



HAL
open science

Probabilistic modelling of *Escherichia coli* concentration in raw milk under hot weather conditions

Rodney Feliciano, Géraldine Boué, Fahad Mohssin, Mohammed Mustafa
Hussaini, Jeanne-Marie Membré

► To cite this version:

Rodney Feliciano, Géraldine Boué, Fahad Mohssin, Mohammed Mustafa Hussaini, Jeanne-Marie Membré. Probabilistic modelling of *Escherichia coli* concentration in raw milk under hot weather conditions. Food Research International, 2021, 149, pp.1-10. 10.1016/j.foodres.2021.110679 . hal-03566746

HAL Id: hal-03566746

<https://hal.inrae.fr/hal-03566746v1>

Submitted on 16 Oct 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

Probabilistic modelling of *Escherichia coli* concentration in raw milk under hot weather conditions

Rodney Feliciano¹, Géraldine Boué¹, Fahad Mohssin², Mohammed Mustafa Hussaini² and Jeanne-Marie Membré¹†

(1) Secalim, INRAE, ONIRIS, Nantes, France, (2) AlSafi Danone, Al-Kharj, Saudi Arabia

† Corresponding author : Jeanne-Marie Membré

Secalim, INRAE, Oniris, Site de la Chantrerie, CS 40706, 44307 Nantes Cédex 3, France

Jeanne-Marie.Membre@oniris-nantes.fr

+33 (0)240684058

<https://orcid.org/0000-0001-6751-4426>

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

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31

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 2nd 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

32 1. Introduction

33

34 The global average temperature is forecasted to increase to more than 2.0 °C due to climate
35 change, and this led to several international efforts be undertaken, to curb the greenhouse gas emission
36 of world economies (Raftery, Zimmer, Frierson, Startz, & Liu, 2017). The change in temperature in
37 Europe is dependent on the Representative Concentration Pathways (RCP) which is projected to be 1-
38 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
39 (European Environment Agency, 2017). In metropolitan France, the projected increase in temperatures
40 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
41 reference period (Météo-France, 2021). The associated changes with these are the increase in
42 precipitation levels and more frequent occurrence of extremely high-temperature periods during
43 summer (European Environment Agency, 2017).

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

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

61 Dairy milk farming in France is at present a mixture of small, medium, and large-scale dairy
62 farming with small-scale being the most common whereas in hot climate countries such as in the
63 Middle East, large-scale dairy farming is commonly used. In this latter system, husbandry conditions
64 are characterized by the presence of highly mechanized equipment and a strict application of hygienic
65 conditions. This set-up extends from cow rearing to the transportation of raw milk: application of good
66 veterinary practices, control of milk quality, maintenance of cold chain, etc. This system is the reason
67 for high milk productivity, safe milk, and steady supply of dairy products to the market especially in
68 regions previously considered unsuitable for milk production (Alqaisi, Ndambi, Uddin, & Hemme,

69 2010). Such countries with hot weather conditions might help understanding what might occur in the
70 future for some of European countries currently undergoing temperature shifts due to climate change.
71 In this respect, studies on the current microbiological status of foods from hot weather conditions can
72 be used as a proxy or representative for the potential future impacts on food safety.

73 In France, raw milk intended for human consumption is currently regulated by the French
74 Ministry of Agriculture through an administrative order (Ministère de l'agriculture de
75 l'agroalimentaire et de la forêt, 2012). This decree specifies the product form in which raw milk may
76 be sold, the time frame from milking to consumption, and how the cold chain must be maintained. In
77 France, raw milk is available to consumers in packaged form or sold through vending machines. These
78 rules are designed to meet the hygiene criteria for raw milk against microbial hazards such as *E. coli*
79 which is among the most common contaminant in raw milk and widely used indicator of hygiene
80 criteria (EFSA, 2015; Martin, Trmčić, Hsieh, Boor, & Wiedmann, 2016). The seasonal effect on *E.*
81 *coli* in cattle has been reported in several studies including Fairbrother & Nadeau (2006); Hussein &
82 Sakuma (2005) and Ranjbar, Safarpour Dehkordi, Sakhaei Shahreza, & Rahimi (2018). Moreover, in
83 their longitudinal risk factor analysis conducted on multiple ranches located on the California Central
84 Coast, Benjamin, Jay-Russell, Atwill, Cooley, Carychao, Larsen, & Mandrell, (2015) observed a
85 positive increase of *E. coli* O157 with the soil temperature (from 21°C to 26.1°C). According to the
86 hygiene criteria, based on three class attribute sampling plans, *E. coli* concentration in raw milk cannot
87 exceed 2 log CFU/mL (Ministère de l'agriculture de l'agroalimentaire et de la forêt, 2012). In
88 addition to this, an internal hygiene criterion is observed by French dairy farmers selling raw milk at
89 local markets, where the *E. coli* concentration in raw milk is limited to 1 log CFU/mL prior to retailing
90 (information provided by a French raw milk farming Expert).

91 In this context, the aim of this paper was to build a probabilistic model to quantify the
92 concentration of *E. coli* in raw milk and explore what may happen to raw milk sold in France under
93 climate change conditions. Probabilistic modelling approaches are highly valuable because they allow
94 the modelling of scenarios, taking uncertainty and variability into account (Koutsoumanis & Aspidou,
95 2016; Nauta, 2000). Probabilistic modelling has been applied in pasteurized milk to assess safety from
96 spoilage organisms (Schaffner, Mcentire, Duffy, Montville, & Smith, 2003) and *E.coli* O157:H7
97 (Clough, Clancy, & French, 2006). In raw milk this modelling approach has been used to assess safety
98 from microbiological hazards such as *Listeria monocytogenes* (Latorre et al., 2011) and chemical
99 hazards such as SEA toxin (Crotta et al., 2016; Heidinger, Winter, & Cullor, 2009). Risk assessments
100 of *E. coli* O157:H7 in raw milk were performed to determine the infections after the consumption of
101 raw milk using probabilistic modelling techniques (Giacometti et al., 2012; Grace et al., 2008). These
102 studies reflect two different retailing scenarios: Giacometti et al. (2012) have performed a risk
103 assessment on vended raw milk while Grace et al. (2008) evaluated the informally marketed raw milk.

104 Therefore, the first novelty of the study presented here lies in having built a farm-to fork
105 probabilistic assessment model to evaluate the *E. coli* concentration under hot weather conditions. For

106 this purpose, an original dataset from a large-scale farm in Kingdom of Saudi Arabia have been
107 collected and analysed. Next, the current raw milk handling practices in France has been introduced in
108 the model to run realistic scenario. The second novelty of this study is to present a 2nd order Monte
109 Carlo model, separating uncertainty and variability, applied to raw milk consumption and the
110 interpretation of its outputs by sensitivity analysis.

