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Quantitative Risk Assessment of Haemolytic and Uremic Syndrome Linked to O157:H7 and Non-O157:H7 Shiga-Toxin Producing *Escherichia coli* Strains in Raw Milk Soft Cheeses

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Shiga-toxin producing *Escherichia coli* (STEC) strains may cause human infections ranging from simple diarrhea to Haemolytic Uremic Syndrome (HUS). The five main pathogenic serotypes of STEC (MPS-STEC) identified thus far in Europe are O157:H7, O26:H11, O103:H2, O111:H8, and O145:H28. Because STEC strains can survive or grow during cheese making, particularly in soft cheeses, a stochastic quantitative microbial risk assessment model was developed to assess the risk of HUS associated with the five MPS-STEC in raw milk soft cheeses. A baseline scenario represents a theoretical worst-case scenario where no intervention was considered throughout the farm-to-fork continuum. The risk level assessed with this baseline scenario is the risk-based level. The impact of seven preharvest scenarios (vaccines, probiotic, milk farm sorting) on the risk-based level was expressed in terms of risk reduction. Impact of the preharvest intervention ranges from 76% to 98% of risk reduction with highest values predicted with scenarios combining a decrease of the number of cow shedding STEC and of the STEC concentration in feces. The impact of postharvest interventions on the risk-based level was also tested by applying five microbiological criteria (MC) at the end of ripening. The five MCs differ in terms of sample size, the number of samples that may yield a value larger than the microbiological limit, and the analysis methods. The risk reduction predicted varies from 25% to 96% by applying MCs without preharvest interventions and from 1% to 96% with combination of pre- and postharvest interventions.

KEY WORDS: Microbiological criteria; preharvest intervention; quantitative risk assessment model; raw milk soft cheese; STEC

1. INTRODUCTION

Shiga-toxin producing *Escherichia coli* (STEC) strains are *E. coli* strains that produce Shiga-toxins (Stx1 and Stx2), toxins very similar to the one produced by *Shigella dysenteriae* type 1, encoded by the *stx* genes.

Although not all STEC are pathogenic for humans, some strains may cause human infections. The most pathogenic for humans may cause severe illnesses, such as the Haemolytic and Uremic Syndrome (HUS), which is the leading cause of renal failure in young children. STEC strains isolated from

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HUS cases are Enterohaemorrhagic *E. coli* (EHEC) strains. Five main pathogenic serotypes of STEC (MPS-STEC) have been identified up until now in Europe: O157:H7, O26:H11, O103:H2, O111:H8, and O145:H28, but a large number of other STEC serotypes that are less frequently involved in human cases or outbreaks are also known as STEC serotypes O21 and O121, which belong to the six most common non-O157:H7 serogroups in the United States.[1]

In 2011, the total number of reported STEC cases in the European Union was 9,478 (overall notification rate of 1.97 per 100,000). Among the confirmed STEC cases, 3,861 were attributed to the atypical STEC serotype O104:H4 that caused a large outbreak in Germany.[2,3]

In France, monitoring of STEC infections is conducted through the surveillance of HUS in children up to 15 years old. In 2009, 109 cases of HUS were identified (0.91 cases per 100,000 children up to 15 years old) and STEC infection was confirmed in 60% of the 106 tested cases.[4] In France, even though outbreaks linked to STEC are mostly associated with the consumption of ground beef,[5–8] in 2005 one outbreak associated with the consumption of cheese was reported. This outbreak included 16 confirmed cases of HUS associated with the consumption of raw milk soft cheese contaminated with *E. coli* O26 and O80.[9]

Human infection may be acquired through the consumption of contaminated food or water, or by direct transmission from person to person, or from infected animals to humans. Ruminants, especially cattle, have been implicated as a principal reservoir of STEC.[10]

Overall, the reported prevalence of healthy carriage of STEC in cattle ranged from 0% to 71% of animals and 0% to 100% of herds.[10,11]

The reported prevalence of STEC strains in fecal samples and in milk and dairy products is highly variable. STEC O157:H7 has been isolated from 0% to 41.5% of fecal samples collected from healthy calves or cattle.[12–14] The STEC strains isolation in milk and dairy products in Europe varied from 0% to 13%, while total gene *stx* prevalence was from 0% to 31%.[15]

Before comparing data from different studies, it is important to consider the differences in sampling strategies and applied analytical methods. First, the researches of *stx* genes and the STEC strain isolation have to be distinguished. Since *stx* genes can be present in nonpathogenic organisms, in phage, or be unlinked to a bacterium, *stx* gene observed prevalence is likely to overestimate the actual cattle STEC excretion prevalence. Second, the most commonly used isolation method targets only the O157:H7 serotype, while fewer investigations have been conducted with analytical methods aiming at detecting all or selected non-O157 serotypes of STEC.

Raw milk could be contaminated by direct excretion into the milk as a result of clinical or subclinical mastitis in dairy cows and sheep. This intramammary source of STEC is still debated, some observations indicating possible udder infection with STEC strains that were found in milk samples.[16] In contrast, none of the 123 *E. coli* strains responsible for clinical mastitis cases in France carried the *stx* gene.[17] Extramammary contamination occurring due to fecal contamination of teats is more common.[11]

The different cheese technologies may influence the likelihood of contamination with, and the growth and survival of STEC. Control of contamination depends on good practices at the farm and manufacturing level, and the growth or survival of STEC present in the products depends on measurable parameters such as time, temperature, pH, and water activity. Control on these parameters may be based on critical control point establishment. STEC strains can survive or grow during cheese making in some processing technologies particularly in soft and semisoft cheeses.[18–22]

Currently, raw milk cheeses in Europe and especially in France constitute an important economic niche in which its producers have the benefit of competitive advantage. As an indication, of the 181,351 tons raw cow milk cheeses produced in France in 2010, approximately 13% were raw milk soft cheeses.[23] In 1998, the European Union began the enforcement of two directives (92/46 and 92/47) establishing the conditions in which raw milk cheese production would continue to be permitted and mandating cheese producers themselves to play a significant role in monitoring the safety of their products. Strategies for reducing human exposure to STEC from consumption of raw milk cheeses may include a set of preharvest and postharvest interventions.[15]

A stochastic quantitative microbial risk assessment (QMRA) model was developed to evaluate the effect of pre- and postharvest interventions on public health risks associated with consumption of raw milk soft cheeses in France. The QMRA model was driven by the availability of data. New data on STEC

serotype prevalence in dairy cows, growth and survival of STEC during cheese processing and storage, and dose-response models were used. The impact on the risk of HUS was assessed for different scenarios combining pre- and postharvest interventions, with a set of potential specifications of microbiological criteria (MC) including different detection tools.

2. MATERIALS AND METHODS

2.1. Model Overview

The model considers typical raw milk soft cheese dairy plants collecting milk from 31 dairy herds. A flowchart (Fig. 1) describes the soft cheese production system. The mathematical model is based on previous QMRA studies.[24,26] The model combines three modules: farm module, cheese production module, and consumer module.

The outcome of the first module is the probability distribution of the level of milk concentration ($\log_{10}$ CFU/mL), which was assessed using data on within-herd animal shedding prevalence, number of colony-forming units (CFU) shed by infected animals, amount of milk produced per herd, and indirect data about the amount of fecal contamination in bulk tank milk per milking. The second module was constructed from the level of milk tank contamination and considers a fixed production size of 23,000 raw milk soft cheeses (equivalent of 50,000 L of milk). For each iteration, the module estimates the probability of a portion of 25 g of cheese being contaminated with STEC or one of MPS-STEC strains and, given that a portion is contaminated, the distribution of the number of organisms at the end of production. The third module assesses the probability and the level of contamination at time of consumption and the associated risk of HUS.

The model was run for different scenarios: baseline scenario without management intervention and a set of risk management scenarios combining pre- and postharvest interventions. The outputs of the risk management scenario are compared to the result of the baseline in term of risk reduction. All the modules were implemented using SAS software (SAS version 9.3 TS).

2.2. Farm Module

The likelihood of STEC contamination in milk by the intramammary route is considered to be negligible: lactating dairy animals carry STEC in their intestinal tracts, excrete it in their feces, which in turn soils the teats, and the milk could be subsequently contaminated during the milking process. The total CFU (FTM_{id}) in the bulk tank belonging to farm i on day d can be estimated using the model established by Clough, Clancy, and French[25] described in Equation (1):

$$FTM_{id} = \sum_{j=1}^{k_{id}} \frac{STECf_{ijd} \times FM_{ijd}}{1000}, \qquad (1)$$

where

- k_{id} is the number of infected animals in herd i at day d. k_{id} is modeled by a Binomial distribution as $k_{id} \approx Binomial\ (N_{\text{cow}}, p_i)$ with N_{cow} the number of lactating cows in herd i and p_i the prevalence of cows shedding STEC strains in feces in herd i.
- $STECf_{ijd}$ ~Weibull ($a = 0.264$, $b = 16.288$) is the number of STEC (in CFU) per gram of feces from infected cow j in herd i on day d.
- FM_{ijd} ~Gamma (α, β) is the amount of fecal material (milligrams) entering the bulk tank from infected cow j in herd i at day d; with shape parameter $\alpha = 0.05$ and scale parameter $\beta = 600$.

