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Quantitative Risk Assessment of Human Salmonellosis and Listeriosis Related to the Consumption of Raw Milk in Italy

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ABSTRACT

Two quantitative risk assessment (RA) models were developed to describe the risk of salmonellosis and listeriosis linked to consumption of raw milk sold in vending machines in Italy. Exposure assessment considered the official microbiological records monitoring raw milk samples from vending machines performed by the regional veterinary authorities from 2008 to 2011, microbial growth during storage, destruction experiments, consumption frequency of raw milk, serving size, and consumption preference. Two separate RA models were developed: one for the consumption of boiled milk and the other for the consumption of raw milk. The RA models predicted no human listeriosis cases per year either in the best or worst storage conditions and with or without boiling raw milk, whereas the annual estimated cases of salmonellosis depend on the dose-response relationships used in the model, the milk storage conditions, and consumer behavior in relation to boiling raw milk or not. For example, the estimated salmonellosis cases ranged from no expected cases, assuming that the entire population boiled milk before consumption, to a maximum of 980,128 cases, assuming that the entire population drank raw milk without boiling, in the worst milk storage conditions, and with the lowest dose-response model. The findings of this study clearly show how consumer behavior could affect the probability and number of salmonellosis cases and in general, the risk of illness. Hence, the proposed RA models emphasize yet again that boiling milk before drinking is a simple yet effective tool to protect consumers against the risk of illness inherent in the consumption of raw milk. The models may also offer risk managers a useful tool to identify or implement appropriate measures to control the risk of acquiring foodborne pathogens. Quantification of the risks associated with raw milk consumption is necessary from a public health perspective.

The sale of raw milk for human consumption in self-service vending machines has been allowed in Italy since 2004. This study addresses the safety of raw milk consumption in relation to two microorganisms of concern and in compliance with microbiological criteria stipulated in the national legal requirements *(19)*, such as *Salmonella* and *Listeria monocytogenes*, based on data collected from 2008 to 2011.

Nontyphoidal *Salmonella* are important foodborne pathogens that cause gastroenteritis; symptoms are often mild, and most infections are self-limiting. In 2011, the European Union member states reported a total of 97,897 salmonellosis cases, with 6,662, 5,715, 4,752, and 3,344 confirmed cases reported in Italy during the years 2008 to 2011, respectively, with a continuously decreasing European Union trend from 2008 to 2011 *(8)*. Listeriosis is a disease caused by a relatively rare infection by *Listeria monocytogenes*; severe symptoms may result primarily in elderly people, immunocompromised individuals, pregnant women, and newborns. In healthy individuals, the infection may be asymptomatic or present with a mild cases of febrile illness or diarrhea. In 2011, the European Union member states reported 1,476 confirmed human cases of listeriosis, with 118, 109, 137, and 100 confirmed cases reported in Italy during the years 2008 to 2011, respectively *(8)*. Most

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cases (all the cases in Italy) with available data regard hospitalized patients, probably due to a focus on invasive cases. An increased incidence in the elderly in the 2008 to 2011 period was already apparent in the group older than 65 years of age *(7)*.

Milk and milk products were implicated in 1 to 5% of the total bacterial foodborne outbreaks in industrialized countries, with 39.1% attributed to milk, 53.1% to cheese, and 7.8% to other milk products *(6)*. Considering only raw cow's milk consumption as the source of human outbreaks, the significance of *Salmonella* and *L. monocytogenes* in the reported literature is very low. Only 2 outbreaks were reported for *L. monocytogenes,* both outside Europe, whereas 5 outbreaks were reported for *Salmonella* in Europe, with 39 worldwide *(5)*. No human cases or outbreaks for salmonellosis and listeriosis in Italy have been related to the consumption of raw cow's milk, even though both microorganisms have been found in raw cow's milk for human consumption *(3, 13, 11),* and official control data report a prevalence in raw milk, varying from 0 to 0.96% for *Salmonella* and 0.21 to 1.63% for *L. monocytogenes*. No substantial difference in the occurrence of these two pathogens in raw milk has been observed over the years, and the number of positive samples is low but appears irreducible. Despite these reports and a warning issued by the Italian Ministry of Health in 2008 *(20)* that all raw milk vending machines must display the notice ''milk must be consumed after boiling,'' many consumers still drink raw milk *(11, 12, 14)*. Quantitative microbial risk assessment (RA) provides a methodological framework to integrate pathogen monitoring data and epidemiological data to quantify the potential risk to humans (often defined as the probability of infection) associated with various exposure pathways.

The main objective of this study was to estimate the risk of salmonellosis and listeriosis attributed to consumption of raw cow's milk purchased from vending machines located in seven Italian regions. The quantitative RA (QRA) considered during a 4-year period, the presence of these pathogens in raw cow's milk at the delivery point to consumers (vending machines), consumer habits, and the behavior of pathogens throughout the food chain and reflects the current trends in raw milk consumption in Italy.

