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Retrospective analysis of a *Listeria monocytogenes* contamination episode in raw milk goat cheese using quantitative microbial risk assessment tools

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Abstract

In 2005, the Belgian authorities reported a *Listeria monocytogenes* contamination episode in cheese made from raw goat's milk. The presence of an asymptomatic shedder goat in the herd caused this contamination. On the basis of data collected at the time of the episode, a retrospective study was performed using an exposure assessment model covering the production chain from the milking of goats up to delivery of cheese to the market. Predictive microbiology models were used to simulate the growth of *L. monocytogenes* during the cheese process in relation with temperature, pH and water activity. The model showed significant growth of *L. monocytogenes* during chilling and storage of the milk collected the day before the cheese production (median increase of 2.2 log CFU/ml) and during adjunction of starter and rennet to milk (median increase of 1.2 log CFU/ml). The *L. monocytogenes* concentration in the fresh unripened cheese was estimated to be 3.8 log CFU/g (median). This result is consistent with the number of *L. monocytogenes* in the fresh cheese (3.6 log CFU/g) reported during the cheese contamination episode. A variance-based method sensitivity analysis identified the most important factors impacting the cheese contamination, and a scenario analysis then evaluated several options for risk mitigation. Thus, by using Quantitative Microbial Risk Assessment (QMRA) tools, this study provides reliable information to identify and control critical steps in a local production chain of cheese made from raw goat's milk.

The safety of soft cheese made from raw milk is debated with regards to several micro-organisms of concern such as *Salmonella*, enterohemorrhagic *E. coli*, toxin-producing *Staphylococcus aureus* and *Listeria monocytogenes (9)*. Cheese made from raw milk may be an important source of human listeriosis *(16, 30, 31)*.

In 2005, a *Listeria monocytogenes* contamination episode in goat cheese made from raw milk was reported by the Belgian Federal Food Agency for the Safety of the Food Chain (FASFC). Using the collected information, we have undertaken a retrospective study based on a quantitative microbial risk assessment (QMRA) method. QMRA is a scientifically based method for modelling the fate of pathogenic micro-organisms along the food chain and for assessing the associated risk of developing adverse effects for the consumer *(27)*. Selected QMRA tools could be used to focus only on the food process and to provide options to reduce the level of contamination of the final product.

Field and laboratory collected data were used to implement the exposure assessment model from the milking of the goats up to the storage of end products in the farm. The final output is the *L. monocytogenes* contamination of goat cheese made from raw milk, due to the presence in the herd of an asymptomatic milk-shedder goat. The model was established in accordance with the *Codex Alimentarius* Commission guidelines *(13)*. Dynamic predictive microbial models were used to follow the bacterial population during food processing by taking into account the temperature, pH and water activity *(38)*. Sensitivity analysis was performed to identify the most important factors impacting *L. monocytogenes* concentration in the cheese *(40)*. Finally, valuable options for risk mitigation were proposed and evaluated using scenario analysis.

MATERIALS AND METHODS

Description of the herd. The herd is composed of 350 goats from the "Alpine" breed. The farm is located in Wallonia (southern part of Belgium). The feed distributed to the goats is mainly composed of hay and grass silage with low moisture and made from herbages stored by the farmer himself. The goats' milking yield is estimated to average 3.1 litres per goat per day (fat content and average total protein content of 3.1% and 3.4%, respectively).

The farmer and the veterinarian have suspected cases of listeriosis among the goats, especially in winters with extremely cold conditions or when molds were observed on hay or silages. The following symptoms were observed in the animals: nervous signs (e.g. ataxia), blindness or reduction of sight ability and spontaneous abortions. No analyses were performed on clinical specimens to confirm the diagnosis.

The cheese making process. The cheese production is based on several steps as shown in Figure 1. Tables 1 and 2 describe the inputs and the calculations used in the model. The first step is to refrigerate and cool the evening milk production from 39.5°C to 10°C during 14h. During this step, the growth of *L. monocytogenes* is possible, and the step was simulated attempting to replicate the temperature evolution, pH (6.63) and water activity (1) of the milk.

As a second step, the milk of the morning is collected at 39.5°C, and stored during 1h. *L. monocytogenes* growth is also possible during this second step. The evening production is then mixed with the morning production and the raw milk mixture is allowed to settle during 1h at room temperature in order to achieve an internal temperature of 21-22 °C. A commercial starter culture (PAL Bioprotect D and Pal LC Mix 6, Standa, Caen, France) is added to the raw milk without heating. The starter is received by the processor in the form of a powder that is reconstituted by mixing 200 g in 1 litre of milk to form the "stock solution". Milk is seeded by adding 20 ml of the "stock solution" to 100 litres of raw milk and is then kept at 22°C for a duration of 2h in order to start the fermentation process. In the fifth step, commercial rennet

(présure simple Berthelot®, laboratories Abia, Meursault, France) is added to the fermented milk (15 ml/100 litres raw milk). The mixture is then allowed to settle at 22°C for an additional period of 22h. The next step consists of draining off the water by ladling the fresh cheese (the curds) into plastic molds at 22°C. Molds are turned over three hours later. After 0,5h, the cheeses are separated from the molds, placed on metallic racks and stored in a dryer room with an air temperature of between 14.5 and 18°C and relative humidity of 70 to 75%. The seventh step is salting. This operation is repeated twice. Cheeses are first manually turned over and hand-salted on their external surfaces at a temperature of 20°C during 24h. They are replaced back in the dry room, after which they are salted on the other side during 48h at 16°C. The final products are cooled during 48h in the chilling room (with a relative humidity of close to 100%) at 1°C until distribution.

The main cheese production on this farm is of unripened fresh cheese, but other productions are sometimes performed. For non-fresh cheese, the previous diagram is completed by a ripening step that may last 6 to 7 days, during which cheeses are turned over daily. Some cheeses are dried off in an automatic apportioner or distributor. The drying off may also take place in cellulose bags or in a cheese strainer. The cheeses' final presentations may also vary.

The number of portions of 100g-cheeses produced in a week is estimated to be 5,000 cheeses in average, from which 95 % are fresh (not ripened). Around five liters are necessary to produce 1 kg of fresh cheese (due to the loss of whey during the process).

Technological analysis performed by the farmer. The farmer uses the Dornic Acidity as a quality indicator during the production process. One Dornic acid degree (°D) is equivalent to 1 mg of lactic acid in 10 ml of milk or 0.1 g/L *(23)*. This test is performed on the milk before fermentation to gain a rough assessment of its hygienic quality and on the curds to monitor the decrease in pH.

Microbiological analysis. Once the contamination episode was identified by the FASFC, several microbiological investigations were conducted. An external accredited laboratory performed these analyses. *L. monocytogenes* was detected on milk and cheese samples using the horizontal method NF EN ISO 11290-1 *(3)*. The method NF EN ISO 11290-2 was used to quantify *L. monocytogenes* in the samples *(4)*.

Technological analysis. To characterize changing factors during the cheese making process, several technological parameters were measured at different stages done at the time of the outbreak by the accredited laboratory. These parameters were:

- pH: Laboratory method derived from the ISO 2917:1999 *(2)*, using a Knick 765 Laboratory pH meter (Escolab, Kruibeke, Belgium).
- Water-activity (a_w): method based on the ISO 21807:2005 *(5)* using a Novasina TH200 water activity meter (Lachen, Switzerland).
- Salt content: Laboratory own method adapted the ISO 1841-1:1996 *(1)*.

Further characterization of isolates. After identification of the species, the Belgian national reference lab serotyped the isolates ((Institute of Public Health (IPH), Brussels, Belgium) according to a standard protocol using a commercial agglutination test (Denka Seiken, Tokyo, Japan) based on antibodies specifically reacting with somatic (O) and flagellar (H) antigens *(46)*.

Susceptibility of the strains to ten antibiotics was determined (Etest, AB BIODISK). Susceptibility to arsenic and cadmium was also performed by the method described in the literature *(33)*.