Data on the amount of feces arriving in the bulk tank milk are sparse; Clough, Clancy, and French[25] reflect this amount of feces using a Gamma distribution established by expert elicitation. The variability between herds in relation to the implementation of housing and milking hygiene practices is not modeled on this gamma distribution. A reliable method to directly determine the amount of fecal matter in milk is not available. We hence developed an indirect approach based on collected data on *E. coli* and the assumption that *E. coli* and STEC strains follow the same fecal routes. The modified model that considers the concentration of *E. coli* (λ_{id}^{EC}) and STEC (λ_{id}^{STEC}) in bulk tank milk is described by Equation (2) (see Appendix A for the mathematical derivation):

$$\lambda_{id}^{STEC} = \overline{STECf}_{ijd} \times \frac{\lambda_{id}^{EC}}{\overline{ECf}_{ijd}}, \qquad (2)$$

where $\frac{\lambda_{id}^{EC}}{\overline{ECf}_{ijd}}$ represents the quantity of feces (g) contaminating 1 mL of milk in the bulk tank.

At herd level, the arithmetic mean of STEC concentration in fecal materials in herd i ($\overline{STECf}_{ijd}$)

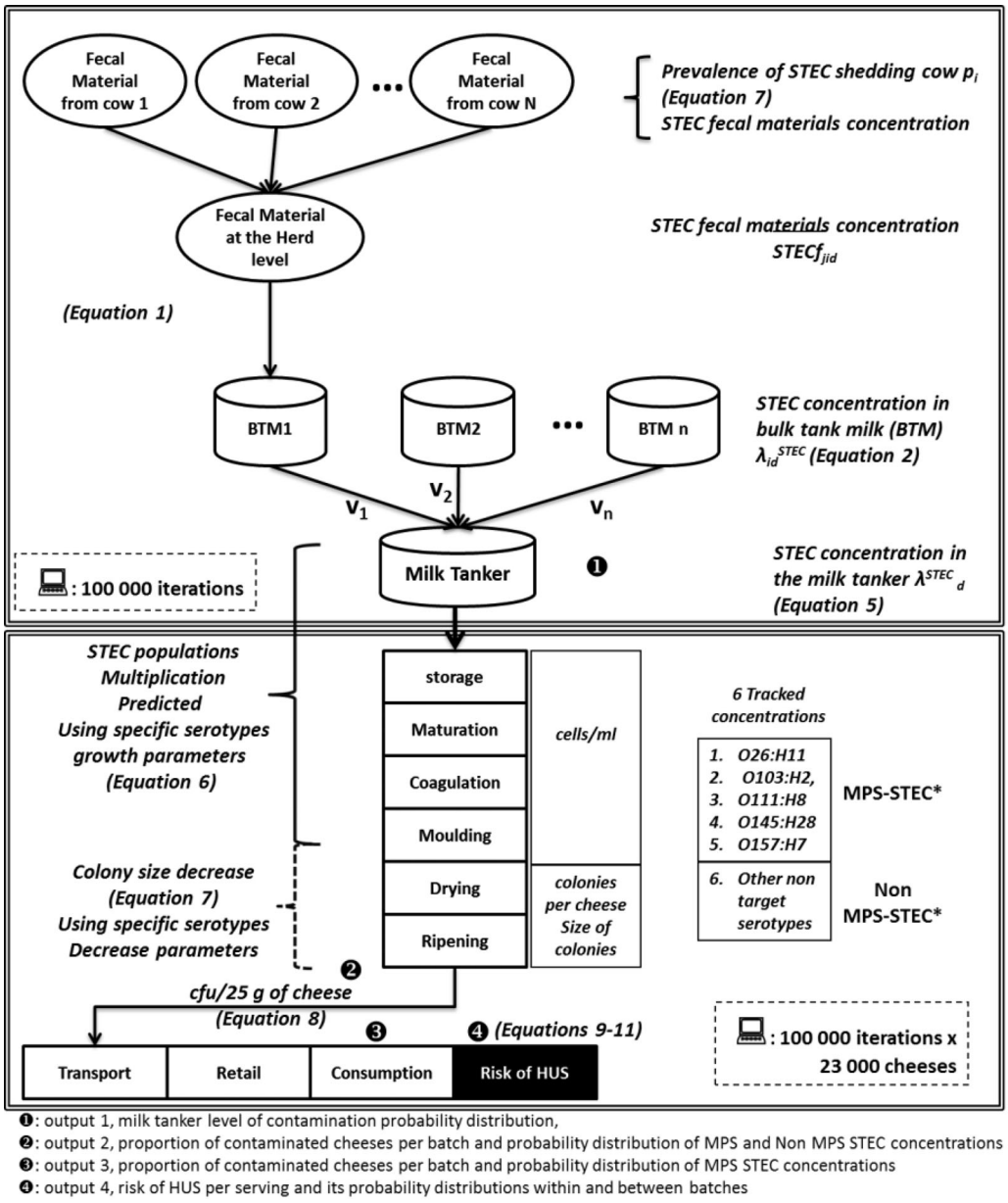

Fig. 1. Flowchart of the quantitative risk assessment model for the five major pathogenic serotypes of STEC (MPS-STEC): O26:H11, O103:H2, O111:H8, O145:H28, and O157:H7 in raw milk soft cheese.

is assessed using the total number of animals in the herds (N_{cow}), the number of infected animals (K_{id}), and the STEC concentration in fecal material from an infected cow ($STECf_{ijd}$).

K_{id} was determined from a Binomial distribution using the prevalence of cows shedding STEC strains (p_i) as probability and the number of cows (N_{cow}) as the number of trials.

The prevalence p_i was estimated using data extracted from the study of Raynaud *et al.*[17] Fecal samples were collected from 115 dairy farms and *stx* genes were detected in 705 fecal samples (35%). A statistical analysis was therefore performed in order to assess the distribution of within-herd daily prevalence of cows shedding STEC in their fecal materials. Data at herd level (number of

positive cows and number of sampled animals) were used to fit the following mixed logistic regression model:

$$\begin{aligned} &\text{logit}(p_i) = \log\left(\frac{p_i}{1-p_i}\right) = \beta_0 + u_i, \\ &u_i \approx Normal\left(0, \sigma^2\right) \end{aligned} \tag{3}$$

where β_0 is the logarithm of the odds of the overall prevalence and u_i is a random parameter describing the "between-herd variability" assumed normal at logit scale. The model was fitted using SAS NLMIXED procedure (SAS Institute Inc., Cary, NC, USA V9.3). The maximum likelihood estimate of β_0 was -0.927, which corresponds to an overall prevalence of 28.3% and σ^2 estimate was 2.173.

Then, K_{id} individual STEC concentrations in fecal materials ($STECf_{ijd}$) were drawn from the Weibull distribution.[25] The individual concentrations were summed and divided by N_{cow}.

This procedure assumes that the fecal materials that contaminate the milk do not come from a particular group of animals. In other words, the contribution of a particular cow to the total amount of feces contaminating the milk is independent of its STEC shedding status.

The same procedure was used to assess the arithmetic mean of the number of *E. coli* per gram of feces ($\overline{ECf_{ijd}}$), assuming that all lactating cows shed *E. coli* at a level assumed to follow a normal distribution ($\mu;\sigma$) at the logarithm scale ($\mu = 6$ $\log_{10}$/g and $\sigma = 0.3$ $\log_{10}$/g).[27–31]

As raw milk intended for production of raw milk cheese is intensively monitored, daily levels of bulk tank milk contamination by *E. coli* were collected from 31 dairy herds over a period of two years (data not shown). A hierarchical model that predicts the daily concentration of *E. coli* in bulk tank milk (λ_{id}) was built using these data:

$$\begin{aligned} &x_{id} \,|\lambda_{id} \approx \text{Poisson}\left(\lambda_{id}\right) \\ &\log\left(\lambda_{id}\right) = \alpha_i + e_d \\ &e_d \approx normal\left(0, \sigma^2\right), \end{aligned} \tag{4}$$

where x_{id} denotes the observed number of *E. coli* CFU/mL of bulk tank milk in dairy farm i on day d. The model specifies different intercepts, α_i, for each dairy farm i that indicates the level of hygiene of the farm i, and the random effect e_d is an instrument used to account for daily milking condition variability. This model was fitted using the SAS-NLMIXED procedure.

After estimating all the needed farm model inputs, the number of STEC in the tanker was assessed:

$$\lambda_d^{STEC} = \sum_{i=1}^{31}\left(\lambda_{id}^{STEC} \times \frac{V_{id}}{\sum_{i=1}^{31} V_{id}}\right), \tag{5}$$

where V_{id} is the volume produced in a herd i on day d.

