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Quantitative risk assessment of human listeriosis from consumption of soft cheese made from raw milk

N. Bemrah[a], M. Sanaa[a,*], M.H. Cassin[b], M.W. Griffiths[c], O. Cerf[a]

[a] *Epidemiology and Animal Health Management Laboratory, Alfort Veterinary School, Maisons-Alfort, France*
[b] *Decisionalysis Risk Consulting, Cambridge, UK*
[c] *Department of Food Science, University of Guelph, Guleph, Ontario, Canada*

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Abstract

Microbial hazards have been identified in soft cheese made from raw milk. Quantification of the resulting risk for public health was attempted within the frame of the Codex Alimentarius Commission, 1995 approach to quantitative risk assessment, using Monte Carlo simulation software. Quantitative data could only be found for *Listeria monocytogenes*. The complete process of cheese making was modeled, from milking to consumption. Using data published on the different sources of milk contamination (environment and mastitis) and bacterial growth, distributions were assumed for parameters of the model. Equations of Farber, J.M., Ross, W.H., Harwing, J. (1996) for general and at-risk populations were used to link the ingested dose of *L. monocytogenes* to the occurrence of listeriosis. The probability of milk contamination was estimated to be 67% with concentration ranging from 0 to 33 CFU ml^{-1}. The percentage of cheese with a predicted concentration of *L. monocytogenes* greater than 100 CFU g^{-1} was low (1.4%). The probability of consuming a contaminated cheese serving was 65.3%. Individual annual cumulative risk of listeriosis, in a population each consuming 50 servings of 31 g, ranged from 1.97×10^{-9} to 6.4×10^{-8} in a low-risk sub-population and $1.04\ 10^{-6}$ to $7.19\ 10^{-5}$ in a high-risk sub-population. The average number of expected cases of listeriosis per year was 57 for a high-risk sub-population and one for a low-risk healthy sub-population. When the frequency of environmental milk contamination was reduced in the model and *L. monocytogenes* mastitis was eliminated, the expected incidence of listeriosis decreased substantially; the average number of expected cases was reduced by a factor of 5. Thus the usefulness of simulation to demonstrate the efficiency of various management options could be demonstrated, even if results should be interpreted with care (as many assumptions had to be made on data and their distributions).

Keywords: Cattle-microbiological diseases; Quantitative risk assessment; *Listeria monocytogenes*; Soft cheese; Milk; Monte Carlo simulation

* Corresponding author. Tel.: +33 1 4396 7026; fax: 33 1 4396 7150; e-mail: sanaa@vet-alfort.fr

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1. Introduction

Safety of soft cheese made from raw milk is debated, and while it can be made and sold within the European Union, it is still subject to discussion within the Codex Committee for Food Hygiene (CCFH) of the Codex Alimentarius Commission, 1995; Anonymous, 1996. Based on the identification of hazards and epidemiological evidence (IFST, 1998), microorganisms of concern are *Listeria monocytogenes*, *E. Coli* O157 : H7, *Salmonella*, and toxin-producing *Staphylococcus aureus* which may contaminate milk and grow in cheese when milk is not pasteurized. Yet estimation of the consequences for public health of the consumption of raw milk soft cheeses has not been published, to our knowledge. A quantitative risk assessment (QRA) of risks linked to the hazards as listed above – made according to the recommendations of the CCFH – could allow for an objective approach.

A bibliographic study showed that quantitative data of raw milk contamination and growth in cheese are available for *L. monocytogenes* but not for the other bacteria mentioned above. The present paper will therefore deal only with this microorganism, in an attempt intended at testing how QRA can be done and used. A generic model of soft cheese will be considered, as is expected, for a risk analysis for the purpose of the establishment of rules for international trade. Data on consumption per capita will be arbitrary, but plausible in countries where raw milk soft cheeses are consumed along with a much larger proportion of pasteurized cheeses. Finally, it will be shown how the QRA can be used to simulate the consequences of a few alternative options of risk management such as farm-level interventions to reduce *Listeria* shedding in milk or decrease of consumption by populations at risk.

2. Material and methods

Risk assessment is a methodology used to organize and analyze scientific information to estimate the risk (defined as the probability and severity of an adverse event resulting from a hazard). Risk assessment is the scientific basis for risk-management and risk-communication activities, which together make up the risk-analysis process (National Research Council, 1983).

In our study, the hazard is *L. monocytogenes* and the risk qualifies the probability of human listeriosis and death associated with the consumption of soft cheese made from unpasteurized milk.

Fig. 1 summarizes the risk-assessment model which is detailed below.

2.1. Hazard identification

L. monocytogenes is frequently isolated from the environment (soil, leaf litter, sewage, silage, dust and water) and can cause a serious food-borne illness: listeriosis. The bacterium often passes through an animal's intestinal tract without causing illness. It has also been found in many domestic and wild animals, including birds and fishes.

Healthy people rarely contract this disease, but listeriosis can be severe for certain groups of people (especially the elderly, newborn, pregnant women and those with a