Pulsed-Field Gel Electrophoresis (PFGE) was applied in accordance with the US PulseNet protocol describing PFGE after DNA digestion with the enzymes *Apa*I and *Asc*I. PFGE enables the cutting of genomic DNA into a number of fragments comprised between 10 and 20, that facilitates the computer analysis. The regular change of the current direction in the gel

142 allows the migration of DNA fragments *(26)*. Analysis of banding patterns was performed
143 with an ImageMaster video documentation system (Amersham Pharmacia Biotech) and
144 Fingerprinting II Informatix software (Bio-Rad).

145 **QMRA applied to *L. monocytogenes* in raw goat's milk cheese – hazard**
146 **identification.** In this study, the hazard is *L. monocytogenes* and the final output of the
147 exposure assessment model is the level of contamination of raw goat's milk cheese.
148 *L. monocytogenes* is ubiquitous and is described as a short rod, catalase-negative, Gram
149 positive micro-organism with a special motility at 25°C *(29)*. In animals this bacterium has
150 been observed since 1926 and has been recognized as a major food borne pathogen since the
151 1980s. It is an intracellular pathogen that can cause a sometimes fatal human disease named
152 "listeriosis", especially prevalent among high-risk populations, namely the elderly (>60) and
153 immuno-compromised patients. In particular, *L. monocytogenes* can cause spontaneous
154 abortion in pregnant women as well as meningitis and septicaemia in newborn infants and
155 immuno-compromised people. The case-fatality risk can reach 34% *(9)*.

156 According to the report of the European Food Safety Authority (EFSA), the number of
157 reported cases of confirmed human listeriosis was estimated to be 1,381 in 2008 *(21)*. De
158 Buyser et al. *(16)* have reviewed the relationship between food borne diseases outbreaks and
159 milk products in France for the period from 1988 to 1997. This study showed that, when the
160 food vehicle was precisely known, milk products accounted for 6% of the outbreaks caused
161 by food borne pathogens.

162 In 1995 "Brie de Meaux" cheese was identified as the source of 36 listeriosis human
163 cases (including 11 deaths), while in 1997 Livarot Pont-L'évêque cheese was implicated in 14
164 cases *(16, 30)*.

165 In Belgium, many cases of listeriosis are not reported in the official statistics since most
166 cases of human listeriosis cause mild to moderate self-limited disease, and the patient does

not automatically consult a physician. Moreover, it remains difficult to assess the number of human listeriosis caused specifically by the ingestion of contaminated cheese made from raw goat's milk. Vanholme et al. *(47)* reported that the number of cases of listeriosis officially reported in Belgium in 2005 was 40, but different food sources were involved including beef, pork, dairy products, fish and ready-to-eat products (RTE). It was therefore not possible to estimate the number of listeriosis claerly attributable to cheese made from raw goat's milk. However, a serotyping comparison was possible. In 2005 serotypes 1/2a caused 55 % of cases of listeriosis reported in Belgium and serotype 4b caused 42.5% *(47)*.

QMRA applied to *L. monocytogenes* in raw goat's milk cheese – exposure assessment. Fresh unripened cheese was chosen for the exposure assessment model because it is the most sold product. Furthermore, data for this product are available to support a retrospective investigation. The principles of the Modular Process Risk Model (MPRM) methodology were used to break down the food production chain into modules *(36)* and to follow the bacteriological concentration of the pathogen throughout the process, including the eight modules presented in Figure 1: (1) storage of the evening milk, (2) storage of the morning milk, (3) mixing of the morning and evening milk, (4) adjunction of the starter to the milk, (5) adjunction of rennet to the milk, (6) draining off of curds, (7) salting at ambient temperature and (8) cooled storage. Each module generates an output that is used as an input for the next module. The simulated events are identified for each module: growth, mixing and/or partitioning. Input values are classified as process inputs, microbiological or food characteristics. Table 1 describes parameters as fixed values or probability distributions reflecting the natural variability.

Growth was simulated using primary and secondary predictive microbiology models.

A three phase linear model without lag was used to simulate the growth of *L. monocytogenes* as a function of time *(11)* as shown in Equation 1.

193

$$\begin{aligned} \ln(N_{t_k}) &= \ln(N_{t_{k-1}}) + \mu_{\max_{i(k)}} \Delta t_k \quad &&, \text{if } N_{t_k} < N_{max} \\ &= \ln(N_{\max}) &&, \text{if } N_{t_k} \geq N_{max} \end{aligned} \qquad \textbf{Equation 1}$$

195

196 where i is one of the eight modules of the process with i = 1 to 8

197 k is the recorded parameter index in the stage i with k = 1,...,n

198 Δt_k is the time interval with Δt_k = 1 hour

199 Nt_k is the bacterial population at time t_k (CFU.ml-1 or CFU.g-1)

200 $N_{\max}$ is the maximal bacterial population (CFU.ml-1 or CFU.g-1)

201 The effects of temperature, pH and water activity on the maximum growth rate $\mu_{\max}$ of

202 *L. monocytogenes* were modelled by a multiplicative function with interaction (Equation 2)

203 derived from the cardinal model (Equation 3 and 4) *(8, 14)*:

204 $$\mu_{\max i(k)} = \mu_{opt} CM_2(T_{i(k)}) CM_1(pH_{i(k)}) CM_1(a_{wi(k)}) \xi(T_{i(k)}, pH_{i(k)}, a_{wi(k)}) \qquad \textbf{Equation 2}$$

205

$$CM_n(X) = \begin{cases} 0, & X \leq X_{\min} \\ \dfrac{(X - X_{\max})(X - X_{\min})^n}{(X_{opt} - X_{\min})^{n-1}\left[(X_{opt} - X_{\min})(X - X_{opt}) - (X_{opt} - X_{\max})\left((n-1)X_{opt} + X_{\min} - nX\right)\right]} & X_{\min} < X < X_{\max} \\ 0, & X \geq X_{\max} \end{cases} \qquad \textbf{Equation 3}$$

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207

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$$\xi(\varphi(T, pH, a_w) = \begin{cases} 1, \psi \leq \theta \\ 2(1-\psi), \theta < \psi < 1 \\ 0, \psi \geq 1 \end{cases} \quad \text{with} \quad \psi = \sum_i \frac{\omega(X_i)}{2 \cdot \prod_{j \neq i} (1 - \omega(x_j))} \;,$$

209

210

$$\omega(X) = \left(\frac{X_{opt} - X}{X_{opt} - X_{\min}} \right)^3 \text{ and } \theta = 0{,}5. \qquad \textbf{Equation 4}$$

211

212

213 where $\mu_{max\ i(k)}$ is the bacterial growth rate following the environmental factors at time t_k

214 $T_{i(k)}$ is the recorded temperature at time t_k (°C)

215 $pH_{i(k)}$ is the recorded pH at time t_k

216 $a_{wi(k)}$ is the recorded water activity at time t_k

X_{min}, X_{opt} *and* X_{max}, are the minimal, optimal and maximal temperature, pH and water activity of growth for *L. monocytogenes.*

Table 2 gives the calculation details to assess the final number of *L. monocytogenes* in a typical serving of fresh goat cheese.

The starting point of the model is the initial concentration of *L. monocytogenes* in the milk from the right part of the mammary gland of the contaminated goat ($4.3.10^2$ CFU/ml or 2.63 log CFU/ml ; source: FASFC). This concentration is used in the first module to calculate the *L. monocytogenes* number per milking and to deduce the concentration of *L. monocytogenes* in the tank before the overnight storage of the evening milking. It is assumed that this concentration is a Poisson distribution and that the milk temperature decreases linearly overnight between the beginning and the end of the cooling. There is no heat exchanger plate in the food process. The temperature of the evening milk decreases slowly in the tank overnight. Predictive microbiology models simulate the growth of *L. monocytogenes* during this storage period.

