was built in R using the $2^{nd}$ order Monte Carlo mc2d package to propagate the uncertainty and analysed its impact independently of the variability. The initial microbial counts were obtained from a dairy farm located in Saudi Arabia to reflect the impact of hot weather conditions. This country was taken as representative of what might happen in Europe and therefore in France in the future due to climate change. A large dataset containing 622 data points was analysed. They were fitted by a Normal probability distribution using the fitdistrplus package. The microbial growth was determined across various scenarios of time and temperature storage reflecting the raw milk supply-chain in France. Existing growth rate data from literature and ComBase were analysed by the Ratkowsky secondary model. Results were interpreted using the nlstools package.

The mean *E. coli* initial concentration in raw milk was estimated to be 1.31 [1.27; 1.35] log CFU/ mL and was found to increase at the end of the supply chain as a function of various time and temperature conditions. The estimations varied from 1.73 [1.42; 2.28] log CFU/mL after 12 h, 2.11 [1.46; 3.22] log CFU/mL after 36 h, and 2.41 [1.69;3.86] log CFU/mL after 60 h of consumer storage. The number of milk packages exceeding the 2-log French hygiene criterion for *E. coli* increased from 10% [8;12%] to 53% [27;77%] during consumer storage. In addition, the most significant factors contributing to the uncertainty of the model outputs were identified by running a sensitivity analysis. The results showed that the uncertainty around the Ratkowsky model parameters contributed the most to the uncertainty of *E. coli* concentration estimates.

Overall, the model and its outputs provide an insight on the possible microbial raw milk quality in the future in France due to higher temperatures conditions driven by climate change.

**Keywords:**

Raw milk, exposure assessment, food safety, climate change, coliform, probabilistic modelling

## 1. Introduction

The global average temperature is forecasted to increase to more than 2.0 °C due to climate change, and this led to several international efforts be undertaken, to curb the greenhouse gas emission of world economies (Raftery, Zimmer, Frierson, Startz, & Liu, 2017). The change in temperature in Europe is dependent on the Representative Concentration Pathways (RCP) which is projected to be 1-4.5 °C for RCP 4.5 and 2.5-5.5 °C for RCP 8.5 by 2071-2100 relative to 1971-2005 temperatures (European Environment Agency, 2017). In metropolitan France, the projected increase in temperatures range from 1.6 °C-2.7 °C (RCP 4.5) and 3.2-4.9 °C (RCP 8.5) by 2071-2100 with 1976-2005 as the reference period (Météo-France, 2021). The associated changes with these are the increase in precipitation levels and more frequent occurrence of extremely high-temperature periods during summer (European Environment Agency, 2017).

These projected changes have implications on food systems in terms of food security and food safety (FAO, 2020; WHO, 2019). These include the dairy supply chain, especially its farming stage where higher average temperatures and occasional extreme hot conditions (e.g. heatwaves) influence the occurrence of heat stress in cows (for temperatures >25°C) (Kekana, Nherera-Chokuda, Muya, Manyama, & Lehloenya, 2018), reduction in cow milk production (Chari & Ngcamu, 2017; Mauger, Bauman, Nennich, & Salathé, 2015; St-Pierre, Cobanov, & Schnitkey, 2003), and increase in the microbial load of milk products (Summer, Lora, Formaggioni, & Gottardo, 2019; van der Spiegel, van der Fels-Klerx, & Marvin, 2012). This effect on the microbiological properties may pose challenges to the efficiency of existing food safety controls.

Raw milk is currently consumed in several European countries (e.g. Italy, Slovakia, Austria, France and others) and is usually sold to consumers in packaged form or through vending machines while local cheesemakers use it to make artisanal raw milk cheeses. However, it is undeniable that raw milk poses a risk to human health. Several foodborne illnesses and outbreaks have been linked to the consumption of raw milk (EFSA, 2015) and artisanal cheeses due to *Escherichia coli* (Yoon, Lee, & Choi, 2016). Several studies have highlighted the contamination pathways of this pathogen in the early stages of raw milk production and its growth under favourable conditions throughout the milk supply chain (Perrin et al., 2015).

Dairy milk farming in France is at present a mixture of small, medium, and large-scale dairy farming with small-scale being the most common whereas in hot climate countries such as in the Middle East, large-scale dairy farming is commonly used. In this latter system, husbandry conditions are characterized by the presence of highly mechanized equipment and a strict application of hygienic conditions. This set-up extends from cow rearing to the transportation of raw milk: application of good veterinary practices, control of milk quality, maintenance of cold chain, etc. This system is the reason for high milk productivity, safe milk, and steady supply of dairy products to the market especially in regions previously considered unsuitable for milk production (Alqaisi, Ndambi, Uddin, & Hemme, 2010). Such countries with hot weather conditions might help understanding what might occur in the future for some of European countries currently undergoing temperature shifts due to climate change. In this respect, studies on the current microbiological status of foods from hot weather conditions can be used as a proxy or representative for the potential future impacts on food safety.

In France, raw milk intended for human consumption is currently regulated by the French Ministry of Agriculture through an administrative order (Ministère de l'agriculture de l'agroalimentaire et de la foret, 2012). This decree specifies the product form in which raw milk may be sold, the time frame from milking to consumption, and how the cold chain must be maintained. In France, raw milk is available to consumers in packaged form or sold through vending machines. These rules are designed to meet the hygiene criteria for raw milk against microbial hazards such as *E. coli* which is among the most common contaminant in raw milk and widely used indicator of hygiene criteria (EFSA, 2015; Martin, Trmčić, Hsieh, Boor, & Wiedmann, 2016). The seasonal effect on *E. coli* in cattle has been reported in several studies including Fairbrother & Nadeau (2006); Hussein & Sakuma (2005) and Ranjbar, Safarpoor Dehkordi, Sakhaei Shahreza, & Rahimi (2018). Moreover, in their longitudinal risk factor analysis conducted on multiple ranches located on the California Central Coast, Benjamin, Jay-Russell, Atwill, Cooley, Carychao, Larsen, & Mandrell, (2015) observed a positive increase of *E. coli* O157 with the soil temperature (from 21°C to 26·1°C). According to the hygiene criteria, based on three class attribute sampling plans, *E. coli* concentration in raw milk cannot exceed 2 log CFU/mL (Ministère de l'agriculture de l'agroalimentaire et de la foret, 2012). In addition to this, an internal hygiene criterion is observed by French dairy farmers selling raw milk at local markets, where the *E. coli* concentration in raw milk is limited to 1 log CFU/mL prior to retailing (information provided by a French raw milk farming Expert).

In this context, the aim of this paper was to build a probabilistic model to quantify the concentration of *E. coli* in raw milk and explore what may happen to raw milk sold in France under climate change conditions. Probabilistic modelling approaches are highly valuable because they allow the modelling of scenarios, taking uncertainty and variability into account (Koutsoumanis & Aspridou, 2016; Nauta, 2000). Probabilistic modelling has been applied in pasteurized milk to assess safety from spoilage organisms (Schaffner, Mcentire, Duffy, Montville, & Smith, 2003) and *E.coli* O157:H7 (Clough, Clancy, & French, 2006). In raw milk this modelling approach has been used to assess safety from microbiological hazards such as *Listeria monocytogenes* (Latorre et al., 2011) and chemical hazards such as SEA toxin (Crotta et al., 2016; Heidinger, Winter, & Cullor, 2009). Risk assessments of *E. coli* O157:H7 in raw milk were performed to determine the infections after the consumption of raw milk using probabilistic modelling techniques (Giacometti et al., 2012; Grace et al., 2008). These studies reflect two different retailing scenarios: Giacometti et al. (2012) have performed a risk assessment on vended raw milk while Grace et al. (2008) evaluated the informally marketed raw milk.