111

112 **2. Materials and Methods**

113

114 *2.1. Model description*

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

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

126

127 *2.2. Module 1: Raw milk contamination level in bulk milk tanks at farm setting*

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

131 The average temperature in Alkharj, where the farm was located, in 2019 varied between
132 13.9°C (January, the coldest month) and 36.9°C (August, the hottest month). In comparison, in France
133 (average values from 30 different locations), the temperature during summer reached 20.1°C (June
134 2019), 23°C (July 2019) and 21.8°C (August 2019). This average temperature included daily
135 fluctuations; during the hottest period of the day (midday and beginning of afternoon), the
136 temperatures fluctuated between 25 to 27°C with several peaks above 30°C observed in France during
137 July 2019.

138 The *E. coli* counts in raw milk were obtained by performing the colony count method based on
139 the norm NF ISO 4832 (updated in 2006). An undiluted 1 mL of raw milk sample were transferred to
140 Petri dishes while 10-12 mL of violet red bile agar (VRBA) (Oxoid, Ltd., UK) (cooled into 45 ± 1 °C)
141 was also added and solidified as the initial layer. An overlay of 3-5 mL of VRBA was then

142 subsequently added to the original basal-sample medium. The plates were then incubated at 37 ± 1 ° C
143 for 24 h. Colonies showing purplish red color with a reddish zone of precipitated bile (≥ 0.5 mm
144 diameter) were enumerated.

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

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

157

158 2.3. Module 2: Packaging of raw milk

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

165 2.4. Module 3: Retailing

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

171 2.5. Determination of growth kinetic parameters

172 The growth parameters of *E. coli* in milk were obtained from the literature and Combase. First,
173 the literature search was done in Web of Science using the combination of the topic terms: growth and
174 (raw and milk), and (*Escherichia* and *coli*) and (Temperature). These terms yielded 77 research
175 articles and were filtered based on their titles to keep only milk as the suspending medium (i.e. raw
176 milk cheese studies were discarded). Moreover, challenge test studies which included *E. coli* in the

177 presence of antimicrobials were excluded. When the growth studies were done in one temperature
178 value, the article was also discarded. Three research papers were retained from this search, all coming
179 from one research laboratory (Ačai, Valík, Medved'ová, & Rosskopf, 2016; Medved'ová, Györiová,
180 Lehotová, & Valík, 2020; Medved'ová, Rosskopf, Liptáková, & Valík, 2018). These papers have
181 utilized only one strain of *E. coli* which have been isolated from a raw milk cheese. Growth studies
182 obtained from these papers were strictly below 30°C.

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

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

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

$$199 \quad \sqrt{\mu_{max}} = \text{Slope} \times \text{Temperature} + \text{Intercept} \quad (1)$$

$$200 \quad T_{min} = (-\text{Intercept}/\text{Slope}) \quad (2)$$

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

$$204 \quad \Delta \log N = \mu_{max} \times \text{Time} \quad (3)$$

205 2.6. Module 4: Refrigeration before consumption

206 The conditions during the consumer refrigeration stage were simulated in order to determine
207 its influence on the microbial concentration in packaged raw milk products. The refrigeration
208 temperatures obtained by Roccato *et al.* (2017) for countries located in Northern Europe (N: 6.1, 2.8),
209 which France is part of, was used in the assessment model. The duration of refrigeration, chosen as
210 realistic scenarios were 12, 36 and 60 h. These different scenarios complete the allowable period of

211 time for human consumption set to a maximum of 72h in France (information provided by a French
212 raw milk farming Expert).

213

214 2.7 Modelling

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

220

221 2.8 Uncertainty analysis

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

228

229 3. Results

230

231 3.1. Module 1: Initial microbial load in bulk milk tank

232 The initial microbial concentration (namely, logN₀) was obtained from the one-year operation
233 in a dairy farm in Saudi Arabia. The data were fitted by normal, log normal, and, gamma distributions
234 and the results were compared based on the AIC value (**Table 3**). The normal distribution provided the
235 best fit (AIC=903). A bootstrap procedure was then performed to estimate the uncertainty around the
236 normal distribution parameters (**Fig.1a**). This resulted in an estimated mean value of 1.31 log CFU/mL
237 with a confidence interval of 1.27-1.35, and, a standard deviation of 0.53 with a confidence interval of
238 0.50-0.57.

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

244

245 3.2. Module 2: Packaging of raw milk

246 The packaging of raw milk from bulk milk tank into a 1L pack is a partitioning process. This
247 follows the Poisson distribution of the microbial counts across the packaged products per batch. The
248 number of packaged products exceeding the two hygiene criteria for raw milk namely, 2-log limit
249 (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).
250 These values were the same as the previous module, showing here that partitioning did not have effect
251 on the concentration level, likely to be linked with the relatively high initial *E. coli* count in raw milk.

252

253 3.3. Module 3: Retailing

254 3.3.1. Determination of growth parameters

255 The microbial growth rates extracted from the literature and Combase were from different
256 strains of the pathogenic *E. coli*. For the literature search, we obtained three papers that have used the
257 same strain which is isolated from a Slovakian cheese (Ačai et al., 2016; Medved'ová et al., 2020,
258 2018). These studies performed growth studies in milk with a total of 34 temperature data. As such,
259 the growth parameters obtained from these were compiled into the *E. coli* BR strain (**Table 2**). The
260 search in Combase has yielded records from four different strains of *E. coli* all from one study (Kauppi
261 et al., 1996).

262 The square root of the μ_{max} was then plotted at function of temperatures, along with the
263 adjusted model (**Fig.2**). The parameters namely, slope and intercept, determined from a linear
264 regression using the Ratkowsky model are reported in **Table 2**. The slope and intercept estimates were
265 used to determine the T_{min} values obtained for each strain. The range of the T_{min} value estimated from
266 the literature and combase is also visible in **Fig.2**, it was between 4 to 6 °C. The strain variability was
267 captured by building a uniform distribution from the strain having the highest T_{min} up to the strain
268 having the lowest T_{min} values. These strains were *E. coli* O111-NM str 403 (5.60°C) and *E. coli* BR
269 (4.07°C) for the highest and lowest T_{min} value, respectively. The strain uncertainty was captured in a
270 Normal distribution using the standard error around the slope estimates (and the intercept,
271 respectively) of the strain having the highest and lowest T_{min} : $slopemax$ and $slopemin$ (interceptmax
272 and interceptmin, respectively). For instance, the lowest slope estimate was fitted by the Normal
273 distribution $N(0.039, 0.005)$.