The second module is dedicated to the storage of the morning milking, where the *L. monocytogenes* concentration in the tank before the storage of the morning milk is assessed and implemented in predictive microbiology models to simulate the pathogen evolution in the tank after the morning storage. The initial temperature of this second module corresponds to the temperature at the end of the milking, 39.5°C, while the final temperature obtained after one hour storage is sampled among a Pert distribution, with a most likely final temperature of 22°C and minimum and maximum final temperatures of 20 and 24°C, respectively. It is explained by the mixing of the evening and the morning milk in the same tank at the end of the storage of the morning milk.

In the third module, the concentration of the pathogen in the tank after mixing is deduced from the concentrations of *L. monocytogenes* in the tank before and after storage to

242 be implemented in modules 4 and 5 representing the steps of starter and rennet adjunction to
243 milk. Using predictive microbiology models (equations 1 to 4), the *L. monocytogenes*
244 concentration in milk before draining off the curdles is calculated. It is assumed that the
245 distribution of the pathogen is heterogeneous during the curdling of milk. Following Bemrah
246 et al. *(9)*, the *Listeria* cells concentrates at a level of 90 % in the curds and 10 % in the whey.
247 The start and end temperatures of this step are sampled among a Pert distribution, with a most
248 likely value of 22°C and minimum and maximum values of 20 and 24°C, respectively. The
249 pH is considered to decrease linearly between the start and the end of the fermentation
250 process according to equation 5.

251 $$pH = -0.1005t + pH_i$$ **Equation 5**

252 This linear relation is based on the evolution of pH and Dornic acidity with time in the
253 fermented milk (data not shown). A correlation was made between the values of Dornic
254 acidity measured by the farmer and the pH measurements in the laboratory.

255 The concentration in milk before draining off the curdles is used in module 6 to assess
256 the amount of pathogen per cheese before storage and salting. Finally, in modules 7 and 8,
257 predictive microbiology models are used to characterize the number of *L. monocytogenes* per
258 serving of cheese, taking into account the effects of temperature, pH and a_w as shown in
259 Figure 1.

260 Technological parameters measured in the milk and at different stages of ripening of
261 the final product (2 measurements per sample) are used according to Table 1 and 2.

262 The model was developed using @Risk 4.5.5 (Palisade, Ithaca, N.Y.), an add-in for
263 Microsoft Excel. Input values of each module were implemented as estimated distributions of
264 probability, to describe the natural variability associated with input factors. We used 50,000
265 iterations with the latin hypercube sampling (LHS) method to obtain stochastic estimates of
266 the output variables *(32)*. Finally, the estimated median concentration of *L. monocytogenes* in

267 a serving of cheese was compared with the concentration measured in the fresh cheese by the
268 FASFC.

269 **QMRA applied to *L. monocytogenes* in raw goat's milk cheese – sensitivity analysis.** In order
270 to identify the subset of the most important factors of the exposure assessment model, a global
271 sensitivity analysis (SA) was performed using the Saltelli method *(40)*. This is a numerical
272 based procedure for computing first order indices, S_i, and total effect indices, St_i, for all the
273 factors i (i=1,…,k) of the studied model. Each first order index S_i provides an estimate of the
274 relative importance of the factor X_i taken singularly, while the total effect index St_i reflects the
275 cooperative effects of the factor X_i and its non linear interactions with the other factors *(40,*
276 *41)*. The method is fully described in the literature *(40-44)*. Its implementation for microbial
277 growth models is provided in Ellouze et al. *(20)*, and recently this method was applied to a
278 QMRA of *L. monocytogenes* in deli meats *(12)*.

279 To calculate these indices, a characterization of the range of variation of the several
280 factors of the model is necessary. These factors are presented in Table 3 and are composed of
281 two categories of factors. The first category includes factors related to the milk production
282 such as the number of goats and the quantity of milk per milking. The second category
283 includes factors representing the characteristics of the pathogen such as its cardinal values, its
284 optimum growth rates in milk and cheese, etc.

285 A total set of 35 input factors was thus identified for the exposure assessment model.
286 Their ranges of variation were obtained directly from experimental data provided by the
287 farmer (minimum and maximum observed values) or from the 1st and 99th percentiles of the
288 distributions characterizing their variability.

289 Once the ranges of variation of the different input factors were characterized, their indices
290 were computed according to the following procedure. Two matrices A and B of N lines
291 corresponding to the N simulation runs (N=5.10^4) and k columns corresponding to the k

studied factors (k=35) were generated using the LHS method as a space filing design. The matrices A and B were filled with respect to the range of variation of each factor (Table 3) and the model was run on each row of the two matrices to provide the response vectors Y_A and Y_B. Then, k matrices C_i, i=1,…,k, were generated, containing all the columns of matrix B except the i^{th} column which was replaced by the i^{th} column of matrix A, and the global model was run again to provide the vectors Y_{Ci}. Finally, the first order indices, S_i, and total effect indices, St_i, were calculated according to the following formula *(40)*:

$$S_i = \frac{\frac{1}{N}\sum_{u=1}^{N} Y_A^{(u)} Y_{Ci}^{(u)} - g_0}{\frac{1}{N}\sum_{u=1}^{N} Y_A^{(u)} Y_A^{(u)} - f_0^2}$$

$$St_i = 1 - \frac{\frac{1}{N}\sum_{u=1}^{N} Y_B^{(u)} Y_{Ci}^{(u)} - f_0^2}{\frac{1}{N}\sum_{u=1}^{N} Y_A^{(u)} Y_A^{(u)} - f_0^2} \qquad \textbf{Equation 6}$$

with $f_0 \quad \frac{1}{N}\sum_{u=1}^{N} Y_A^{(u)}$

$g_0 \quad \frac{1}{N}\sum_{u=1}^{N} Y_A^{(u)} Y_B^{(u)}$

The bootstrap method *(18)* was used to assess the confidence intervals of these indices through reliable estimates without additional computational effort *(6)*. Values obtained for the response vectors were sampled with replacement for 10^4 bootstrap replicates, and, for each replicate, the indices S_i and St_i were calculated, leading to a bootstrap estimate of the distribution of the sensitivity indices. The 95% confidence intervals of the indices were thus defined using the 5th and 95th percentiles and were used to identify the most important factors as those for which the total effect indices were significantly different from 0.

QMRA applied to *L. monocytogenes* in raw goat's milk cheese – Scenario analysis. The effect of some variables in the exposure assessment model was assessed using simulation scenarios to provide valuable information on possible ways of reducing the concentration of

pathogens in a final serving *(17, 48)*. This assessment was achieved by selecting various combinations of input variables.

This procedure is commonly known as a "what if scenario" *(49)*. In the present study, a first run of the model without modifications was performed to provide the baseline results of the selected outputs. Three scenarios were tested in order to look for ways of reducing the risk, and one worst-case scenario was tested to assess the magnitude of the risk increase in such a case:

- Scenario 1: Install a heat exchanger plate to obtain a temperature of 7 °C directly after milking and maintain a constant temperature during the overnight storage.
- Scenario 2: Reduce pH by 0.5 units at the start of adjunction of ferment and rennet. This could be achieved, for example, by adjunction of a common food acid such as lactic acid or glucono delta lactone.
- Scenario 3: Increase efforts during production by combining Scenarios 1 and 2.
- Scenario 4: Two shedder goats in the herd each excreting the same amount of *L. monocytogenes* as the goat on the farm studied.

Decontamination treatments, such as pasteurization, are not considered to respect the initial characteristics of the product. The results are displayed as the concentration of *L. monocytogenes* in a cheese serving.

RESULTS

Alert investigations. Table 4 summarizes the information in relation to the anamnesis of the case.

The serotyping results of the first external laboratory showed that the strain isolated from the cheese belongs to serotype 1/2a with a characteristic β-hemolysis. Three days later another external laboratory analyzed the pools of milk collected from the goats. One positive pool

(milk collected from twenty goats) was detected, and from the sources of that milk one clearly positive goat was identified in the herd.