Therefore, the first novelty of the study presented here lies in having built a farm-to fork probabilistic assessment model to evaluate the *E. coli* concentration under hot weather conditions. For this purpose, an original dataset from a large-scale farm in Kingdom of Saudi Arabia have been collected and analysed. Next, the current raw milk handling practices in France has been introduced in the model to run realistic scenario. The second novelty of this study is to present a $2^{nd}$ order Monte Carlo model, separating uncertainty and variability, applied to raw milk consumption and the interpretation of its outputs by sensitivity analysis.

## 2. Materials and Methods

### *2.1. Model description*

The model describes the level of contamination of packaged raw milk from dairy farms up to consumer place in France. The sale of raw milk on local market within few hours after milking is allowed under French regulation (Ministère de l'agriculture de l'agroalimentaire et de la foret, 2012) considering the followings conditions: storage temperature lower than 8°C along the whole supply-chain and a consumption within 72 hours maximum (information provided by a French raw milk farming Expert).

The current steps that raw milk undergoes prior to the consumption were used to split the model into four modules (**Table 1**). For each module, inputs and latent variables (i.e. not directly observed or measured but used in the model) are also presented. As the total duration of time from milking until consumption was 72 h maximum, the duration of scenarios in each of the modules were set in order to meet this time frame.

### *2.2. Module 1: Raw milk contamination level in bulk milk tanks at farm setting*

The initial contamination levels of *E. coli* in raw milk, as representative of hot weather conditions, were obtained from a set of data collected in bulk milk tank in 2019 at AlSafi-Danone, AlKharj, Kingdom of Saudi Arabia.

The average temperature in Alkharj, where the farm was located, in 2019 varied between 13.9°C (January, the coldest month) and 36.9°C (August, the hottest month). In comparison, in France (average values from 30 different locations), the temperature during summer reached 20.1°C (June 2019), 23°C (July 2019) and 21.8°C (August 2019). This average temperature included daily fluctuations; during the hottest period of the day (midday and beginning of afternoon), the temperatures fluctuated between 25 to 27°C with several peaks above 30°C observed in France during July 2019.

The *E. coli* counts in raw milk were obtained by performing the colony count method based on the norm NF ISO 4832 (updated in 2006). An undiluted 1 mL of raw milk sample were transferred to Petri dishes while 10-12 mL of violet red bile agar (VRBA) (Oxoid, Ltd., UK) (cooled into 45 ± 1 °C) was also added and solidified as the initial layer. An overlay of 3-5 mL of VRBA was then subsequently added to the original basal-sample medium. The plates were then incubated at 37 ± 1 ° C for 24 h. Colonies showing purplish red color with a reddish zone of precipitated bile (≥ 0.5mm diameter) were enumerated.

The *E. coli* counts represented 1695 data points taken from the operations for the year 2019 in different farm units. The dataset was checked and cleaned. Only the farm unit containing the most number of data (622 data points) was selected for further analysis since mixing data from the different farm units would have brought additional variability. The data were fitted to Normal, Gamma, and Lognormal distributions using the R package fitdistrplus. The final probability distribution was selected based on its fitting in the Cullen and Frey diagram and statistical performance in terms of Akaike Information Criterion (AIC). A bootstrap procedure was subsequently performed to quantify the uncertainty and build a confidence interval around the distribution parameter estimates.

In this module, the temperature in the milk tank was assumed to follow the cold chain requirements of the French standard in raw milk production, i.e. ≤ 4°C. This assumption was confirmed by data (temperature probe in the tank). Therefore, significant microbial growth of *E. coli* was not considered in this module.

### *2.3. Module 2: Packaging of raw milk*

The packaging of raw milk (in 1L-pack) is a partitioning process that follows the Poisson process as described by Nauta, (2005). The unit operations within this module (e.g. volumetric filling and packaging) were assumed to be in-compliance with the French standard of maintaining temperatures 2-4°C of raw milk during packaging (Ministère de l'agriculture de l'agroalimentaire et de la foret 2012). Therefore, during this procedure, any significant additional microbial contamination and growth was not considered.

### *2.4. Module 3: Retailing*

Packs of raw milk were assumed to be sold in the farm or nearby markets and sold to consumers within the period of 12 h (i.e. maximal time between milking and selling raw milk allowable in France). The retailing temperature conditions should be between 2-4 °C but in practice it could reach 8°C (information provided by a French raw milk farming Expert). This value was then chosen as maximal and worst-case scenario.

### *2.5. Determination of growth kinetic parameters*

The growth parameters of *E. coli* in milk were obtained from the literature and Combase. First, the literature search was done in Web of Science using the combination of the topic terms: growth and (raw and milk), and (*Escherichia* and *coli*) and (Temperature). These terms yielded 77 research articles and were filtered based on their titles to keep only milk as the suspending medium (i.e. raw milk cheese studies were discarded). Moreover, challenge test studies which included *E. coli* in the presence of antimicrobials were excluded. When the growth studies were done in one temperature value, the article was also discarded. Three research papers were retained from this search, all coming from one research laboratory (Ačai, Valík, Medved'Ová, & Rosskopf, 2016; Medveďová, Györiová, Lehotová, & Valík, 2020; Medveďová, Rosskopf, Liptáková, & Valík, 2018). These papers have utilized only one strain of *E. coli* which have been isolated from a raw milk cheese. Growth studies obtained from these papers were strictly below 30°C.

Second, the results from Combase were also used to obtain the growth kinetics of *E. coli* in raw milk with the following search criteria: microorganism (*E. coli*), food (milk), Aw (0.95-1.00), Temperature (< 30°C). This yielded 24 records but four growth curves were discarded because *E. coli* was grown in fermented milk. This form of milk might contain metabolites produced by lactic acid bacteria (LABs) that could have exerted inhibitory properties during the growth of the other microorganisms. The 20 growth curves that were retained came from one research paper (Kauppi, Tatini, Harrell, & Feng, 1996).

The list of *E.coli* strains obtained from both resources (i.e. literature and ComBase), its origins and the temperature conditions are presented in **Table 2**.

The μmax obtained from the literature and Combase were all estimated by the researchers through the use of the Baranyi and Roberts model. Next, to take into account the strain variability, each strain dataset was analysed separately. The square root of the maximum growth rates (μmax) were fitted against temperature values. An equation derived from the Ratkowsky model (Eq. (1)) was used to estimate the parameters, as the temperature values were sub-optimal (<30°C) (Ratkowsky, Lowry, McMeekin, Stokes, & Chandler, 1983). The slope and the intercept of the straight line were estimated through linear regression in R using the lm function to finally obtain the $T_{min}$, Eq. (2).

$$\sqrt{\mu\mathrm{max}} = \mathrm{Slope} \times \mathrm{Temperature} + \mathrm{Intercept} \quad (1)$$

$$T_{min} = (-\mathrm{Intercept}/\mathrm{Slope}) \quad (2)$$

To determine the potential growth of *E. coli* ($\Delta$ log N) after different storage time values in the retailing and consumer modules, the exponential model was used, considering no lag phase Eq. (3) (Nauta, Litman, Barker, & Carlin, 2003).

$$\Delta \log N = \mu\mathrm{max} \times \mathrm{Time} \quad (3)$$

*2.6. Module 4: Refrigeration before consumption*

The conditions during the consumer refrigeration stage were simulated in order to determine its influence on the microbial concentration in packaged raw milk products. The refrigeration temperatures obtained by Roccato *et al.* (2017) for countries located in Northern Europe (N: 6.1, 2.8), which France is part of, was used in the assessment model. The duration of refrigeration, chosen as realistic scenarios were 12, 36 and 60 h. These different scenarios complete the allowable period of time for human consumption set to a maximum of 72h in France (information provided by a French raw milk farming Expert).