274 The results of the 2nd order Monte Carlo simulation analysing the uncertainty and variability of
275 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%
276 confidence interval of [1.8; 7.6]°C. This large confidence interval around the mean value reflects the
277 uncertainty in the estimation process due to lack of data and model misfit when applying the
278 Ratkowsky secondary model. Its influence on the final output will be assessed by sensitivity analysis
279 hereafter. Besides, T_{min} variability is also large with variation from a 5th percentile estimated to
280 3.4°C [-0.3; 6.5]°C up to a 95th percentile estimated to 6.1°C [3.2; 9.8]°C.

281

282 3.3.2. Microbial growth during retailing period

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

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

293

294 3.4. Module 4: Refrigeration before consumption

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

299 The consumer scenario of storage for 12 h resulted in a probability of 31.0 % with a
300 confidence interval of 15.0-61.0% of exceeding the 2-log hygiene criterion while a much higher
301 probability is achieved with the more stringent 1-log criterion (88.0% with a confidence interval of
302 77.0-97.0%). The 1-log criterion was provided by a French raw milk farming Expert as the maximal
303 acceptable limit for *E. coli* in milk foreseen to be consumed without any heating step.

304 The changes with the microbial concentration from the initial microbial load in bulk milk
305 tanks (logN₀) to the end of consumer's storage (logN₃) are depicted in the cumulative distribution
306 graphs (**Fig.1c-e**). In these figures, it can be seen that the changes in the distribution of values shift
307 towards higher microbial counts while the uncertainties surrounding the predicted values also increase
308 across the dairy supply chain.

309 As indicated in **Table 1** the inputs containing uncertainty namely, initial *E. coli* concentration
310 (mean, LogN_{0_mean_U} and standard deviation, LogN_{0_sd_U}), slope (minimum value of slope,
311 slopemin and maximum value of slope, slopemax) and the intercept (minimum value, interceptmin
312 and maximum value, interceptmax) were presented. These uncertainties were then propagated in the
313 model during the computation of the latent variables. The impact of uncertainty on the output (logN₃)
314 was then assessed using sensitivity analysis. The output of these were shown in the tornado plots that
315 captured all the uncertainties and reflected their impact on the uncertainty of the estimates during
316 consumer storage (**Fig.4a-c**).

317 Unsurprisingly, as already highlighted when describing the T_{min} estimated values, most of the
318 uncertainty came from the characterisation of the intercept and slope associated with the strain growth

319 parameters: the uncertainties generated to estimate interceptmin and interceptmax, slopemin and
320 slopemax were the major source of uncertainty around the 95th percentile of logN₃ probabilistic
321 distribution. This result was observed across the three consumer refrigeration scenarios. On the other
322 hand, uncertainties from logN₀ parameters (i.e. logN0_mean_U and logN0_sd_U) had a limited
323 contribution to the uncertainty around the 95th percentile of logN₃ probabilistic distribution. A slight
324 difference could be observed for the 60h-consumer-storage scenario (**Fig.4c**) where logN0_mean_U
325 contributed more to the uncertainty of the output than logN0_sd_U, in contrast to what was observed
326 in the previous two scenarios.

327

328 4. Discussion

329

330 4.1. *The probabilistic assessment model*

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

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

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

355 such as prevalence of *E. coli* in the herd, lactating cows and the faeces contamination of the tank and
356 contamination during milking (Clough et al., 2009), in-line filter counts (Perrin et al., 2015), and
357 faecal contamination of raw milk and counts from raw milk in vending machines (Giacometti et al.,
358 2013).

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

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

373 On the opposite the model outputs showed further increase of *E. coli* during the different
374 consumer refrigeration scenarios (Δ log N consumer) where the estimated mean concentration grew to
375 0.2 log (12 h), 0.58 (36h) and 0.88 (60h) log CFU/mL. Since a probabilistic assessment was
376 performed, it is also possible to interpret the result considering the 95th percentile of the distribution: in
377 that case, the growth reached up to 0.35 (12 h), 1.45 (36h) and 2.75 (60h) log CFU/mL. Regarding the
378 domestic temperature variation, there are two distinct phenomena: the variation in refrigerator
379 temperature, from home to home (Roccatto et al. 2017) and for a given home refrigerator, the variation
380 of temperature during the day (Evans & Redmond, 2016) if for instance the consumer opens the
381 refrigerator to serve himself/herself a glass of milk. The first source of variability was integrated in the
382 model but not the second due to a lack of data to build a dynamic fluctuation of temperature without
383 introducing too much uncertainty. It can be assumed that the daily temperature fluctuation would have
384 a negative effect on the final contamination level, leading here to an underestimation of the exposure
385 level.

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

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

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

414 4.2. *The use of hot weather conditions and E. coli as test organism in understanding the future of raw* 415 *milk consumption*

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

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

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

452 The dairy farming systems such as the one used in this study are raising Holstein breed cows
453 that are kept inside large, naturally ventilated farm buildings, where they do not go outside or for very
454 limited time during the day because cows suffer from heat stress when they are exposed to temperature
455 above 25°C (information provided by a French veterinary expert). Although these systems can be seen
456 in European countries, adoption to these farming conditions varies. This is particularly true in France
457 where the dairy farms are medium-scale farms and with the widespread use of production machinery
458 (Poczta, Średzińska, & Chenczke, 2020). Nevertheless, the shift to this system is taking place in
459 southern France, where its adoption has been accelerated by the regular occurrence of heat waves
460 during the summer period (information provided by a French veterinary expert). Another challenge to
461 its widespread adoption is the shift towards sustainability with efficient use of resources,
462 implementation of recovery mechanisms and pressure from consumers to devolve to localized farms
463 (Thorpe, Schmalzried, & Fallon, 2010). These barriers to acceptance may hinder present adoption but

464 may not completely prevent it given the intensification of climate change effects. Overall, it is hoped
465 that the implication of the results obtained in this study may be useful in understanding the impact of
466 climate change driven hot weather conditions on the microbial quality of raw milk which is expected
467 to be more apparent in the future.

468

469 **Acknowledgement:**

470 The authors would like to acknowledge the inputs provided by Mrs Florence Daviaud, a
471 French raw milk farming expert (Foix) and Prof. Nathalie Baraille, a French veterinary expert from
472 BIOEPAR, Oniris (Nantes). This research has received funding from the European Union's Horizon
473 2020 research and innovation programme under Marie Skłodowska Curie grant agreement No.
474 813329.

