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Research Note

Quantitative Risk Assessment of Verocytotoxin-Producing *Escherichia coli* O157 and *Campylobacter jejuni* Related to Consumption of Raw Milk in a Province in Northern Italy

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ABSTRACT

A quantitative risk assessment was developed to describe the risk of campylobacteriosis and hemolytic uremic syndrome (HUS) linked to consumption of raw milk sold in vending machines in Northern Italy. Exposure assessment considered the microbiological status of dairy farms, expected milk contamination, storage conditions from bulk tank to home storage, microbial growth during storage, destruction experiments, consumption frequency of raw milk, age of consumers, serving size, and consumption preference. The differential risk between milk handled under regulation conditions (4°C throughout all phases) and the worst field handling conditions was considered. The probability of *Campylobacter jejuni* infection was modeled with a single-hit dose-response beta-Poisson model, whereas for HUS an exponential dose-response model was chosen and two probabilities were used to model the higher susceptibility of children younger than 5 years old. For every 10,000 to 20,000 consumers each year, the models predicted for the best and worst storage conditions, respectively, 2.12 and 1.14 campylobacteriosis cases and 0.02 and 0.09 HUS cases in the 0- to 5-year age group and 0.1 and 0.5 HUS cases in the >5-year age group. The expected pediatric HUS cases do not differ considerably from those reported in Italy by the Minister of Health. The model developed may be a useful tool for extending the assessment of the risk of campylobacteriosis and HUS due to raw milk consumption at the national level in Italy. Considering the epidemiological implications of this study, the risk of illness linked to raw milk consumption should not be ignored and could be reduced by the use of simple measures. Boiling milk before consumption and strict control of temperatures by farmers during raw milk distribution have significant effects on campylobacteriosis and HUS and are essential measures for risk management.

Raw milk for human consumption has been sold by automatic self-service vending machines in Italy since 2004. Raw milk has been implicated worldwide as a vehicle for milkborne disease outbreaks of caused by several pathogenic microorganisms *(7, 8, 10, 12, 20, 21, 29, 33),* but only *Campylobacter jejuni* and verocytotoxin-producing *Escherichia coli* infections have been associated with raw milk consumption in Italy.

According to Italian legislation, raw milk sold in vending machines must be refrigerated at a temperature of 0 to 4°C as soon as possible after milking and must be maintained at this temperature during transport to and storage in vending machines until delivery to the consumer. After a case report of hemolytic uremic syndrome (HUS) related to the consumption of raw milk *(35),* the Italian Health Ministry (10 December 2008) ordered that vending machines should bear the notice ''Milk must be consumed after boiling'' and fixed the milk expiration date at 3 days after delivery to the consumer.

The development and implementation of a quantitative risk assessment (QRA) model is one means to ensure food safety control. In Italy, no attempts have been made to develop simulation models even though several disease outbreaks were reported following raw milk consumption: two outbreaks of *C. jejuni* infection and two of *E. coli* O157:H7 infection in the Emilia Romagna Region in 2008 and 2009 *(3),* one of *C. jejuni* infection in the Veneto Region *(1),* and another of *C. jejuni* infection in the Marche Region *(39).*

Scientific reports on risk factors for human infection indicate that *C. jejuni* and *E. coli* O157:H7 can contaminate various foodstuffs, including raw milk and dairy products, and that raw milk has been responsible for major disease outbreaks *(13).*

No official data exist on the incidence of *Campylobacter* infection in Italy, but several incidents of campylobacteriosis were associated with raw milk *(24).* The importance of milk as a source of human *Campylobacter* infections was

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confirmed by the European Union summary report on foodborne disease outbreaks *(13),* and when milk is consumed without further heat treatment, it may pose a risk for human health. Verocytotoxin-producing *E. coli* (VTEC) strains are an important cause of uncomplicated or bloody diarrhea and its most important sequela, HUS, in humans in many countries. *E. coli* O157:H7 seems to be responsible for roughly half of the reported HUS cases, and patients with *E. coli* O157:H7 infection are more likely to develop severe clinical manifestations such as HUS than are patients with non-O157:H7 infections *(9).* A variety of foods have been implicated in disease outbreaks, but raw or undercooked foods of bovine origin are primarily responsible. In 2008, the reported levels of VTEC contamination in various food categories were low except for raw milk from cows, where a threefold increase in the proportion of positive samples was observed compared with the data from 2007 *(13).*

The aim of this study was to carry out a QRA for *C. jejuni* and *E. coli* O157:H7 in raw milk sold in one province of the Emilia Romagna Region in Northern Italy. The QRA considered the presence of these pathogens in dairy farms, the field handling conditions of raw milk during distribution and delivery to the consumer, consumer habits, and the behavior of pathogens throughout the food chain.