The goat was transferred to the Faculty of Veterinary medicine. The results of milk samples were as follows:

- 2.6 log *L. monocytogenes* /ml in the milk collected from the right part of the mammary gland
- absence of *L. monocytogenes* in 25 g of the milk collected from the left part of the mammary gland

Enumerations were also made on the cheeses with the following results:

- concentration of *L. monocytogenes* in fresh not ripened goat cheese: 3.6 log CFU/g
- concentration of *L. monocytogenes* in ripened goat cheese: 3.8 log CFU/g
- concentration of *L. monocytogenes* in ripened goat cheese coated with charcoal: 3.7 log CFU/g

The *L. monocytogenes* isolates collected from the milk and cheese were sensitive to the 10 investigated antibiotics and were sensitive to arsenic and cadmium.

The pulsotyping results showed that isolates from the milk of the isolated goat and from the contaminated cheese belonged to the same pulsovar A (data not shown). In 2005, the Belgian *Listeria* reference laboratory received 40 strains of *L. monocytogenes* of human clinical origin: 16 strains were of serovar 1/2a, of which 7 strains were arsenic and cadmium sensitive. However, pulsotyping excluded genetic matching for these 7 strains with the cheese and milk isolates from the goat farm (data not shown). This means that no cases of human listeriosis could be traced back to the consumption of the contaminated goat cheese.

QMRA model – baseline results. Table 5 gives the base line results of the exposure assessment and the risk characterization modules. Since these models were built to take into account the natural variability associated with the different input factors, the results are

365 expressed as distributions. The median estimates (50^{th} percentile) associated with the 5^{th} and
366 95^{th} percentiles presented in Table 5 give a good assessment of the results. *L. monocytogenes*
367 concentrations results are converted to a logarithmic scale (base 10).

368 The modular exposure assessment model shows a significant growth of *L. monocytogenes*
369 during chilling and storage of the milk collected the day before the cheese production (an
370 increase of 2.2 log CFU/ml for the median). Figure 2a gives the pathogen evolution at this
371 step with dynamic temperature conditions. During the storage of the evening milking
372 overnight, the milk is slowly chilled from 39.5°C to 10°C. The growth rate of
373 *L. monocytogenes* is directly related to the temperature, which explains the observed brake of
374 microbial growth during the chilling process.

375 A less important increase (1.2 log CFU/ml for the median) was obtained after the starter
376 and rennet adjunction to milk. This result is explained by the pH drop in the milk due to the
377 fermentation activity, which gradually decreased the pH down to 4.41. Figure 2b shows the
378 evolution of *L. monocytogenes* after the adjunction of ferment and rennet. At the end of the
379 fermentation, the pH value was close to the minimum pH for *L. monocytogenes* growth
380 (pH_{min}=4.19), which can explain the limited growth of the pathogen after the fermentation.

381 The estimated median *L. monocytogenes* concentration in a serving of cheese (Table 5)
382 was equal to 3.8 log CFU/g. This estimate was in compliance with the concentration of
383 *L. monocytogenes* reported in the fresh cheese by the FASFC during the contamination
384 episode, which was equal to 3.6 log CFU/g. The model gives satisfactory results by
385 comparison with the data provide by the FASC.

386 **Sensitivity analysis.** The global sensitivity analysis results are depicted in Table 6, which
387 presents the first order and total effect indices of each factor with their confidence bootstrap
388 intervals.

389 Total effect indices and first order indices are especially powerful when performing SA in
390 cases of non additive and non linear models *(6)* such as the exposure assessment part of the
391 model used in this study. In fact, as can be deduced from Table 6, the sum of the non negative
392 first order indices (S_i), which account for the individual contribution of each factor into the
393 variance of the output, is less than 1, which means that the variance of the output cannot be
394 explained solely by the sum of the individual effects of each factor, but is also attributed to
395 the effects of interactions.

396 This is also confirmed by the relatively substantial difference observed between the St_i
397 and the S_i for all the important factors, which indicates the significant role of interactions.
398 Ranking the 35 factors of the model according to their total effect indices identified the most
399 important factors. Total effect indices were chosen as ranking criteria because they indicate
400 the effect of each studied factor and its interactions with the other factors. They were
401 therefore preferred to the first order indices, which only reflect the relative importance of the
402 factor taken singularly.

403 The confidence intervals associated with the total effect indices were examined, and the
404 factors with confidence intervals significantly different from 0 were identified as the most
405 important factors. Four factors were thus selected: the duration of the first salting step
406 ($D_{salting1}$), the minimum pH for *L. monocytogenes* growth pH_{min}, the optimal growth rate of
407 *L. monocytogenes* in milk (μ_{opt_milk}) and the initial *L. monocytogenes* concentration (N_0).

408 **Scenario analysis**. The results of the scenario analysis are displayed in Table 7. The
409 outputs are the amount of *L. monocytogenes* per cheese serving. The results obtained for the
410 first scenario show a reduction of 1.5 log CFU/g compared with the baseline results and could
411 be a good alternative for risk mitigation. The results obtained for the second scenario prove
412 that a reduction of 0.5 pH units could only reduce by 0.2 log CFU/g the median concentration
413 compared with the baseline results. The results obtained for the third scenario, which

414 combines Scenarios 1 and 2, show a reduction of 1.8 log CFU/g of the median concentration
415 compared with the baseline results. The last scenario shows a significant increase of 0.4 log
416 *L. monocytogenes*/g in a cheese serving compared with the baseline results.

417 # DISCUSSION

418 Few QMRA concerning cheese contaminated with *L. monocytogenes* have been published
419 *(9, 22)*. This is probably due to the difficulty of obtaining valuable data to develop a complete
420 QMRA of the cheese production chain. In fact, a frequently heard criticism of QMRA is that
421 it is extremely data hungry *(27)*, and its final results depend heavily on the quality of the input
422 data, particularly when variability and uncertainty are taken into account.

423 In an attempt to simulate the entire production chain of cheese made from raw goat‘s
424 milk, an exposure assessment that takes into account different sources of natural variability
425 was developed in this study. The results showed a significant growth of *L. monocytogenes*
426 during the cheese manufacturing process, especially after the evening milk storage (median
427 increase of 2.2 log CFU/ml) and during the steps of starter and rennet adjunction to milk
428 (median increase of 1.2 log CFU/ml).

429 However, it is thought that the acidification process experienced during cheese making is
430 not favourable to pathogen growth. In fact, Schvartzman et al. *(45)* have shown that there was
431 no growth of *L. monocytogenes* 4b isolated from cow faeces during the process of cheese
432 making from raw cow’s milk, but growth was observed on the same process when pasteurized
433 milk was used, which is probably due to the absence of a competitive flora. Some studies
434 have already reported the importance of the presence and/or the level of the competitive flora
435 on the growth/no growth of *L. monocytogenes* in several foods *(25, 34)*. The model developed
436 in this study did not specifically include the effect of the competitive flora, but the simulated
437 median result for the *L. monocytogenes* concentration in the fresh cheese was equal to 3.8 log
438 CFU/g which was consistent with the concentration of *L. monocytogenes* (3.6 log CFU/g)

439 detected by the FASFC in the contaminated fresh cheese. This result does not constitute a
440 validation, it means only that the model seems to have a good behaviour with the collected
441 data.

442 Due to the lack of data, this exposure assessment model like any other may suffer
443 descriptive errors that represent incorrect or insufficient information *(24)*. In fact, the lack of
444 data sometimes made it necessary to build in several assumptions. First, the storage
445 temperature of the evening milking was considered to decrease linearly during the night.
446 Some authors have attempted to model the temperature evolution during the cheese making
447 process *(28)*, but as the linear decrease in temperature gave satisfactory simulated
448 temperatures compared to the observed temperatures, this assumption was adopted to avoid
449 over-parameterization of the exposure assessment model. Second, the excretion of
450 *L. monocytogenes* by the contaminated shedder goat was considered to be the same for each
451 production day. Third, *L. monocytogenes* distribution was supposed to be heterogeneous
452 during the curdling of milk with a level of 90 % in the curds and 10 % in the whey. This last
453 assumption was based on the study of Bemrah et al. *(9)*.