MATERIALS AND METHODS

Exposure assessment. Data were collected from a previous survey that gathered together microbiological records of official controls monitoring raw milk samples from self-service vending machines in seven regions of Italy performed by the regional veterinary authorities from 2008 to 2011 *(11)*. The seven regions, Emilia Romagna, Lazio and Tuscany (pooled data), Lombardy, Marche, Piedmont, Sicily, and Veneto, account for a total of 1,239 vending machines, i.e., 87.73% of the 1,411 vending machines registered in Italy. The Integrated National Annual Report of the Italian Ministry of Health states that these vending machines are supplied by 1,032 dairy herds *(21)*; each herd can supply one or more vending machines. The number of vending machines in each region and the number of samples collected from vending machines may differ among regions, but in all regions, at least one sample was tested from each vending machine every year.

Prevalence of *Salmonella* and *L. monocytogenes* in raw milk in vending machines and estimation of their concentration in raw milk. Previous prevalence data on positive samples of *Salmonella* and *L. monocytogenes* in 30,684 raw milk samples from self-service vending machines were included in the present study *(11)*. The samples were analyzed in accordance with microbiological criteria stipulated in the national legal requirements of the State-Regions Agreement for *Salmonella* and *L. monocytogenes* using the official International Organization for Standardization cultural methods 6579:2002/C1:2004 *(16)* and 11290-1:1996/AM1:2004 *(17)*. All samples were processed at the Experimental Institutes for Zooprophylaxis in the different regions; all the laboratories and test procedures are accredited according to International Organization for Standardization method 17025:2005 *(18)* by Accredia, the Italian accreditation body. *Salmonella* and *L. monocytogenes* were detected in 18 (0.11%) of 15,420 and 83 (0.54%) of 15,264 raw milk samples, respectively. The minimum detectable level of culture presence-absence tests is usually estimated in 1 CFU in 25 g/ml or 0.04 cells per g per ml in standard cultures.

The beta function was used to model the variability and uncertainty introduced by sampling on the true prevalence estimation of *Salmonella* and *L. monocytogenes* in raw milk samples. Assuming that the true prevalence in a given population of raw milk samples collected from vending machines is P, the number of positive raw milk samples, S, in N raw milk samples tested is binomial (N, P). If we assume a priori a uniform [0,1] prior distribution for P (the probability of being positive) and find that S of N sampled vending machines have one or more positive raw milk samples, the posterior distribution of raw milk prevalence P is Beta ($S + 1, N - S + 1$).

Following this consideration, the probability P^+ of finding a positive sample was modeled as

$$P^+ = \text{beta}\ (S+1;\ N-S+1) \quad (1)$$

where S is the number of the positive raw milk samples and N is the total number of raw milk samples analyzed ($n = 15{,}420$ for *Salmonella* and 15,264 for *L. monocytogenes*).

Assuming that pathogens follow a Poisson distribution in milk samples, the probability that no *Salmonella* or *L. monocytogenes* are detected during microbiological analysis in a samples volume (V_{sample}) is

$$P^- = \exp\left(-C \times V_{\text{sample}}\right) \quad (2)$$

Following this assumption, the mean concentration of *Salmonella* and *L. monocytogenes* is therefore

$$C = -\text{Ln}(1-P^+)/V_{\text{sample}} \quad (3)$$

Assuming the level of pathogens was lognormally distributed in raw milk, the mean (m of the lognormal distribution) was estimated by a Monte Carlo simulation (with practical value of 100,000 iterations) using @Risk, version 4.5.2 (Palisade Corporation, Newfield, NY). Once the distribution of the mean of the lognormal distribution was obtained, the standard deviation(s) was fixed to match the fraction of positive samples (0.04 CFU/ml) actually observed in the official controls.

Time-temperature history of raw milk from vending machine to consumption and pathogens' growth model. Data from a previous study *(14)* were used for the time-temperature history and the pathogens' growth model: briefly, time-temperature conditions of raw milk from 33 farms authorized to produce and sell this product were investigated together with consumer habits. Field handling temperatures were recorded from the bulk tank milk of the producers, during transport to the vending machine, storage in the vending machines, home transportation, and home storage until consumption. The best (4°C as fixed by law) and worst temperature storage conditions detected were simulated by a challenge test, and the doubling time (T_d) of the two pathogens in the two different conditions was calculated. T_d values were recalculated for this study to consider the different sampling points (the farm in the previous studies and the vending machine in the present study). A triangular distribution was chosen, with the most likely value fixed as equal to the experimental mean T_d observed; minimum and maximum parameters in the triangular distribution were calculated considering the standard deviation observed experimentally:

$$T_{d\,(Salmonella\ 4^{\circ})} = \text{triangular}\ (35.1, 61.46, 88.22)\ \text{h} \quad (4)$$

$$T_{d\,(Salmonella\ \Delta T)} = \text{triangular}\ (13, 22, 31)\ \text{h} \quad (5)$$

$$T_{d\,(L.\ monocytogenes\ 4^{\circ})} = \text{triangular}\ (31.88, 69.7, 107.52)\ \text{h} \quad (6)$$

$$T_{d\,(L.\ monocytogenes\ \Delta T)} = \text{triangular}\ (24, 28, 32)\ \text{h} \quad (7)$$