MATERIALS AND METHODS

Nomenclature of distributions. Normal (μ, σ) stands for normal distribution with mean of μ and a standard deviation of σ. Triangular (x, y, z) stands for the triangular distribution with minimum of x, a most likely value of y, and a maximum of z. Beta (a, b) stands for the beta distribution, with parameters a and b.

Exposure assessment. Data were collected in one province of the Emilia Romagna Region from all farms authorized to produce and sell raw cow's milk. These farms served 60 vending machines and together sold about 3,000 liters of raw milk daily. The province was used as the epidemiologic unit because the direct sale of raw milk is allowed only for the local area, i.e., the province where the raw milk is produced and the neighboring provinces. The province has a population of around 995,000 people with a surface area of around 370,000 ha.

Data on prevalence in dairy herds and estimation of *C. jejuni* and VTEC O157:H7 levels in raw milk. Data on the prevalence of *C. jejuni* and VTEC O157:H7 in milk filters described in a previous study were included in this study *(16).* In that study, 378 in-line milk filters were collected from 27 farms authorized for the production and sale of raw milk (14 filters for each farm) and evaluated qualitatively (presence or absence) by culturing. VTEC O157:H7 was detected in four samples from two farms, and *C. jejuni* was detected in eight samples from three farms, indicating intrafarm variability during the survey period.

Examination of in-line milk filters is 3 to 10 times more sensitive than standard culturing of milk *(27, 28, 34, 42, 43),* and the minimum detectable level of culture presence-absence tests is usually 1 CFU in 25 g or 0.04 cells per g in standard cultures. Hence, in our case the minimum detectable level from the presence-absence tests for the milk filter examinations was 0.004 CFU/ml.

The beta function was used to model the intrafarm variability of VTEC O157:H7 and *C. jejuni* in milk filters. The beta distribution was chosen because it describes uncertainty surrounding the prevalence introduced by sampling. Assuming that the true filter prevalence in a given population of a herd is P, the number of positive filters S in a sample of N filters 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 herds have one or more positive filters, the posterior distribution of herd prevalence P is Beta ($S + 1$, $N - S + 1$).

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

$$P^{+}_{\text{filter}} = \text{Beta}(S_{\text{filter}} + 1; N_{\text{filter}} - S_{\text{filter}} + 1)$$

where S_{filter} is the number of positive filters and N_{filter} is the total number of filters analyzed ($n = 378$).

Assuming a Poisson distribution of bacteria in milk, the probability that no VTEC O157:H7 (or *C. jejuni*) are present in a sample of in-line filters, i.e., a volume (V) of 250 ml (10-fold increase from the standard culture examination) of milk and a test sensitivity close to 100%, is estimated by

$$P^{-}_{\text{filter}} = e^{(-C \times V)}$$

where C is the pathogen level and V is the volume of sample analyzed.

Following these assumptions and estimations, we modeled the distribution of the foodborne pathogens in the milk sold by farms located in the province. Assuming the level of pathogens was lognormally distributed

$$C = (\log\ \mu; \sigma)$$

and considering data from the filter survey *(16)* modeled as described above, the mean lognormal distribution is

$$\mu = -\ln(1 - P^{+}_{\text{filter}})/V$$

A Monte Carlo simulation (with practical value of 100,000 iterations) was carried out for μ using @Risk, version 4.5.2 (Palisade Corporation, Newfield, NY), and 100,000 iterations were done for practical reasons. Once the mean of the lognormal distribution was obtained, the standard deviation (σ) was calculated to match the fraction of positive samples (>0.004 CFU/ml) actually observed in the survey of in-line milk filters.

Time-temperature history of raw milk and growth model. Data from a previous study *(18)* on raw milk handling by farmers and consumers were used for the time-temperature model. The raw milk temperature was recorded at all farms authorized to produce and sell raw milk; milk temperatures were recorded in the bulk tank during transportation from farm to vending machine, during storage in the vending machine, and at the time of delivery to consumers. One hundred consumers were interviewed on how much milk they bought weekly, how the milk was transported from vending machine to home, how long the milk was stored at home, and whether the milk was boiled before consumption. The temperature of transportation from the vending machine to the consumer's home was experimentally simulated *(18),* and the temperature of home storage was based on published data *(4).* Several episodes of thermal abuse were detected in the raw milk chain from bulk tank storage to consumption, and the data collected were used to simulate the behavior of the two foodborne pathogens in two experimental conditions: the optimum storage condition (4°C throughout all phases) and the worst field handling conditions detected (ΔT). An increase in *E. coli* O157:H7 under conditions of thermal abuse was shown, and the doubling time (T_d) for *E. coli* O 157:H7 was calculated *(18).* Considering that the cell population was naturally variable in T_d and data collected during experimental determination confirm differences in pathogen behavior, a triangular distribution was chosen with the most likely value fixed as equal to the experimental mean T_d observed, and minimum and maximum parameters in the triangular distribution were calculated considering the standard deviation observed experimentally:

$$T_{d\,(E.coli\ 4^\circ\mathrm{C})} = \mathrm{Triangular}(34.2, 45.1, 56)\ \mathrm{h}$$

where x is 34.2 h, y is 45.1 h, and z is 56 h for constant storage at 4°C and

$$T_{d\,(E.coli\ \Delta T)} = \mathrm{Triangular}(14.21, 15.95, 17.68)\ \mathrm{h}$$

where x is 14.21 h, y is 15.95 h, and z is 17.68 h for variable storage temperatures.

C. jejuni counts decreased during both experimental storage conditions *(18)*; therefore, the decimal reduction time (DRT) was calculated and expressed in hours: 624 h, 19 min at 4°C and 132 h, 39 min in the worst field handling conditions detected (ΔT). Similarly, to consider the variability in cell population observed during experimental determination, a triangular distribution was chosen with the most likely value equal to the experimental mean DRT observed, and minimum and maximum parameters were calculated considering the standard deviation observed experimentally:

$$\mathrm{DRT}_{(Camp\ 4^\circ\mathrm{C})} = \mathrm{Triangular}(225.1,\ 624.31,\ 1023.53)\ \mathrm{h}$$

where x is 225.1 h, y is 624.31 h, and z is 1023.53 h for constant storage at 4°C and

$$\mathrm{DRT}_{(Camp\ \Delta T)} = \mathrm{Triangular}(113.35,\ 132.65,\ 151.95)\ \mathrm{h}$$

where x is 113.35 h, y is 132.65 h, and z is 151.95 h for variable storage temperatures.

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

$$T\ (\mathrm{h}) = \mathrm{Triangular}(0.5, 24, 120)\ \mathrm{h}$$

where x is 0.5 h, y is 24 h, and z is 120 h.

Data obtained from experiments in which the milk was boiled *(18)* were used to model the survival of VTEC O157:H7 and *C. jejuni* 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:

$$D_{\mathrm{boil}} = \mathrm{Triangular}(2, 4, 6)\ \mathrm{log\ reduction}$$

On the basis of data collected in a previous study *(18),* 57% of consumers boiled the raw milk before consumption; therefore, 43% of consumers did not apply an effective heat treatment. This distribution of consumer habits was used in the final model:

$$\mathrm{Boil} = \mathrm{Bernoulli}(0.57) \times D_{\mathrm{boil}}$$

The distribution of raw milk serving size (S_i) was characterized by values obtained from consumer interview and the values of raw milk consumed *(18)*; S_i was modeled by the triangular distribution as:

$$S_i = \mathrm{Triangular}(100, 250, 1{,}000)\ \mathrm{ml}$$

Pathogen dose per serving size. For each pathogen considered, two dose output models were achieved: one for the best and the other for the worst storage milk chain scenarios:

$$\text{Dose } E.\ coli\ \mathrm{O157{:}H7}_{4^\circ\mathrm{C}} = 10^\wedge(\log[10^C \times 2^{(T(h)/T_d(E.\ coli\ 4^\circ\mathrm{C}))}] - \mathrm{Boil}) \times S_i$$

$$\text{Dose } E.\ coli\ \mathrm{O157{:}H7}_{\Delta T} = 10^\wedge(\log[10^C \times 2^{(T(h)/T_d(E.\ coli\ \Delta T))}] - \mathrm{Boil}) \times S_i$$

$$\text{Dose } Campylobacter_{4^\circ\mathrm{C}} = 10^\wedge\ (C - [\mathrm{DRT}(Camp\ 4^\circ\mathrm{C}) \times T(\mathrm{h})] - \mathrm{Boil}) \times S_i$$

$$\text{Dose } Campylobacter_{\Delta T} = 10^\wedge\ (C - [\mathrm{DRT}(Camp\ \Delta T) \times T(\mathrm{h})] - \mathrm{Boil}) \times S_i$$