Not all STEC strains can be considered pathogenic. The proportion of pathogenic STEC was estimated using data from a recent survey conducted in France.[32] A total of 1,318 fecal samples in six slaughterhouses including 337 dairy cows were collected between October 2010 and June 2011. The study does not show any seasonal variability of proportion of pathogenic STEC nor of serotypes distribution. Among the 337 dairy cows analyzed, 70% shed the *stx* gene and 1.8% (95% CI: 0.9%–3.8%) carried at least one strain belonging to one of the five MPS-STEC. In other words, 2.5% (95% CI: 1.3%–5.5%) of the cows shedding the *stx* gene shed STEC strains belonging to one of the five MPS-STEC. Because of the low number of isolated MPS-STEC from dairy cow samples, the proportion of each of the five MPS-STEC was assessed from all the samples including dairy and nondairy cows. The percentages of isolation from *stx* positive samples were: O157:H7 strains: 1.9% (95% CI: 1.2%–2.9%), O103:H2:0.8% (95% CI: 0.5%–1.6%), O26:H11:0.3% (95% CI: 0.1%–0.9%), O145:H28:0.2% (95% CI: 0.07%–0.7%), and O111:H8:0.2% (95% CI: 0.07%–0.7%).

Hence, the assessment of milk contamination levels with one of the five MPS-STEC was obtained by multiplying the number of total STEC strains by the proportion of MPS-STEC and by the specific proportion of each of the five serotypes.

A total of 100,000 iterations of the farm-level model were performed, resulting in an empirical distribution of the concentration of total STEC strains and of the five MPS-STEC strains in the tanker milk. A log-normal distribution was fitted.

2.3. Cheese Processing Module

2.3.1. Growth and Survival of STEC Characteristics

A specific microbiological challenge testing was conducted in order to qualitatively describe the behavior of STEC strains in raw milk soft cheeses

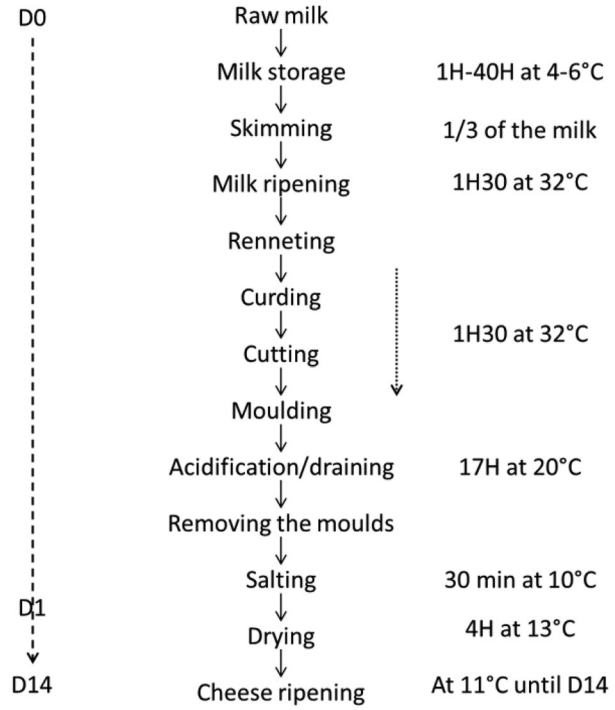

Fig. 2. Diagram of raw milk soft cheese manufacturing process.

during production, storage, and distribution and to assess the parameters needed to model this behavior (growth and/or decrease rate) according to the challenge-testing results.

- Challenge-testing protocol

A volume 480 L of raw milk was used for this testing and divided into 12 different vats of 40 L. Each of the 12 raw milk vats were inoculated with 10^2 CFU/mL of one of the following serotypes: O157:H7, O26:H11, O103:H2, and O145:H28. All the STEC strains used in this experiment were isolated from raw milk products and carried virulence genes *eae* and either *stx1* or *stx2*.

Cheeses were made following the cheese production scheme described in Fig. 2 and the STEC strains were inoculated just before the starter culture.

During cheese making, ripening (14 days) and storage (42 days: 14 days at 4 °C and 28 days at 8 °C) samples were taken from milk and from cheese and analyzed for STEC strains, moisture, pH, water activity (aw), temperature, and L-lactic acid contents. During cheese production samples were taken at the end of milk ripening and curdling, during molding, 20 hours after salting. Cheese samples were taken during the ripening at days 1.5, 7, and 14. During storage, samples were taken on days 28, 42, and 56. Each cheese was sampled both in the core and on the surface. STEC strains were prepared and counted using the same protocol described by Miszczycha *et al.*[33]

- Assessment of the predictive microbiological models' parameters

At the end of the first 24 hours of cheese making, the STEC concentrations reached high levels (between 4 and 5 $\log_{10}$ CFU/g). Following this, STEC strains levels progressively decreased. Serotype O157:H7 was less persistent than the other serotypes during cheese ripening. Data were grouped in two sets.

The first covers the growth phase during cheese making from the inoculation of STEC strains to the drying step. The second set corresponds to the decrease of STEC population during the ripening and storage over 42 days.

STEC population growth phase. The first set of data was used to model the growth of STEC strains in raw milk soft cheese using primary and secondary growth models. Primary models describe growth or decay kinetics over time. The main parameter of such a model is the maximum exponential growth rate (μ_{max}) of the bacteria.[34] A logistic primary growth model was used:

$$\frac{dy}{dt}(t) = \mu_{max}(t) \times y(t) \times \left(1 - \frac{y(t)}{y_{max}}\right), \qquad (6)$$

where $\mu_{max}(t)$ is the maximum growth rate (in h^{-1}), $y(t)$ the STEC strain population at time t, expressed in natural log CFU/g, and y_{max} is a parameter that represents the hypothetical maximum population of STEC strain in milk or cheese. It was set at 10^9 CFU/mL of milk and 10^5 CFU/g of cheese.

The secondary cardinal model with interactions developed by Augustin *et al.*[35] was used to estimate the effect of the physicochemical parameters (temperature [T], pH, and aw) on μ_{max}. The maximum growth rate is expressed as the multiplicative effect between the optimum growth rate μ_{opt} (the growth rate qualified at optimum conditions) and the modular functions of the different physicochemical parameters.

The cardinal values of STEC strains (X_{min}, X_{opt}, and X_{max}, for $X = T$, pH, or aw) used for the secondary model were assessed using growth data from Combase database. Two hundred twenty-one kinetics data and 421 growth rates observed in broth

Table I. Cardinal Parameters of STEC Strains Estimated in Culture Medium (622 Sets of Data)

Estimates	T_{min}	T_{opt}	T_{max}	pH_{min}	pH_{opt}	pH_{max}	aw_{min}	aw_{opt}
Mean	5.5	40.6	48.1	3.9	6.25	14	0.9533	0.999
Standard error	0.72	0.27	0.24	0.06	0.2	0	0.002	0

Table II. Growth Rates and Decrease Rates Estimated from Challenge-Testing Data

	Growth rate (μ_{opt}).		Decrease rate (ρ_s)	
Serotypes	Estimates	SE	Estimates	SE
O157H:7	2.03	0.626	0.14[a]	0.015
O103H:2	1.98	0.400	0.03[b]	0.003
O26H:11	1.94	1.362	0.03[b]	0.007
O145H:28	1.43	0.291	0.04[b]	0.007

No significant statistical difference between growth rates assessed on the challenge-testing results. a, b: no significant statistical differences between estimates with the same letter.

media with known static physiochemical conditions were extracted (T, pH, aw). First, the primary growth model was fitted in the 221 growth kinetics to assess the corresponding growth rate for each one. Second, growth rate estimates were pooled with the 421 growth rates extracted from the database. The secondary growth model was then fitted using the 622 growth rates to assess the cardinal values. The fitting process was carried out with SAS software (SAS Institute Inc., Cary, NC, USA. V9.2). The estimated cardinal values are shown in Table I. The used data included different STEC serotypes. For the purpose of our analysis, the cardinal values were assumed not to vary between serotypes. However, the optimal growth was assessed by serotypes to capture the possible serotype effect.

The μ_{opt} of STEC strains during cheese making was then assessed using the challenge-testing data, the estimated cardinal parameters and dynamic form of the primary growth model (Equation (6)). The latter allows variation of μ_{max} when physiochemical parameters (T, pH, and aw) vary during time. As no analytical solution was available for this type of dynamic system, a numerical resolution was obtained by using SAS Model procedure (SAS Institute Inc., Cary, NC, USA. V9.1), which permits fitting this type of complex time-dependent model on growth data in dynamic physiochemical conditions with the full information maximum likelihood estimation method.[36] Results of the estimates per serotype are shown in Table II. No significant statistical differences were observed between the serotypes' optimal growth rates.

STEC population decrease phase. During the ripening period a survival/decline period that lasted until the end of the ripening and storage of cheeses was observed. A log-linear mixed model was used to analyze the second data set of the challenge testing:

$$\begin{aligned} &y_{si}(t) = y_{si}(t_{max}) - (\rho_s + u_i) \times (t - t_{max}) + \varepsilon_{sit} \\ &\varepsilon \approx normal\left(0, \sigma^2\right) \\ &u_i \approx normal\left(0, \sigma_{u_i}^2\right), \end{aligned} \quad (7)$$

where y_{si} *(t)* is the STEC population in $\log_{10}$ CFU/g at time *t* for strain STEC *s* and replication *i*. $y_{si}(t_{max})$ is the STEC population observed before decrease ($\log_{10}$ CFU/g), ρ_s represents the decrease rate ($\log_{10}$ CFU/day) for strain *s*, and u_i is a random effect that describes the between-cheese-production variability. The model parameters were estimated using SAS-NLMIXED procedure. O157:H7 strains had a higher decrease rate than the non-O157 serotypes ($p < 0.004$). No significant statistical differences were observed between the non-O157 serotypes (Table II).