*2.7 Modelling*

The exposure assessment model was implemented in R software (R Core Team, 2019). The bootstrap procedures were carried out using the bootdistcens package of the fitdistrplus (Delignette-Muller & Dutang, 2015). The second order Monte Carlo procedure was used to propagate uncertainty and variability separately using the mc2d package (Pouillot, Kelly, & Denis, 2016). The number of iterations performed for uncertainty was 1000 and for variability 100,000.

*2.8 Uncertainty analysis*

A sensitivity analysis was performed to evaluate the impact of uncertainty on the main model output, i.e. the microbial concentration at the consumer level (log N3). The tornadounc function of the mc2d package was used with the Spearman rank correlation method. The results obtained from this analysis determined the influence of the input uncertainties on the uncertainty around the $95^{th}$ percentile of log N3. This percentile was chosen as representative of the upper tail of the distribution of *E. coli* concentration.

## 3. Results

*3.1. Module 1: Initial microbial load in bulk milk tank*

The initial microbial concentration (namely, $\log N_0$) was obtained from the one-year operation in a dairy farm in Saudi Arabia. The data were fitted by normal, log normal, and, gamma distributions and the results were compared based on the AIC value (**Table 3**). The normal distribution provided the best fit (AIC=903). A bootstrap procedure was then performed to estimate the uncertainty around the normal distribution parameters (**Fig.1a**). This resulted in an estimated mean value of 1.31 log CFU/mL with a confidence interval of 1.27-1.35, and, a standard deviation of 0.53 with a confidence interval of 0.50-0.57.

The probability of the milk tanks exceeding the *E. coli* criteria was also determined (**Table 4**). In this assessment, the number of bulk milk tanks that exceed the 2-log was estimated to 10.0% with a confidence interval of 8.0-12.0% while probability to exceed 1-log was estimated to 72.0% with a confidence interval of 69.0 -75.0%. The impact of this initial microbial concentration on the final concentration prior to consumption is reflected in the next modules.

*3.2. Module 2: Packaging of raw milk*

The packaging of raw milk from bulk milk tank into a 1L pack is a partitioning process. This follows the Poisson distribution of the microbial counts across the packaged products per batch. The number of packaged products exceeding the two hygiene criteria for raw milk namely, 2-log limit (10.0%, CI: 8.0-12.0) and the 1-log limit (72.0%, CI: 69.0-75.0) were in high numbers (Table 4). These values were the same as the previous module, showing here that partitioning did not have effect on the concentration level, likely to be linked with the relatively high initial *E. coli* count in raw milk.

*3.3. Module 3: Retailing*

*3.3.1. Determination of growth parameters*

The microbial growth rates extracted from the literature and Combase were from different strains of the pathogenic *E. coli*. For the literature search, we obtained three papers that have used the same strain which is isolated from a Slovakian cheese (Ačai et al., 2016; Medveďová et al., 2020, 2018). These studies performed growth studies in milk with a total of 34 temperature data. As such, the growth parameters obtained from these were compiled into the *E. coli* BR strain (**Table 2**). The search in Combase has yielded records from four different strains of *E. coli* all from one study (Kauppi et al., 1996).

The square root of the μmax was then plotted at function of temperatures, along with the adjusted model (**Fig.2**). The parameters namely, slope and intercept, determined from a linear regression using the Ratkowsky model are reported in **Table 2**. The slope and intercept estimates were used to determine the $T_{min}$ values obtained for each strain. The range of the $T_{min}$ value estimated from the literature and combase is also visible in **Fig.2**, it was between 4 to 6 °C. The strain variability was captured by building a uniform distribution from the strain having the highest $T_{min}$ up to the strain having the lowest $T_{min}$ values. These strains were *E. coli* O111-NM str 403 (5.60°C) and *E. coli* BR (4.07°C) for the highest and lowest $T_{min}$ value, respectively. The strain uncertainty was captured in a Normal distribution using the standard error around the slope estimates (and the intercept, respectively) of the strain having the highest and lowest $T_{min}$: slopemax and slopemin (interceptmax and interceptmin, respectively). For instance, the lowest slope estimate was fitted by the Normal distribution N (0.039, 0.005).

The results of the 2$^{nd}$ order Monte Carlo simulation analysing the uncertainty and variability of the $T_{min}$ is presented in **Fig.3**. The different strains of *E. coli* have a mean value of 4.7°C with a 95% confidence interval of [1.8; 7.6]°C. This large confidence interval around the mean value reflects the uncertainty in the estimation process due to lack of data and model misfit when applying the Ratkowsky secondary model. Its influence on the final output will be assessed by sensitivity analysis hereafter. Besides, $T_{min}$ variability is also large with variation from a 5$^{th}$ percentile estimated to 3.4°C [-0.3; 6.5]°C up to a 95$^{th}$ percentile estimated to 6.1°C [3.2; 9.8]°C.

*3.3.2. Microbial growth during retailing period*

The growth parameters estimated by analysing data from both the literature and Combase were used to predict the growth rate of *E. coli* under specific temperature conditions and then to determine the microbial concentration during retailing (log N2). The microbial load during retailing depends on temperature but also on duration of retailing on local markets. The maximal duration was set to 12 hours (i.e. maximal time between milking and selling raw milk allowable in France).

The *E. coli* concentration (1.53 [CI:1.30; 2.11] and sd 0.55 [CI:0.51; 0.67] log CFU/mL) in raw milk after 12h at 8°C (**Fig.1b**) was greatly higher than the *E. coli* concentration in the farm just after milking (**Fig.1a**). The probability to exceed 1-log was estimated to be around 83.0 %, with a confidence interval of 71.0-97.0 and the probability to exceed 2-log was estimated to 19.0 %, with a confidence interval of 9.0-57.0 (**Table 4**).

*3.4. Module 4: Refrigeration before consumption*

Three refrigeration times during storage at consumer's place were considered in the consumer module model. The refrigeration temperatures were those determined by (Roccato et al., 2017) for countries located in Northern Europe. The *E. coli* concentration in raw milk is provided in **Table 4** along with the probability to exceed the hygiene criteria.

The consumer scenario of storage for 12 h resulted in a probability of 31.0 % with a confidence interval of 15.0-61.0% of exceeding the 2-log hygiene criterion while a much higher probability is achieved with the more stringent 1-log criterion (88.0% with a confidence interval of 77.0-97.0%). The 1-log criterion was provided by a French raw milk farming Expert as the maximal acceptable limit for *E. coli* in milk foreseen to be consumed without any heating step.

The changes with the microbial concentration from the initial microbial load in bulk milk tanks ($logN_0$) to the end of consumer's storage ($logN_3$) are depicted in the cumulative distribution graphs (**Fig.1c-e**). In these figures, it can be seen that the changes in the distribution of values shift towards higher microbial counts while the uncertainties surrounding the predicted values also increase across the dairy supply chain.