Dose response. The most frequently used dose-response model for *Campylobacter* is the beta-Poisson *(30, 38)* based on the data of a volunteer study *(5)*. According to the beta-Poisson model the probability of human infection *(15)* can be defined by

$$P_{\mathrm{inf}} = 1 - (1 + \mathrm{Dose}/\beta) - \alpha$$

which expresses the probability of raw milk consumer infection provided that $\beta >> \alpha$, where α and β are parameters of the beta-Poisson dose-response model. In the case of *Campylobacter* infection, the parameters of the beta-Poisson model estimated *(37)* are $\alpha = 0.145$ and $\beta = 7.589$. The infection status linked to consumption of raw milk (the presence or absence of infection) was simulated as

$$\mathrm{Inf}_{Camp}(1\ \text{or}\ 0) = \mathrm{Bernoulli}(P_{\mathrm{inf}})$$

where Inf_{Camp} is the *Campylobacter* infection status of raw milk consumers and is indicated as 1 when infected and 0 otherwise.

Regarding the estimation of the probability of campylobacteriosis given the infection, the approach used by Nauta et al. *(32)* was followed, assuming a constant probability of illness equal to 0.33. Consequently, consumer illness can be calculated as

$$\mathrm{Ill}_{Camp} = \mathrm{Inf}_{Camp} \times \mathrm{Bernoulli}(0.33)$$

where Ill_{Camp} is consumer illness, in this case campylobacteriosis.

Regarding the estimation of the probability of illness from *E. coli* O157:H7 infection, two dose-response models were used for two age groups: 0 to 5 years and >5 years *(31)*. The incidence of *E. coli* O157:H7 infection and HUS is reported to be higher among children younger than 5 years, with a median of around 2 years for children with HUS *(26, 40, 41)*. As proposed *(11),* the probability of HUS (P_{HUS}) as a function of the ingested dose (Dose) by a single-hit model was modeled:

$$P_{\mathrm{HUS}} = 1 - (1 - r)^{\mathrm{Dose}}$$

where P_{HUS} is the probability of illness given an exposure to dose (Dose), Dose is the dose (number) of organisms, and r is the dose-response parameter per organism, i.e., the probability of developing HUS after infection with a single cell. Delignette-Muller and Cornu *(11)* proposed using the same approach by defining different values of r for two age groups. For each age group, based on the distribution performed by Delignette-Muller and Cornu *(11),* r was estimated from the median of its distribution and its 95% confidence interval from the 2.5th to the 97.5th quartile. Values of 1.2×10^{-3} for the 0- to 5-year group and 2.4×10^{-4} for the >5-year group were used.

Simulation. The models were implemented with software for Monte Carlo simulation @Risk, version 4.5.2, and 100,000 iterations were done.

Risk output. The objective of the model was to estimate the probability of illness from a single exposure. The median risk obtained after the simulation was used to estimate the number of cases expected yearly. To obtain this estimation, data from a