475

476 Declarations of interest: None.

477 References:

- 478 Ačai, P., Valík, L., Medved'ová, A., & Roszkopf, F. (2016). Modelling and predicting the
479 simultaneous growth of *Escherichia coli* and lactic acid bacteria in milk. *Food Science and*
480 *Technology International*, 22(6), 475–484. <https://doi.org/10.1177/1082013215622840>
- 481 Alqaisi, O., Ndambi, O. A., Uddin, M. M., & Hemme, T. (2010). Current situation and the
482 development of the dairy industry in Jordan, Saudi Arabia, and Syria. *Tropical Animal Health*
483 *and Production*, 42(6), 1063–1071. <https://doi.org/10.1007/s11250-010-9553-y>
- 484 Benjamin, L. A., Jay-Russell, M. T., Atwill, E. R., Cooley, M. B., Carychao, D., Larsen, R. E., &
485 Mandrell, R. E. (2015). Risk factors for *Escherichia coli* O157 on beef cattle ranches located
486 near a major produce production region. *Epidemiology and Infection*, 143(1), 81-93. doi:
487 <https://doi.org/10.1017/S0950268814000521>
- 488 Boor, K. J., Brown, D. P., Murphy, S. C., Kozłowski, S. M., & Bandler, D. K. (1998). Microbiological
489 and Chemical Quality of Raw Milk in New York State. *Journal of Dairy Science*, 81(6), 1743–
490 1748. [https://doi.org/10.3168/jds.S0022-0302\(98\)75742-X](https://doi.org/10.3168/jds.S0022-0302(98)75742-X)
- 491 Chari, F., & Ngcamu, B. S. (2017). An assessment of the impact of disaster risks on dairy supply chain
492 performance in Zimbabwe. *Cogent Engineering*, 4(1).
493 <https://doi.org/10.1080/23311916.2017.1409389>
- 494 Clough, H. E., Clancy, D., & French, N. P. (2006). Vero-cytotoxigenic *Escherichia coli* O157 in
495 pasteurized milk containers at the point of retail: A qualitative approach to exposure assessment.
496 *Risk Analysis*, 26(5), 1291–1309. <https://doi.org/10.1111/j.1539-6924.2006.00825.x>
- 497 Clough, H. E., Clancy, D., & French, N. P. (2009). Quantifying exposure to Vero-cytotoxigenic
498 *Escherichia coli* O157 in milk sold as pasteurized: A model-based approach. *International*
499 *Journal of Food Microbiology*, 131(2–3), 95–105.
500 <https://doi.org/10.1016/j.ijfoodmicro.2008.12.036>
- 501 Crotta, M., Rizzi, R., Varisco, G., Daminelli, P., Cunico, E. C., Luini, M., ... Guitian, J. (2016).
502 Multiple-strain approach and probabilistic modeling of consumer habits in quantitative microbial
503 risk assessment: A quantitative assessment of exposure to staphylococcal enterotoxin A in Raw
504 Milk. *Journal of Food Protection*, 79(3), 432–441. [https://doi.org/10.4315/0362-028X.JFP-15-
505 235](https://doi.org/10.4315/0362-028X.JFP-15-235)
- 506 Delignette-Muller, M. L., & Dutang, C. (2015). fitdistrplus: An R package for fitting distributions.
507 *Journal of Statistical Software*, 64(4), 1–34. <https://doi.org/10.18637/jss.v064.i04>
- 508 Duqué, B., Canon, J., Haddad, N., Guillou, S., & Membré, J. M. (2021). Quantitative approach to
509 assess the compliance to a performance objective (PO) of *Campylobacter jejuni* in poultry meat
510 in France. *International Journal of Food Microbiology*, 336.
511 <https://doi.org/10.1016/j.ijfoodmicro.2020.108916>
- 512 EFSA. (2015). Scientific Opinion on the public health risks related to the consumption of raw drinking
513 milk. *EFSA Journal*, 13(1), 3940. <https://doi.org/10.2903/j.efsa.2015.3940>
- 514 European Environment Agency. (2017). *Climate change, impacts and vulnerability in Europe 2016 an*
515 *indicator based report*. <https://doi.org/10.2800/534806>
- 516 Evans, E. W., & Redmond, E. C. (2016). Time-Temperature Profiling of United Kingdom Consumers'
517 Domestic Refrigerators. *Journal of Food Protection*, 79(12), 2119-2127. doi:
518 <https://doi.org/10.4315/0362-028x.Jfp-16-270>
- 519 Fairbrother, J. M., & Nadeau, É. (2006). *Escherichia coli*: on-farm contamination of animals. *Revue*
520 *Scientifique et Technique de l'OIE*, 25(2), 555–569. <https://doi.org/10.20506/rst.25.2.1682>
- 521 FAO. (2020). *Climate change: Unpacking the burden on food safety*. <https://doi.org/10.4060/ca8185en>