2.3.2. *STEC Prevalence and Concentration in Cheese Predictive Model*

From milk storage to molding, the growth of STEC and MPS-STEC strains was simulated using the dynamic form of the logistic growth model without delay (Equation (6)), the secondary cardinal growth model with interactions,[35] the optimal growth rates (Table II), and cardinal parameters (Table I). The variations of temperature, pH, and aw during processing from industrial data are described in Table III.

At the end of molding, each cell of STEC was assumed to be immobilized by the cheese matrix and gave rise to one colony. The number of colonies was derived from:

$$\begin{aligned} NC &= Poisson\left(10^{y(\text{moulding})} \times V_{\text{cheese}} \times 0.90\right) \\ NC_s &= Poisson\left(10^{y_s(\text{moulding})} \times V_{\text{cheese}} \times 0.90\right) \end{aligned} \quad (8)$$

Table III. Temperature, pH, and Water Activity (aw) Variations During Cheese Processing Simulations Steps Corresponding to the Growth Phase of STEC and MPS-STEC Strains

Step processing	Duration (H)	Temperature (°C)	pH	aw
Milk storage before processing	Triangular (1, 12, 40)	Uniform (4, 6)	6.5	0.99
Premolding steps	3	32	$6.5–0.148 \cdot t$	0.99
Draining	17	$32–5.459 \cdot t + 0.643 \cdot t^2$ if $t \leq 4$ $20.46–0.197 \cdot t$ if $t > 4$	$6.06–0.246 \cdot t$ if $t \leq 5$ $5.08–0.05 \cdot t$ if $5 < t \leq 10$ $4.64–0.006 \cdot t$ if $10 < t \leq 17$	0.99
Salting and drying	4.5	$17.1–2.325 \cdot t$ if $t \leq 2$ 12.45 if $2 < t \leq 4.5$	4.52	0.99

where NC and NC_s are, respectively, the number of colonies of all STEC strains and for the specific serotype s, y(molding) and y_s(molding) are, respectively, the total STEC and the specific STEC serotype s concentrations at the end of molding derived from the dynamic growth model described in Section 2.3.1. V_{cheese} is the milk volume need to produce one cheese (2,500 mL) and the factor 0.90 is used to account for the fraction of STEC cells eliminated in cheese whey (10%). The number of cells per colony was then calculated using the dynamic growth model (Equation (6)) until the end of the growth phase and with an initial population equal to 1 cell per colony. The number of cells per colony decline was assessed using the model described in Equation (7).

At the end of production, which corresponds to day 14, the prevalence and concentration of 25 g portions were extracted for the simulation results.

2.4. Consumer Module

Thanks to a cheese consumption survey commissioned by the French Dairy Board, the distribution of raw milk soft cheese consumption per age group was assessed. Combining this distribution with the French demographic data,[37] the proportion of cheese servings (25 g) that are expected to be consumed per age group (in years) was determined. The result was that 5.6% of raw milk cheeses were consumed by children up to 14 years old. The distribution of the percent of raw milk soft cheeses consumed by children under the age of 14 is shown in Fig. 3.

Cheeses are assumed to be consumed at a minimum cheese age of 18 days, maximum 42 days, and most probably 21 days. The cheese age at consumption was considered independent of the age of consumer.

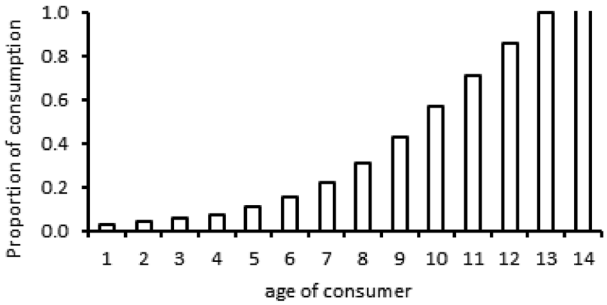

Fig. 3. Distribution of the proportion of raw milk soft cheeses consumed by children up to 14 years old.

At the end of decrease phase using Equation (7), which corresponds to the time of consumption, the prevalence and concentration of 25 g portions were extracted to assess the consumer exposure. The associated risk to the consumer was then estimated using the following relationship:

$$P_{\text{HUS}} = 1 - (1 - r_{\text{age}})^D$$
$$r_{\text{age}} = r_0 \times e^{-k \times \text{age}} \quad (9)$$
for ages from 0 to 15,

where P_{HUS} is the probability of HUS associated with the ingestion of a dose of D (CFU) of one of the major pathogenic serotype of STEC. The two model parameters (r_0 and k) were assessed by Delignette-Muller *et al.*[38] using the exponential trend from French surveillance data excluding babies (less than one year old), and data from the largest community-wide outbreak of O157:H7 STEC in France:[5,39] k = 0.38 (95% CI = [0.33, 0.43]) and $\log_{10}(r_0)$ = −2.33 (95% CI = [−2.58, −2.09]). The dose-response model is described in Appendix B. For consumers over 14 years old, r_{age} parameter was assumed constant and equal to r_{15}.

The risk of HUS was assessed per batch. A typical batch was assumed to be a production of one day using a total volume of 50,000 L of raw milk.

The number of cheeses (250 g) produced per batch is around 22,000 and 23,000. The distribution of pathogenic STEC number per 25-g cheese portion (D) is assessed per batch. The risk of HUS per batch was then derived as follows:

$$R_{\text{batch}} = \sum_{\text{age}=1}^{15} g(age) \int_0^{\infty} \left[1-(1-r_{\text{age}})^D\right] \cdot f(D)\, dD, \tag{10}$$

where $g(age)$ is the proportion of cheeses consumed by age group (14 groups: 1 to 14 years old and one group over the age of 14). $f(D)$ is the probability density function of D. As the risk of HUS per batch is conditional on the initial level of milk contamination with pathogenic STEC, the overall risk of HUS is calculated by:

$$R = \int_0^{\infty} R_{\text{batch}} \cdot h(c)\, dc, \tag{11}$$

where $h(c)$ is the probability density functions of the initial concentration of pathogenic STEC in milk.

2.5. Management Options

2.5.1. Preharvest Interventions

The primary preventive measures against STEC contamination of milk are all interventions that are expected to decrease prevalence or concentration of STEC in feces. Different interventions were suggested, such as detection and isolation of high shedders of STEC, vaccination, use of probiotics, antimicrobials, sodium chlorate, or bacteriophages or altering diet.[15]

If an intervention has an impact on STEC in feces, then the prevalence of animal shedding STEC postintervention is calculated based on the following formula:

$$p_{\text{post}} = \frac{OR \times p_{\text{pre}}}{1 - p_{\text{pre}} + OR \times p_{\text{pre}}}, \tag{12}$$

where p_{pre} is the prevalence preintervention, p_{post} is prevalence postintervention, and OR is the odds ratio measuring the efficacy of the intervention: odds of the prevalence after intervention divided by the odds of the prevalence before intervention. Fig. 4 shows the impact of different OR on the cumulative probability distribution of intraherd prevalence.

In the absence of accurate quantitative estimates of the efficiency of the preharvest interventions, we simulated the public health impact associated with odds ratio values $k = 0.10$, 0.15, and 0.29 according to the expected effects of use of probiotics or vaccines reviewed by Sargeant *et al.*[40] and Snedecker *et al.*[41] In addition to prevalence reduction, the use of probiotics or vaccines is also expected to decrease the concentration of STEC in fecal material (between 0.5 and 1.5 log decimal reductions).

The secondary prevention measures are linked to milking hygiene, resulting in limiting fecal contamination of the teats. To assess this type of intervention, the 31 dairy farms were ranked according to their level of hygiene as described by the parameters α_i in Equation (4). The milk of the top five farms was not included in the tanker milk concentration calculation (see Section 2.2).

2.5.2. Postharvest Interventions

There is no specific intervention at processing level. The impact of a MC for STEC at the end of the ripening phase of the cheese process was evaluated. Setting a MC at this step allows food business operators to manage the safety of their products before distribution to retailers.

The MC definition should include a precise definition of the sampling protocol and microbiological analysis of the samples (CAC/GL 21–1997). Subsequently, per cheese batch it defines (i) the sample size (n), (ii) the maximum concentration (m), (iii) the number of samples (c) that may yield a value larger than m, and (iv) the microbiological analysis method.

Different MCs were evaluated, as defined by a series of possible values for n, c, and the microbiological analysis method, with $m = 1$ CFU/25 g. Five MCs were considered: MC1: a composite sample of 25 g (from five different cheeses) analyzed for total *stx*; MC2: a composite sample of 25 g (from five different cheeses) analyzed to determine the presence of one of the MPS-STEC; MC3: five samples of 25 g analyzed for *stx* without tolerance ($c = 0$); MC4: five samples of 25 g analyzed for *stx* with a tolerance of one positive sample ($c = 1$); and MC5: five samples of 25 g analyzed to determine the presence of one of the MPS-STEC without tolerance ($c = 0$).