454 In spite of these limitations, this study attempts to simulate the contamination flow of raw
455 goat cheese produced on a local dairy farm and to relate the final concentration of
456 *L. monocytogenes* in cheese with observed epidemiological and microbiological data made in
457 a contamination alert episode in 2005.

458 This study also suggests risk mitigation scenarios to reduce the concentration of
459 *L. monocytogenes* at the end of the cheese process. The first scenario considered a faster
460 chilling of the milk from the initial temperature of 39.5 °C down to 7 °C. At this temperature,
461 the growth rate of *L. monocytogenes* is considerably reduced during the storage of the milk
462 over night, resulting in a reduction of 1.5 log CFU/g in the final concentration of the pathogen
463 in the cheese. The second scenario evaluated a pH reduction of 0.5 units at the start of the

464 adjunction of ferment and rennet. This intervention could be simply achieved by lactic acid
465 adjunction, for example, and could reduce the median growth of *L. monocytogenes* by 0.2 log
466 CFU/g. The third scenario, which is a combination of the previous two, showed a reduction of
467 1.8 log CFU/g of the median *L. monocytogenes* concentration compared with the model
468 baseline results. The last scenario, involving two shedder goats in the herd, showed a
469 significant increase of 0.4 log *L. monocytogenes* concentration in a serving cheese compared
470 with the base line results.

471 The results of the sensitivity analysis gave complementary information and identified four
472 factors significant for their impact on the concentration of *L. monocytogenes* in cheese.
473 Among the four significant factors, $pH_{\min}$ and μ_{opt_milk} are characteristics of the pathogen for
474 which it is therefore impossible to control. However, the sensitivity analysis also uncovered
475 the potential to act on technological parameters, such as the salting time to reduce the number
476 of contaminated cheeses. It is also feasible to perform effective actions to reduce the initial
477 contamination level N_0. Efficient and frequent monitoring of the pathogen in the food chain
478 could significantly reduce its concentration in the end product and could be easily achieved by
479 means of a more stringent sampling plan for the raw milk and cheese.

480 In the future it would be interesting to identify sources of *L. monocytogenes*
481 contamination. A few studies have explored sources of contamination on farms. Danielsson-
482 Tham et al. *(15)* studied an outbreak of gastro-intestinal listeriosis affecting 120 humans in
483 Sweden after consumption of raw-milk cheese produced in a summer farm composed of dairy
484 cattle and goats. The authors traced back the origin of contamination in the cheese by
485 investigating the different sources in the summer farm and in the cheese production facilities.
486 The most likely hypothesis, although not strictly confirmed, was the presence of a goat in the
487 herd with a subclinical mastitis which led to the contamination of equipment in the cheese
488 production facilities, specifically a wooden bench and the home-made brine. Nightingale et al.

489 *(37)* explain that the patterns of contamination on farms are not very clear: is the feed the
490 main source of infection in animals and hence in the milk products or is the animal itself?
491 Grazing seems not to be the predominant route of infection, but poorly made silages (e.g. corn
492 silages with a high end-pH value) may lead to an amplification of the levels of *Listeria*
493 species in animals. Comparing the prevalence of *Listeria monocytogenes* on case farms
494 (farms with a history of animal listeriosis) and on control farms (farms with no reports of
495 animal listeriosis) they observed that for small ruminants the prevalence was higher on case–
496 farms. Yet, for cattle the prevalence was statistically equivalent between case and control
497 farms.

498 Faecal contamination of the animals may explain the greater prevalence of listeriosis in
499 small ruminants on case farms. If it is the cause, then faecal contamination may also increase
500 the likelihood of *Listeria monocytogenes* contamination of raw milk. The fact that patterns of
501 contamination appear quite different between the cattle and the goat populations led
502 Danielsson-Tham et al. *(15)* to conclude that some ribotypes may be able to persist in the
503 environment and others not. Wiedmann et al. *(51)* also concluded that multiple ribotypes may
504 exist on a farm at the same time, colonizing differently depending on environmental factors,
505 because the researchers observed in only one case a relationship between the strains isolated
506 in clinical samples of animals and strains isolated from silages. In our study, we assume that
507 the contamination of raw milk occurred mainly through the milk, even though *Listeria*
508 *monocytogenes* could have been recovered from the faecal matter of the asymptomatic
509 shedder goat.

510 Meyer-Broseta et al. *(35)*, using different sampling strategies, noticed that *Listeria*
511 *monocytogenes* occurs at a very low level in the tank. When the bulk tanker in the milk
512 processing industry was contaminated (collecting different deliveries from cattle farms), the
513 average prevalence for positive farms was 7.7% with contamination levels for

514 *L. monocytogenes* below 3 CFU/ml and a median of between 5.10^{-2} and 1.10^{-1} CFU/ml.
515 Several authors have noticed that it is important to have a final pH-value in the silages that is
516 lower than 5 in order to decrease the risk of listeriosis in ruminants *(50, 51)*. In our case, the
517 farmer observed a significant decrease of listeriosis cases in the goats after he had adapted his
518 machine for producing hay and low-moisture grass-silages. He began cutting the grass higher,
519 which may have reduced the contamination of the hay and silages by dirt and dust from the
520 soil. This decrease in contamination may explain the decrease in observed cases of listeriosis
521 in the goats. The farmer also observed that the remaining cases of listeriosis were more
522 frequent during the winter season, which is consistent with findings in the scientific literature
523 *(10)*.

524 In conclusion, this retrospective study aims to simulate the fate of *L. monocytogenes*
525 throughout a raw goat's milk cheese making process from milking to delivery to the market,
526 using quantitative microbial risk assessment methodology. The results were satisfactory when
527 compared with the epidemiological and microbiological observations of the alert
528 investigations. The most important factors of the *L. monocytogenes* contamination were
529 identified, and risk mitigation scenarios were evaluated to identify the most efficient
530 strategies to reduce the risk of listeriosis in respect of the food characteristics.

531 Due to the lack of data for some parameters, the exposure assessment was based on
532 several assumptions. Although continued work is needed to better evaluate risks to
533 consumers, this study clearly demonstrated that QMRA tools and predictive modelling can be
534 useful to increase food safety and should be gradually implemented in the coming years.