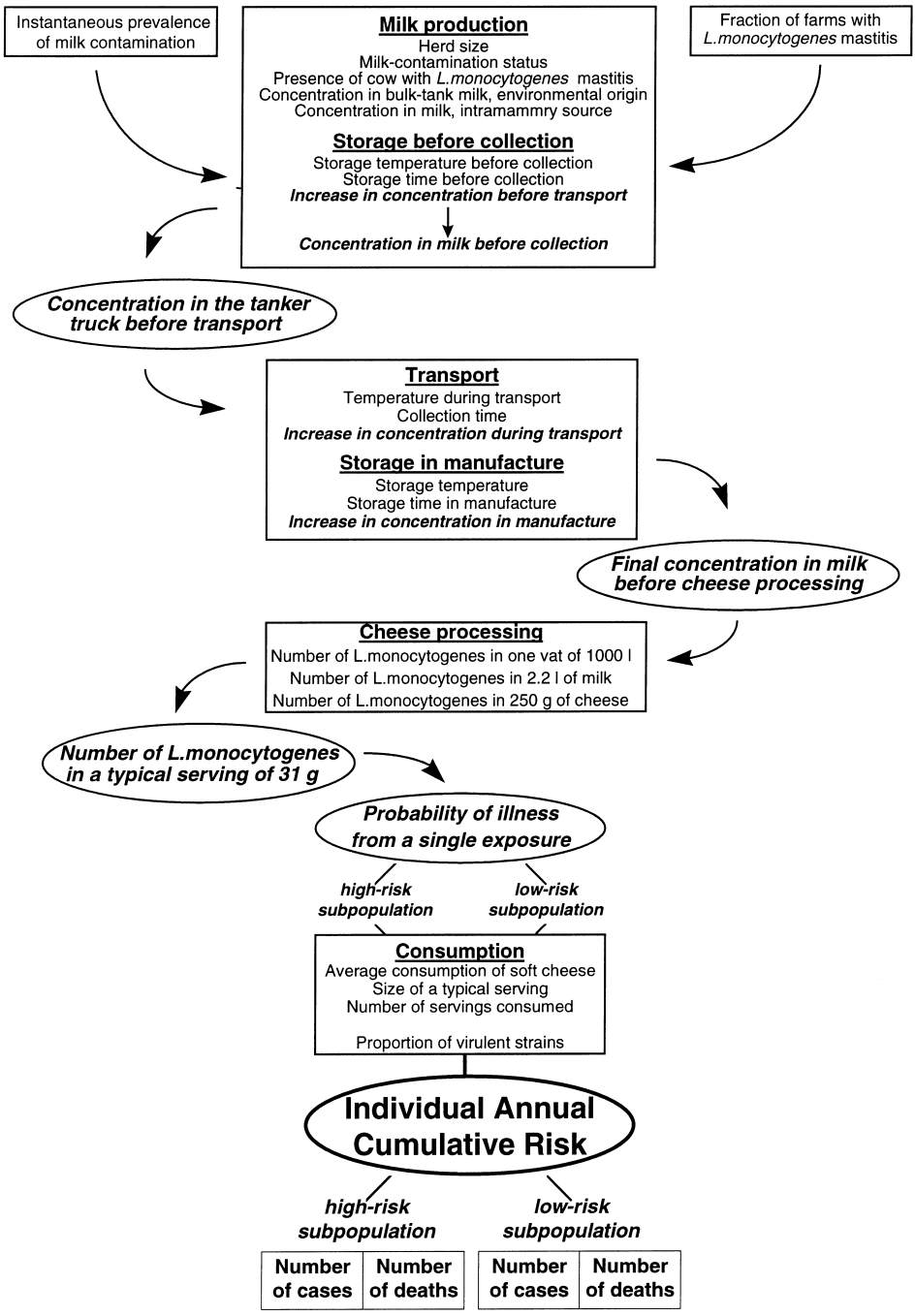

Fig. 1. Model of risk assessment for human listeriosis from consumption of soft cheeses.

weakened immune system). *L. monocytogenes* can cause abortion in pregnant women as well as meningitis and septicaemia in newborn infants and immunocompromized people; the case-fatality risk can reach 34%.

The investigation of several outbreaks has demonstrated that epidemic listeriosis was caused by food-borne transmission of *L. monocytogenes* (Farber and Peterkin, 1991). Coleslaw, milk and dairy products (particularly soft cheeses) were implicated in these outbreaks. Schuchat et al. (1992) and Pinner et al. (1992) clarified the role of food-borne transmission in sporadic listeriosis in the USA; sporadic individual cases of listeriosis were associated with soft cheese, undercooked poultry, hot dogs not thoroughly reheated and food purchased from delicatessen counters.

In North America, the published rates showed annual incidences of listeriosis of six cases per million population in USA (Altekruse et al., 1997) and between 1.7 and 4.5 per million in Canada (Farber et al., 1996). The annual incidence of sporadic listeriosis which was about seven cases per million in France before 1995 (Jacquet et al., 1995), but has now decreased to 5.9 per million in 1995 and 3.8 per million in 1996 (Rocourt et al., 1997), showing the same tendency as observed in the USA (Tappero et al., 1995). According to Schuchat et al. (1992), most cases are sporadic and food-related. However, in the last 15 years, eight outbreaks have been associated with the consumption of cheese (Linnan et al., 1988; Bille, 1990; Goulet et al., 1995). Cheeses have been the vehicles for four major outbreaks of listeriosis. The first in USA (California) associated with a Mexican-style soft cheese (case-fatality risk 34%) (Linnan et al., 1988; James et al., 1985), the second in Switzerland (case-fatality risk 27%) where Vacherin Mont d'Or-type cheese appeared to be the principal source. Two outbreaks occurred in France, linked to the consumption of Brie de Meaux (Goulet et al., 1995) and soft cheeses from Normandy (RNSP, 1997).

2.2. Exposure assessment

L. monocytogenes has been found in a wide range of dairy products. The prevalence of the bacterium in cheese ranges from 0.5 to 10% (Farber and Peterkin, 1991; Pierre and Veit, 1996), depending on survey design, type of cheeses and isolation methods. However, these results are not easily interpretable because of the absence of information about the sampling frame.

In order to assess the risk of human listeriosis associated with the consumption of soft cheese made from raw milk, we attempted to estimate the potential exposure to *L. monocytogenes* in a single serving. The exposure was characterized by the probability distribution of *L. monocytogenes* colony-forming units (CFUs) in 31-*g* servings of cheese at the time of consumption. This mass represents one-eighth of a 250-*g* cheese (representative of most cheeses made from raw milk). A list of variables was identified and distribution was assumed for each variable (Table 1).

2.2.1. Milk production

To estimate the frequency and the distribution of *L. monocytogenes* concentration in raw milk, we used data from a French study on the origin of bovine raw milk contamination by *L. monocytogenes* (Sanaa, 1993; Sanaa et al., 1993, 1996). The study

Table 1
Description and distribution of variables and models from milk contamination to cheese processing