For each of the defined MCs, the probability of compliance with the MC for all the produced cheese batches was calculated (Equation (13)), as well as

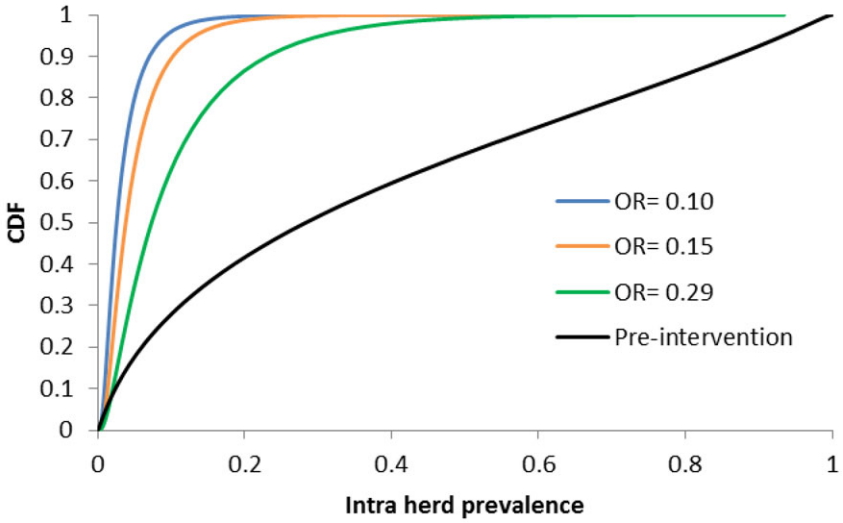

Fig. 4. Pre- and postintervention intraherd prevalence distribution. Preintervention intraherd prevalence derived from model presented in Equation (7). OR (odds ratio): expected effect of the intervention.

the probability of HUS for all batches complying and batches not complying with the MC. All the considered MCs batches with no compliance were assumed to have been destroyed.

$$P_{NC} = \begin{cases} 1 - \exp\left(-\frac{L_{\text{STEC}}}{f}\right) \\ 1 - \exp\left(-\frac{\sum_{1}^{5} L_s}{f}\right) \end{cases}, \qquad (13)$$

where P_{NC} is the probability of compliance for a specific batch, s is one of the five MPS-STEC, L_{STEC} is the expected number of all STEC colonies at the end of ripening, and L_s is the number of serotype s STEC colonies at the end of ripening, both in one 250 g cheese. L_{STEC} and L_s are derived from Equation (7) after subtracting colonies reaching size zero during the decline phase. f represents a sampling factor equal to 10 if a composite sample (5 × 5 g) is analyzed and to 2 if five different samples of 25 g are analyzed. In the case where $n = 5$ and $c = 1$, a Poisson cumulative distribution with parameter L_{STEC}/f or L_S/f was used to assess the probability of at least two positive samples.

For the two microbiological analysis methods the test will be positive if the analyzed sample contains at least one cell of the target STEC. The two methods include a selective enrichment step.

Finally, the expected risk reduction that can be achieved by setting a MC was assessed, under the assumptions that all batches are tested, and that all noncompliant batches are rejected.

3. RESULTS

The model was run to assess milk contamination just before starting cheese production (output of the farm-level model), cheese contamination at time of consumption, and the associated public health risk (output of the cheese-processing level mode). Several scenarios were applied to evaluate no intervention (baseline scenario) and preharvest or postharvest interventions. The baseline scenario represents a theoretical, worst-case scenario where no intervention is considered throughout the entire farm-to-fork continuum. It is clear that this scenario is not representative of the current situation in France and especially not in the context of raw milk cheese production. The model output considering the baseline scenario should not be used in an absolute manner but solely as a working reference point for demonstrating relative risk reductions or increases.

3.1. Raw Milk Contamination (Baseline Scenario)

The concentrations of *E. coli* and total STEC strains were assessed based on 100,000 iterations of the model, in the bulk tank milk contamination at farm level (one iteration producing 31 tank milk contamination estimates) and in the milk tanker. The results are summarized in Table IV.

Table IV. Baseline Results of QRMA Model: Summary Statistics of the Predicted Concentration of STEC and *E. coli* in Milk ($\log_{10}$ CFU/mL)

Outputs	Notation[a]	Mean (median)	std (ϕ)[b]	$q_{.05}$ [c]	$q_{.95}$ [c]
STEC in bulk tank milk	λ_{id}^{STEC}	−4.80 (−4.7)	1.3 (0.28)	−6.90	−2.80
E. coli in bulk tank milk	λ_{id}^{EC}	−0.23 (−0.25)	0.93 (0.38)	−1.73	1.35
STEC in milk tanker	λ_{d}^{STEC}	−3.98 (−3.80)	0.80 (NA)	−5.11	−2.48
E. coli in milk tanker	λ_{d}^{EC}	0.28 (.27)	0.74(NA)	−0.94	1.49

[a]STEC (total STEC), EC (*E. coli*), i and d represent, respectively, the collected farms ($i = 1$ to 31) and days of milk collection ($d = 1$ to 100,000).
[b]ϕ represents the intraclass correlation (between dairy farm variance divided by the total variance).
[c]$q_{0.05}$ and $q_{0.95}$ represent the 5th and 95th percentile, respectively.

By simulating the detection of *stx* gene in a milk sample of 25 mL, the predicted total STEC apparent prevalence using the distribution's characteristics in Table IV was 1.05% and 1.88%, respectively, for bulk tank milk and tanker milk.

3.2. Cheese Contamination (Baseline Scenario)

On average, the predicted percentage of contaminated cheese servings was 16.31% for the total STEC and 1.75% for the five MPS-STEC. The outputs revealed a disparity in the percentage of contaminated servings per batch: percentages ranged from 0% to 100% with 95% of batches showing a percentage of contaminated servings lower than 70% and 10%, respectively, for total STEC and MPS-STEC.

The level of total STEC contamination of servings expressed in CFU/g varied from 0.04 to 13.76 with a mean of 0.12 and standard deviation (*SD*) of 0.02. Similarly, the MPS-STEC contamination ranged in the same order of magnitude, from 0.04 to 9.88 with a mean of 0.21 and *SD* of 0.30.

3.3. Risk of HUS (Baseline Scenario)

The average probability of HUS per serving of 25 g of cheese was 4.2×10^{-6} (Table V). This probability provides an indication of the overall risk per serving among cheeses from different batches. It could be used to directly calculate the number of expected cases of HUS associated with a volume of produced cheeses.

Although the average risk of HUS can be considered low, it is important to recognize that is integrating variability between and within batches: 95% of batches were expected to be associated with an average probability of HUS per serving varying between 2.1×10^{-8} and 3.6×10^{-5}, 50% of batches were associated with a probability of HUS greater than 8.7×10^{-7}, and 1% of batches were linked with a probability of HUS greater than 7.2×10^{-5}.

If one considers a risk of HUS less than 4.4×10^{-6} (1 case/227,273 portions, $227{,}273 = 10 \times 50{,}000$ L of milk/2.2 L per cheese) as an acceptable level of protection (ALOP), only 80% of batches are expected to achieve this ALOP in this baseline scenario.

It is important to note that 99.6% of the overall risk of HUS per serving of cheese is attributable to non-O157:H7 STEC serotypes.

3.4. Impact of Preharvest Interventions

Eight scenarios were applied: scenario 1 (S1) is a scenario where no interventions are applied (baseline), scenarios 2 (S2) to 7 (S7) are considering probiotics treatment or cow vaccination with different sets of expected reduction of the prevalence of cows shedding STEC and the concentration of STEC in feces, and scenario 8 (S8) corresponds to the exclusion of the dairy farms with the poorest farm and milking hygiene practices (excluding five farms among the 31 collected farms with the highest α_i; see Equation (8)).

The average probability of HUS per serving of cheese following the application of preharvest interventions was reduced relatively to the baseline scenario by 76.28% to 98.42% (Table V). The highest risk reductions were observed when treatments or vaccinations were expected to significantly reduce the concentration of STEC in cow feces ($d = -1.5$ $\log_{10}$ CFU/g) with scenarios S5, S6, and S7.