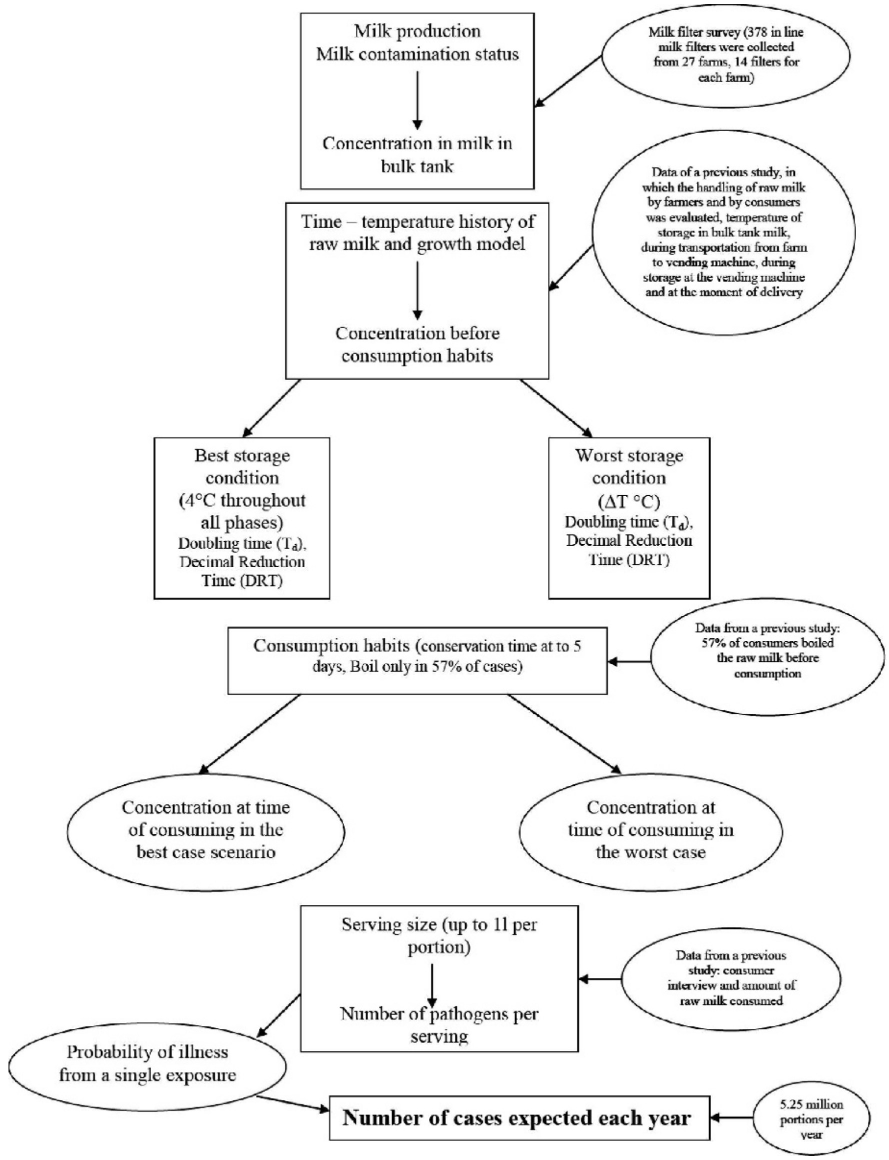

FIGURE 1. *Flow chart of the model used to estimate the probability of illness from a single exposure to contaminated raw milk and the number of illness cases expected each year.*

previous study *(18)* were used: 93% of consumers usually drink 1 to 2 liters of raw milk per week, and 3.57% of the consumers are younger than 5 years of age. In the province, 3,022 liters were sold daily; therefore, the number of consumers was estimated at 10,577 to 21,154. Considering a median portion size of about 210 ml, 14,390 portions were sold daily and 5.25 million portions were sold per year. Figure 1 shows the flow chart of the model used to estimate the probability of illness from a single exposure and hence the number of cases expected each year.

RESULTS

The estimated lognormal distribution of *C. jejuni* and *E. coli* O157:H7 levels in bulk tank milk is shown in Figures 2 and 3, with $\mu = -4.396$ log CFU/ml and $\sigma = 0.859$ log CFU/ml for *E. coli* O157:H7 and $\mu = -4.093$ log CFU/ml and $\sigma = 0.825$ log CFU/ml for *C. jejuni*.

The estimated levels of *C. jejuni* and *E. coli* O157:H7 in milk under the best and worst storage conditions and after boiling are listed in Table 1. The estimated *E. coli* O157:H7 level increased in the worst storage scenario, whereas estimated *C. jejuni* levels decreased because of the more pronounced decrease in *C. jejuni* in raw milk stored at higher temperatures *(18)*. Boiling greatly reduces the estimated levels of both *C. jejuni* and *E. coli* O157:H7. The risks per serving for campylobacteriosis and HUS with the simulated percentile distribution are listed in Table 2. The risk per serving for campylobacteriosis was higher in the best versus worst storage scenarios, whereas the contrary was estimated for HUS because of the different behaviors of *C. jejuni* and *E. coli* O157:H7 at the different storage temperatures *(18)*.

Considering the median risk of *Campylobacter* infection per serving of raw milk, the calculated numbers of cases of infection expected per year were 6.64 and 3.48 (per 10,000 to 20,000 consumers) for the best and worst storage conditions, respectively. The expected cases of campylobacteriosis (per 10,000 to 20,000 consumers) calculated per year were 2.12 and 1.14 for the best and worst storage conditions, respectively.

Considering the median risk for HUS and the fact that 3.57% of the consumers of raw milk are younger than 5 years of age, we expected the following numbers of HUS