As indicated in **Table 1** the inputs containing uncertainty namely, initial *E. coli* concentration (mean, LogN0_mean_U and standard deviation, LogN0_sd_U), slope (minimum value of slope, slopemin and maximum value of slope, slopemax) and the intercept (minimum value, interceptmin and maximum value, interceptmax) were presented. These uncertainties were then propagated in the model during the computation of the latent variables. The impact of uncertainty on the output ($logN_3$) was then assessed using sensitivity analysis. The output of these were shown in the tornado plots that captured all the uncertainties and reflected their impact on the uncertainty of the estimates during consumer storage (**Fig.4a-c**).

Unsurprisingly, as already highlighted when describing the $T_{min}$ estimated values, most of the uncertainty came from the characterisation of the intercept and slope associated with the strain growth parameters: the uncertainties generated to estimate interceptmin and interceptmax, slopemin and slopemax were the major source of uncertainty around the 95th percentile of $logN_3$ probabilistic distribution. This result was observed across the three consumer refrigeration scenarios. On the other hand, uncertainties from $logN_0$ parameters (i.e. logN0_mean_U and logN0_sd_U) had a limited contribution to the uncertainty around the 95th percentile of $logN_3$ probabilistic distribution. A slight difference could be observed for the 60h-consumer-storage scenario (**Fig.4c**) where logN0_mean_U contributed more to the uncertainty of the output than logN0_sd_U, in contrast to what was observed in the previous two scenarios.

# **4.** Discussion

## *4.1. The probabilistic assessment model*

The probabilistic modelling tools were demonstrated to be useful in estimating accurately the level of concentration of *E. coli* in raw milk at the time of consumption. The model was constructed to determine the possible impact of current raw milk practices in France under climate change conditions. To this end, the initial microbial load was obtained from a dairy farm located in a hot region to represent to a certain extent the effect of higher temperatures on the microbial load of raw milk. At the farm, it was assumed that the temperature of the milk cooling tank complied with the legislation (≤4°C). This assumption seemed realistic for a scenario in France because the farm facilities allow for a permanent and efficient refrigeration system. Nevertheless, if the temperature was higher than 4°C at (small) farms in France, the quality of the milk at the time of consumption would be even worse than estimated in this study. Therefore, it can be said that the "4°C-assumption" leads to an underestimation of the exposure level.

Next, by modelling, the concentration of *E. coli* in raw milk at retail and after consumer refrigeration was estimated. The modelling method adopted here aimed to analyse uncertainty independently of variability; it was implemented with *E coli* but it is sufficiently generic and straightforward to be re-used for other spoilage or pathogenic bacteria in the dairy supply-chain.

The distribution fit of *E.coli* observed in this study follows a normal distribution while it was not the case in several risk assessments where researchers described *E. coli* O157:H7 raw milk counts using different distributions such as uniform distribution (Clough, Clancy, & French, 2009), lognormal distribution (Giacometti et al., 2012), Poisson distribution (Perrin et al., 2015), or even Beta distribution to describe the prevalence in raw milk from vending machines in Northern Italy (Giacometti et al., 2013). The distribution fit we found is different from these studies because the model was built with *E. coli* counts from bulk milk tanks obtained as part of regular quality control monitoring of dairy farm while in these previous studies the pathogenic *E.coli* strains were described. The authors have not analysed an original set of data but derived their estimates from existing data such as prevalence of *E. coli* in the herd, lactating cows and the faeces contamination of the tank and contamination during milking (Clough et al., 2009), in-line filter counts (Perrin et al., 2015), and faecal contamination of raw milk and counts from raw milk in vending machines (Giacometti et al., 2013).

The packaging phase which is a partitioning process was described using the Poisson distribution as recommended by Nauta, (2005). It should be noted that the possible variation of the conditioning volume (depending on the type of equipment available on the farm) has not been taken into account; this could have had an influence if the contamination had been much lower. Nonetheless, more generally, partitioning is an important step to keep in mind when building a farm-to-fork model.

During retailing and consumer storage, some *E. coli* strains have the ability to continue growing in raw milk even within the cold chain as the temperature is not strictly kept at values lower than 4°C and a tolerance up to 8°C is accepted for selling raw milk in French local markets (information provided by a French raw milk farming Expert). The current conditions during the retailing have shown that the difference in the estimated mean concentration between packaging and after retailing of 12 h resulted to a 0.22 log CFU/mL growth (0.23 log CFU/mL at 95$^{th}$ percentile) (Δlog N retail). This shows the importance of the French policy on maintaining the cold chain during the retailing of raw milk (8°C maximum, 12h maximum) in controlling the *E. coli* concentration levels.

On the opposite the model outputs showed further increase of *E. coli* during the different consumer refrigeration scenarios (Δlog N consumer) where the estimated mean concentration grew to 0.2 log (12 h), 0.58 (36h) and 0.88 (60h) log CFU/mL. Since a probabilistic assessment was performed, it is also possible to interpret the result considering the 95$^{th}$ percentile of the distribution: in that case, the growth reached up to 0.35 (12 h), 1.45 (36h) and 2.75 (60h) log CFU/mL. Regarding the domestic temperature variation, there are two distinct phenomena: the variation in refrigerator temperature, from home to home (Roccato et al. 2017) and for a given home refrigerator, the variation of temperature during the day (Evans & Redmond, 2016) if for instance the consumer opens the refrigerator to serve himself/herself a glass of milk. The first source of variability was integrated in the model but not the second due to a lack of data to build a dynamic fluctuation of temperature without introducing too much uncertainty. It can be assumed that the daily temperature fluctuation would have a negative effect on the final contamination level, leading here to an underestimation of the exposure level.

Overall, if the *E. coli* concentration observed in hot weather conditions became the norm in the future for metropolitan France, raw milk consumption might be of concern. This is mainly because, as shown by the current probabilistic model, the initial *E. coli* contamination level will lead to non-compliance of packaged raw milk to the 2-log limit even if the cold chain was maintained. Having said that, the maximum storage of 72h might be questioned in the future as it brings an additional burden to the final contamination.

The model developed was also able to show that the influence of uncertainty and variability in the predicted outcomes. Using 2nd order Monte Carlo technique, uncertainty from the inputs should be propagated across the model independently of variability to make the output estimate more accurate (Duqué, Canon, Haddad, Guillou, & Membré, 2021). As a result, the estimates of the model (i.e. the probability distribution descriptors such mean, 95th percentile, probability to exceed 1 or 2 log CFU/mL) are presented with their confidence intervals reflecting uncertainty. Also, it was demonstrated here that the separation of uncertainty and variability is relatively easy to implement. However, this comes at the cost of requiring more details about the data. It is hoped that this will lead to more exposure assessment papers implementing the separation of uncertainty and variability in their models in the future. Nonetheless, it was shown here that $T_{min}$ had both a large variability and uncertainty range. The large variability range reflected the fact that *E.coli* strains were capable of growing within a wide temperature range. In this respect, our assessment model is on the safe-side as it covers pathogenic and non-pathogenic *E.coli* strains; indeed it has been reported that pathogenic *E. coli* strains have the ability to grow and survive lower temperatures better than the non-pathogenic ones (Farrokh et al., 2013; Vidovic, Mangalappalli-Illathu, & Korber, 2011).