By law, raw milk has a storage life of 3 days, but on the basis of interview answers *(13)*, milk was consumed up to 5 days after purchase. For this reason, the shelf life T (h) was modeled by the triangular distribution:

$$T\ (\text{h}) = \text{triangular}\ (0.5, 24, 120)\ \text{h} \quad (8)$$

Therefore, the number of pathogen replications (dn) after storage in vending machines, home transportation, and home storage is

$$\text{dn} = T\ (\text{h})/T_d \quad (9)$$

We modeled the concentration of each pathogen in raw milk after home storage both for the best and the worst storage raw milk scenario as

$$C_{\text{after storage}} = \left(2^{\text{dn}}\right) \times \left(10^{C}\right) \quad (10)$$

Data obtained from experiments in which the milk was boiled *(14)* were used to model the survival of *Salmonella* and *L. monocytogenes* in milk after an effective heat treatment. No viable pathogenic bacteria were recovered from boiled milk, but to consider possible undertreatment of milk (microwave treatment or insufficient boiling), the log reduction count was modeled using the triangular distribution:

$$D_{\text{boil}\ Salmonella} = \text{triangular}\ (6, 7, 8)\ \text{log reduction} \quad (11)$$

$$D_{\text{boil}\ L.\ monocytogenes} = \text{triangular}\ (6, 7, 8)\ \text{log reduction} \quad (12)$$

On this basis, the potential concentration of each pathogen in raw milk after boiling was calculated for the best and the worst storage raw milk scenario

$$C_{\text{after boiling}} = 10^{(\log C_{\text{after storage}} - D_{\text{boil}})} \quad (13)$$

The distribution of raw milk serving size (S_i) was characterized by a previous survey *(12)*; S_i was modeled by the triangular distribution as

$$S_i = \text{triangular}\ (100, 250, 1{,}000)\ \text{ml} \quad (14)$$

Pathogen dose per serving size. The percentages of consumers not boiling milk before consumption, ranging from 13.9 to 43% in three previous investigations *(2, 12, 13)*, were considered in this study. Regarding the fraction of consumers correctly boiling milk before drinking, it is difficult to obtain an accurate estimate from questions on consumption habits. Given the importance of this estimate in the results of the RA, we developed two separate RA models: one for the consumption of boiled milk (assuming that the entire population boils raw milk) and the other for the consumption of raw milk without boiling (assuming that the entire population does not boil raw milk). In addition, each dose output model was achieved for the best and the worst storage milk scenarios as

$$\text{dose} = C_{\text{after storage (after boiling)}} \times S_i \quad (15)$$

Dose response. For *Salmonella*, this RA model used two dose-response (D-R) relationships, the D-R proposed by the World Health Organization and Food and Agriculture Organization of the United Nations (WHO/FAO) in 2002 *(30)* and the D-R proposed by Teunis et al. in 2010 *(25)*. The beta-Poisson WHO/FAO *(30)* D-R relationship directly calculates the probability of salmonellosis (p_{ill}) due to the ingested dose:

$$p_{\text{ill}} = 1 - (1 + \text{dose}/b)^{-a} \quad (15)$$

where a = triangular (0.0763, 0.1324, 0.2274) and b = triangular (38.49, 51.45, 57.96). In this D-R model, the ID_{50} (the dose that produces a probability of 0.5 to become infected) was equal to about 10^4 CFU of *Salmonella*.