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List of tables

Table 1: Characterization of the model inputs

Cheese making process	Parameter	Description	Values / Distributions	Units
0. Characterization of the milk production	N_{goat}	Number of goats	350	goats
	$N_{goat\ prod}$	Number of goats for the cheese production	$P(160;170;180)$	goats
	$N_{contaminated.goat}$	Number of contaminated goats for the cheese production	1	goats
	$Q_{milk/goat/day}$	Quantity of milk per goat per day	$P(2.5;3;3.5)$	litres/day
	$N_{milking/day}$	Number of milkings per day	2	times/day
	L_{chesse}	Numbers of litres per 1 Kg of cheese	$P(4;5;5.5)$	litres
	W_{cheese}	Weight of cheese	$P(95;100;105)$	g
	$N_{days.prod}$	Number of days for cheese production per week	5	days
	$N_{week.prod}$	Number of weeks of production with the contaminated milk	19	weeks
	$N_{servings/cheese}$	Number of servings per cheese	2	servings
1. Storage of the evening milk	$T_{start.tank.evening}$	Initial temperature of milk collected in the evening	39.5	°C
	$T_{end.tank.evening}$	Final temperature of milk collected in the evening	$P(9;10;12)$	°C
	$D_{tank.evening}$	Duration of the evening milk storage overnight	$P(13;14;15)$	h
2. Storage of the morning milk	$T_{start.tank.morning}$	Initial temperature of milk collected in the morning	39.5	°C
	$T_{end.tank.morning}$	Final temperature of milk collected in the morning	$P(20;22;24)$	°C
	$D_{tank.morning}$	Duration of the morning milk storage before mixing	1	h
3. Mixing	$T_{end.mix}$	Milk temperature after mixing	$P(20;22;24)$	°C
	D_{mix}	Duration of the step mixing of the morning and evening milk	1	h
4. Adjunction of starter	$T_{starter}$	Milk temperature at the step adjunction of starter	$P(20;22;24)$	°C
	$D_{starter}$	Duration of the step adjunction of starter	2	h
	$pH_{starter}$	pH at the end of the step adjunction of starter	6.3	pH units
5. Adjunction of rennet	T_{rennet}	Milk temperature at the adjunction of rennet	$P(20;22;24)$	°C
	D_{rennet}	Duration of the step adjunction of rennet	22	h
	pH_{rennet}	pH at the end of the step adjunction of rennet	4.41	pH units
6. Draining off curdles	$T_{curdles}$	Temperature at the step draining off curdles	$P(20;22;24)$	°C
	$D_{curdles}$	Duration of the step draining off curdles	0.5	h
7. Saltings 1 and 2	$T_{salting1}$	Temperature at the step Salting 1	$P(19;20;21)$	°C
	$D_{salting1}$	Duration of the step Salting 1	24	h
	$pH_{salting1}$	Final pH obtained after the step Salting 1	4.28	pH units
	$T_{salting2}$	Temperature at the step Salting 2	$P(14;16;18)$	°C
	$D_{salting2}$	Duration of the step Salting 2	48	h
	$pH_{salting2}$	Final pH obtained after the step Salting 2	4.42	pH units
8. Cooled storage	$T_{storage}$	Temperature of the cooled storage	$P(0;1;2)$	°C
	$D_{storage}$	Duration of the cooled storage	48	h

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***L. monocytogenes* / matrices characteristics**	**Parameter**	**Description**	**Values / Distributions**	**Units**	**References**
L. monocytogenes characteristics	T_{min}	Minimum temperature for growth (°C)	N(-1.8;0.72)	°C	*(7, 19, 29)*
	T_{opt}	Optimal temperature for growth (°C)	N(38.2;0.76)	°C	
	T_{max}	Maximal temperature for growth (°C)	N(43.3;1.2)	°C	
	pH_{min}	Minimum pH for growth	N(4.19;0.12)	pH units	
	pH_{opt}	Optimal pH for growth	7	pH units	
	pH_{max}	Maximum pH for growth	9.61	pH units	
	$a_{w\,min}$	Minimum a_w for growth	N(0.922;0.009)	a_w units	
	$a_{w\,opt}$	Optimal a_w for growth	0.997	a_w units	
	$a_{w\,max}$	Maximal a_w for growth	1	a_w units	
Growth characteristics in milk	$\mu_{opt.milk}$	Optimal growth rate in milk	N(0.75; 0.13)	h^{-1}	*(8, 39)*
	N_{max}	Maximum concentration in milk	7	log CFU/ml	
	pH_{milk}	pH of the milk	6.63	pH units	(measured)
	$a_{w\,milk}$	a_w of the milk	0.99	a_w units	(measured)
Growth characteristics in cheese	$\mu_{opt.cheese}$	Optimal growth rate in cheese	NT(0.21;0.19;0.02;0.6)	h^{-1}	*(8, 39)*
	N_{max}	Maximum concentration in cheese	7	log CFU/g	
	pH_{cheese}	pH of the cheese	4.28	pH units	(measured)
	$a_{w\,cheese}$	a_w of the cheese	0.977	a_w units	(measured)

713 *N (m; s),* normal distribution with expected value *m* and standard deviation *s*.
714 *NT (m; s; a; b)* normal distribution with expected value *m* and standard deviation *s* truncated on [*a; b*].
715 *P(a ; b; c)*= Pert distribution with the minimum *a*, most likely *b* and maximum values *c*

716 **Table 2:** Calculation details to assess the contamination of a serving of cheese

Parameters	Description	Calculations	Units
Milk production			
$Q_{milk/milking}$	Quantity of milk per milking ($Q_{milk.morning\ or}$ $Q_{milk.evening}$)	$Q_{milk/milking}= Q_{milk/goat/day} /N_{milking/day}$	litres/milking
$N_{cheese/batch}$	Number of cheeses per batch	$N_{cheese/batch}= N_{goat\ prod}*Q_{milk/goat/day} /L_{chesse}$ *1000/W_{cheese}	units
$N_{cheese/prod}$	Number of cheeses produced with the contaminated milk	$N_{cheese/prod}=N_{cheese/batch}* N_{days.prod}*N_{week.prod}$	units
$N_{cont.servings}$	Number of contaminated servings	$N_{cont.servings} =N_{cheese/prod}*N_{servings}$	servings
Storage of the evening milking (module 1)			
$C_{ini.conc}$	Concentration of *L. monocytogenes* in the milk from the right mammary gland of the shedder goat	$4.3.10^2$ (i.e. 2.63 log CFU/ml)	CFU/ml
$N_{milking}$	Number of *L. monocytogenes* from the infected goat per milking	$N_{milking}=C_{ini.conc}$*1000*$Q_{milk/milking}$/2	CFU/milking
$C_{tank\ evening\ milk}$	Concentration of *L. monocytogenes* in a tank before storage over night of the evening milk	$C_{\text{tank evening milk}}= Pois(N_{milking}/(N_{goat\ prod}*Q_{milk/milking}*1000))$	CFU/ml
$C_{tank\ night\ stor}$	Concentration of *L. monocytogenes* in a tank after storage over night of the evening milk	Dynamic predictive microbial model, equations 1 to 4 (table 1)	CFU/ml
Storage of the morning milking, mixing of milkings and adjunction of ferment and rennet (modules 2, 3, 4 and 5)			
$C_{tank\ morning\ milk}$	Concentration of *L. monocytogenes* in a tank before storage of the morning milk	$C_{tank\ morning\ milk}= Pois(N_{milking}/(N_{goat\ prod}*Q_{milk/milking}*1000))$	CFU/ml
$C_{tank\ morning\ stor}$	Concentration of *L. monocytogenes* in a tank after storage of the morning milk	Dynamic predictive microbial model, equations 1 to 4 (table 1)	CFU/ml
$C_{after\ mix}$	Concentration of *L. monocytogenes* in a tank after mixing	$C_{after\ mix}=(C_{tank\ night\ sto}/2)+(C_{tank\ morning\ stor}/2)$	CFU/ml
$C_{ferment,rennet}$	Concentration in milk before draining off the curdles	Dynamic predictive microbial model, equations 1 to 4 (table 1)	CFU/ml
Draining off of curdles, storage and salting, cooled storage and wrapping (modules 6, 7 and 8)			
C_{cheese}	Concentration of *L. monocytogenes* per cheese before storage and salting	$C_{cheese=}$ ($C_{ferment,rennet}$*1000* L_{chesse}*(90/100))/1000	CFU/g
N_{cheese}	Number of *L. monocytogenes* per cheese	$N_{cheese}= C_{cheese}*W_{cheese}$	CFU/cheese
$C_{serving}$	Number of *L. monocytogenes* per cheese at presentation to customers	Dynamic predictive microbial model, equations 1 to 4 (table 1)	CFU/g
$N_{serving}$	Number of *L. monocytogenes* per serving of cheese	$N_{serving}= C_{serving}*W_{cheese}/N_{servings/cheese}$	CFU/serving

717 $Pois(\lambda)$=Poisson distribution with the lambda value

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723 **Table 3:** Identification of input factors for the sensitivity analysis and their respective
724 ranges of variation