Variable	Description	Unit	Distribution/Model
N	Number of farms per collection	Herds	Triangular [20, 30, 40]
P	Prevalence of farms with contaminated milk	–	Pert [0.01, 0.03, 0.1] or Pert [0.01, 0.02, 0.05]
F	Fraction of infected farms with *L. monocytogenes* mastitis	–	Beta [4, 31]
n	Herd size	Cows	Normal truncated [34, 17, 10, 100]
MCS	Milk contamination status	–	Bernoulli [P]
LMM	Presence of a cow with *L. monocytogenes* mastitis	–	Bernoulli [P × F] or zero
CEN	Concentration in the bulk tank milk, extramammary source	CFU ml^{-1}	0.02 + Poisson [2.25]
CQ	Concentration in milk from an infected quarter	CFU ml^{-1}	$10^{\text{Normal [4, 0.2]}}$
CM	Concentration in milk, intramammary source	CFU ml^{-1}	0.25 × CQ
θ_1	Storage temperature before collection	°C	Triangular [4, 5, 6]
t_1	Storage time before collection	h	Triangular [18, 24, 30]
IC_1	Increase in concentration in the bulk tank before transport	–	Function (θ_1, t_1)[a]
c	Concentration in milk before collection	CFU ml^{-1}	$c = IC_1 \times (CEN \times MCS + (CM/n) \times LMM)$
c	Concentration in the tanker truck before transport	CFU ml^{-1}	$C=(\Sigma n \times c/\Sigma n)$
θ_2	Temperature during transport	°C	Triangular [5, 8, 10]
t_2	Collection time	h	Triangular [4, 6, 8]
IC_2	Increase in concentration during transport	–	Function (θ_2, t_2)[a]
θ_3	Storage temperature during storage after transport	°C	Triangular [3, 4, 5]
t_3	Storage time in manufacture	h	Triangular [18, 24, 30]
IC_3	Increase in concentration in manufacture	–	Function (θ_3, t_3)[a]
FC	Final concentration in milk before cheese processing	CFU ml^{-1}	$FC = C \times IC_2 \times IC_3$
NLM_{vat}	Number of *L. monocytogenes* in one vat	CFU/1000 l	Poisson [$FC \times 10^6$]
$NLM_{2.2\ l}$	Number of *L. monocytogenes* in 2.2 l of milk (the amount needed to make a cheese of 250 g)	CFU/2.2 l	Poisson [NLM_{vat} (2.2×10^{-3})]
NLM_{cheese}	Number of *L. monocytogenes* in 250 g of cheese	CFU/250 g	Poisson [$0.9 \times NLM_{2.2\ l}$]
$NLM_{serving}$	Number of *L. monocytogenes* in a typical serving of 31 g (1/8)	CFU/serving	Poisson [($NLM_{cheese}/250$) × 31)]

[a] Function of temperatures (θ) and times (t) proposed by Peeler and Bunning (1994).

was conducted in a limited geographical area in France in 1989–1992, including a group of 2000 dairy farms. Sixty-four dairy farms with contaminated bulk milk were followed for 2 years. The presence of *L. monocytogenes* was investigated in bulk raw milk, quarter milk and from environmental samples such as silage, feces, teats and water in 33 of the 64 selected herds. The results of this study suggested that the principal source of raw milk

contamination was the environment. The risk of raw milk contamination on dairy farms increased with poor silage quality and insufficient housing and milking hygiene. *L. monocytogenes* mastitis was rare, but was associated with a persistent infection and a high concentration of *L. monocytogenes* in milk.

According to the findings of this study (Sanaa, 1993; Sanaa et al., 1993, 1996), a model of the production process was developed. It is a function of the 17 variables presented in Table 1 and is explained below.

2.2.1.1. L. monocytogenes in milk from infected cows. Bovine *L. monocytogenes* mastitis represented an important source of contamination even though there was never more than one infected cow per farm. According to many authors, one infected quarter can shed between 1000 and 10^6 *L. monocytogenes* ml^{-1} of milk (Sanaa, 1993; Sanaa et al., 1996). For our model analysis, the decimal logarithm of the concentration of *L. monocytogenes* from an infected quarter (CQ) was assumed to be normally distributed with a mean of 4 and a standard deviation of 0.2.

The fraction of herds with *L. monocytogenes* mastitis (F) observed by Sanaa et al. (1996) was 10% (3/33). Using Bayes' theorem where no prior knowledge of F is assumed, the fraction of herds with *L. monocytogenes* mastitis was assumed to have a Beta distribution with $\alpha = 3 + 1 = 4$, $\beta = 33 - 3 + 1 = 31$ (Vose, 1996).

2.2.1.2. L. monocytogenes contamination of milk from environmental sources. On dairy farms, raw milk may be contaminated from environmental sources during milking, storage and transport. This manner of contamination is frequent (90% of herds with contaminated milk) but the concentration is low; generally, less than 1 CFU ml^{-1} in bulk tank milk (Sanaa, 1993; Sanaa et al., 1996). Wilesmith and Gitter (1986) found that the incidence of listeriosis increased with the introduction of silage feeding, since poorly-ensiled silage can contain more than 10^7 cells g^{-1}. Animal feces often contain *L. monocytogenes* (Husu, 1990; Husu et al., 1990), and three studies (Fenlon, 1986; Skovgaard and Morgen, 1988; Sanaa, 1993) have shown that there is a relationship between poor-quality silage and the presence of *L. monocytogenes* in feces. Bad hygienic practices and poorly cleaned milking lines are also risk factors for milk contamination (Sanaa et al., 1993).

Out of the 2000 farms studied, 3% were positive ($\geq$1 CFU/50 ml in bulk-tank milk), ranging from 0% to 8%, with a seasonal variation peaking in winter (Sanaa, 1993). This prevalence (P) was taken as the mode of an assumed Pert distribution (Vose, 1996), with conservative minimum of 1% and maximum of 10%.

Based on Sanaa (1993), the concentration in bulk tank milk of the pathogen from environmental sources (CEN) was assumed to have a Poisson distribution (as usual for low microorganism concentration) with a mean of 2.25. We introduced the term 0.02 to avoid the zero concentration. This distribution does not account for the few cases where *L. monocytogenes* concentration was higher than 10.

2.2.1.3. Growth of L. monocytogenes. After milking, milk is stored in farm bulk tanks, transported in tanker trucks and stored again in the manufacturer's silos. During these three steps, growth of *Listeria* in milk can take place. Increase in count (IC_1, IC_2, IC_3)

was estimated from the published curves of Peeler and Bunning (1994). The increase depends on both holding times (t_1, t_2, t_3) and temperatures (θ_1, θ_2, θ_3). These factors were assumed to have triangular distributions (minimal value, most probable value, maximal value), the parameters of which are summarized in Table 1. The increase in concentration is equal to $2^{(\text{holding time/generation time})}$.

2.2.1.4. Simulation. In order to simulate the distribution of *L. monocytogenes* concentration in milk, @Risk software (version 3.5d, Palisade Corporation, Newfield, NY, USA) was used. The simulation included 10 000 iterations with Latin Hypercube sampling. Each iteration was started by randomly generating three parameters (according to Table 1) to characterize one randomly selected milk collection in the tanker truck. These parameters are the number of farms (N), the instantaneous prevalence of milk contamination (P) and the fraction of farms with *L. monocytogenes* mastitis (F). For each of the N farms, parameters such as herd size, milk contamination status, presence of *L. monocytogenes* mastitis, concentrations in milk, storage times and temperatures were generated. Resulting values were used to calculate the concentrations of *L. monocytogenes* in bulk-tank milk before collection for each farm. The concentration in the tanker truck (C) was the mean of N concentrations weighted by herd size. Time and temperature during transport and storage in manufacture were generated for the collection unit in the tanker truck and used to calculate the increase in concentration. The final concentration in raw milk (FC) was calculated as the product of the increase in concentration factors (according to times and temperatures during transport and storage in manufacture) and the concentration of *L. monocytogenes* in the collection unit in the tanker truck (C) before transport.