The D-R proposed by Teunis et al. *(25)* took into account the risk of infection p_{inf} and the probability to be ill when infected due to the ingested dose $p_{\text{ill}} = p_{\text{inf}} \times p_{\text{ill inf}}$. Teunis et al. *(25)* kindly provided a set of 5,000 values of the four parameters proposed by their model: the obtained parameters set for an unstratified model in which the $p_{\text{ill 50}}$ was equal to about 36 CFU of *Salmonella* used in this RA model. The number of cases of salmonellosis due to the p_{ill} was simulated as

$$\text{salmonellosis (1 or 0)} = \text{Bernoulli}\ (p_{\text{ill}}) \quad (16)$$

For *L. monocytogenes*, the exponential model used meets the recommendations of the draft proposed by the FAO/WHO in 2003 *(10)*. This D-R model calculates the probability of severe listeriosis, as a function of the ingested CFU of the microorganisms

$$p_{\text{ill}} = 1 - \exp\ (r \times \text{dose}) \quad (17)$$

Values for r were obtained from the "Joint FAO/WHO Expert Consultation on Risk Assessment of Microbiological Hazards in Foods" proposed in 2001 *(9, 29)*. For more susceptible populations (immune-deficient patients or organ transplant recipients, bladder cancer, dialysis, gynecological cancer patients, elderly older than 65 years, and perinatal exposure), the r value used is 1.06×10^{-12}. For the normal population, the r value used is 2.37×10^{-14}.

The number of cases of severe listeriosis due to the p_{ill} was simulated as

$$\text{severe listerioris case (1 or 0)} = \text{Bernoulli}\ (p_{\text{ill}}) \quad (18)$$

Output of the models. The models were implemented with software for Monte Carlo simulation @Risk, version 4.5.2, and 100,000 iterations were done for each simulation. The first output of the model was the lognormal distribution of the milk concentration of *Salmonella* and *L. monocytogenes*, estimated on the bases of official control results on vending machines. For each hazard assessed, four different simulation models were run: two for the best and worst scenarios in raw milk storage, considering all milk servings were correctly boiled or not boiled by consumers. In addition, the *Salmonella* model considered two different D-R relationships *(25, 30)*.

Figure 1 shows the flow chart of the model used to estimate the probability of illness from a single exposure to contaminated raw milk and the number of illness cases expected each year. The number of expected cases was calculated on the basis of the total amount of raw milk sold in vending machines estimated by

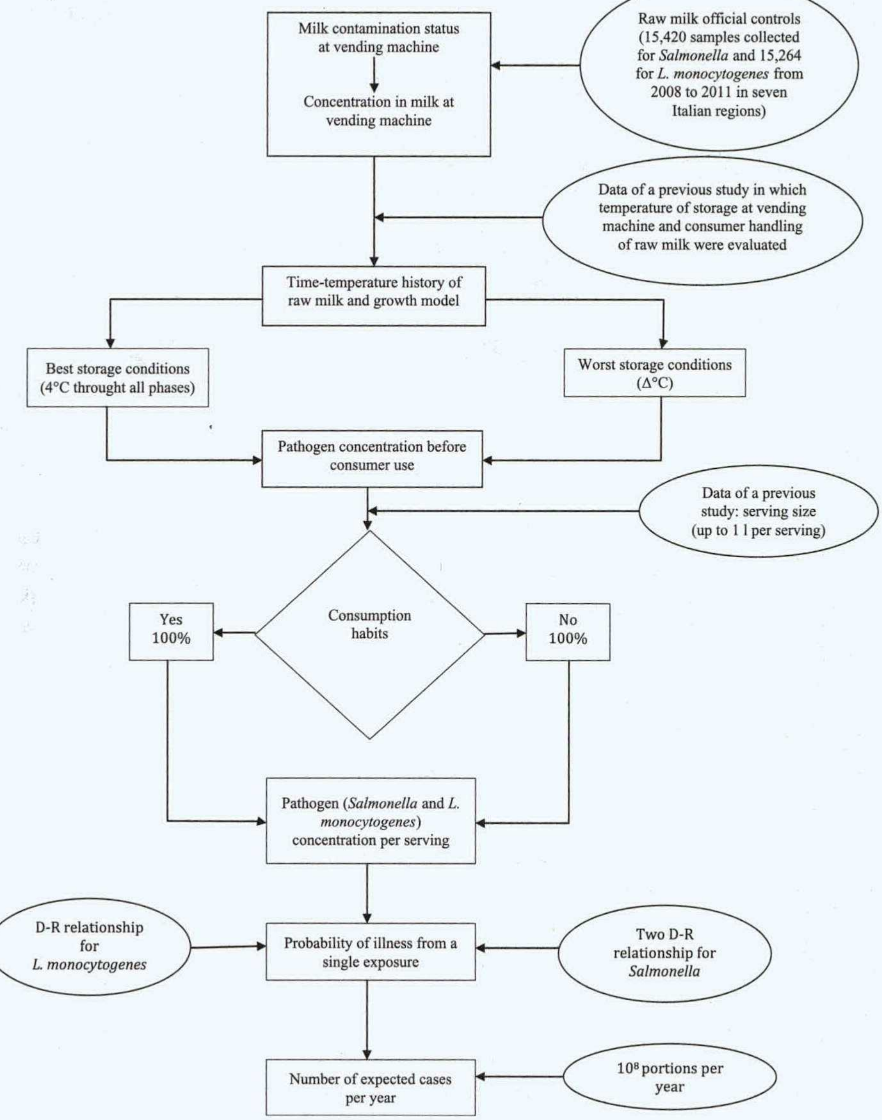

FIGURE 1. *Flow chart of the model used to estimate the probability of illness from a single exposure to contaminated raw milk and the number of illness cases expected each year.*

economic considerations (about 50 liters/day to be economically sustainable for the farmers). A simple Excel document describing the model has been prepared and is available from the authors.