- 522 Farrokh, C., Jordan, K., Auvray, F., Glass, K., Oppegaard, H., Raynaud, S., ... Cerf, O. (2013).
523 Review of Shiga-toxin-producing *Escherichia coli* (STEC) and their significance in dairy
524 production. *International Journal of Food Microbiology*, 162(2), 190–212.
525 <https://doi.org/10.1016/j.ijfoodmicro.2012.08.008>
- 526 Giacometti, F., Serraino, A., Bonilauri, P., Ostanello, F., Daminelli, P., Finazzi, G., ... Rosmini, R.
527 (2012). Quantitative risk assessment of verocytotoxin-producing *Escherichia coli* O157 and
528 *Campylobacter jejuni* related to consumption of raw milk in a province in Northern Italy. *Journal*
529 *of Food Protection*, 75(11), 2031–2038. <https://doi.org/10.4315/0362-028X.JFP-12-163>
- 530 Giacometti, Federica, Serraino, A., Peli, A., Fustini, M., Rosmini, R., Bonilauri, P., ... Bolzoni, G.
531 (2013). Four-Year monitoring of foodborne pathogens in raw milk sold by vending machines in
532 Italy. *Journal of Food Protection*, 76(11), 1902–1907. [https://doi.org/10.4315/0362-028X.JFP-](https://doi.org/10.4315/0362-028X.JFP-13-213)
533 13-213
- 534 Grace, D., Omore, A., Randolph, T., Kang'ethe, E., Nasinyama, G. W., & Mohammed, H. O. (2008).
535 Risk assessment for *Escherichia coli* O157:H7 in marketed unpasteurized milk in selected East
536 African countries. *Journal of Food Protection*, 71(2), 257–263. [https://doi.org/10.4315/0362-](https://doi.org/10.4315/0362-028X-71.2.257)
537 028X-71.2.257
- 538 Heidinger, J. C., Winter, C. K., & Cullor, J. S. (2009). Quantitative microbial risk assessment for
539 *Staphylococcus aureus* and *Staphylococcus enterotoxin a* in raw milk. *Journal of Food*
540 *Protection*, 72(8), 1641–1653. <https://doi.org/10.4315/0362-028X-72.8.1641>
- 541 Hussein, H. S., & Sakuma, T. (2005). Invited review: Prevalence of Shiga toxin-producing
542 *Escherichia coli* in dairy cattle and their products. *Journal of Dairy Science*, 88(2), 450–465.
543 [https://doi.org/10.3168/jds.S0022-0302\(05\)72706-5](https://doi.org/10.3168/jds.S0022-0302(05)72706-5)
- 544 Kauppi, K. L., Tatini, S. R., Harrell, F., & Feng, P. (1996). Influence of substrate and low temperature
545 on growth and survival of verotoxigenic *Escherichia coli*. *Food Microbiology*, 13(5), 397–405.
546 <https://doi.org/10.1006/fmic.1996.0046>
- 547 Kekana, T. W., Nherera-Chokuda, F. V., Muya, M. C., Manyama, K. M., & Lehloeny, K. C. (2018).
548 Milk production and blood metabolites of dairy cattle as influenced by thermal-humidity index.
549 *Tropical Animal Health and Production*, 50(4), 921–924. [https://doi.org/10.1007/s11250-018-](https://doi.org/10.1007/s11250-018-1513-y)
550 1513-y
- 551 Koutsoumanis, K. P., & Aspidou, Z. (2016). Moving towards a risk-based food safety management.
552 *Current Opinion in Food Science*, 12, 36–41. <https://doi.org/10.1016/j.cofs.2016.06.008>
- 553 Latorre, A. A., Pradhan, A. K., Van Kessel, J. A. S., Karns, J. S., Boor, K. J., Rice, D. H., ...
554 Schukken, Y. H. (2011). Quantitative risk assessment of listeriosis due to consumption of raw
555 milk. *Journal of Food Protection*, 74(8), 1268–1281. [https://doi.org/10.4315/0362-028X.JFP-10-](https://doi.org/10.4315/0362-028X.JFP-10-554)
556 554
- 557 Martin, N. H., Trmčić, A., Hsieh, T.-H., Boor, K. J., & Wiedmann, M. (2016). The Evolving Role of
558 Coliforms As Indicators of Unhygienic Processing Conditions in Dairy Foods. *Frontiers in*
559 *Microbiology*, 7(September), 1–8. <https://doi.org/10.3389/fmicb.2016.01549>
- 560 Mauger, G., Bauman, Y., Nennich, T., & Salathé, E. (2015). Impacts of Climate Change on Milk
561 Production in the United States. *The Professional Geographer*, 67(1), 121–131.
562 <https://doi.org/10.1080/00330124.2014.921017>
- 563 Medved'ová, A., Györiová, R., Lehotová, V., & Valík, Ľ. (2020). Co-cultivation growth of *Escherichia*
564 *coli* and *staphylococcus aureus* as two common dairy contaminants. *Polish Journal of Food and*
565 *Nutrition Sciences*, 70(2), 151–157. <https://doi.org/10.31883/pjfn/116395>
- 566 Medved'ová, A., Roszkopf, F., Liptáková, D., & Valík, L. (2018). Prediction of temperature effect on
567 growth of two raw milk cheese isolates of *Escherichia coli* in milk. *Journal of Food and*
568 *Nutrition Research*, 57(2), 141–150. Retrieved from

- 569 <https://vup.sk/index.php?mainID=2&navID=36&version=2&volume=57&article=2096>
- 570 Météo-France. (2021). *Les nouvelles projections climatiques de référence DRIAS 2020 pour la*
571 *métropole*. Retrieved from [http://www.observatoireclimat-hautsdefrance.org/Les-](http://www.observatoireclimat-hautsdefrance.org/Les-ressources/Ressources-documentaires/Les-nouvelles-projections-climatiques-de-referance-DRIAS-2020-pour-la-metropole)
572 [ressources/Ressources-documentaires/Les-nouvelles-projections-climatiques-de-referance-](http://www.observatoireclimat-hautsdefrance.org/Les-ressources/Ressources-documentaires/Les-nouvelles-projections-climatiques-de-referance-DRIAS-2020-pour-la-metropole)
573 [DRIAS-2020-pour-la-metropole](http://www.observatoireclimat-hautsdefrance.org/Les-ressources/Ressources-documentaires/Les-nouvelles-projections-climatiques-de-referance-DRIAS-2020-pour-la-metropole).
- 574 Ministère de l'agriculture de l'agroalimentaire et de la forêt. (2012). *Arrêté du 13 juillet 2012 relatif*
575 *aux conditions de production et de mise sur le marché de lait cru de bovinés, de petits ruminants*
576 *et de solipèdes domestiques remis en l'état au consommateur final* (p. 11990). p. 11990.
577 Retrieved from <https://www.legifrance.gouv.fr/eli/arrete/2012/7/13/AGRG1229148A/jo/texte>
- 578 Nauta, M. J. (2000). Separation of uncertainty and variability in quantitative microbial risk assessment
579 models. *International Journal of Food Microbiology*, 57(1–2), 9–18.
580 [https://doi.org/10.1016/S0168-1605\(00\)00225-7](https://doi.org/10.1016/S0168-1605(00)00225-7)
- 581 Nauta, M. J. (2005). Microbiological risk assessment models for partitioning and mixing during food
582 handling. *International Journal of Food Microbiology*, 100(1–3), 311–322.
583 <https://doi.org/10.1016/j.ijfoodmicro.2004.10.027>
- 584 Nauta, M. J., Litman, S., Barker, G. C., & Carlin, F. (2003). A retail and consumer phase model for
585 exposure assessment of *Bacillus cereus*. *International Journal of Food Microbiology*, 83(2),
586 205–218. [https://doi.org/10.1016/S0168-1605\(02\)00374-4](https://doi.org/10.1016/S0168-1605(02)00374-4)
- 587 Perrin, F., Tenenhaus-Aziza, F., Michel, V., Miszczycha, S., Bel, N., & Sanaa, M. (2015). Quantitative
588 Risk Assessment of Haemolytic and Uremic Syndrome Linked to O157: H7 and Non-O157: H7
589 Shiga-Toxin Producing *Escherichia coli* Strains in Raw Milk Soft Cheeses. *Risk Analysis*, 35(1),
590 109–128. <https://doi.org/10.1111/risa.12267>
- 591 Poczta, W., Średzińska, J., & Chenczke, M. (2020). Economic situation of dairy farms in identified
592 clusters of european union countries. *Agriculture (Switzerland)*, 10(4).
593 <https://doi.org/10.3390/agriculture10040092>
- 594 Pouillot, R., & Delignette-Muller, M. L. (2010). Evaluating variability and uncertainty separately in
595 microbial quantitative risk assessment using two R packages. *International Journal of Food*
596 *Microbiology*, 142(3), 330–340. <https://doi.org/10.1016/j.ijfoodmicro.2010.07.011>
- 597 R Core Team. (2019). *R: A language and environment for statistical computing*. Retrieved from
598 <https://www.r-project.org/>
- 599 Raftery, A. E., Zimmer, A., Frierson, D. M. W., Startz, R., & Liu, P. (2017). Less than 2 °c warming
600 by 2100 unlikely. *Nature Climate Change*, 7(9), 637–641. <https://doi.org/10.1038/nclimate3352>
- 601 Ranjbar, R., Safarpour Dehkordi, F., Sakhaei Shahreza, M. H., & Rahimi, E. (2018). Prevalence,
602 identification of virulence factors, O-serogroups and antibiotic resistance properties of Shiga-
603 toxin producing *Escherichia coli* strains isolated from raw milk and traditional dairy products.
604 *Antimicrobial Resistance and Infection Control*, 7(1), 1–11. [https://doi.org/10.1186/s13756-018-](https://doi.org/10.1186/s13756-018-0345-x)
605 [0345-x](https://doi.org/10.1186/s13756-018-0345-x)
- 606 Ratkowsky, D. A., Lowry, R. K., McMeekin, T. A., Stokes, A. N., & Chandler, R. E. (1983). Model
607 for bacterial culture growth rate throughout the entire biokinetic temperature range. *Journal of*
608 *Bacteriology*, 154(3), 1222–1226. <https://doi.org/10.1128/JB.154.3.1222-1226.1983>
- 609 Roccato, A., Uyttendaele, M., & Membré, J. M. (2017). Analysis of domestic refrigerator
610 temperatures and home storage time distributions for shelf-life studies and food safety risk
611 assessment. *Food Research International*, 96, 171–181.
612 <https://doi.org/10.1016/j.foodres.2017.02.017>
- 613 Schaffner, D. W., Mcentire, J., Duffy, S., Montville, R., & Smith, S. (2003). Monte Carlo Simulation
614 of the Shelf Life of Pasteurized Milk as Affected by Temperature and Initial Concentration of