Although our model was a farm-to-fork model, it is important to keep in mind that climate change is a multi-faceted phenomenon that can affect the other parts of the dairy supply chain. As such other possible effects of climate change may also be seen (e.g. higher temperature during transportation, disruption of the supply chain due to flooding). These events may have consequential impact on food safety and quality such as allowing or supporting *E. coli* growth. Therefore, once these are determined, ways on how to incorporate these in the probabilistic model developed can be further explored in the future.

### *4.2. The use of hot weather conditions and E. coli as test organism in understanding the future of raw milk consumption*

The current probabilistic model has shown that raw milk consumption might pose microbial food quality concerns in the future under hot weather conditions brought by climate change. In order to understand the possible impact of hot weather conditions on raw milk, data from a dairy farm in Saudi Arabia was obtained. These were considered to be representative of what initial microbial counts might look in the future for countries undergoing shifts in high temperature due to climate change. The selection of this farm allowed an insight to a certain extent on what microbial quality might look like in the future under hot weather conditions. The comparison with the farms in France is possible because in the farm selected in our study, Holstein breed cows (a very common breed in France for milk production) are raised. Also, the best practices in dairy farming such as good veterinary practices (GVP) and good hygiene practices (GHP) applied at the farm are comparable with the ones being applied elsewhere with the difference only in its location and hot weather conditions.

The data used are *E. coli* counts from bulk milk tanks, collected and analysed as part of routine operations. These were used to assess the raw milk contamination just after the milking step. This approach supports the notion that the contamination pathway of *E. coli* in the dairy supply-chain starts in the early stages of raw milk supply chain (Perrin et al., 2015). *E. coli* was used in this study because aside from being a microbial hazard commonly linked with raw milk consumption it is also a microorganism that is foreseen to pose a concern in the future for the raw milk produced under hot weather conditions (Fairbrother & Nadeau, 2006). *E. coli* has been widely reported to survive and proliferate in hot weather conditions and during summer season (Hussein & Sakuma, 2005; Ranjbar, Safarpoor Dehkordi, Sakhaei Shahreza, & Rahimi, 2018). In addition, it is known for its prevalence within farms that is facilitated by increased cow shedding and growth in feeds which are both highly occur during hot weather conditions (Fairbrother & Nadeau, 2006).

As such, the results of the model built here have shown that the current practice of drinking raw milk in France might need to be revisited since the current hygiene criteria for packaged raw milk might be difficult to meet in the future if hotter conditions become the standard. Indeed, the estimated mean value at the initial concentration (log $N_0$) was estimated to 1.33 log CFU/mL, however the 95$^{th}$ percentile reached 2.19 log CFU/mL. This is not in line with the hygiene criterion of 2-log limit for the *E. coli* in France (Ministère de l'agriculture de l'agroalimentaire et de la foret, 2012): it was estimated that 10% of the raw milk package exceed the criterion. Nevertheless, this estimated value seems to be consistent with the results in other places such as in New York state (23% of the milk producers had more than 2-log) (Boor, Brown, Murphy, Kozlowski, & Bandler, 1998). It is important to keep in mind that these results do not represent a safety concern but a hygienic concern. The presence of high amounts of *E. coli* signifies faecal contamination, which is an indicator of hygiene and associated veterinary practices at the farm level (Martin et al., 2016). It was reported that the pathogenic strains Shiga-toxin producing *E. coli* was isolated in 0.4-1.7% in raw milk from the EU (during 2005-2008) while in France the isolates were around 3.4-15 % of the samples (Farrokh et al., 2013).

The dairy farming systems such as the one used in this study are raising Holstein breed cows that are kept inside large, naturally ventilated farm buildings, where they do not go outside or for very limited time during the day because cows suffer from heat stress when they are exposed to temperature above 25°C (information provided by a French veterinary expert). Although these systems can be seen in European countries, adoption to these farming conditions varies. This is particularly true in France where the dairy farms are medium-scale farms and with the widespread use of production machinery (Poczta, Średzińska, & Chenczke, 2020). Nevertheless, the shift to this system is taking place in southern France, where its adoption has been accelerated by the regular occurrence of heat waves during the summer period (information provided by a French veterinary expert). Another challenge to its widespread adoption is the shift towards sustainability with efficient use of resources, implementation of recovery mechanisms and pressure from consumers to devolve to localized farms (Thorpe, Schmalzried, & Fallon, 2010). These barriers to acceptance may hinder present adoption but may not completely prevent it given the intensification of climate change effects. Overall, it is hoped that the implication of the results obtained in this study may be useful in understanding the impact of climate change driven hot weather conditions on the microbial quality of raw milk which is expected to be more apparent in the future.

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**Table 1.** Model inputs and latent variables implemented in the model. When the input is deterministic, the value is given. When it is pure variability, the distribution is given. However, when the inputs included both uncertainty and variability, its structure is more complex, it is given in the core document but not in this Table.

| Name | Abbreviation | Description | Unit | Uncertainty | Variability | Determinsitic | Latent/input |
|---|---|---|---|---|---|---|---|
| **Module 1: Bulk milk tank** | | | | | | | |
| **Bulk milk tank concentration** | $logN_0$ | Normal distribution + Bootstrap to assess uncertainty | log CFU/mL | x | x | | Input |
| **Module 2: Packaging of raw milk** | | | | | | | |
| **Volume per pack** | Vp | Deterministic | mL | | | 1000 | Input |
| **Concentration of microorganisms per pack** | N1 | Poisson ($10^{logN0} \times$ Vp) | CFU/ pack | x | x | | Latent |
| **Concentration of microorganisms per mL** | logN1 | $log_{10}$ (N1/pack) | log CFU/mL | | | | Latent |
| **Module 3: Growth at Retailing** | | | | | | | |
| **Secondary model Ratkowsky Slope** | Slope | Uniform in the Variability dimension, Normal in the Uncertainty dimension | $h^{-1/2}.°C^{-1}$ | x | x | | Input |
| **Secondary model Ratkowsky Intercept** | Intercept | Uniform in the Variability dimension, Normal in the Uncertainty dimension | $h^{-1/2}$ | x | x | | Input |
| **Secondary model Ratkowsky Tmin** | Tmin | Probabilistic as result of calculation (i.e. - Intercept/Slope) | °C | x | x | | Latent |
| **Temperature at retail (local market)** | $Temperature_R$ | Deterministic | °C | | | 8.0 | Input |
| **Square root of growth rate (square root of $\mu max_R$)** | | Probabilistic as result of calculation (i.e. Slope × ($Temperature_R$-Tmin)) | $h^{-1/2}$ | x | x | | Latent |
| **Time at retail (between milking and selling at local market)** | $Time_R$ | Deterministic | h | | | 12 | Input |
| **Concentration after retailing** | logN2 | Probabilistic as result of calculation (i.e. log N1 + $\mu max_R$× $Time_R$) | logCFU/mL | x | x | | Latent |
| **Module 4: Growth during consumer storage** | | | | | | | |
| **Temperature of consumer refrigerators** | $Temperature_C$ | Normal | °C | | N (6.1, 2.8) | | Input |
| **Square root of growth rate (square root of $\mu max_C$)** | | Probabilistic as result of calculation (i.e. Slope×($Temperature_C$-Tmin)) | $h^{-1/2}$ | x | x | | Latent |
| **Time before consumption scenarios** | $Time_C$ | Deterministic | h | | | 12, 36, 60 | Input |
| **Concentration at consumption** | logN3 | Probabilistic as result of calculation (i.e. logN2 + $\mu max_C$ × $Time_C$) | logCFU/mL | x | x | | Output |