Table V. Interventions' Impact on Raw Milk Contamination, Prevalence in Cheese Serving, and Risk of HUS per Serving of Cheese

Scenarios		Tanker milk contamination level (log_{10} CFU/mL)		Prevalence in 25-g cheese serving (95th percentiles)		Average risk per 1 million servings (25g)	Risk reduction in percent
		Mean	*SD*	Total STEC	MPS-STEC		
S1: Baseline		−3.98	0.80	16.31% (69.0%)	1.75% (10.0%)	4.21	0.00
Prevalence reduction (OR) and level of fecal shedding reduction (d in log_{10} CFU/g)							
S2: OR = 0.29	$d = -0.5$	−4.51	0.85	5.57% (32.0%)	0.44% (2.6%)	1.00	76.28
S3: OR = 0.15	$d = -0.5$	−4.65	0.88	4.58% (27.0%)	0.36% (2.0%)	0.80	80.93
S4: OR = 0.10	$d = -0.5$	−4.77	0.90	3.88% (24.0%)	0.30% (1.7%)	0.67	84.19
S5: OR = 0.29	$d = -1.5$	−5.46	0.83	0.75% (4.4%)	0.05% (0.3%)	0.10	97.55
S6: OR = 0.15	$d = -1.5$	−5.62	0.86	0.58% (3.4%)	0.04% (0.2%)	0.08	98.11
S7: OR = 0.10	$d = -1.5$	−5.81	0.92	0.48% (2.8%)	0.03% (0.2%)	0.07	98.42
Increase in farm and milking hygiene							
S8: Excluding top five farms		−4.65	0.78	3.55% (19.8%)	0.25% (1.4%)	0.55	86.97

The exclusion of farms repeatedly delivering raw milk with the highest concentration of *E. coli* among the 31 collected dairy farms (S8) is expected to reduce the risk of HUS by almost 87% comparatively to the baseline scenario.

Fig. 5 shows the cumulative distribution of probability of the average risk of HUS per serving between batches. The probability of exceeding a risk of 4.4×10^{-6} (theoretical ALOP as defined above) was less than 0.5% for the three most effective scenarios. It is worthwhile to observe a significant reduction of the probability of batches exceeding the theoretical ALOP from almost 20% in the baseline scenario to almost 3% in S8, the latter representing one of the dominant current practices applied in France to prevent the presence of STEC in cheese. Fig. 6 shows the cumulative distribution of probability of the prevalence of contaminated serving of cheese per batch. In addition to the overall prevalence reduction shown in Table V, the 95 percentiles of serving total STEC contamination prevalence were 69%, 32%, 27%, 24%, 4.4%, 3.4%, 2.85%, and 19.8%, respectively, for scenarios 1 to 8. Similarly, when considering the MPS-STEC, the 95 percentiles were 10%, 2.6%, 2%, 1.7%, 0.3%, 0.2%, 0.17%, and 1.4%, respectively, for scenarios 1 to 8.

3.5. Impact of End-Production Testing

The expected public health impact associated with the application of the five MCs depends on the contamination level of batches. Considering the baseline scenario the average probability of HUS per serving of cheese following the application of the five MCs was reduced by 89%, 25%, 96%, 94%, and 53%, respectively, for MC1 to MC5. The most effective MCs include those testing for total *stx*. Notwithstanding the important risk reductions, the more effective MCs were coupled to high proportions of destroyed batches: the expected probabilities of noncompliance were 0.52, 0.03, 0.79, 0.63, and 0.12, respectively, for MC1 to MC5.

The risk reduction and proportion of noncompliant batches following the application of MCs were evaluated in association with the interventions scenario (Fig. 7). For the clarity of the illustration, Fig. 7 presents only four scenarios. Note that the nonpresented scenarios (S2 and S4) showed figures close to S3. Similarly, the nonpresented scenarios S5 and S7 had figures close to S6.

If one targets a risk reduction higher than 50% and no more than 25% of noncompliance, the operative curves in Fig. 7 readily provide the more effective MC. For instance, when considering the

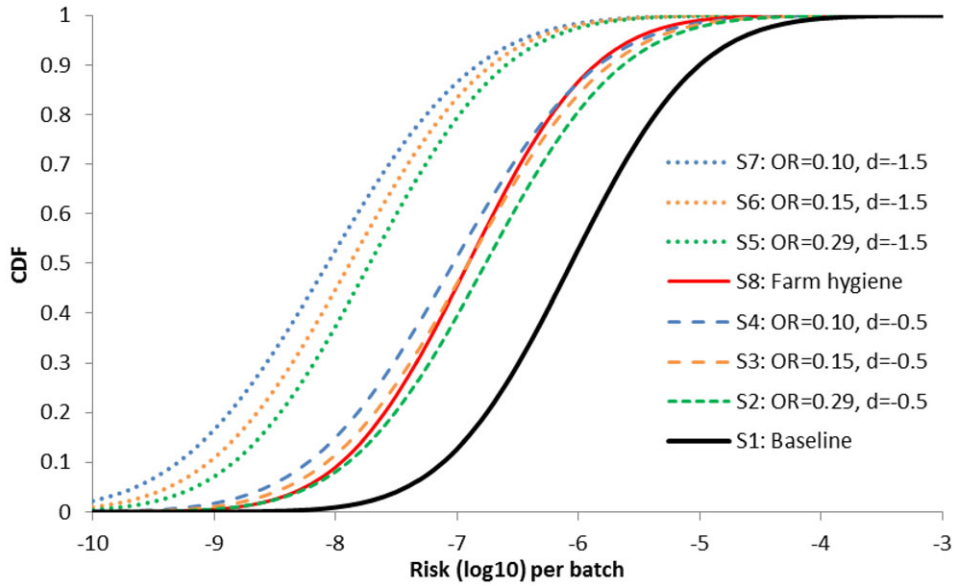

Fig. 5. Cumulative distribution of average probability of HUS per serving of cheese (25 g) between batches.

baseline scenario MC5 ($n = 5$, $c = 0$, with isolation of one of the MPS-STEC) is the MC that satisfied the two constraints. However, taking into consideration scenario 8 (farm hygiene), MC1 (1 composite sample with only *stx* screening) in the more effective MC. Similarly, MC1 was found to be adapted to scenario 3 (OR = 0.15, $d = -0.5$). In contrast, for scenario 6 (OR = 0.15, $d = -1.5$), the more appropriate MC was MC3 ($n = 5$, $c = 0$ and *stx* screening).

4. DISCUSSION

The model described in this article was built to characterize prevalence, concentration, and behavior of pathogenic STEC at preharvest, soft cheese processing, and ripening. This article also provides the first quantitative risk assessment of HUS linked to the contamination of raw milk soft cheeses with O157 and non-O157 STEC serotypes. Outputs are directly influenced by the quality of the model inputs and assumptions, and how accurately the model construction and structure represents the current system that rules the occurrence and behavior of pathogenic STEC from farm to fork. The model estimates reflect the current variability surrounding the quantification of the prevalence, concentration, and behavior of STEC.

The model provides an original tool for assessing the risk of HUS from consumption of soft cheeses made in one dairy plant from raw milk collected in its milkshed. Furthermore, the provided tool can be used to assess the relative impacts of possible interventions and MC on public health risk from the consumption of pathogenic STEC in raw milk cheese products. It can also be applied to multiple dairy plants collecting from one or different milksheds, to benchmark their performances or to individually or collectively derive the most effective combinations of interventions and evaluate the impact of the choice of the MC. The results presented in this article are specific to a dairy plant. Before applying the model to other dairy plants, specific data need to be collected. They include daily or weekly data on *E. coli* counts in farm bulk tank milk covering a time period of at least one year, milk yields per farm, number of milked cows per farm, and data describing cheese production and ripening (pH, aw, temperature kinetics). A challenge testing is not needed unless the cheese production conditions differ significantly from the one described in this article.

The model started with estimating the STEC raw milk contamination that was assumed to follow the same pathway as the daily monitored *E. coli* in bulk tank milk. The predicted prevalence of STEC is lower than the majority of reported prevalence in milk.[15] This discrepancy may be explained by the specific sample of dairy farms included in the study, which were specific to raw milk cheese production. These dairy farms were therefore selected by dairy