- 615 Spoilage Organisms. *Food Protection Trends*, 23(12), 1014–1021.
- 616 St-Pierre, N. R., Cobanov, B., & Schnitkey, G. (2003). Economic losses from heat stress by US
617 livestock industries1. *Journal of Dairy Science*, 86(SUPPL. 1), E52–E77.
618 [https://doi.org/10.3168/jds.S0022-0302\(03\)74040-5](https://doi.org/10.3168/jds.S0022-0302(03)74040-5)
- 619 Summer, A., Lora, I., Formaggioni, P., & Gottardo, F. (2019). Impact of heat stress on milk and meat
620 production. *Animal Frontiers*, 9(1), 39–46. <https://doi.org/10.1093/af/vfy026>
- 621 Thorpe, L., Schmalzried, H. D., & Fallon, L. F. (2010). Proposed Mega-Dairies and Quality-of-Life
622 Concerns: Using Public Health Practices to Engage Neighbors. *Public Health Reports*, 125(5),
623 754–758. <https://doi.org/10.1177/003335491012500518>
- 624 van der Spiegel, M., van der Fels-Klerx, H. J., & Marvin, H. J. P. (2012). Effects of climate change on
625 food safety hazards in the dairy production chain. *Food Research International*, 46(1), 201–208.
626 <https://doi.org/10.1016/j.foodres.2011.12.011>
- 627 Vidovic, S., Mangalappalli-Illathu, A. K., & Korber, D. R. (2011). Prolonged cold stress response of
628 *Escherichia coli* O157 and the role of rpoS. *International Journal of Food Microbiology*, 146(2),
629 163–169. <https://doi.org/10.1016/j.ijfoodmicro.2011.02.018>
- 630 WHO. (2019). *Food safety, climate change and the role of WHO* (pp. 1–7). pp. 1–7. Retrieved from
631 [http://www.who.int/globalchange/publications/quantitative-
632 %0Ahttps://www.who.int/foodsafety/publications/all/Climate_Change_Document.pdf?ua=1](http://www.who.int/globalchange/publications/quantitative-%0Ahttps://www.who.int/foodsafety/publications/all/Climate_Change_Document.pdf?ua=1)
- 633 Yoon, Y., Lee, S., & Choi, K. H. (2016). Microbial benefits and risks of raw milk cheese. *Food*
634 *Control*, 63, 201–215. <https://doi.org/10.1016/j.foodcont.2015.11.013>
- 635

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	Deterministic	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	V _p	Deterministic	mL			1000	Input
Concentration of microorganisms per pack	N1	Poisson ($10^{\log N_0} \times V_p$)	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 μmax_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 + μmax _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 μmax_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 + μmax _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

Strain	Information collected from literature or ComBase			Estimated growth kinetic parameters generated in this present study				
	Origins	Temperature (°C)	Reference	Slope	Sd slope	Intercept	sd Intercept	Tmin
<i>Escherichia coli</i> BR	Isolated from Slovakian Brydzna cheese	8,10,12,15,18,21,25,30 °C	Medvedova et al., 2018					
<i>Escherichia coli</i> BR	Isolated from Slovakian Brydzna cheese	6,12,15,18,21,25,30 °C	Medvedova et al., 2020	0.0392*	0.005	-0.1598α	0.088	4.07
<i>Escherichia coli</i> BR	Isolated from Slovakian Brydzna cheese	10,12,15,18,21,25,30 °C	Acai et al., 2015					
<i>Escherichia coli</i> 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
<i>Escherichia coli</i> O111-NM str 403	USFDA collection	6.5,7.5,8.5,9.5 °C	Kauppi et al.1996	0.0388**	0.007	-0.2176αα	0.055	5.60
<i>Escherichia coli</i> 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
<i>Escherichia coli</i> 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
<i>Escherichia coli</i> 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

α and αα values used to build the probability distribution regarding the intercept

Table 3. Results of the initial microbiological concentration ($\log N_0$ 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, 95th 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	95th 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 25th and 75th percentiles of the uncertainty.

Fig. 2. The square root of the μ_{max} of the different *E.coli* strain (markers), collected at various temperature values, with the adjusted values of square root of the μ_{max} (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 25th and 75th percentiles of the uncertainty.