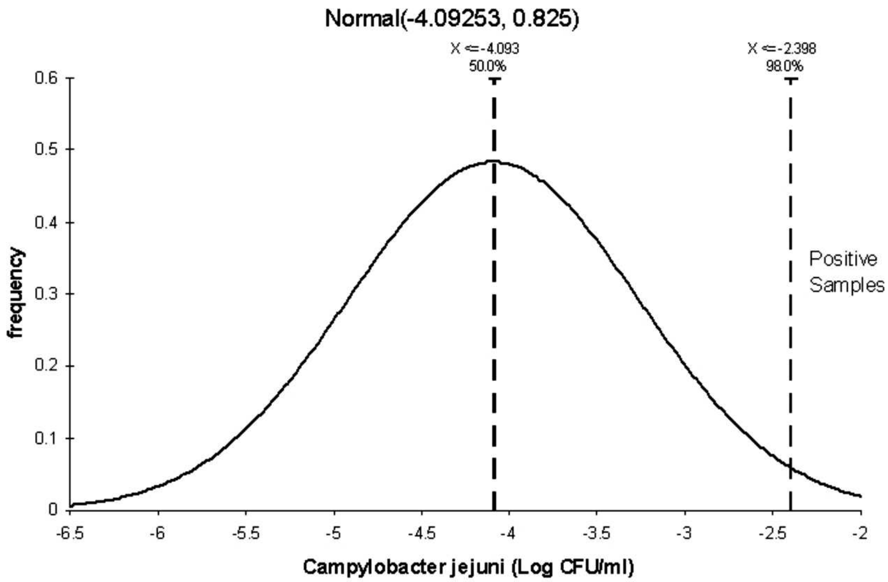

FIGURE 2. *Estimation of* Campylobacter jejuni *levels (log CFU per milliliter) in bulk tank raw milk.*

cases per year linked to consumption of raw milk based on 10.000 to 20.000 consumers and 5.25 million portions: (i) 0.09 cases in the 0- to 5-year age group for the worst storage scenario, (ii) 0.02 cases in the 0- to 5-year age group for the best storage scenario, (iii) 0.5 cases in the >5-year age group for the worst storage scenario, and (iv) 0.1 cases in the >5-year age group for the best storage scenario.

DISCUSSION

This study is the first in Italy to adopt a farm-to-table risk analysis approach for raw milk consumption using specific local area data as model inputs. This QRA model has the distinctive feature of considering storage phases (both at the farm and in the vending machine), transportation from farm to vending machines, sale and consumption patterns, storage at home, and consumer habits. Many uncertainties were highlighted in the model; the main pitfall was the level of pathogens in raw milk samples (*C*), which was calculated on the basis of qualitative data from milk filter sampling. Using the data from milk filter prevalence allowed us to model the effect of raw milk handling by farmers on pathogen growth. The estimated normal distribution of the *E. coli* O157:H7 and *C. jejuni* levels in the present work (Figs. 2 and 3) is in agreement with that found previously *(17)* on the same farms in the same province. The results of that study revealed that raw milk contamination by *E. coli* O157:H7 and *C. jejuni* can be estimated for units per liter. A comparison of the results of the survey of in-line milk filters *(16)* with those of the survey of raw milk collected from vending machines *(17)* revealed that *E. coli* O157:H7 and *C. jejuni* prevalences were similar (1 and 2%, respectively). However, we examined 210 ml of raw milk collected from vending machines instead of the 25 ml usually analyzed by standard methods. These results confirm that the decision to consider the sensitivity of the in-line milk filter analysis to be 10-fold the sensitivity of the standard cultural method was appropriate.

The pathogen level in raw milk was calculated considering a lognormal homogeneous distribution of pathogens and then calculating the mean assuming a test sensitivity very close to 100%, meaning that the analytical method was able to detect a single cell in the volume sample. However, this assumption is difficult to justify in a

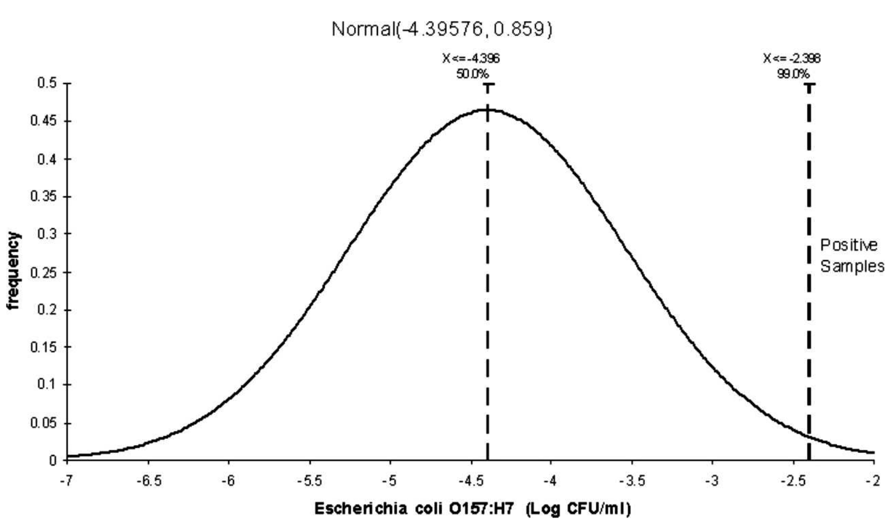

FIGURE 3. *Estimation of* Escherichia coli *O157:H7 levels (log CFU per milliliter) in bulk tank raw milk.*