RESULTS

Concentration of *Salmonella* and *L. monocytogenes* in raw milk. For *Salmonella,* the model provided a mean concentration of pathogen per milliliter of milk equal to 4.931×10^{-5} CFU/ml and standard deviation 1.13×10^{-5} CFU/ml. To match the fraction of positive samples detected in official controls at the vending machines, the concentration (expressed in logarithm) of *Salmonella* was modeled as normal (−4.307; 0.955). The distribution includes 99.9% of milk with a *Salmonella* concentration below the detection limit of microbiological methods applied during official controls (0.04 CFU/ml; see Fig. 2). For *L. monocytogenes,* the model provided a mean concentration of pathogen per milliliter of milk equal to 2.207×10^{-4} CFU/ml and standard deviation 2.408×10^{-5} CFU/ml. To match the fraction of positive samples detected in official controls at the vending machines, the concentration (expressed in logarithm) of *L. monocytogenes* was modeled as normal (−3.656; 0.888). The distribution includes 99.4% of milk with *L. monocytogenes* concentration below the detection limit of microbiological methods applied during official control (0.04 CFU/ml; see Fig. 3).

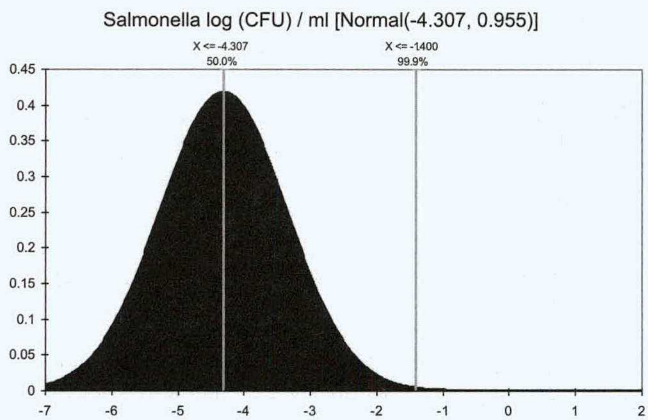

FIGURE 2. *Normal distribution in logarithmic scale of modeled* Salmonella *contamination in raw milk collected from vending machines during official controls.*

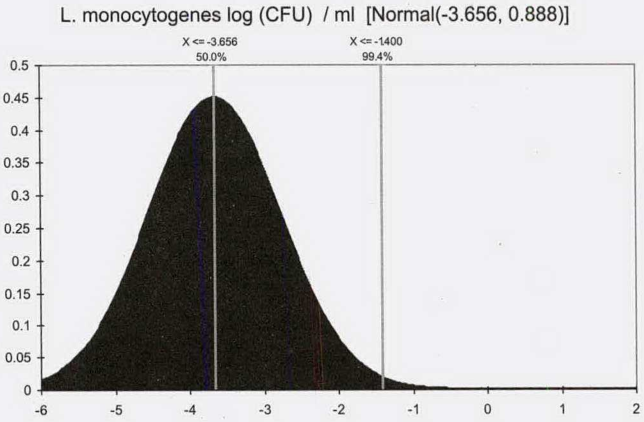

FIGURE 3. *Normal distribution in logarithmic scale of modeled* Listeria monocytogenes *contamination in raw milk collected from vending machines during official controls.*

The estimated levels of *Salmonella* and *L. monocytogenes* in raw milk under the best and worst storage conditions at retail in vending machines and after boiling are listed in Table 1.

Risk characterization. The following was used for *Salmonella* boiled milk servings: in simulations in which all consumers boil raw milk before drinking, maximum doses of 6.808×10^{-5} and 2.283×10^{-3} CFU were calculated for best and worst storage scenarios, respectively. The median (50th percentile) of p_{ill} applying the WHO/FAO *(30)* D-R models was 5.63×10^{-12} and 2.66×10^{-11}, for best and worst scenarios, respectively. Considering the 1,238 vending machines and considering that each vending machine works 365 days/year and sells at least 50 liters/day, corresponding to 240 servings of 210 ml of milk (one mug), a total of 1.08×10^8 servings per year could be estimated, and considering this number of services, we expect 0.001 and 0.003 cases per year, for best and worst storage scenarios, respectively. By applying the Teunis et al. *(25)* D-R model, the median (50th percentile) of p_{ill} was 8.85×10^{-17} and 2.09×10^{-15} that do not allow any salmonellosis cases to be expected in a reasonable number of years.