2.2.2. Cheese processing

A typical process of soft-cheese making is described here, as the one studied by Ryser and Marth (1987). These authors observed no net growth of *L. monocytogenes* during the first month after milk curdling. This period included ripening for 10 days at 15–16°C and 20 days at 6°C. Data relative to processing and post-processing contamination are not available, so we will not include them in the assessment and will only estimate the risk of cheese contamination by the raw milk itself. It was also assumed that no temperature abuse occurred at distribution and consumption stages.

About 2.2 l of milk are needed to make a cheese of 250 g. We assumed that 90% of the *Listeria* cells are transferred to the curd and 10% of them to the whey (Richard, J., National Institute of Agricultural Research - INRA, pers. commun.). Milk assigned to cheese manufacture is stored in vats of 1000 l. We assumed that the number of *L. monocytogenes* in one cheese vat (NLM_{vat}) has a Poisson distribution with a mean equal to 10^6 times the concentration in milk. In the 2.2 l needed to make one cheese, the same distribution was assumed with a mean equal to: $NLM_{2.2\ l} = NLM_{vat}\ (2.2 \times 10^{-3})$. A Poisson distribution was also attributed to the number of cells transferred to 250 g of cheese with a mean equal to: $NLM_{cheese} = 0.9\ NLM_{2.2\ l}$.

An analysis was performed to determine the influence of the instantaneous prevalence of infected farms and bovine *L. monocytogenes* mastitis on the concentration of the

pathogen in cheese. Four scenarios were studied according to the presence or absence of mastitis with two different prevalences in each case.

2.2.3. Consumption

The number of *L. monocytogenes* in a cheese serving was assumed to be a random variable following a Poisson distribution and the ingested dose was dependent on the number of *L. monocytogenes* present in 250 g of cheese (output of the previous step) and the amount ingested. Accurate data about individual consumption patterns are not available. However, data from Centre Interprofessionel de Documentation et d'Information Laitières (CIDIL, Paris) allow us to estimate the consumption of ripened soft cheeses of any type made from raw milk to be close to 50 servings (each of 31 g) per capita per year.

In addition, the influence of the number of consumed servings per capita and per year on the incidence of human listeriosis was studied. In two scenarios, we used a constant number of servings (20 and 50 /capita/year) and in one scenario we assumed that the number of servings per capita and per year followed a triangular distribution (10, 20, 50).

2.3. Dose-response assessment

The dose-response model used to estimate the probability of illness resulting from a single exposure was the Weibull–Gamma equation suggested by Farber et al. (1996). This flexible model predicts the percentage of the population which responds to a particular dose D of *L. monocytogenes*:

$$\mathrm{PI} = 1 - [1 + (D^b)/\beta]^{-\alpha} \tag{1}$$

where PI denotes the probability of illness for an individual exposed to the dose D of *L. monocytogenes* cells; α, β and b are the model parameters. The model is an extension of the Weibull model (Krewski and Van Ryzin, 1980) taking into account the host/pathogen heterogeneity. The parameter related to the probability of illness given exposure to a single *Listeria* cell is assumed to follow a γ distribution (with parameters α and β).

In the establishment of this relationship, one has to consider factors such as the pathogenicity of the strain and the vulnerability of the host. Healthy people (the low-risk sub-population) rarely develop clinical listeriosis symptoms after eating contaminated food. However, the high-risk sub-population can develop the disease. In the USA, the latter currently represents almost 20% of the total population (Gerba et al., 1996). The same percentage was assumed in the present simulation. For both sub-populations, $\alpha = 0.25$, $b = 2.14$; the low-risk populations' β of $10^{15.26}$ was decreased to $10^{10.98}$ for the high-risk population (Ross, W.H., Bureau of Biostatistics and Computer Applications, Health Protection Branch, Health Canada, pers. commun.).

2.4. Risk characterization

The risk of listeriosis relative to the consumption of raw milk cheese was estimated using the results of the previous steps.

The individual annual cumulative risk (CR) of listeriosis associated with consumption of raw-milk cheese is a combination of the probability of illness linked to the consumption of one cheese serving (R_i) and the number of servings/capita/year (C)

$$CR = 1 - \prod_{i=1}^{C}(1 - R_i) = 1 - \prod_{i=1}^{C}(1 - V \times PI) \quad (2)$$

where V is the proportion of virulent *Listeria* strains, PI the probability of illness for dose D_i.

The probability of illness (R_i) was calculated using Eq. (1) times the probability that the consumed strains of *L. monocytogenes* are virulent.

The proportion of virulent strains was assumed to be $V = 0.1$, the upper limit of the range suggested by Farber et al. (1996).

As for the milk and cheese contamination, we carried out 10 000 iterations using Latin Hypercube sampling implemented with @Risk software, to estimate the final risk of listeriosis, as described above.

Distribution of variables and models for the risk of human listeriosis are presented in Table 2.

3. Results

3.1. Milk contamination

A simulated distribution of the concentration of *L. monocytogenes* in milk before cheese processing was obtained (Fig. 2) and ranged from 0 to 32.68 CFU ml^{-1} with a mean of 1.29 and a median of 0.32 CFU ml^{-1}. The probability of milk being contaminated was estimated to be 67%.