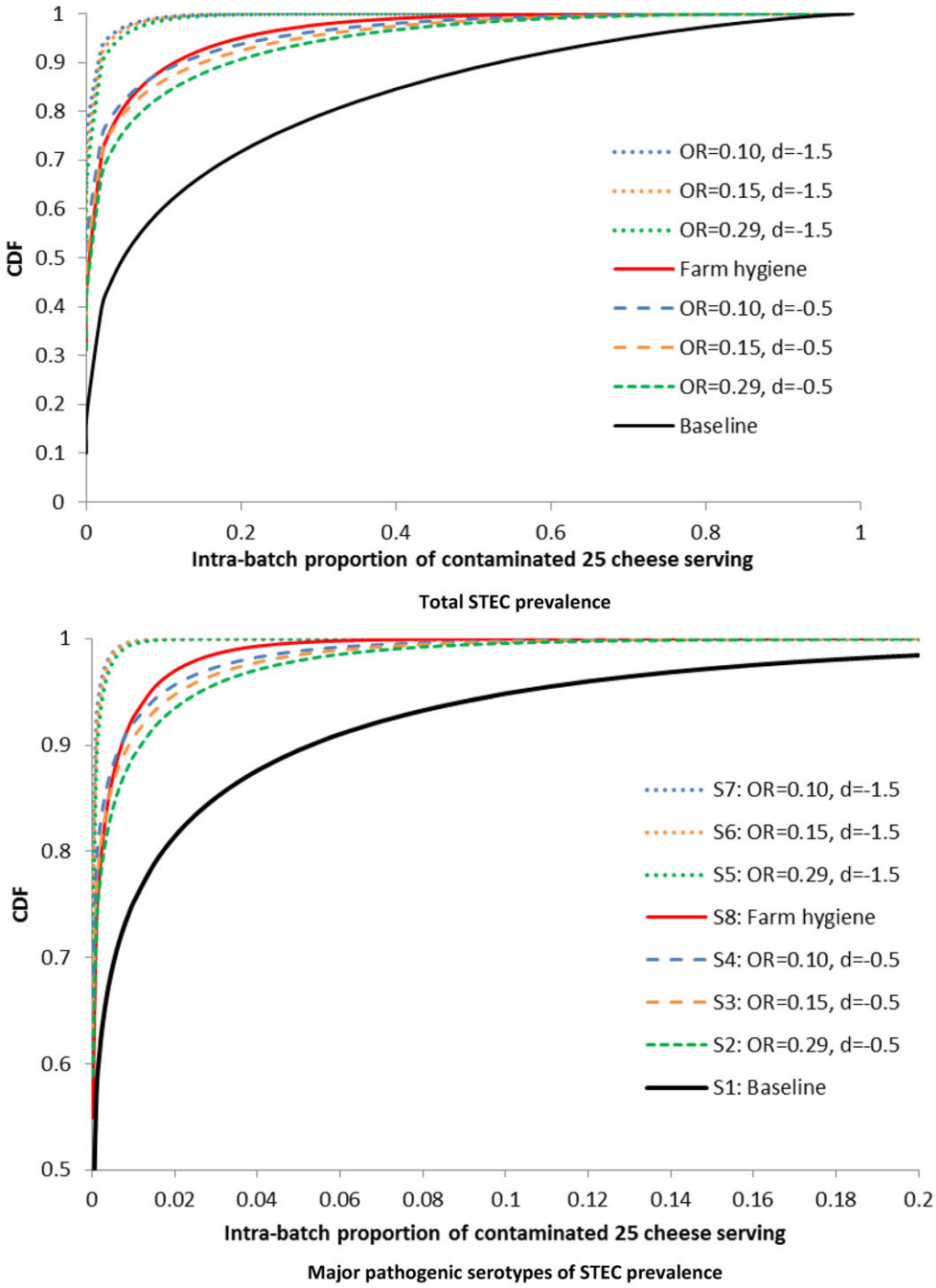

Fig. 6. Probability distribution of the prevalence of contaminated serving of cheese per batch.

plants for the good sanitary quality of their milk based on their historical performances regarding to *E. coli* and pathogenic bacteria, such as *Salmonella* spp. and *Listeria monocytogenes*. It is crucial to understand that the results obtained in our study are specific to this described context.

The estimated prevalence of contaminated cheeses falls within the ranges reported in France

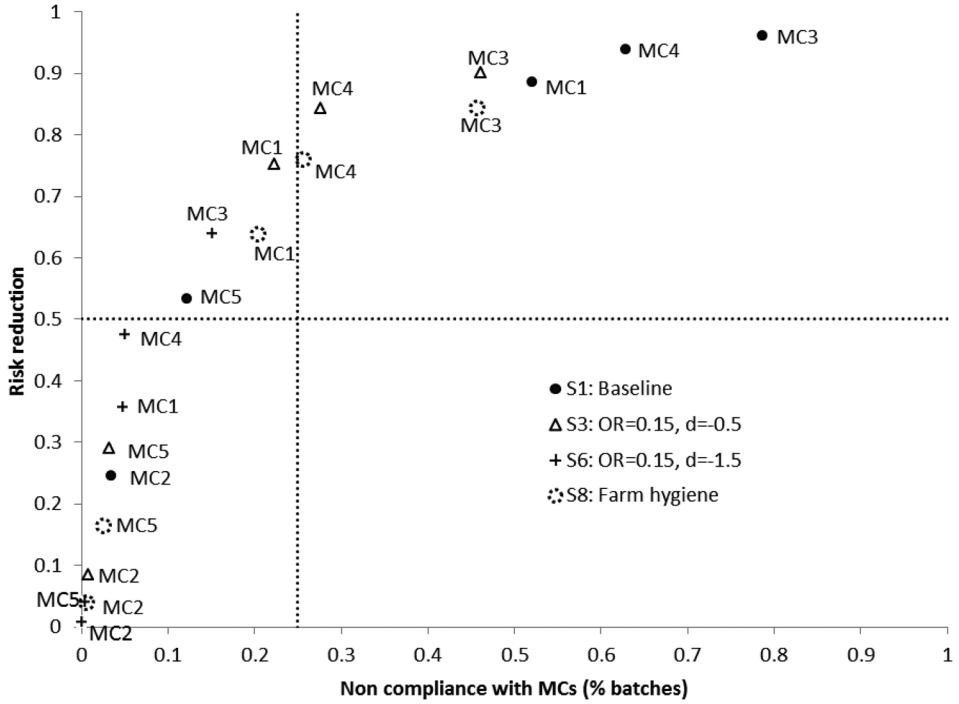

Fig. 7. Microbial criteria (MCs) operating curves illustrating the expected risk reduction and the proportion of batches that will not comply with the evaluated series of MCs.
MC1: a composite sample of 25 g (from five different cheeses) analyzed for total *stx*; MC2: a composite sample of 25 g (from five different cheeses) analyzed to determine the presence of one of the MPS-STEC; MC3: five samples of 25 g analyzed for *stx* without tolerance ($c = 0$); MC4: five samples of 25 g analyzed for *stx* with a tolerance of one positive sample ($c = 1$); and MC5: five samples of 25 g analyzed to determine the presence of one of the MPS-STEC without tolerance ($c = 0$).

5.5%–30% and 1%–13%, respectively, for total *stx* prevalence and major pathogenic STEC serotypes.[42–46]

Presently, O157:H7 STEC is the more often monitored and tested serotype because it is still perceived as the most recurrently implicated serotype of STEC in outbreaks linked to dairy products. However, a thorough analysis of 50 reviewed outbreaks[15] that have been linked to milk and dairy products showed that O157 STEC serotype outbreaks were mainly associated with raw milk and fresh products and in contrast non-O157 STEC serotypes were more closely linked to more elaborate cheese products. In this study, and in order to better assess the behavior of STEC strains during cheese production and ripening, a challenge testing including four serotypes (O157:H7, O26:H11, O103:H2, and O145:H28) was conducted. The results showed a significant difference between O157 and non-O157 serogroups. Serotypes O26:H11, O103:H2, and O145:H28 have more of a chance of surviving during ripening than serotype O157:H7. The survival probability after two weeks ripening was 1%, 34%, 37%, and 27%, respectively, for O157:H7, O103:H2, O26:H11, and O145:H28. This finding is in line with the results obtained in a recent study including other cheese types.[33]

The cheese contamination was assessed at time of testing, which is performed before the products are put into the market, namely, at 14 days of age, and at time of consumption. The baseline estimate of risk of HUS per serving of cheese provided an expected number of HUS cases, much higher than the number of reported cases in France. Caution must be taken when interpreting this latter estimate because the baseline scenario represents a worst-case scenario as it ignores the current control measures applied by dairy plants. It is obvious that this scenario is not representative of the current situation in France. This estimate should not be used in an absolute manner but exclusively as an operational reference point for demonstrating relative risk reductions or increases.

To our knowledge, this is the first time that the risk of HUS per serving of raw milk soft cheese has been assessed. Therefore, our risk estimates cannot be compared to the outputs of similar risk assessment models. However, despite the baseline scenario overestimation, the estimated risk per serving of cheese is lower than the ones assessed for O157:H7 STEC from the consumption of ground beef, which vary between 3.2×10^{-7} and 2.6×10^{-4}.[47–51]

The probability of HUS following the consumption of one of the five MPS-STEC was assessed thanks to an original dose-response model including age as a covariable.[38] The dose-response model was fitted using French HUS epidemiological monitoring data and a large set of outbreak investigation data as described in Appendix B.

In previous STEC risk assessments, different dose-response models were used. Surrogate dose-response models were first used: the *Shigella* dose-response model[52] and O157:H7 STEC dose-response model using animal experimentation data (rabbits as surrogate hosts).[53] Thereafter, dose-response models incorporating data from foodborne and environmental outbreaks were published.[54,55]

The more recent dose-response model was based on a series of outbreaks involving O157:H7 STEC.[55] None of the published dose-response models took into account the age of exposed hosts and the use of the beta-binomial model does not really capture the age effect.

This is why we believe that the model proposed by Delignette-Muller *et al.* (2010) is a closer match to the epidemiological context of the target end point (HUS). However, in our risk assessment we assume the same dose-response model for all the MPS-STEC. This assumption is probably overestimating the risk of HUS.

Application of preharvest intervention provides a significant reduction of the probability of HUS from the consumption of raw milk soft cheeses. Our model showed that preharvest interventions only providing a reduction in numbers of shedding cows will result in less significant beneficial impacts to public health compared to interventions associated with a decrease of STEC concentration in cows' fecal material (Table V). The latter can be explained by the fact that low numbers of high shedding cows could maintain sufficient concentration of milk tanker. Although there are several studies evaluating the efficacies of preharvest interventions, quantifying their effects is difficult because different studies used different experimental or observational designs.[15] Generally, interventions aiming at reducing fecal contaminations are based on challenge trials where enrolled cattle were exposed to high doses of STEC O157 in order to enable decrease measurement. The results obtained from this type of studies may be biased and not accurately represent the reality where cattle are shedding different STEC serotypes at a level lower than those met in challenge trials. Randomized controlled trials need to be conducted to better assess the efficiencies of preharvest interventions.