TABLE 1. *Estimated* C. jejuni *and* E. coli *O157:H7 levels in raw milk under the best and worst storage conditions and after boiling*[a]

Measure[b]	Estimated level (CFU/ml)[c]							
	E. coli O157:H7				*C. jejuni*			
	BS	WS	BBS	BWS	BS	WS	BBS	BWS
Minimum	1.02×10^{-8}	1.06×10^{-8}	1.35×10^{-13}	1.95×10^{-13}	1.52×10^{-8}	3.44×10^{-9}	1.34×10^{-13}	4.08×10^{-14}
Mean	6.69×10^{-4}	4.90×10^{-3}	2.97×10^{-7}	1.99×10^{-6}	6.66×10^{-5}	3.49×10^{-5}	6.66×10^{-9}	3.49×10^{-9}
Maximum	7.84×10^{-1}	12.14×10^{0}	3.05×10^{-4}	3.33×10^{-3}	5.06×10^{-2}	7.19×10^{-1}	3.87×10^{-4}	1.64×10^{-4}
5th	3.07×10^{-6}	8.16×10^{-6}	9.15×10^{-11}	2.61×10^{-10}	2.89×10^{-6}	1.40×10^{-6}	8.33×10^{-11}	4.07×10^{-11}
50th	8.50×10^{-5}	3.18×10^{-4}	8.48×10^{-9}	3.24×10^{-8}	6.67×10^{-5}	3.48×10^{-5}	6.59×10^{-9}	3.48×10^{-9}
95th	2.36×10^{-3}	1.49×10^{-2}	8.08×10^{-7}	4.43×10^{-6}	1.53×10^{-3}	8.61×10^{-4}	5.30×10^{-7}	2.95×10^{-7}

[a] Only 53% of consumers boiled the raw milk before consumption.
[b] Values are minimum, maximum, and mean estimated bacterial levels and 5th, 50th, and 95th percentiles of the distribution of levels.
[c] BS, best storage conditions; WS, worst storage conditions; BBS, best storage conditions and boiling; BWS, worst storage conditions and boiling.

biological system because foodborne pathogen cells may be stressed or sublethally injured. Other weak points of the model are that the serving size was estimated (rather than measured) on the basis of the weekly purchase data declared by consumers (1 to 2 liters/week) and that the actual pathogen reduction due to domestic boiling was uncertain. Experimental data on the reduction of pathogen counts achieved by boiling may not be reproducible in the domestic setting; for this reason a triangular distribution was assumed.

The predicted probability of at least one HUS case and one campylobacteriosis case per year may appear low, but when we consider the small geographical area investigated, the small volume of raw milk purchased (about 3,000 liters/day), and the estimated number of raw milk consumers (about 10,000 to 20,000), the low risk of illness linked to raw milk consumption becomes more apparent. No official data on the incidence of *Campylobacter* infection in Italy are available because the Italian reporting system for human infectious diseases does not distinguish episodes of gastroenteritis caused by *Campylobacter* from those caused by other agents listed in the national legislation *(6)*, and there are no literature reports on QRA of *C. jejuni* related to raw milk consumption.

Only one HUS risk assessment has been conducted for *E. coli* O157:H7 in marketed unpasteurized milk, that by Grace et al. in Africa *(19)*. Those authors reported a best estimate of 2,402 to 2,835 cases of symptomatic VTEC infections per 10,000 servings of unpasteurized milk consumed. These results differ substantially from our data; for 5.25 million milk portions under the worst and best storage conditions, the expected numbers of HUS cases per year were 0.09 and 0.02, respectively, for the 0- to 5-year age group and 0.5 and 0.1, respectively, for the >5-year age group. This difference between study results is not negligible and could result from two methodological differences. First, our study included only HUS cases, which usually represent 10% of total symptomatic VTEC infections *(19)*. Second, the epidemiological status of the two studies differed in several ways.

(i) The two reported prevalence rates (both 1 to 2%) are only apparently similar because Grace et al. tested milk by pour plating in violet red bile agar *(2)*, whereas we tested 210 ml of milk by enrichment *(17)*. The amount of milk analyzed by pour plating can be assumed to account for 0.1 to 1 ml of milk, so we deduced that the milk tested was more frequently contaminated by *E. coli* O157:H7 at a higher level.