The following was used for *Salmonella* raw milk servings: in simulations in which no consumers boil raw milk before drinking, a mean dose of 0.248 CFU (50th percentile 1.946×10^{-2} CFU; maximum simulated 452 CFU) and a mean dose of 2.051 CFU (50th percentile 0.091 CFU; maximum simulated 7,396 CFU) were obtained from the model for best and worst storage scenarios, respectively. By applying the WHO/FAO *(30)* D-R model, the median (50th percentile) of p_{ill} was 5.65×10^{-5} and 2.64×10^{-4}, for best and worst storage scenarios. Considering 1.08×10^8 servings per year, the expected number of cases of salmonellosis were 6,455 and 27,973, respectively. By applying the Teunis et al. *(25)* D-R model, the median (50th percentile) of p_{ill} was 1.39×10^{-3} and 9.10×10^{-3}, predicting about 149,547 and 980,128 cases of salmonellosis per year for best and worst storage scenarios.

The following was used for *L. monocytogenes* boiled servings: in simulations in which all consumers boil raw milk before drinking, maximum doses of 3.64×10^{-4} and

TABLE 1. *Estimated concentrations of* Salmonella *and* Listeria monocytogenes *in milk after the best and worst storage conditions and after boiling: minimum, mean, and maximum estimated concentrations and 5th, 50th, and 95th percentiles of distribution of concentrations*[a]

	Estimated concn (CFU/ml) of *Salmonella*				Estimated concn (CFU/ml) of *Listeria monocytogenes*			
	BS	WS	BBS	BWS	BS	WS	BBS	BWS
Minimum	4.42×10^{-7}	2.90×10^{-6}	1.33×10^{-16}	8.19×10^{-16}	4.58×10^{-8}	1.09×10^{-7}	1.26×10^{-15}	2.35×10^{-15}
Mean	0.25	2.05	8.53×10^{-11}	7.33×10^{-10}	3.18×10^{-3}	7.53×10^{-3}	4.82×10^{-10}	1.15×10^{-9}
Maximum	560	5,323	1.31×10^{-7}	6.44×10^{-6}	7	15	5.04×10^{-7}	1.53×10^{-6}
5th	4.97×10^{-4}	1.83×10^{-3}	9.56×10^{-14}	3.60×10^{-13}	1.22×10^{-5}	2.16×10^{-5}	8.75×10^{-13}	1.56×10^{-12}
50th	2.02×10^{-2}	9.43×10^{-2}	4.99×10^{-12}	2.33×10^{-11}	3.65×10^{-4}	7.24×10^{-4}	3.65×10^{-11}	7.30×10^{-11}
95th	0.799	5.32	2.51×10^{-10}	1.60×10^{-9}	1.10×10^{-2}	2.52×10^{-2}	1.53×10^{-9}	3.51×10^{-9}

[a] BS, best storage conditions; WS, worst storage conditions; BBS, best storage conditions and boiling; BWS, worst storage conditions and boiling.

TABLE 2. *Distribution of the expected cases of listeriosis and salmonellosis linked to consumption of raw milk in relation to consumer habits, milk storage conditions, and D-R models used in the QRA*

	Illness:					
			Salmonellosis			
	Listeriosis		WHO/FAO *(30)*		Teunis et al. *(25)*	
Consumer habits	Best storage scenario	Worst storage scenario	Best storage scenario	Worst storage scenario	Best storage scenario	Worst storage scenario
Boiled milk						
100%	No expected cases	No expected cases	0.001	0.003	No expected cases	No expected cases
Raw milk						
13.9%[a]	No expected cases	No expected cases	839	3,636	19,441	127,417
26%[a]	No expected cases	No expected cases	1,678	7.272	38,882	254,833
43%[a]	No expected cases	No expected cases	2,775	12,028	64,305	421,454
100%	No expected cases	No expected cases	6,455	27,973	149,547	980,128

[a] Estimated cases considering the percentage of consumers that do not boil milk before consumption collected in three previous investigations.

8.14×10^{-4} CFU were obtained from the model, for best and worst storage scenarios, respectively. The model did not predict any cases of severe listeriosis linked to the consumption of raw milk correctly boiled before drinking (0 cases in 1,000,000 iterations).