In addition to interventions aiming to reduce the prevalence and the level of STEC shedding in fecal materials, we evaluated the impact of intervention reducing the probability of milk being soiled by fecal materials (farming and milking hygiene improvement). The model describing the variability of bulk tank milk contamination with *E. coli* (Equation (4)) provides a quantitative indicator summarizing the farm hygiene level. Based on this indicator, if we exclude the five least best farms in our example we observed a risk reduction of approximately 87%. This reduction was higher than the risk reduction associated with the scenario with a 10-fold reduction of the odds of prevalence of shedding animal and half-log decimal decrease of STEC fecal concentration. This finding clearly showed that farming and specifically milking hygiene, resulting in limiting fecal contamination of the teats, should continue to be considered as one of the key defenses against STEC contamination of milk. In cases where the likelihood of contamination from the environment is high, hygienic milking can be insufficient and a number of additional interventions aiming to decrease fecal shedding should help in controlling milk contamination.

In the same way as the Nauta, Sanaa, and Havelaar model,[56] we showed once more how risk-based MC can be derived without the consideration of other food safety risk targets like Food Safety Objectives and Performance Objectives. We used a similar approach that offers a tool for food safety risk managers to choose a MC on the basis of the predicted health benefit (HUS risk reduction) and the predicted proportion of cheese batches that will not comply with the MC. This is well illustrated in Fig. 7 from which the risk manager can choose a point that offers the best balance between the potential health benefits and the potential costs of noncomplying batches.

Our analysis shows that microbiological analysis for STEC is likely to deliver meaningful HUS reduction. Thus, the end-product testing for STEC would,

in some circumstances, significantly reduce the public health risk but with a cost to pay in terms of loss of production, which could jeopardize the sustainability of this type of cheese production. In order to reduce the cost induced by MCs application, the latter should be combined with preharvest control options such as detection and isolation of high shedders of STEC, vaccination, or use of probiotics.

In addition to public health benefits and risk reduction, the total cost associated with MCs (including sampling, testing, and loss of production) has to be compared with the benefits linked to the prevention of withdrawal or recall of products and related loss of contracts.

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APPENDIX A: CONCENTRATION OF STEC IN BULK TANK MILK

The modified model is based on the following Equations that consider the concentration of *E. coli* ($\lambda^{EC}{}_{id}$) and STEC ($\lambda^{STEC}{}_{id}$) in bulk tank milk (CFU/mL):

$$\lambda_{id}^{EC} = \frac{\sum_{j=1}^{N_{\text{cow}}} \frac{ECf_{ijd} \times FM_{ijd}}{1000}}{\sum_{j=1}^{N_{\text{cow}}} V_{jd}},$$

where ECf_{ijd} is the *E. coli* concentration in fecal material (CFU/g) and V_{jd} (mL) is the volume of milk coming from a cow j at day d.

As ECf_{ijd} and FM_{ijd} are independent variables, we can write the following:

$$\lambda_{id}^{EC} = \frac{\overline{ECf}_{ijd} \sum_{j=1}^{N_{\text{cow}}} \frac{FM_{ijd}}{1{,}000}}{\sum_{j=1}^{N_{\text{cow}}} V_{jd}},$$

where $\overline{ECf}_{ijd}$ is the arithmetic mean of ECf_{ijd}. Introducing X as the total amount of feces entering the bulk tank we have:

$$\lambda_{id}^{EC} = \frac{\overline{ECf}_{ijd} \times X}{\sum_{j=1}^{N_{cow}} V_{jd}}.$$

Applying the same formula to STEC concentration we can write:

$$\lambda_{id}^{STEC} = \frac{\overline{STECf}_{ijd} \times X}{\sum_{j=1}^{N_{cow}} V_{jd}},$$

where $\overline{STECf}_{ijd}$ is the arithmetic mean of the number of STEC per gram of feces. Finally we obtain:

$$\lambda_{id}^{STEC} = \overline{STECf}_{ijd} \times \frac{\lambda_{id}^{EC}}{\overline{ECf}_{ijd}}.$$

APPENDIX B: DOSE-RESPONSE MODEL

The development of the dose-response model was based on epidemiological estimates of the incidence rates (IRs) of HUS for different population age groups (King *et al.* 2009) and data collected from a large community-wide outbreak of *E. coli* O157 in France associated with consumption of contaminated frozen ground beef patties in households.[39]

The approach used was first presented by M. L. Delignette-Muller, S. Jaloustre, and H. Bergis, in Non-Clinical Statistics 2010 conference, Statistical Methods for Pharmaceutical Research and Early Development, Lyon, September 27–29, 2010, with original data provided by the ANSES. The model proposed was checked and the dose-response model parameters were recalculated with SAS V9.3.

The model assumes that the probability of HUS follows a binomial process:

$P(HUS|\text{age}) = 1 - (1 - r_{\text{age}})^D$, where

D is the ingested dose and $r_{\text{age}} = r_0 \times \exp(k \times \text{age})$.

The model parameters are assessed in two steps. In the first step k is assessed using epidemiological surveillance data. In the second step r_0 is estimated using the French outbreak data.

Fifteen age groups were defined, from 1 to 15 years. Children less than six months of age were excluded because they are expected to have a very different dietary diversification comparing to other

group of ages. From King *et al.* (2009) we extracted a vector of 16 IRs per 100,000 children: (1.82, 2.88, 2.2, 1.46, 0.91, 0.62, 0.46, 0.36, 0.22, 0.16, 0.19, 0.13, 0.09, 0.08, 0.05, 0.04). Combining the observed IRs and the subpopulation sizes, we estimated the trend of HUS incidence over age using a Bayesian analysis of log-linear Poisson regression model implemented in SAS (GENMOD procedure):

$$\text{Log}(\text{IR}|\text{age}) = \text{IR}_0 + k \times \text{age}.$$

Results obtained for IR_0 and k were: $\text{IR}_0 = -2.21$ with 95% credible interval $[-2.31, -2.11]$ and $k = -0.38$ with 95% credible interval $[-0.40, 0.36]$.

In order to assess r_0, an exposure model was built to describe the outbreak of HUS linked to the consumption of contaminated frozen beef burgers that occurred in France in 2005, covering the food pathway from packaging to consumption.[39] According to the distributor, the estimated total number of consumed ground beef patties was 2,155. The ingested dose for a specific group of age (D_{age} in CFU) depended on the initial level of contamination (C in CFU/g), the consumption preference for each group of age (CP_{age}: raw, rare, medium, or well-done), and the serving size for each group of age (S_{age} in g). D_{age} was assumed to follow a Poisson distribution with a parameter $C \times S_{\text{age}} \times 10- R_{\text{age}}$, where R_{age} is the number of log decimal reduction, which depends on the consumption preference CP_{age}.

The concentration C was estimated from microbial detection and counts performed on 22 frozen patties sampled from the contaminated batch: $C = 5.8$ CFU/g, with 95% credible interval [3.2, 9.4].

The serving size S_{age} was assumed to follow a log normal distribution with mean equal to $\mu_0 + \Delta\mu \times (1 - \exp(-\beta \times \text{age}))$ and $SD\ \sigma$. Values assessed for μ_0, $\Delta\mu$, and β were, respectively, 4.7, 1.1, and -0.18. The value assessed for σ was 0.40.

The proportion CP_{age} was assessed for children under two years old and children of two years old and below and the vectors of proportion of raw, rare, medium, and well-done were, respectively (0, 0.02, 0.38, 0.60) and (0.01, 0.19, 0.52, 0.28). For the CP rare, medium, and well-done, the decimal log reductions were assumed to follow gamma distributions with, respectively, the following means and SDs (0.47, 0.25), (0.98, 0.23), and (2.4, 0.46).[40]

Additionally, the number of consumed ground beef patties in each group of age was estimated between 36 and 39.

The probability of HUS was derived from the following equation:

$$P(HUS|age) = 1 - (1 - r_{age})^{D_{age}}$$

where D_{age} is the ingested dose assessed from the described exposure model and $r_{\text{age}} = r_0 \times \exp(k \times \text{age})$ for age from 1 to 15 years old.

In order to estimate the two parameters of the dose-response model r_0 and k, a Bayesian framework was used using the SAS-MCMC procedure. The model inputs were:

- the distribution of ingested dose per age assessed from the exposure model D_{age},
- the number of consumed ground beef patties for in each group of age,
- the posterior distribution of the parameter k from the Bayesian analysis of log-linear Poisson regression model applied to the epidemiological surveillance system,
- the total number of observed HUS during the 2005 outbreak (16 confirmed cases of HUS).

The obtained results were: $k = 0.38$ with 95% credibility interval $[-0.40, -0.36]$ and $\log_{10}(r_0) = -2.33$ with 95% credibility interval $[-2.56, -2.12]$.

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