Table 2
Description and distribution of variables and models for the risk of human listeriosis from consumption of raw milk cheese

Variable	Description	Unit	Distribution/Model
PI_L	Probability of illness from a single exposure to a virulent strain, in the low-risk sub-population	–	$PI_L = 1 - [1 + (D^{2.14}) / 10^{15.26}] - 0.25$
PI_H	Probability of illness from a single exposure to a virulent, in the high-risk sub-population	–	$PI_H = 1 - [1 + (D^{2.14}) / 10^{10.98}] - 0.25$
Cs	Number of servings consumed	Servings/capita. yr servings/capita/yr	20 or 50 or Triangular [10, 20, 50]
V	Proportion of strains of *L. monocytogenes* that are virulent	–	0.1
R	Probability of illness	–	$V \times PI$
CR	Individual annual cumulative risk	–	$CR = 1 - \prod_{i=1}^{C}(1 - R_i)$

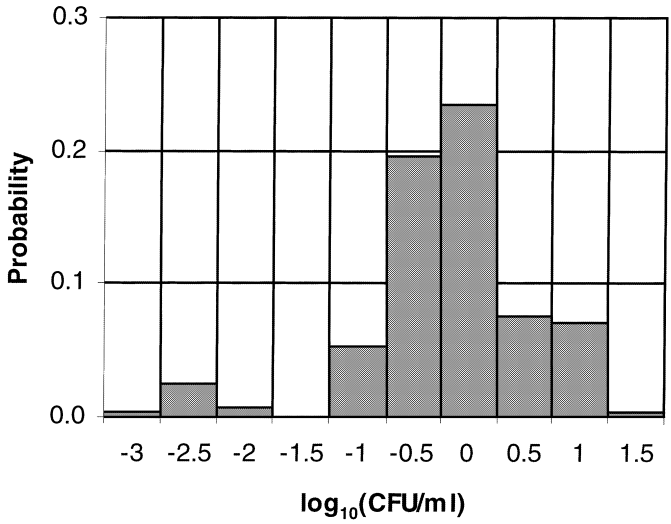

Fig. 2. Simulated frequency distribution for *Listeria monocytogenes* concentration before cheese processing (33% of milk was expected not to be contaminated): 10 000 iterations.

The expected percentages of concentrations exceeding 1, 10 and 100 CFU ml^{-1} were, respectively, 25.1%, 2.5% and less than 0.01% (no value over 100 CFU ml^{-1} was found after 10 000 iterations). The model predicted that 99% of the concentrations before cheese processing would be less than 14 CFU ml^{-1}.

3.2. Cheese contamination

The simulated concentration of *L. monocytogenes* in a 250 g cheese ranged from 0 to 259.6 CFU g^{-1} with a mean of 10.2 and a median of 2.5 CFU g^{-1} (Fig. 3). About 33.2% of cheese was expected to be uncontaminated (0 CFU g^{-1}). The expected percentages of cheese with contamination greater than 1, 10 and 100 CFU g^{-1} were 61.1%, 20.1% and 1.4%, respectively.

For a typical cheese serving of 31 g, the number of bacteria ranged from 0 to 6462 *L. monocytogenes* cells with a mean of 255.5 and a median of 63 cells. The estimated probability of consuming a contaminated cheese serving was 65.3%. However, the estimated probabilities of consuming a dose of *L. monocytogenes* greater than 10^2, 10^3 and 5×10^3 were 41%, 8.3% and 0.08%, respectively.

The results (Table 3) show a decrease in the expected percentage of cheese with a concentration greater than 10 CFU g^{-1} when cows with *L. monocytogenes* mastitis are eliminated from the simulation.

3.3. Risk of listeriosis in humans consuming cheese

The dose-response model predicted a probability of illness associated with the consumption of one cheese serving. This risk varied from 0 to 3.73×10^{-4} with a median of

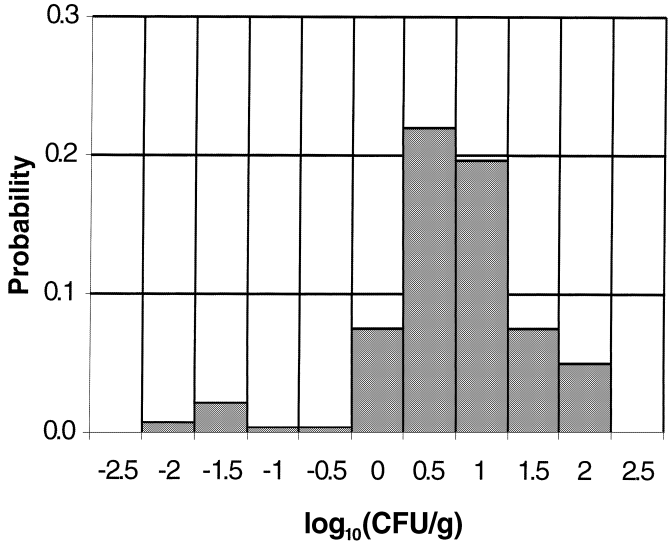

Fig. 3. Simulated frequency distribution for *Listeria monocytogenes* concentration in cheese (33.2% of cheese was expected not to be contaminated): 10 000 iterations.

1.86×10^{-8} for a high-risk sub-population (Fig. 4(a)), and from 0 to 1.96×10^{-8} with a median of 9.74×10^{-13} for a low-risk healthy sub-population (Fig. 4(b)). The risk is equal to zero in 34.7% of the high-risk sub-population and 45.4% of the low-risk sub-population.

Using a constant number of consumed servings (50/capita/year) individual annual cumulative risk of listeriosis (CR) ranged from 2.0×10^{-9} to 6.4×10^{-8} for the low-risk sub-population and 1.0×10^{-6} to 7.2×10^{-5} for the high-risk sub-population. Using 20% as the proportion of the overall population that is high-risk, the number of expected human cases of clinical listeriosis in a country with 50 million inhabitants ranged from 34 to 90 cases (mean 57) and a number of deaths varying from 1 to 23 (mean 12). The same

Table 3
Influence of the instantaneous prevalence of milk contamination and the presence of mastitis on the contamination of soft cheese made from raw milk; expected probabilities and distributions (10 000 iterations)

Outcome	Presence of mastitis		Absence of mastitis	
	Simulation 1[a]	Simulation 2[b]	Simulation 3[a]	Simulation 4[b]
Probability (CFU/g > 0)	0.611	0.516	0.631	0.475
Probability (CFU/g > 10)	0.200	0.106	0.086	0.040
Probability (CFU/g > 100)	0.014	0.068	0.000	0.000
Minimum value (CFU g^{-1})	0	0	0	0
Median (CFU g^{-1})	2.536	0.024	1.876	0
99th percentile (CFU g^{-1})	110.36	87.21	20.35	16
Maximum value (CFU g^{-1})	259.59	203.75	44.89	31.67

[a] Prevalence was assumed to follow Pert (0.01, 0.03, 0.1).
[b] Prevalence was assumed to follow Pert (0.01, 0.02, 0.05).