**Table 2.** *E.coli* strains, temperature conditions used in the growth studies on milk and estimated growth kinetic parameters from linear regression

| Information collected from literature or ComBase | | | | Estimated growth kinetic parameters generated in this present study | | | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Origins | Temperature (°C) | Reference | Slope | Sd slope | Intercept | sd Intercept | Tmin |
| *Escherichia coli* BR | Isolated from Slovakian Brydzna cheese | 8,10,12,15,18,21,25,30 °C | Medvedova et al., 2018 | | | | | |
| *Escherichia coli* BR | Isolated from Slovakian Brydzna cheese | 6,12,15,18,21,25,30 °C | Medvedova et al., 2020 | 0.0392* | 0.005 | -0.1598¤ | 0.088 | 4.07 |
| *Escherichia coli* BR | Isolated from Slovakian Brydzna cheese | 10,12,15,18,21,25,30 °C | Acai et al., 2015 | | | | | |
| *Escherichia coli* O104:H21 str 13A | USFDA collection | 6.5,7.5,8.5,9.5 °C | Kauppi et al.1996 | 0.028 | 0.003 | -0.121 | 0.028 | 4.25 |
| *Escherichia coli* O111-NM str 403 | USFDA collection | 6.5,7.5,8.5,9.5 °C | Kauppi et al.1996 | 0.0388** | 0.007 | -0.2176¤¤ | 0.055 | 5.60 |
| *Escherichia coli* O157:H7 | USFDA collection | 6.5,9.5,12.0 °C | Kauppi et al.1996 | 0.035 | 0.000 | -0.171 | 0.003 | 4.82 |
| *Escherichia coli* O157:H7 str.22 | USFDA collection | 6.5,7.5,8.5,9.5 °C | Kauppi et al.1996 | 0.032 | 0.008 | -0.147 | 0.060 | 4.53 |
| *Escherichia coli* O22:H8 str.406 | USFDA collection | 6.5,7.5,8.5,9.5 °C | Kauppi et al.1996 | 0.031 | 0.004 | -0.143 | 0.036 | 4.64 |

* and ** values used to build the probability distribution regarding the slope

¤ and ¤¤ values used to build the probability distribution regarding the intercept

**Table 3.** Results of the initial microbiological concentration ($logN_0$ in log CFU/mL ) distribution fitting.

| **Normal** | **Log Normal** | **Gamma distribution** |
|---|---|---|
| AIC: 903.13 | AIC: 975.19 | AIC: 907.95 |
| Mean: 1.31 [1.26,1.35] | Meanlog: 0.17 [0.13; 0.21] | Shape: 5.21 [4.67; 5.81] |
| Sd: 0.53 [0.50, 0.57] | Sdlog: 0.48 [0.45; 0.51] | Rate: 3.98 [3.53; 4.48] |

**Table 4.** *E. coli* concentration in bulk milk tank and packaged raw milk: mean value, standard deviation, 95$^{th}$ percentile of the distribution; probability of exceeding the 2-log and 1-log limit at different stages across the dairy supply chain. Results are provided with the median estimate and its uncertainty interval.

| Time | Mean concentration | Standard deviation | 95$^{th}$ percentile of the concentration | Exceeding 2-log CFU/mL | Exceeding 1-log CFU/mL |
|---|---|---|---|---|---|
| | | | Bulk milk tank | | |
| - | 1.31 [1.27; 1.35] | 0.53 [0.50; 0.57] | 2.19 [2.12; 2.26] | 0.10 [0.08; 0.12] | 0.72 [0.69; 0.75] |
| | | | Packaging | | |
| - | 1.31 [1.27; 1.35] | 0.53 [0.50; 0.56] | 2.19 [2.11; 2.25] | 0.10 [0.08; 0.12] | 0.72 [0.69; 0.75] |
| | | | Retailing | | |
| **12 h** | 1.53 [1.30; 2.11] | 0.55 [0.51; 0.67] | 2.42 [2.17; 3.16] | 0.19 [0.09; 0.57] | 0.83 [0.71; 0.97] |
| | | | Consumer refrigeration scenarios | | |
| **12 h** | 1.73 [1.42; 2.28] | 0.62 [0.54; 0.83] | 2.77 [2.36; 3.73] | 0.31 [0.15; 0.61] | 0.88 [0.77; 0.97] |
| **36 h** | 2.11 [1.46; 3.22] | 1.00 [0.58; 2.06] | 3.87 [2.50; 7.33] | 0.45 [0.18; 0.78] | 0.91 [0.78; 0.99] |
| **60 h** | 2.41 [1.69; 3.86] | 1.46 [0.76; 2.89] | 5.17 [2.85; 9.76] | 0.53 [0.27; 0.77] | 0.91 [0.81; 0.98] |

## Figures

**Fig.1**. Cumulative probability distribution of *E. coli* concentration in raw milk across the different modules. (a) Initial microbial concentration and after partitioning, (b) after 12h of retailing, (c) after 12h of consumer refrigeration, (d) after 36 h of consumer refrigeration, (e) after 60 h of consumer refrigeration. The light grey corresponds to the lower and upper limits of the 95% uncertainty interval, the dark grey corresponds to the 25$^{th}$ and 75$^{th}$ percentiles of the uncertainty.

**Fig. 2**. The square root of the µmax of the different *E.coli* strain (markers), collected at various temperature values, with the adjusted values of square root of the µmax (line).

**Fig. 3**. The cumulative probability distribution of the Tmin (°C) estimate, reflecting strain variability and uncertainty including in the estimate. The light grey corresponds to the lower and upper limits of the 95% uncertainty interval, the dark grey corresponds to the 25$^{th}$ and 75$^{th}$ percentiles of the uncertainty.

**Fig.4.** Tornado plot illustrating the sensitivity analysis results: correlation between inputs' uncertainty and uncertainty around the 95$^{th}$ percentile of *E.coli* concentration (log N3) during consumer refrigeration module. (a) 12 h, (b) 36 h and (c) 60h refrigeration times.