	Parameters	Description	Units	Value in the model	Ranges of variation
Cheese making process	$N_{\text{goat prod}}$	Number of goats for the cheese production	goats	P(160; 170; 180)	50-1000
	$N_{\text{contaminated.goat}}$	Number of contaminated goats	goats	1	1-50
	$N_{\text{mamgalnd.ex}}$	Number of excreting mammary glands	glands	1	1-2
	$T_{end.tank.evening}$	Final temperature of milk collected in the evening	°C	P(9;10;12)	5-14
	$D_{tank.evening}$	Duration of the evening milk storage overnight	h	P(13;14;15)	10-18
	$T_{end.tank.morning}$	Final temperature of milk collected in the morning	°C	P(20;22;24)	17-27
	$D_{tank.morning}$	Duration of the morning milk storage before mixing	h	1	1-8
	$pH_{starter}$	pH at the end of the step adjunction of starter	pH units	6.30 *	5.5-7.0
	D_{rennet}	Duration of the step adjunction of rennet	h	22	18-24
	pH_{rennet}	pH at the end of the step adjunction of rennet	pH units	4.41 *	4.0-5.0
	$D_{Curdles}$	Duration of the step draining off curdles	h	0.5	0.25-2.00
	$T_{salting1}$	Temperature at the step Salting 1	°C	P(19;20;21)	17-27
	$D_{salting1}$	Duration of the step Salting 1	h	24	12-72
	$pH_{salting1}$	Final pH obtained after the step Salting 1	pH units	4.28 *	4.00-5.00
	$T_{salting2}$	Temperature at the step Salting 2	°C	P(14;16;18)	10-22
	$D_{salting2}$	Duration of the step Salting 2	h	48	36-96
	$pH_{salting2}$	Final pH obtained after the step Salting 2	pH units	4.42	4.00-5.00
	$T_{storage}$	Temperature of the cooled storage	°C	P(0;1;2)	-1-5
	$D_{storage}$	Duration of the cooled storage	h	48	24-96
L. monocytogenes characteristics	N_0	Initial concentration of *L. monocytogenes*	CFU/ml	$4.3.10^2$	0.00-10000
	T_{min}	Minimum temperature for growth (°C)	°C	N(-1.8;0.72)	-5- -1
	T_{opt}	Optimal temperature for growth (°C)	°C	N(38.2;0.76)	35- 41
	T_{max}	Maximum temperature for growth (°C)	°C	N(43.3;1.2)	41- 45
	pH_{min}	Minimum pH for growth	pH units	N(4.19;0.12)	3.00-5.00
	pH_{opt}	Optimal pH for growth	pH units	7	6.00-8.00
	pH_{max}	Maximum pH for growth	pH units	9.61	8.0-10.0
	$a_{w\ min}$	Minimum a_w for growth	a_w units	N(0.922;0.009)	0.850-0.960
	$a_{w\ opt}$	Optimal a_w for growth	a_w units	0.997	0.98-0.995
	$a_{w\ max}$	Maximum a_w for growth	a_w units	1	0.995-1
	$\mu_{\text{opt.milk}}$	Optimal growth rate in milk	h^{-1}	N(0.75;0.13)	0.50-1.00
	pH_{milk}	pH of the milk	pH units	6.63	6.00-7.00
	$a_{w\ milk}$	a_w of the milk	a_w units	0.99	0.990-1.000
	$\mu_{\text{opt cheese}}$	Optimal growth rate in cheese	h^{-1}	NT(0.21;0.19;0.02;0.6)	0.02-0.61
	pH_{cheese}	pH of the cheese	pH units	4.28	3.80-5.20
	$a_{w\ cheese}$	a_w of the cheese	a_w units	0.977	0.960-0.990

725 *N (m; s),* normal distribution with expected value *m* and standard deviation *s*.
726 *NT (m; s; a; b)* normal distribution with expected value *m* and standard deviation *s* truncated on [*a; b*].
727 *P(a ; b; c)*= Pert distribution with the minimum *a*, most likely *b* and maximum values *c*
728 * measured values
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730 **Table 4:** Anamnesis elements in relation to the carrier animal

Date	Event	Remark / result
22 February 2004	birth of the shedder goat	
20 March 2005	Analyses of final products (goat cheeses made from raw milk) upon request of the FASFC. Results of analyses favourable (i.e. no *L. monocytogenes* found in the products)	no *L. monocytogenes* found in the products
In March 2005	Dropping of the shedder goat and start of the lactation process for this goat.	
11 July 2005	New analyses of final products (goat cheeses) upon request of the FASFC in an external laboratory. The herd is then blocked and the sale of cheese is prohibited by FASFC. The farmer himself performs the recall of products.	Positive results (i.e. *L. monocytogenes* isolated in the samples: presence in 25 g samples).
12 July 2005	Numbers of *L. monocytogenes* in different final products. Serotyping performed	▪ fresh cheese not ripened: $4.3.10^3$ CFU/g ; ▪ goat cheese ripened: $6.5.10^3$ CFU/g ▪ goat cheese ripened and coated with charcoal: $5.1.10^3$ CFU/g Serotype 1/2a with a β-hemolysis.
15 July 2005 to 18 July 2005	Seeking out the excreting goat by analysing pools of 20 samples of milk from goats collected directly after the milking process.	Only 1 pool was positive with identification of only one clearly excreting goat in the herd.
19 July 2005	Transfer of the goat to the faculty of veterinary medicine (Liège University).	No clinical signs were observed in this animal after complete clinical examination.
End of July 2005	Re-start of the fabrication process with mandatory analyses imposed by the competent authority in order to perform a surveillance of *L. monocytogenes* contamination in the final products (5 samples on the 1st batch of the final products, then 1 sample each [for the next] 5 batches of final products – both ripened goat cheese and not ripened).	no *L. monocytogenes* found in the products (in 25-g of analytical samples)
14 September to 22 September 2005	Milk samples taken on the hospitalized goat in the two different parts of the mammary gland.	▪ $4.3.10^2$ CFU *L. monocytogenes*/ml for the right part. [The isolated strain will harbour the following internal lab reference: 05/180 D] ▪ Absence of *L. monocytogenes* in 25 ml for the left part of the mammary gland
4 October 2005	The isolated strain is sent to the National Reference Laboratory for Listeria (NRL) in Brussels (Scientific Institute of Public Health). Meanwhile, another strain (the one isolated from the cheese; analyses performed by another external accredited laboratory) was also sent to the National Reference Laboratory (so the NRL had two strains originating from this farm).	
11 October to 3 November 2005	Serotyping at the National Reference Laboratory, antibiogram and PFGE (Pulse Field Gel Electrophoresis) on one strain isolated in the milk, one strain isolated in the cheese and three strains of human origin (collected from human patients with	confirmation of serotype 1/2a, with a β-hemolysis

	listeriosis).	
September to December 2005	Request of surveillance of final products in the herd at a frequency of 1 analysis/15 days of production	no *L. monocytogenes* found in the products
From January 2006	Request of surveillance of final products in the herd at a frequency of 1 analysis/6 months of production (with the following parameters: *Listeria*, *Salmonella*, *E. Coli* and coagulase – positive *Staphylococci*).	no *L. monocytogenes* found in the products

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733 **Table 5:** Baseline results of the exposure assessment and the risk characterization modules

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Modules	Item	Acronym	Percentiles			Unit
			5th	50th	95th	
Milking	Concentration in a tank before storage over night of the evening milk	$C_{tank\ evening\ milk}$	-5	0	0.47	log CFU/ml
Storage of the evening milk before mixing	Concentration in the tank after storage over night of the evening milk	$C_{tank\ night\ stor}$	-3.2	2.2	3.1	log CFU/ml
Adjunction of ferment and rennet	Concentration before draining off the curdles	$C_{ferment,rennet}$	-2.2	3.2	4.1	log CFU/ml
Draining off the curds	Number of *L. monocytogenes* per cheese	N_{cheese}	0.5	5.8	6.8	log CFU/cheese
Cooled storage and wrapping	Concentration of *L. monocytogenes* in a serving of cheese	$C_{serving}$	-1.5	3.8	4.8	log CFU/g
	Number of *L. monocytogenes* per serving of cheese	$N_{serving}$	0.2	5.5	6.5	log CFU/serving

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738 **Table 6:** Estimates of the significant first order (S_i) and total effect (St_i) indices of the

739 sensitivity analysis and their bootstrap confidence intervals (p5 and p95)