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

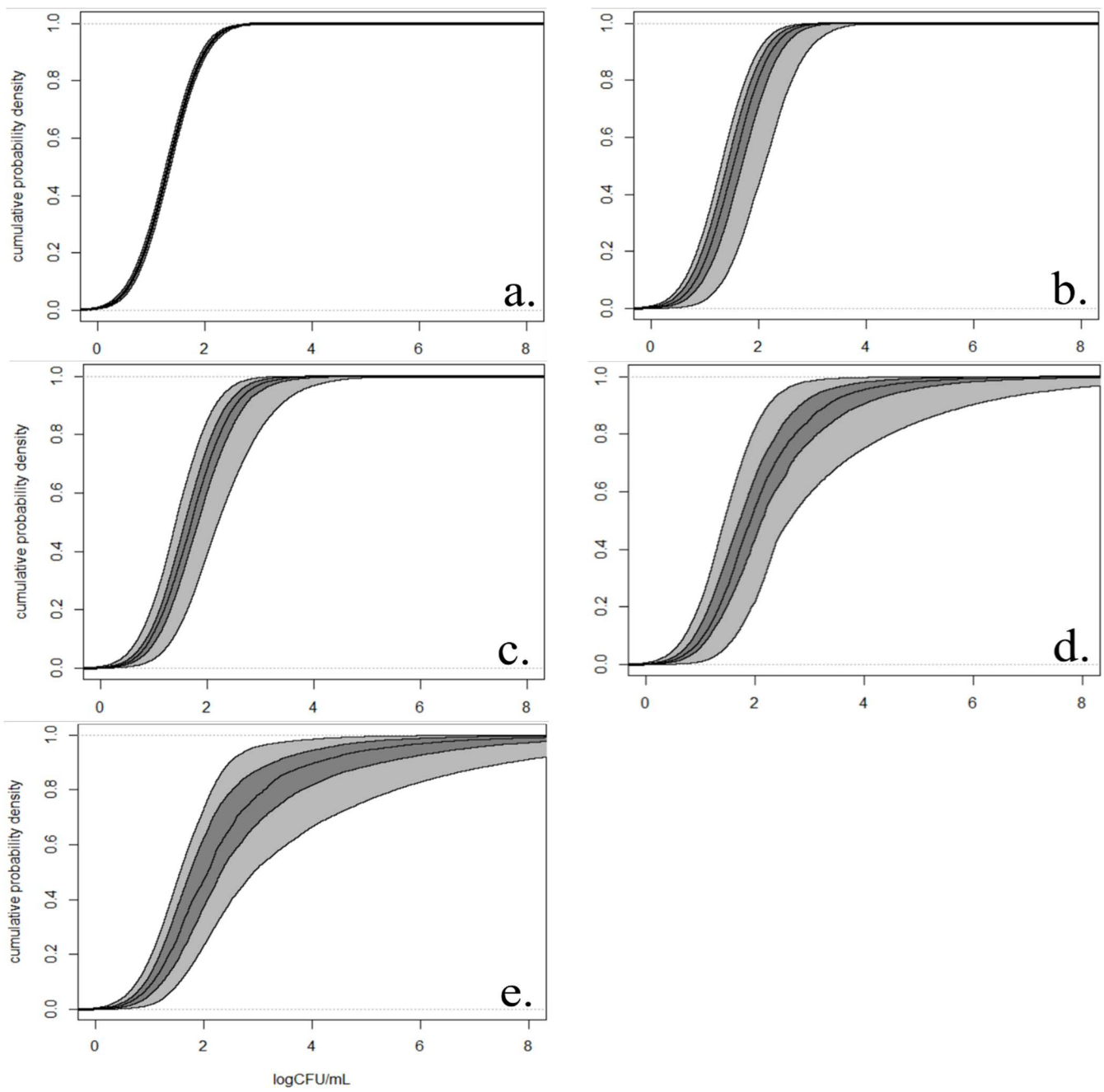


Fig.1.

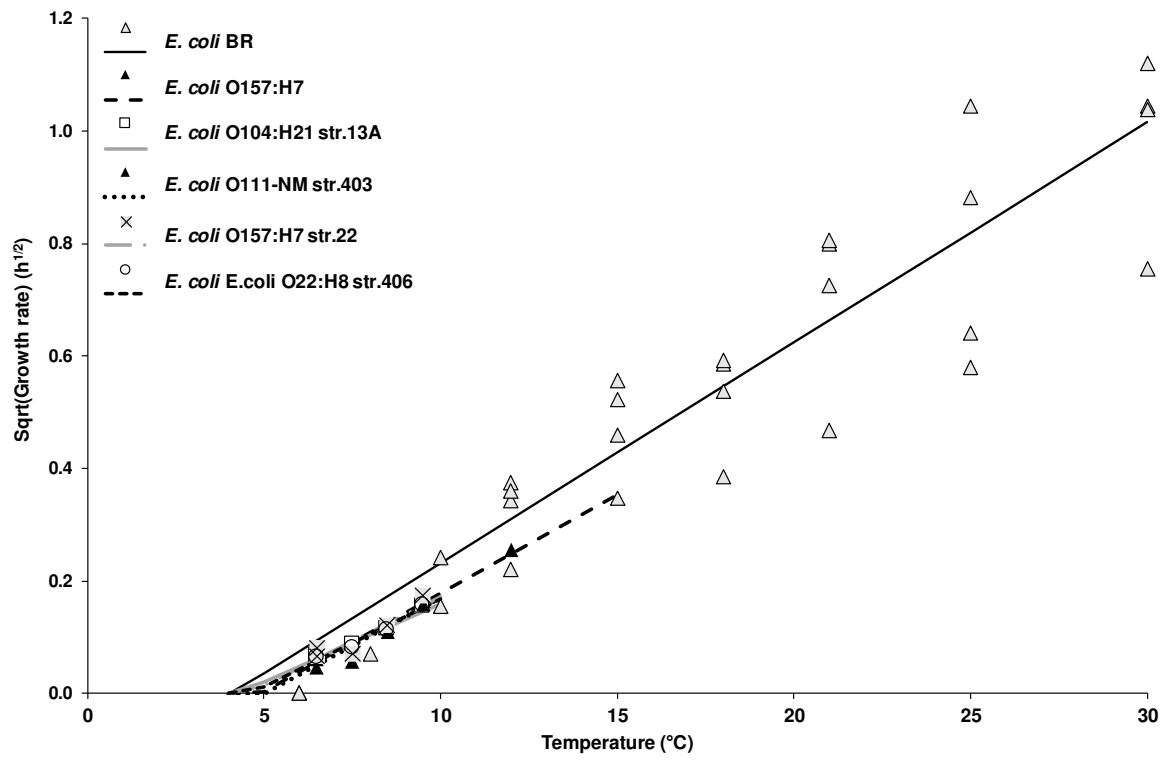


Fig. 2.