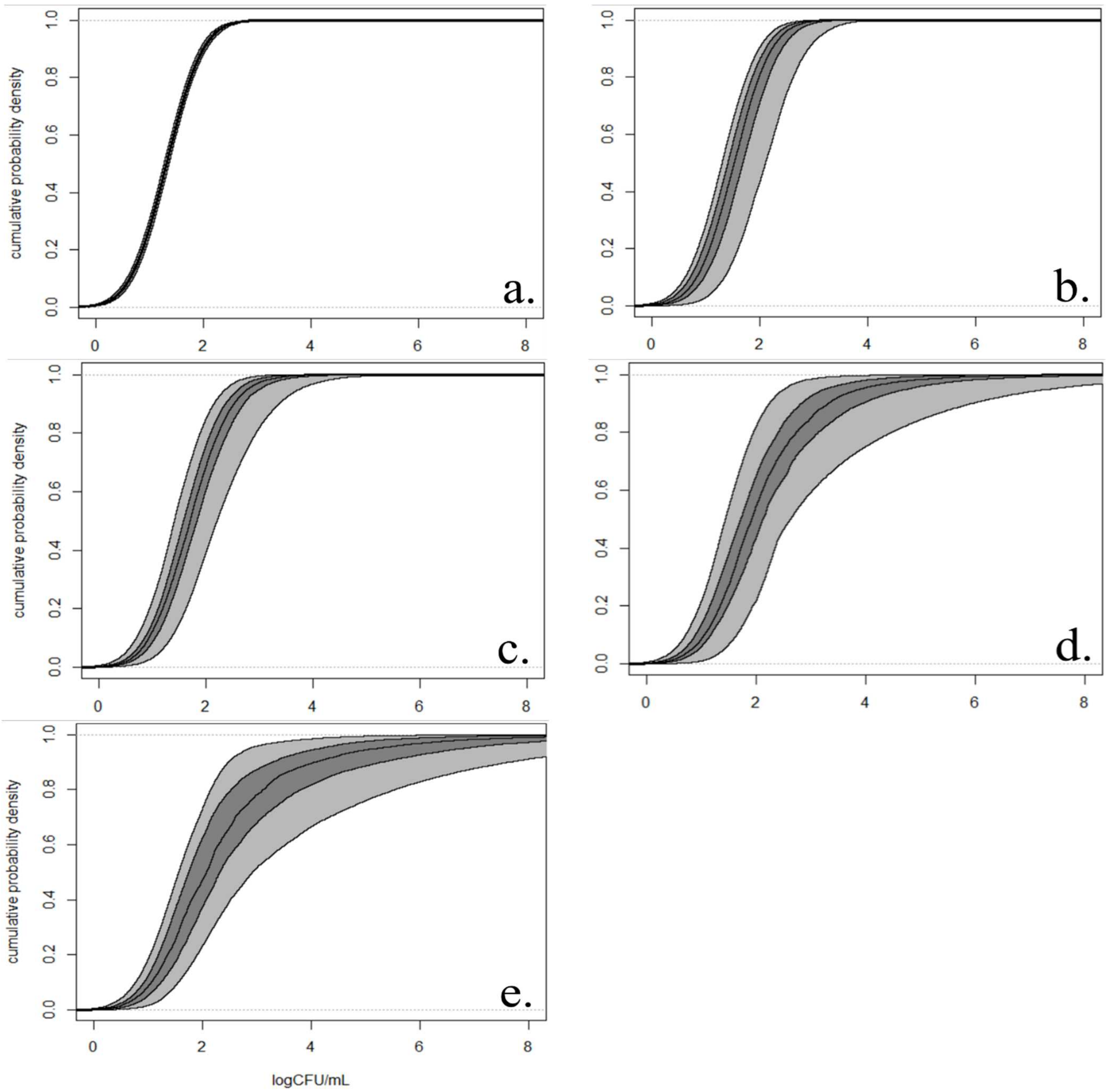

cumulative probability density
0.0
0.2
0.4
0.6
0.8
1.0
0
2
4
6
8
a.
b.
c.
d.
e.
logCFU/mL

**Fig.1**.

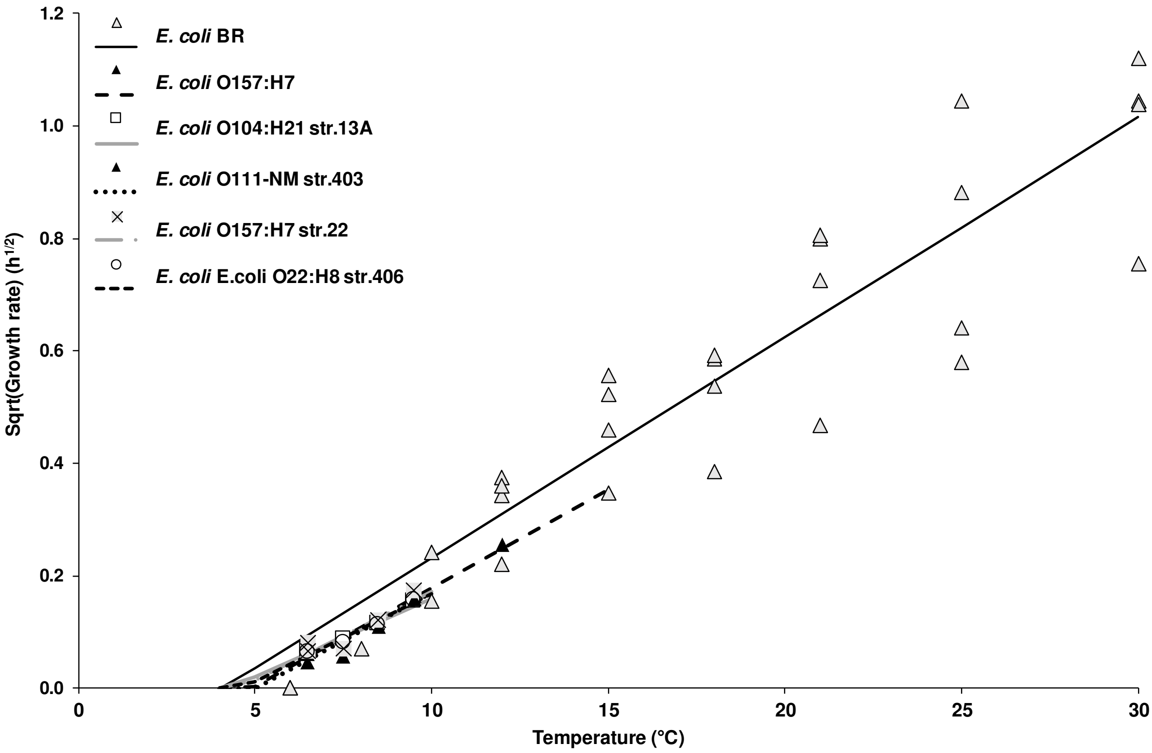

E. coli BR
E. coli O157:H7
E. coli O104:H21 str.13A
E. coli O111-NM str.403
E. coli O157:H7 str.22
E. coli E.coli O22:H8 str.406
Sqrt(Growth rate) (h1/2)
Temperature (°C)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15
20
25
30

**Fig. 2**.

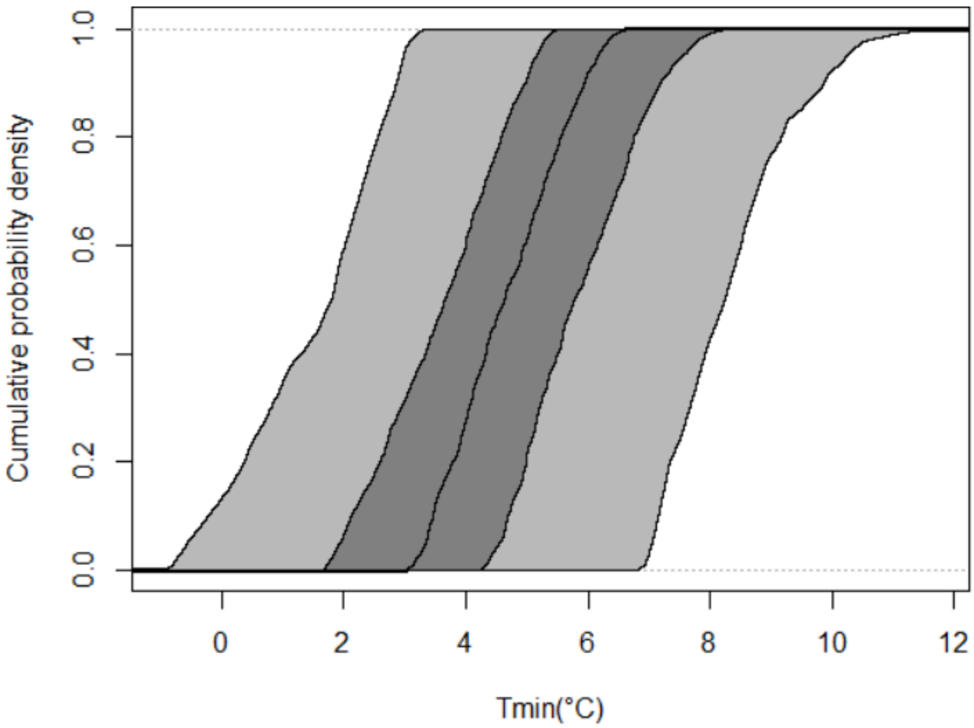

Cumulative probability density
0.0
0.2
0.4
0.6
0.8
1.0
0
2
4
6
8
10
12
Tmin(°C)