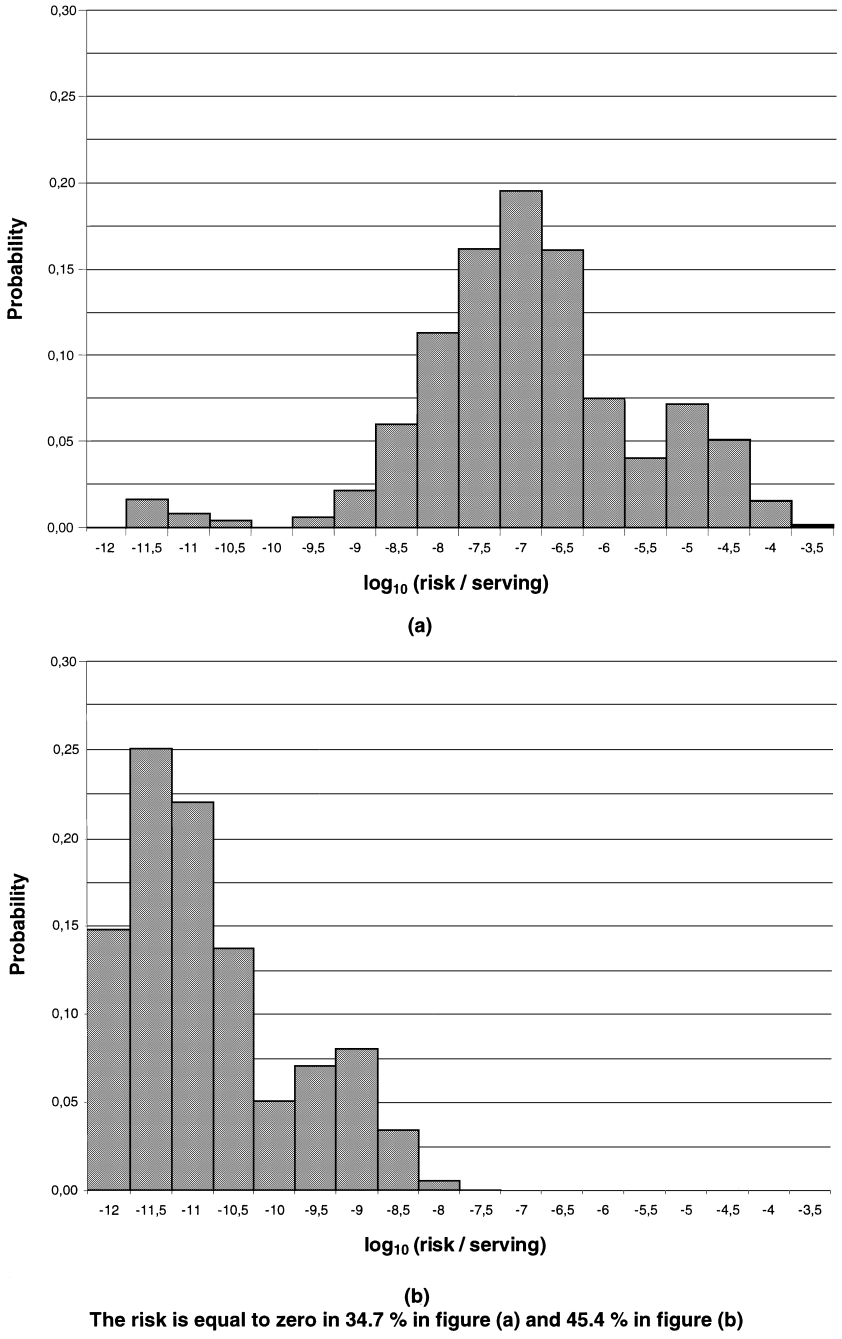

Fig. 4. Risk of illness associated with the consumption of one serving of cheese by (a) people in high-risk and (b) typical healthy populations.

Table 4
Number of expected listeriosis cases and deaths according to different individual consumption patterns

Number of/servings/capita year	Number of cases				Number of deaths			
	Min	Max	Mean	99%	Min	Max	Mean	99%
20[a]	9[c] (0)[d]	38 (3)	23 (0.2)	33 (2)	0 (0)	16 (2)	5 (0.04)	10 (1)
Triangular (10,20,50)[a]	12 (0)	50 (3)	31 (0.2)	44 (2)	1 (0)	15 (2)	7 (0.04)	13 (1)
50[a]	34 (0)	90 (4)	57 (0.5)	74 (3)	1 (0)	23 (3)	12 (0.1)	19 (1)
50 (simulation 4)[b]	3 (0)	25 (2)	11 (0.1)	20 (1)	0 (0)	8 (1)	2 (0.02)	6 (1)

[a] We used risk distribution from output of simulation 1 (Fig. 1 and Table 3).
[b] We used risk distribution from output of simulation 4 (Table 3).
[c] High-risk sub-population.
[d] Low-risk sub-population.

number of expected cases in the low-risk sub-population varies from 0 to 4 (0 to 3 deaths).

The numbers of expected cases of listeriosis were recalculated using parameters of simulation 4 (Table 3), which reduced the prevalence of milk contamination and eliminated the mammary source of *L. monocytogenes*. The number of listeriosis cases ranged, for low-risk sub-population, from 0 to 3 cases per year and for high-risk sub-population, from 3 to 25 cases (mean 11). The number of expected deaths was less than 2 for low-risk sub- two population. The number of expected cases of listeriosis decreased (by inspection) when the number of servings/capita/year was modified (Table 4).

4. Discussion

The Monte Carlo simulation approach was preferred to the method used by Peeler and Bunning (1994). The latter estimated the probability of several scenarios: values for each of the factors were selected by choosing a particular percentile of each variable, and yielded the probability of exceeding particular *L. monocytogenes* concentration in pasteurized milk. This leads to large errors in the estimation of probabilities: Cassin et al. (1996) identified 'compounding conservatism' and 'compounding scenario exclusion' as the two main sources of error.

In lieu of the origin of data and assumptions made, the results of this risk assessment should be interpreted carefully. As regards data, the only ones that were available had been collected more than 6 years ago. They do not reflect improvement of hygiene that occurred since; both the proportion of farms with *L. monocytogenes* from the environment, and the proportion of cows shedding *L. monocytogenes*, have decreased (according to unpublished data). In addition, the time needed for detecting and eliminating infected cows decreased. Despite the fact that newer data reflect great progress, recent data are confidential and could not be used for the present study.

As regards assumptions, they belong to several categories. The first one concerns milk production: number of farms, herd size, and milk volume per cow. We chose to conduct the simulations with a low number of farms, representative of a small cheese plant. We

checked that choosing a higher number of farms gives a lower proportion of the most contaminated servings. Thus, the choice made drives us to an overestimation of the risk. It can be questioned if there is a correlation between the size of herds and milk volume per cow on the one hand and the proportion of infected cows on the other. Available data do not provide an answer to such a question.