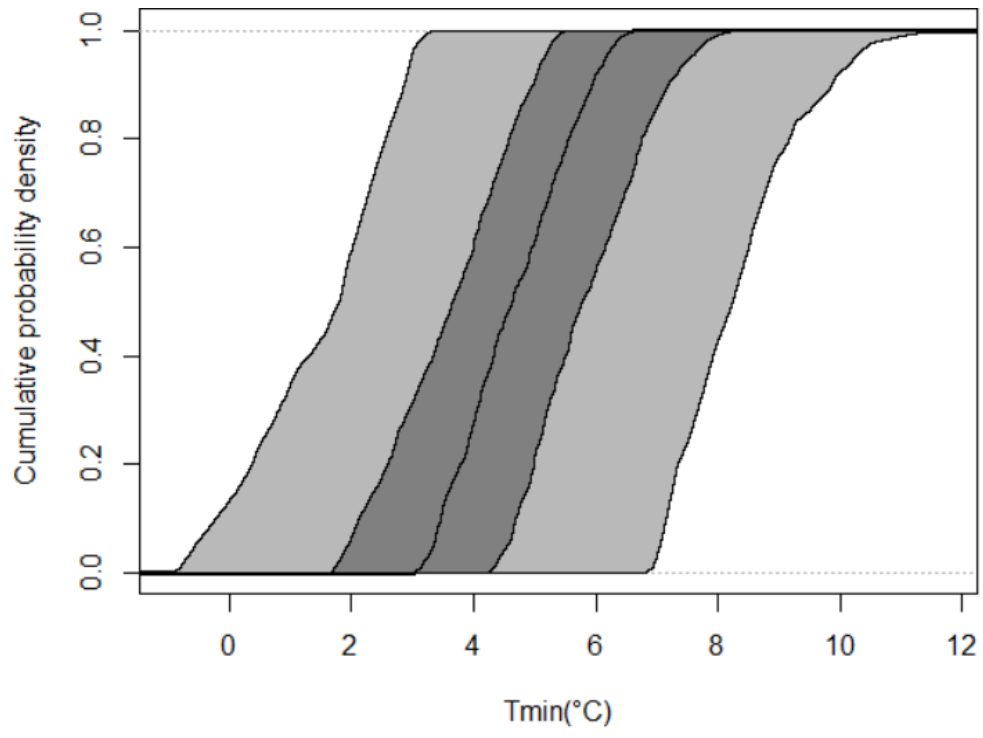
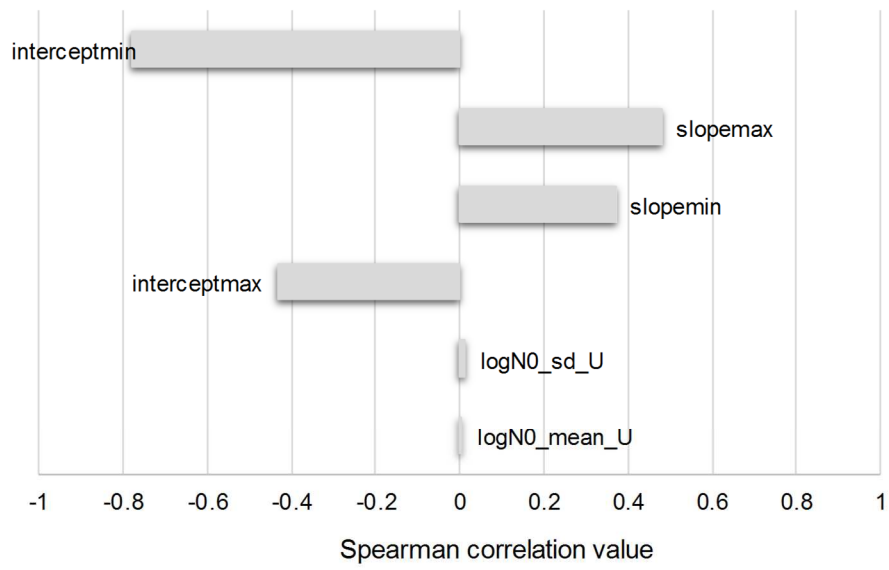
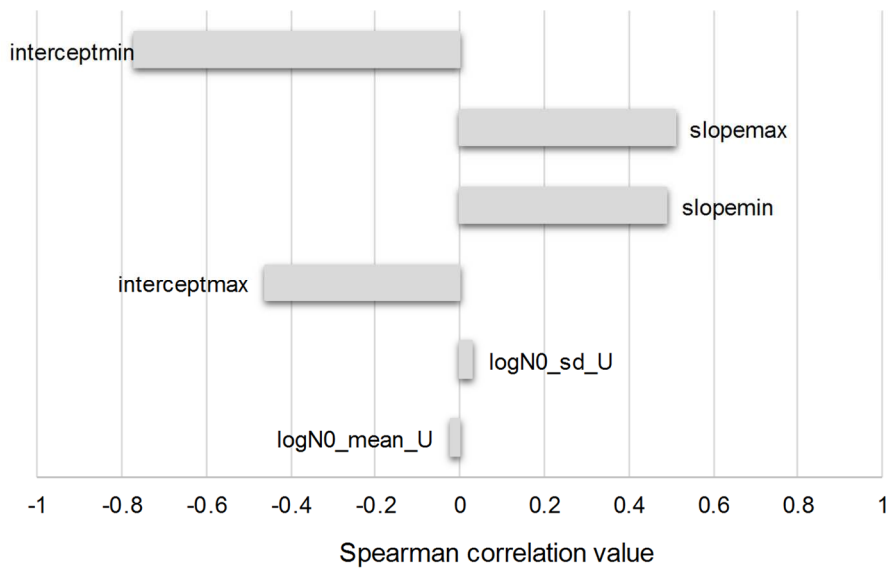


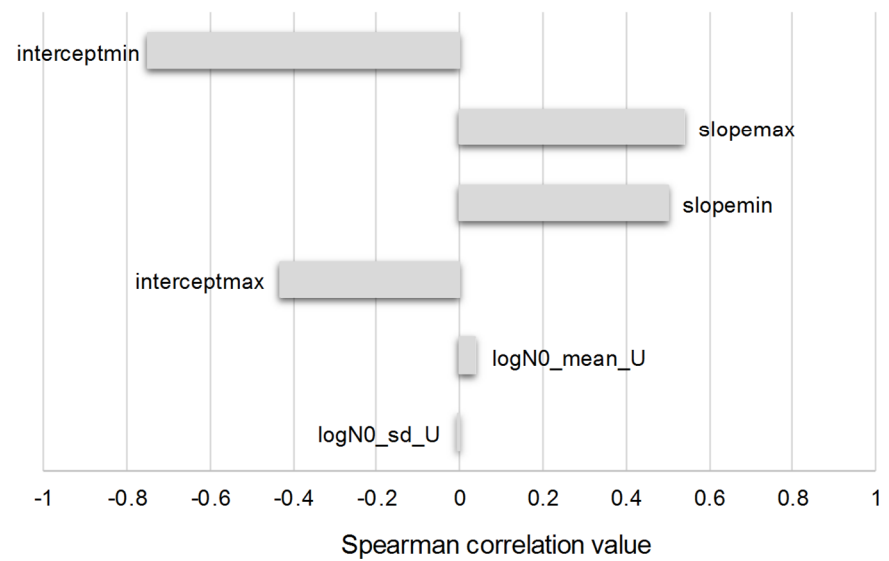
Fig. 3.



a



b



c

Fig.4.