**Fig. 3**.

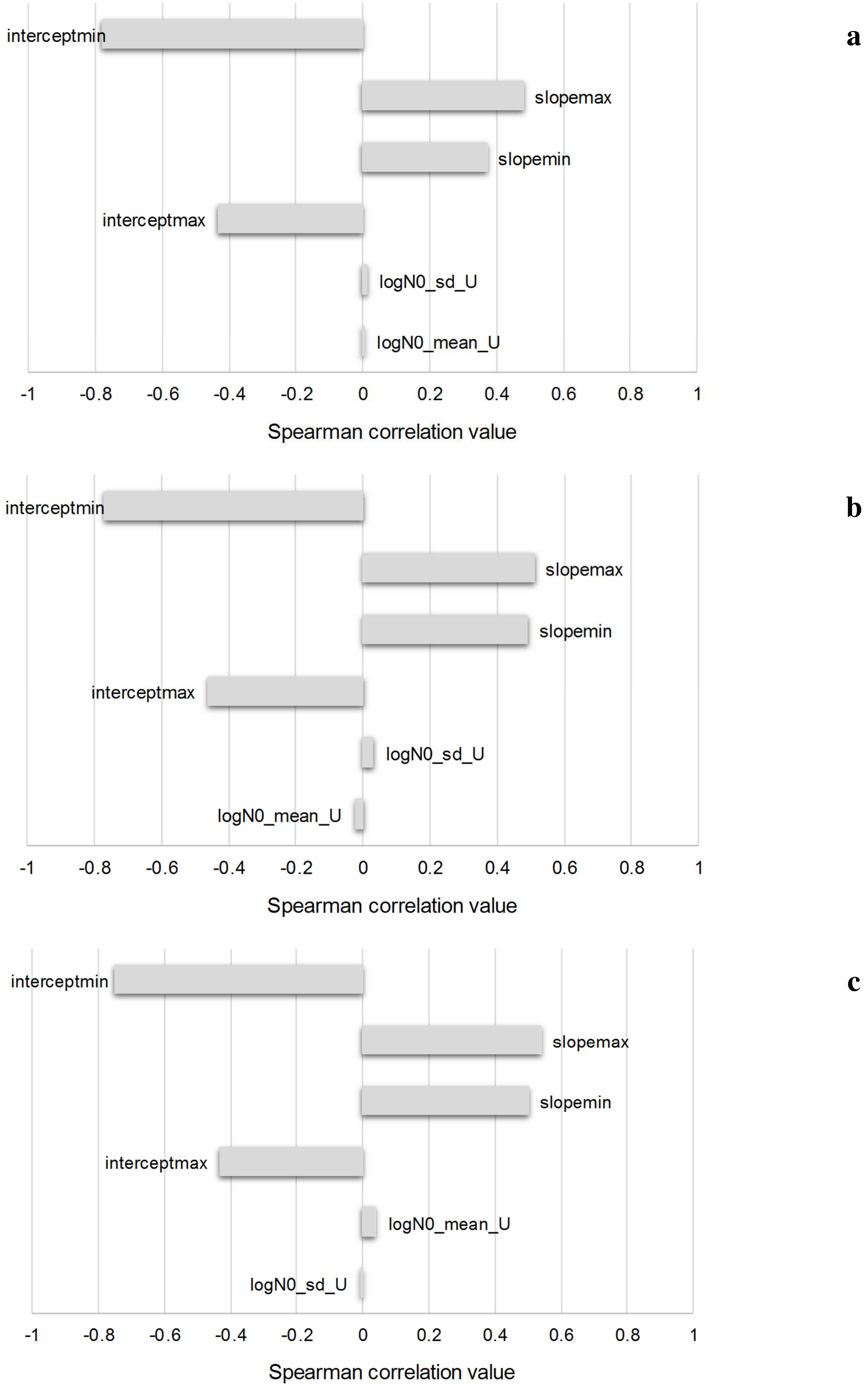
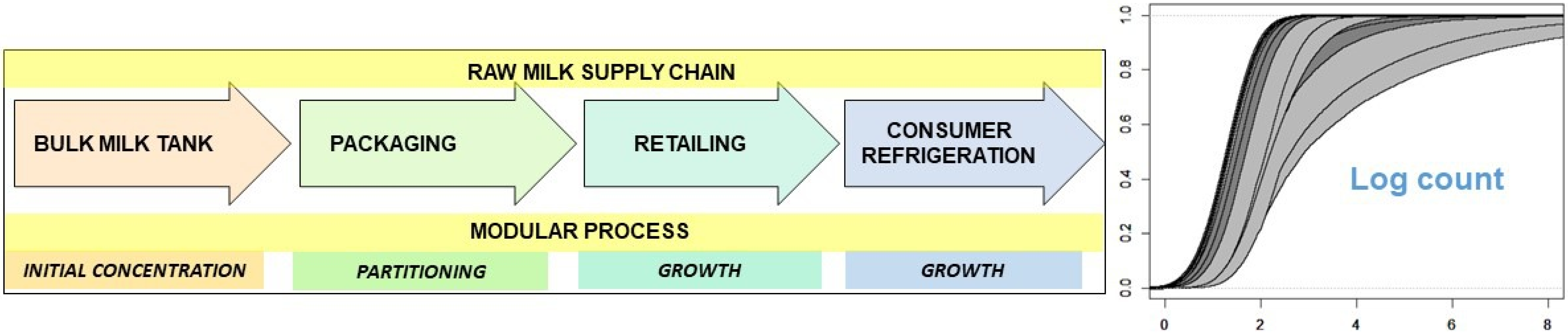

a
interceptmin
slopemax
slopemin
interceptmax
logN0_sd_U
logN0_mean_U
-1 -0.8 -0.6 -0.4 -0.2 0 0.2 0.4 0.6 0.8 1
Spearman correlation value
b
interceptmin
slopemax
slopemin
interceptmax
logN0_sd_U
logN0_mean_U
-1 -0.8 -0.6 -0.4 -0.2 0 0.2 0.4 0.6 0.8 1
Spearman correlation value
c
interceptmin
slopemax
slopemin
interceptmax
logN0_mean_U
logN0_sd_U
-1 -0.8 -0.6 -0.4 -0.2 0 0.2 0.4 0.6 0.8 1
Spearman correlation value

**Fig.4.**


RAW MILK SUPPLY CHAIN
BULK MILK TANK
PACKAGING
RETAILING
CONSUMER REFRIGERATION
MODULAR PROCESS
INITIAL CONCENTRATION
PARTITIONING
GROWTH
GROWTH
Log count
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8