Assumptions of the second category regard the cheese making process. We eliminated from the model any contamination arising during transportation of raw milk, cheese making, ripening, distribution, and in households. Concerning transportation of raw milk we could use the factor calculated by Peeler and Bunning (1994), but we wondered if it was appropriate for the situation described. Neglecting this aspect drove us to a slight underestimation of the risk. Contamination during cheese making and subsequent steps was deliberately ignored: it can be assumed that it is similar when cheese is made from pasteurized milk. This option could be chosen since the scope of the present paper is only the risk created by the raw milk itself, and not the way it is used. We also limited the manufacturing process of cheese to 1 month, and we did not simulate temperature abuse between production and consumption, thus limiting the model to what is good hygienic practice.

The consumption of raw milk cheese belongs to a third kind of assumption. The model considers similar consumption in the susceptible and general populations. Yet, do babies and the elderly, or people in care institutions, eat expensive raw milk cheeses? Assuming an equal number of servings for everybody is likely to dilute the risk among the general population, and to overemphasize it for the population at risk. We cannot estimate the respective weights of these contradictory effects.

A fourth class of assumptions concerns the distributions used. Triangular distributions were used when the exact distribution was not known. According to Vose (1996), ''the triangular shape overemphasizes the tails of the distribution and under-emphasizes the shoulders in comparison with other, more-natural, distributions''. This can lead to overestimating both the best and worse cases – for example, where growth temperature is modeled. A Poisson distribution was taken for the concentration of *L. monocytogenes* in milk of farms with environmental contamination. The actual distribution could not be evaluated (because of the low frequency of contamination, data were too scarce). Therefore, we assumed a Poisson distribution, the best description of microorganisms' distribution in homogeneous liquids. Furthermore, since the farms were defined as 'positive' for the presence of *L. monocytogenes*, and since the probability of getting zero concentration from the Poisson distribution is high, we arbitrarily chose to replace the zero output of the simulation by 0.02.

Finally, it must be realized that the dose-response equation of Farber et al. (1996) are also based on assumptions. We prefer these equations, rather than the one published by Buchanan et al. (1997). While Farber et al. (1996) considered mostly listeriosis caused by pâté and cheese, Buchanan et al. (1997) work was based on data pertaining to the consumption of smoked salmon in Germany. In addition, the latter attributed every case of listeriosis in that country to this sole food, and did not account for differences between the general population and the susceptible fraction of it. Farber et al. (1996) did not provide distributions of parameters of their equations, therefore it must be realized that the spread of the distribution of simulation outputs is most to be likely underestimated.

Farber et al. (1996) noted that infection by *L. monocytogenes* is associated with only a few virulent strains (Farber and Peterkin, 1991; McLauchlin, 1993. Rocourt, 1994). Hence, they suggested to multiply the number of ingested bacterial cells by a factor in the range 0.01–0.1. To stay on the conservative side of the estimation, we used the factor 0.1 in the present work.

In the first step, we modeled the distribution of milk contamination. The expected percentage for milk in cheese production is high: 67%. However, the concentration was predicted to be low: for example, 99% would have a concentration of less than 14 CFU ml^{-1}, whereas Peeler and Bunning (1994) found that the concentration in the upper 95 percentile was 870 CFU ml^{-1}. The difference is due to what we believe is a more realistic integration of mastitis in milk contamination.

In the second step, we modeled the distribution of cheese contamination. The expected percentage of contaminated cheeses is high (66.8%) – however, only 20.1% and 1.4% were predicted to contain, respectively, more than 10 and 100 CFU g^{-1} of cheese. In a survey of contamination of various foods over the period 1994–1995, Pierre and Veit (1996) found that 11% of a sample of 358 mould-ripened soft cheeses were positive for *L. monocytogenes*. The proportion of cheese in the sample made from raw milk was not indicated. Discrepancy between Pierre and Veit (1996) and the present simulation can have one of the following explanations or a combination of them:

- Data in the simulation are no longer valid (collected earlier than 1993), and reflect a sanitary status worse than the one in 1994–1995.
- The detection method in the survey had a high detection threshold (low sensitivity). It is more difficult to detect *L. monocytogenes* in complex food matrices such as cheese than in milk. Also, the culturability of *L. monocytogenes* can be modified by cheese processing and antagonistic species (Farber and Peterkin, 1991; Back et al., 1993; Bachmann and Spahr, 1995).

It is also possible that *L. monocytogenes* is not homogeneously distributed in milk and cheese. Clustering in milk would reduce the number of colony-forming units in milk and cheese, and also reduce the probability of detecting them in cheese.

The results of the model could first be compared to epidemiological evidence. In a population of 50 million people (including 20% susceptible) consuming 50 servings per capita per year, the simulated average number of expected cases of listeriosis was 57 per year. We do not intend either to say if the proportion relative to all causes of listeriosis is low (we would be suspected to be in favor of raw milk cheese) or high (we would be accused to be their enemy).

The simulation of the model can also be used to study the influence on morbidity and mortality of realistic management options, as shown in Table 4. Thus it could be a useful tool for raw milk soft cheese manufacturers as well as for the authority in charge of public health.

Despite the limitations that we underscored, the present work is the first attempt to model the risk of *L. monocytogenes* infection and death from consumption of raw milk soft cheese. It shows how poor the available data are, and what needs to be done to improve them.

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References

Altekruse, S.F., Cohen, N.L., Swerdlow, D.L., 1997. Emerging foodborne diseases. Emerging Infect. Dis. 3(3), 285–293.

Anonymous, 1996. Report of the 28th Session of the Codex Committee on Food Hygiene, ALINORM 97/13, Codex Alimentarius Commission, Rome.

Bachmann, H.P., Spahr, U., 1995. The fate of potentially pathogenic bacteria in Swiss hard and semi-hard cheeses made from raw milk. J. Dairy. Sci. 78, 476–483.

Back, J.P., Langford, S.A., Kroll, R.G., 1993. Growth of *Listeria monocytogenes* in camembert and other soft cheeses at refrigeration temperatures. J. Dairy Res. 60, 421–429.

Bille, J., 1990. Epidemiology of human listeriosis in Europe, with special reference to the Swiss outbreak. In: Miller, A.J., Smith, J.L., Somkuti, G.A. (Ed.), Foodborne listeriosis. Society for Industrial Microbiology. Elsevier, New York.

Buchanan, R.L., Damert, W.G., Whiting, R.C., Van Schothorst, N., 1997. Use of epidemiologic and food survey data to estimate a purposefully conservative dose-response relationship for *Listeria monocytokines* and incidence of listeriosis. J. Food. Prot. 60(8), 918–922.