Parameters	Description	Ranges of variation	St_i	[p5	p95]	S_i	[p5	p9
$D_{salting1}$	Duration of the salting step 1	12-72	0,78	0,68	0,88	-0,14	-0,20	-0,
pH_{min}	Minimum pH for growth	3.00-5.00	0,40	0,30	0,50	-0,14	-0,19	-0,
μ_{opt_milk}	Optimal growth rate in milk	0,50-1,00	0,19	0,08	0,30	-0,20	-0,23	-0,
N_0	Initial concentration of *L. monocytogenes*	0.00-4.00	0,12	0,02	0,23	0,00	-0,03	0,(

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743 **Table 7:** Results of the scenarios analysis (Concentration of *L. monocytogenes* in a
744 cheese (log CFU/g))

Scenarios	Percentiles		
	5th	**50th**	**95th**
Baseline results	-1.5	3.8	4.8
Scenario 1: Installation of a heat exchanger plate to obtain a temperature of 7 °C directly after milking and maintain a constant temperature during the overnight storage.	-2.9	2.3	2.8
Scenario 2: pH reduction of 0.5 units at the start of adjunction of ferment and rennet. This could be achieved, for example, by adjunction of food acid.	-1.7	3.6	4.6
Scenario 3: Increase efforts in the production by combining previous scenarios.	-3.1	2.0	2.6
Scenario 4: 2 shedder goats excreting each 2.6 log *L. monocytogenes*/ml in the right part of the mammary gland.	-1.0	4.2	5.1

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1 List of figures

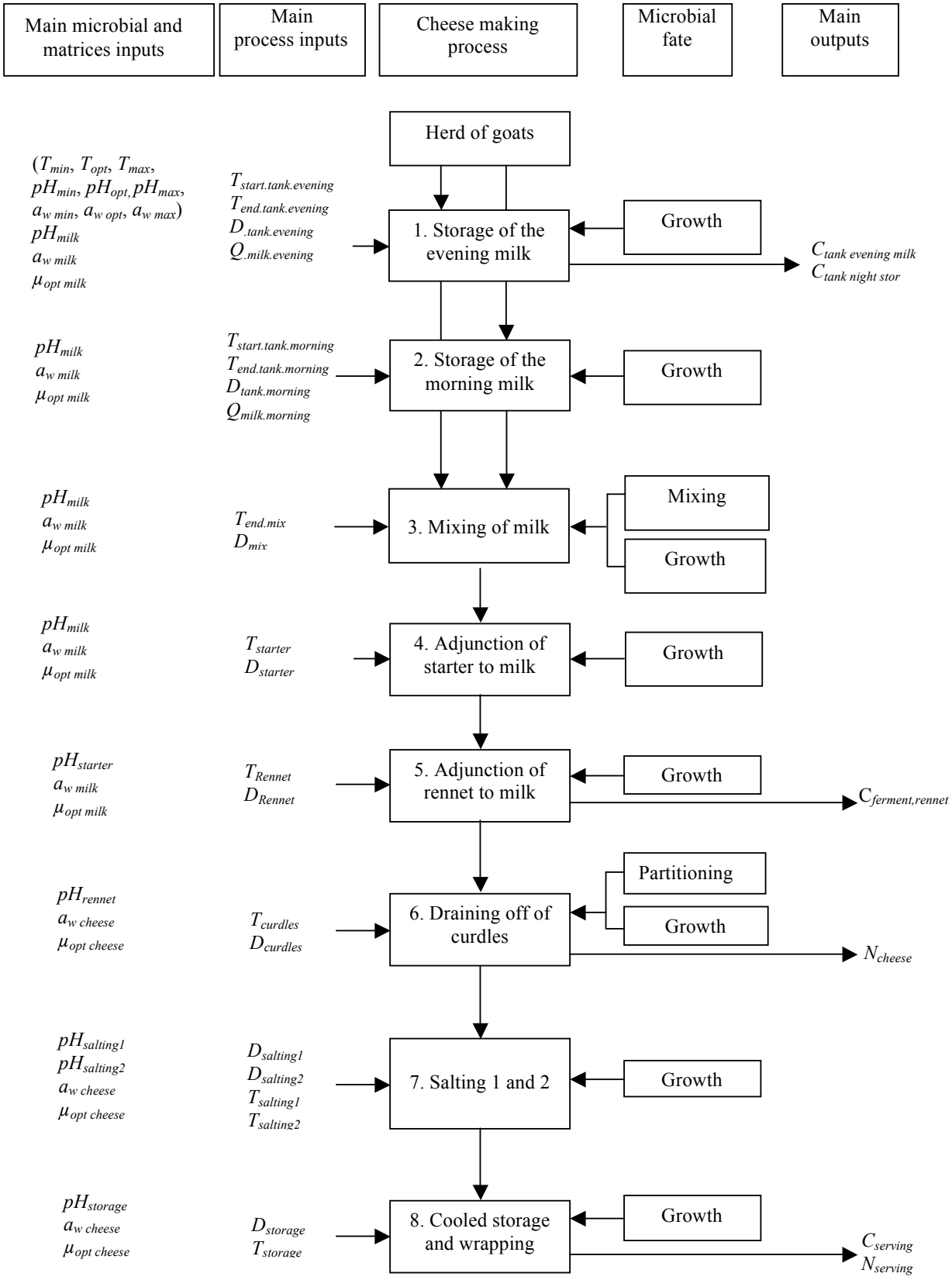

Figure 1: Presentation of the exposure assessment model

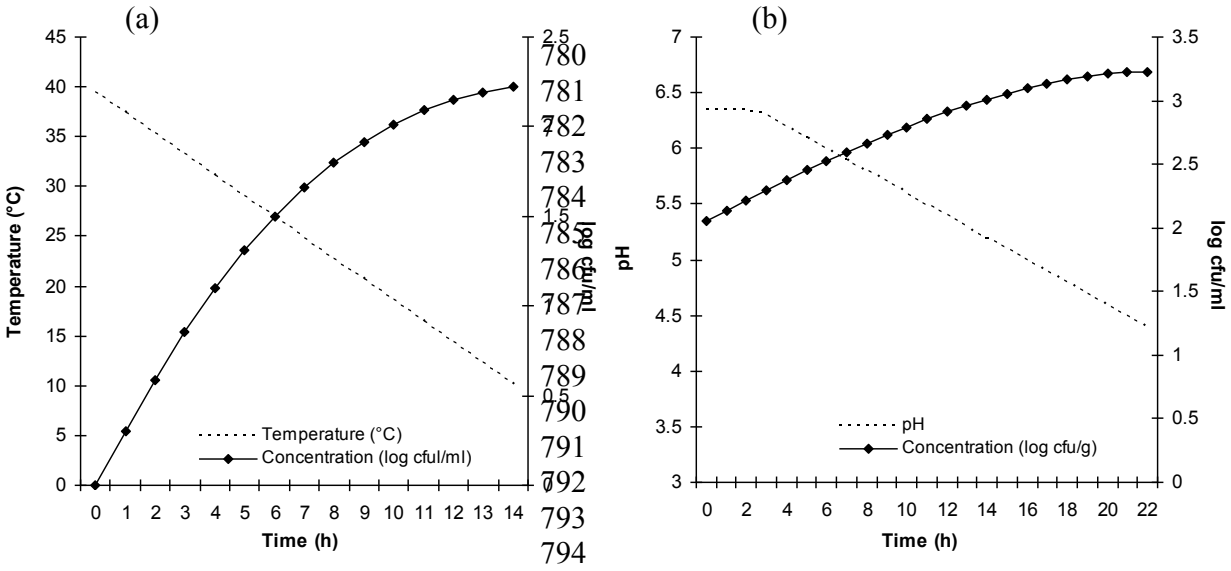

Figure 2: Evolution of *L. monocytogenes* concentration in function (a) of temperature during the storage of the evening milk in the tank and (b) pH after the adjunction of rennet to milk steps