The following was used for *L. monocytogenes* raw milk servings: in simulations in which no consumers boil raw milk before drinking, a mean dose of 1.40 CFU (50th percentile 0.149 CFU; maximum simulated 2,464 CFU) and a mean dose of 3.350 CFU (50th percentile 0.293 CFU; maximum simulated 14,000 CFU) were obtained from the model for best and worst storage scenarios, respectively. Considering 30% of the population as sensitive, we can estimate 3.23×10^7 servings of raw milk drunk by this subpopulation. The model predicted no cases in 1,000,000 iterations, even in the worst storage scenario. The median (50th percentile) of p_{ill} in the worst storage case scenario was 3.13×10^{-13}, so the model does not allow any cases of severe listerioris to be expected in a reasonable number of years.

The overall number of expected salmonellosis and listeriosis cases predicted by the different simulation models is summarized in Table 2, which also addresses different consumer behavior.

DISCUSSION

To our knowledge, this is the first QRA model of salmonellosis associated with the consumption of raw milk. Only two RAs of listeriosis due to consumption of raw milk are available in the literature *(22, 26)*, but unlike previous reports, the proposed *L. monocytogenes* RA models reflect the current trends in raw milk consumption in Italy and use specific national data and consumer habits as model inputs. The prevalence data on *Salmonella* and *L. monocytogenes* raw milk contamination used in our model were based on official controls of raw milk samples collected from vending machines over a 4-year period in seven Italian regions. These regions account for most of the machines registered in Italy and therefore provide a good insight into the true prevalence of the pathogens in raw milk sold in vending machines. Our QRA models address storage in vending machines, sale and consumption patterns, storage at home, and consumer habits, in an attempt to reproduce real-life scenarios. However, the models highlighted many uncertainties: the main pitfall was the lack of data on the amount of raw milk sold in vending machines that was estimated by economic considerations (about 50 liters/day to be economically sustainable for the farmers). In addition, the serving size was estimated, rather than measured, on the basis of the weekly purchase data declared by consumers (1 to 2 liters/week). Considering that the expected cases were associated with the estimated serving sizes, the bias due to the estimation of the amount of raw milk sold in vending machines may produce a bias in the estimated expected cases. Another shortcoming was that the actual pathogen reduction due to domestic boiling may not be reproducible in the domestic setting, and for this reason a triangular distribution was assumed.

Another limitation of our model was the different information on consumer habits reported in the surveys considered: about 100 questionnaire respondents in 2010 and 2011 *(12, 14)* and 550 in 2012 *(2)*. Briefly, 43% of the 100 consumers interviewed in the first survey did not boil the milk before drinking (23% drank raw milk and 20% heated the milk in the microwave), while 57% boiled the raw milk before consumption. In the second survey, 37% did not boil the milk before drinking (26% drank raw milk and 11% heated the milk in the microwave), while 63% boiled the raw milk before consumption. In the last survey, 13.9% did not boil the milk before drinking, 2.6% sometimes boiled the raw milk before consumption, and 81.8% boiled the raw milk before consumption. As consumer habits differed in the three investigations and these data are highly discriminant for RA and not completely definable, we developed two separate RAs: one for the consumption of raw milk without boiling (assuming that the entire population boils raw milk) and the other for the consumption of boiled milk (assuming that the entire population does not boil raw milk). For these reasons, the results of the two separate RAs should be linked to the different consumer behaviors in relation to boiling raw milk or not (see in Table 2). Table 2 clearly shows how consumer habits could affect the probability and number of salmonellosis cases and, in general, the risk of illness.

Overall, this RA model predicted no human listeriosis cases per year due to raw milk consumption in all the conditions simulated (see Table 2), and consequently, the probability of illness was low. These results contrast with the U.S. Food and Drug Administration, Food Safety and Inspection Service's *(22)* relative risk ranking, in which unpasteurized liquid milk consumption was considered at moderate risk (≤5 but ≥1 case per billion servings). They also conflict with the results reported by Latorre et al. *(22)*, who estimated 5.8 to 29 cases of listeriosis per year in the United States when the raw milk is sold at a retail store, which is not comparable to raw milk vending machines in Italy. Instead, in the case of direct selling from farmer to consumer, which is similar to our scenario, Latorre et al. *(22)* considered the probability of listeriosis due to raw milk consumption low (≤1 predicted case of listeriosis per billion of servings), a finding closer to our results. Differences in specific data used in the different RAs, mainly the number and habits of raw milk consumers, must be considered in comparing different studies and might explain this discrepancy in results.

The predicted absence of listeriosis cases due to raw milk consumption, resulting from the D-R relationship used in this study, and the low estimated concentration of *L. monocytogenes* in raw milk is difficult to validate: though highly improbable, the occurrence of listeriosis cases cannot be ruled out completely. Although few listeriosis outbreaks linked to raw milk consumption have been reported, healthy adults may only exhibit flulike or gastrointestinal symptoms that generally do not require medical attention *(22)*, while sporadic cases of illness may far outnumber cases associated with recognized outbreaks *(24)*.