Cassin, M.H., Paoli, G.M., McColl, R.S., Lammerding, A., 1996. A comment on 'Hazard assessment of *Listeria monocytogenes* in the processing of bovine milk'. J. Food Prot. 59(4), 341–343.

Codex Alimentarius Commission, 1995. Food Standards Program Codex Alimentarius Commission. Report of the 28th Session of the Codex Committee on Food Hygiene. Washington DC, 27 November–1 December 1995. ALINORM 97/13.

Farber, J.M., Peterkin, P.I., 1991. Listeria monocytogenes, a food-borne pathogen. Microbiol Rev. 55(3), 476–511.

Farber, J.M., Ross, W.H., Harwig, J., 1996. Health risk assessment of *Listeria monocytogenes* in Canada. Int. J. Food Microbiol. 30, 1–2, 145–156.

Fenlon, D.R., 1986. Growth of naturally occurring *Listeria* spp. in silage: A comparative study of laboratory and farm ensiled grass. Grass Forage Sci. 41, 375–378.

Gerba, P.C., Rose, J.B., Haas, C.N., 1996. Sensitive populations: Who is at the greatest risk?. Int. J. Food Microbiol. 30, 113–123.

Goulet, V., Jacquet, C., Vaillant, V., Rebière, I., Mouret, E., Lorente, C., Maillot, E., Stainer, F., Rocourt, J., 1995. Listeriosis from consumption of raw-milk cheese. Lancet 345, 1581–1582.

Husu, J.R., 1990. Epidemiological studies on the occurrence of *Listeria monocytogenes* in the feces of dairy cattle. J. Vet. Med. B 37, 276–282.

Husu, J.R., Seppanen, J.T., Sivela, S.K., Rauramaa, A.L., 1990. Contamination of raw milk by *Listeria monocytogenes* on dairy farms. J. Vet. Med. Ser. B 37, 268–275.

IFST (Institute of Food Science and Technology), 1998. http://www.easynet.co.uk/ifst/hottop.htm.

Jacquet, C., Michelon, F., Saint-Cloment, C., Rocourt, J., 1995. La listériose humaine en France en 1994. Bull. Epidémiol. Hebdom. 39, 173–174.

James, S.M., Fannin, S.L., Agee, B.A., Hall, B., Parker, E., Vogt, J., Rung, G., Williams, J., Lieb, L., Salminen, C., Prendergrast, T., Werner, S.B., Chin, J., 1985. Listeriosis outbreak associated with Mexican-style cheese. MMWR. 34, 357–359.

Krewski, D., Van Ryzin, J., 1980. Dose-response models for quantal response toxicity data. In: Csorgo, M., Dawson, D.A., Rao, J.N.K., Saleh, A.K.Md.E. (Eds.), Statistics and Related Topics. Proc. Int. Symp, Statistics and Related Topics Ottawa, Canada, 5–7 May, 1980. North Holland, New York, pp 201–231.

Linnan, M.J., Mascola, L., Dong Lou, X., Goulet, V., May, S., Salminen, C., Hird, D.W., Yonekura, L.M., Hayes, P., Weaver, R., Audurier, A., Plikaytis, B.D., Fannin, S.L., Kleks, A., Broome, C.V., 1988. Epidemic listeriosis associated with Mexican-style cheese. N. Engl. J. Med. 319, 823–828.

McLauchlin, J., 1993. Listeriosis and *Listeria monocytogenes*. Environ. Policy Pract. 3, 201–214.

National Research Council (NRC), 1983. Risk assessment in the Federal Government: Managing the process. National Academy Press, Washington DC.

Peeler, J.T., Bunning, V.K., 1994. Hazard assessment of *Listeria monocytogenes* in the processing of bovine milk. J. Food Prot. 57(8), 689–697.

Pierre, O., Veit, P., 5 Novembre 1996. Plan de surveillance de la contamination par Listeria monocytogenes des aliments distribués. Bull Epidémiol. Hebdom 45, 195–197.

RNSP, 1997. Epidémie de listériose 1997, Rapport du Réseau National de Santé Publique, France, http://www.b3e.jussieu.fr/rnsp/publicat/listeriose/index.html.

Rocourt, J., 1994. *Listeria monocytogenes*, the state of the science. Dairy Food Environ. Sanit. 14, 70–82.

Rocourt, J., Jacquet, C., Brouille, F., Saint-Cloment, C., Catimel, B., 1997. La listériose humaine en France en 1995 et 1996. Bull. Epidémiol. Hebdom.41: http://www.b3e.jussieu.fr/rnsp/beh/9741/9741_p2.html.

Ryser, E.T., Marth, E.H., 1987. Fate of *Listeria monocytogenes* during the manufacture and ripening of camembert cheese. J. Food Prot. 50(5), 372–378.

Sanaa, M., 1993. Epidémiologie de la contamination du lait à la ferme par *Listeria monocytogenes*. Thèse Doctorat Univ. Paris XI, p. 207.

Sanaa, M., Poutrel, B., Menard, J.L., Serieys, F., 1993. Risk factors associated with contamination of raw milk by *Listeria monocytogenes* in dairy farms. J. Dairy Sci. 76, 2891–2898.

Sanaa, M., Audurier, A., Poutrel, B., Menard, J.L., Serieys, F., 1996. Origin of bovine milk contamination by *Listeria monocytogenes*. Int. Dairy Fed. 25, 163–179.

Schuchat, A., Deaver, K.A., Wenger, J.D., Plikaytis, B.D., Mascola, L., Pinner, R.W., Reingold, A.L., Broome, C.V., and the *Listeria* Study Group, 1992. Role of foods in sporadic listeriosis I. Case-Control Study Of Dietary Risks Factors. JAMA. 267, vol. 15, pp. 2041–2045.

Skovgaard, N., Morgen, C.A., 1988. Detection of *Listeria* spp. in faeces from animals, in feeds and raw foods of animal origin. Int. J. Food Microbiol. 6(3), 229–242.

Tappero, J.W., Schuchat, A., Deaver, K.A., Mascola, L., Wenger, J.D., 1995. Reduction in the incidence of human listeriosis in the United States. Effectiveness of prevention efforts?. The Listeriosis Study Group. JAMA 273(14), 1118–1122.

Vose, D., 1996. Quantitative Risk Analysis: A Guide To Monte Carlo Simulation Modelling. Wiley, New York, p. 330.

Wilesmith, J.W., Gitter, M., 1986. Epidemiology of ovine listeriosis in great Britain. Vet. Rec. 119, 467–470.

Pinner et al. (1992).