(ii) The unpasteurized milk marketed in urban East Africa is not usually refrigerated at 4°C as it is in Italy.

(iii) The population in the study by Grace et al. contained a relatively high proportion of susceptible individuals (40% were <15 years old and 7% were HIV positive) that drank milk daily, but the widespread practice of boiling milk with tea prior to consumption reduces the level of pathogens consumed.

(iv) In Italy the home storage of raw milk lasts 5 days, and 40% of consumers did not boil raw milk before consumption.

(v) The different hygienic conditions under which raw milk is handled throughout the supply chain must be taken into account.

TABLE 2. *Distribution of the per-serving risk for campylobacteriosis and HUS linked to consumption of raw milk*

Illness type	Risk per milk serving					
	Best storage scenario			Worst storage scenario		
	5th percentile	50th percentile	95th percentile	5th percentile	50th percentile	95th percentile
Campylobacteriosis	1.30×10^{-9}	1.23×10^{-6}	5.29×10^{-3}	6.50×10^{-10}	6.64×10^{-7}	2.95×10^{-3}
HUS						
Children (0–5 yr old)	9.36×10^{-11}	1.08×10^{-7}	4.83×10^{-4}	2.80×10^{-10}	4.99×10^{-7}	2.56×10^{-3}
Older consumers (5 yr old)	1.87×10^{-11}	2.16×10^{-8}	9.66×10^{-5}	5.60×10^{-11}	9.97×10^{-8}	5.13×10^{-4}

Italy does not have a compulsory reporting system for cases of VTEC infection and/or HUS, and only pediatric cases of HUS are reported on a voluntary basis by the national reference center for VTEC. The annual number of reported illness cases in children in Italy is about 50, of which 18 have been linked to raw milk consumption since 2007 *(25)*.

Based on data in previous reports *(22, 23)*, HUS cases caused by *E. coli* O157:H7 account for 35 to 40% of all HUS cases for which it was possible to isolate and characterize the VTEC strain involved. Consequently, we estimated that 6.3 to 7.2 HUS cases connected with raw milk consumption in Italy between 2007 and 2011 were caused by *E. coli* O157:H7.

In Italy, 1,411 vending machines are registered for the sale of raw milk. Extending our risk assessment data for the 60 vending machines authorized in a province of Northern Italy to all vending machines nationwide, under the best and worst storage conditions we expect 0.47 and 2.11 total HUS cases per year, respectively (i.e., the number of predicted HUS cases per year in the province in this study × 1,411/60) and 2.35 and 10.58 total HUS cases from 2007 to 2011, respectively. On the basis of these data, the numbers of HUS cases in children predicted by our model do not differ widely from those reported by the Italian Ministry of Health and hence we consider our model to be accurate reflection of the data.

Risk analysis is now widely accepted as the preferred means of assessing possible links between hazards in the food chain and actual risks to human health *(14)*. QRA does not usually consider storage conditions, distribution, and consumption patterns and thus may not have been useful to risk managers for the identification or implementation of appropriate measures for controlling the risk of acquiring foodborne pathogens. The model developed in this study may be a useful tool for assessing the risk of campylobacteriosis and HUS due to raw milk consumption at a national level on the basis of the official data from farms and vending machines authorized to sell raw milk and the prevalence of *C. jejuni* and *E. coli* O157:H7 in raw milk detected through official monitoring.

The raw milk food chain should enforce transport and storage at temperatures of 0 to 4°C to prevent microbial growth and reduce the pathogen levels *(36)*. As clearly shown by our results, failure to maintain the cold chain carries significant implications for the risk of *E. coli* O157:H7 infection and HUS. When farmers did not maintain correct temperatures throughout the supply chain and when thermal abuse during home transportation and storage were reported, the annual expected cases of infection were higher. Consequently, effective maintenance of low temperatures will reduce the potential risk to consumers, especially when raw milk is consumed without heat treatment. Optimal storage of raw milk by farmers is a simple measure that must be implemented to reduce the risk of *C. jejuni* or VTEC infection for consumers.

Boiling milk before consumption greatly reduces the estimated contamination of raw milk and the consequent risk of acquiring HUS or campylobacteriosis. Because 23% of consumers did not boil raw milk before drinking and 20% did not use an effective heat treatment, there is an urgent need to improve milk hygiene education for consumers.

Pathogen-free raw milk is difficult to obtain, but the risk of illness could be significantly reduced by using simple control measures, including strict monitoring of temperatures by farmers during raw milk distribution and enhanced educational efforts reminding consumers of the importance of boiling raw milk.

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