The annual number of predicted cases of salmonellosis due to raw milk consumption varies widely depending on the D-R relationships used in the model, the storage conditions of milk during its shelf life, and the proportion of consumers not boiling milk before consumption. In view of the different consumer habits, the annual estimated cases ranged between 839 (in the best storage scenario, applying the WHO/FAO D-R) to 421,454 (in the worst storage scenario, applying the Teunis et al. D-R; see Table 2). In any case, the predicted number of cases conflicted with the data of confirmed cases reported in Italy by European Food Safety Authority *(8)*, which accounted for between 3,334 and 6,662 in the period considered; these findings merit several observations.

First, different outputs were obtained in the study using the two different D-R relationships. The D-R relation proposed by WHO/FAO *(30)* considered the majority of *Salmonella* data available in the literature and was built on previous D-R studies predominantly based on human volunteer studies. By contrast, Teunis et al. *(25)* developed a *Salmonella* D-R model based on outbreak data in which estimates of both the dose and the attack rate were known; this model had an ID_{50} of 7 CFU and $p_{ill\ 50}$ of 36 CFU, indicative of much higher infectivity and pathogenicity than studies feeding healthy human volunteers with laboratory-adapted strains *(25)*.

Second, the 18 *Salmonella*-positive samples isolated in raw milk during the 4-year monitoring were identified as *Salmonella* Typhimurium (7), *Salmonella* Bispebjerg (4), *Salmonella* Muenster (2), *Salmonella* Anatum (2), *Salmonella* Ndolo (1), *Salmonella* Kottbus (1), and *Salmonella* Dublin (1). Considering the data of confirmed salmonellosis cases in humans by the 10 most frequent serovars reported by the European Food Safety Authority *(8)*, most of the *Salmonella* serovars isolated in raw milk sold in Italy are not included in this list, with the sole exception of the seven *Salmonella* Typhimurium isolates. Based on the study of Teunis et al. *(25)*, no differences were found in the D-R outbreak models between serotypes and susceptibility categories, but for serotypes other than *Salmonella* Enteritidis or *Salmonella* Typhimurium, results indicate that a minor proportion of individuals will fall ill even at high doses. In fact, Teunis et al. reported that the majority of outbreaks (83%) have doses >100 CFU, which result in clusters of cases, whereas low level exposure (i.e., the raw milk scenario in Italy) may result in sporadic diseases.

A third consideration regards the evaluation of the real burden of human salmonellosis. The reported data on the incidence of specific pathogens causing gastroenteritis are largely based on passive surveillance and underestimate the true incidence: underreporting and underdiagnosis contribute to this problem. Several studies extrapolated "multipliers" of surveillance artifacts (care seeking, stool submission, laboratory testing, and culture sensitivity) to estimate the total number of human infections. Applying the proposed multipliers 38.6 *(27)*, 39 *(4)*, 38 *(23)*, 3.2 *(28)*, and 17 *(15)* and considering that an annual average of 5,118 salmonellosis cases were reported in Italy in the period 2008 to 2011, between 87,006 and 194,484 cases of salmonellosis can be estimated each year in the general population. In addition, an Italian survey by the Regional Reference Center for Foodborne Diseases *(1)* evaluated the underreporting of pathogen-specific human gastroenteritis in the Tuscany region by comparing the results of each culture-confirmed case in the study with those reported by official infectious disease surveillance programs. The findings clearly show that the number of culture-confirmed *Salmonella* cases investigated by the study (15.7 cases per 100,000 inhabitants) was double the number of cases reported by the Regional Authority (7.5 cases per 100,000 inhabitants) and triple the number of cases reported by national passive surveillance (five cases per 100,000 inhabitants). The *Salmonella* notification rate in Europe is 20.36 cases per 100,000 inhabitants) *(8)*. Considering these omissions and applying the previous multiplers, we estimated between 261,018 and 583,452 salmonellosis cases per year in Italy, making the number of cases predicted by the proposed models more comparable; on the other hand, several issues, i.e., the lack of data on the amount of raw milk sold in vending machines, the serving size, and the actual pathogen reduction due to domestic boiling, as well as the D-R relationships used and the identified *Salmonella* serovars, could have led to an overestimation in the predicted salmonellosis cases associated with raw milk consumption.

This study estimated the risks of salmonellosis and listeriosis among raw milk consumers at a national level. The risks associated with raw milk consumption must be quantified from a public health perspective, as the presence of foodborne pathogens in raw milk over the years appears to be stable and a considerable proportion of people still prefer to drink raw milk. Hence, the proposed QRA models emphasize, yet again, that boiling of milk before drinking is a simple but effective tool to protect consumers against the risks of illness inherent in the consumption of raw milk. The models may also be a useful tool for risk managers to identify or implement appropriate measures to control the risk of acquiring foodborne pathogens.

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