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The final published version is available online at:

http://dx.doi.org/10.1016/j.prevetmed.2015.06.009

Human campylobacteriosis related to the consumption of raw milk sold by vending machines in Italy: quantitative risk assessment based on official controls over four years

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Abstract

A quantitative risk assessment (RA) model was developed to describe the risk of campylobacteriosis linked to consumption of raw milk sold in vending machines in Italy. Exposure assessment was based on the official microbiological records of raw milk samples from vending machines monitored by the regional Veterinary Authorities from 2008 to 2011, microbial growth during storage, destruction experiments, consumption frequency of raw milk, serving size, consumption preference and age of consumers. The differential risk considered milk handled under regulation conditions (4°C throughout all phases) and the worst time-temperature field handling conditions detected. Two separate RA models were developed, one for the consumption of boiled milk and the other for the consumption of raw milk, and two different dose-response (D-R) relationships were considered. The RA model predicted no human campylobacteriosis cases per year either in the best (4°C) storage conditions or in the case of thermal abuse in case of boiling raw milk, whereas in case of raw milk consumption the annual estimated campylobacteriosis cases depend on the dose-response relationships used in the model (D-R I or D-R II), the milk time-temperature storage conditions, consumer behaviour and age of consumers, namely young (with two cut-off values of ≤5 or ≤6 years old for the sensitive population) versus adult consumers. The annual estimated cases for young consumers using D-R II for the sensitive population (≤5 years old) ranged between 1013.7/100,000 population and 8110.3/100,000 population and for adult consumers using D-R I between 79.4/100,000 population and 333.1/100,000 population. Quantification of the risks associated with raw milk consumption is necessary from a public health perspective and the proposed RA model represents a useful and flexible tool to perform future RAs based on local consumer habits to support decision-making on safety policies.

Further educational programmes for raw milk consumers or potential raw milk consumers are required to encourage consumers to boil milk to reduce the associated risk of illness.

Keywords: Raw milk, risk assessment, *Campylobacter jejuni*, monitoring

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1. Introduction Campylobacteriosis is a diarrhoeal disease caused by bacteria of the genus *Campylobacter* and represents one of the leading causes of gastrointestinal infections and the most commonly reported zoonosis in humans in the European Union (EU) since 2005. The EU Member States reported an overall incidence of 55.49 cases of human campylobacteriosis per 100,000 inhabitants in Europe in 2012 and 265, 531, 457 and 468 confirmed annual cases in Italy for the years 2008 to 2011 respectively, corresponding to an apparent incidence of 0.44-0.89 cases/100,000 inhabitants per year (EFSA, 2014). There are no official data in Italy on the incidence of *Campylobacter* infection and the true incidence of campylobacteriosis is probably greatly underestimated due to the deficiency of the surveillance system.

Raw cow's milk was reported to be a vehicle of human campylobacteriosis in outbreaks in the United States and Europe (Claeys et al., 2013; Hauri et al., 2013; Longenberger et al., 2013; Taylor et al., 2013; EFSA, 2014). The expert consultation on the global view of campylobacteriosis reported that the most frequent food vehicle in outbreaks of campylobacteriosis in the United States is raw unpasteurized milk, whereas the relative contribution of raw milk accounts for only a small fraction of sporadic cases (WHO, 2013). The European Union summary report on zoonoses, zoonotic agents and food-borne outbreaks in 2012 reports that milk was the second most frequently identified food vehicle in the strong-evidence *Campylobacter* outbreaks, 20.0% of which implicated milk, indicating a well-documented risk of campylobacteriosis (EFSA, 2014).

The sale of raw milk for human consumption by self-service vending machines has been allowed in Italy since 2004. In line with their desire to purchase locally and consume natural unprocessed food, consumers appreciate this distribution channel and can purchase a product that is less expensive than buying pasteurized retail milk.

To meet this demand for "freedom of choice", farmers have increased their sales with self-service automatic vending machines located on farms, outside supermarkets, in public squares, parking lots or along crowded high streets and in cheese factories. The vending machines sell raw milk and usually plastic (PET) or glass bottles, so consumers can buy the bottles or use their own. The sale of raw milk via vending machines is regulated by the State-Regions Agreement (Italian Ministry of Health, 2007) but regional regulations have been developed and are periodically reviewed in cooperation with the Ministry of Health and the local authorities. Farmers who produce milk for human consumption can have more than one vending machine, but a vending machine can only contain the raw milk from one dairy farm. Raw milk can only be sold directly to the public in the province of raw milk production and in neighboring provinces. Farmers intending to sell raw milk via vending machines must comply with specific regulations: biosafety measures and self-checking for producers, microbiological and chemical criteria for milk, and vending machines installation and management specifications (Bianchi et al., 2013). Since December 2008, raw milk vending machines must display the notice "milk must be consumed after boiling" (Italian Ministry of Health, 2008).

The microbiological characteristics of the milk delivered by the vending machines are controlled by the Official Veterinary Services. The sampling frequency may differ from region to region, but, according to Italian law, all regions must test at least one sample a year from each vending machine for the presence of foodborne pathogens including *Campylobacter jejuni*. In case of positivity, the Official Veterinary Services stop the sale of raw milk until two consecutive negative cultures from milk samples are recorded at the dairy farm of production.

In Italy, the results of diagnostic activities performed in different surveys and official control data showed that the prevalence of *C. jejuni* in raw milk samples collected by self-service vending machines varies from 0 to 1.5% (Giacometti et al., 2012b; Bianchi et al., 2013; Giacometti et al., 2013). In addition, two outbreaks of *C. jejuni* linked to the consumption of raw milk have been reported in Italy, one in the Veneto region and the other in the Marche region (Amato et al., 2007; Tonucci, 2011 personal communication).

In this context, a quantitative risk assessment (RA) describing the risk of campylobacteriosis linked to the consumption of raw milk sold by vending machines was previously developed in one province of the Emilia

Romagna region, in Northern Italy (Giacometti et al., 2012a). That study considered data on *C. jejuni* prevalence and consumer habits collected in a single investigation in a small geographical area and therefore cannot be considered representative of the national situation.

The present study performed an updated RA to estimate the risk of campylobacteriosis attributed to the consumption of raw milk purchased from vending machines located in seven Italian regions. The RA considered national data on *C. jejuni* prevalence in raw cow's milk at the time of delivery to consumers by vending machines over a four-year period (2008-2011), data on habits and age of consumers by different investigations over the years and in different geographical areas, and the behaviour of *C. jejuni* throughout the food chain from the time of delivery. Finally, considering the highest risk reported for *Campylobacter* infection in children, two different dose–response (D-R) relationships were used to consider the supposed higher susceptibility of some consumer populations in order to define the most realistic scenario (EFSA, 2014).

2. Materials and methods

2.1 Exposure assessment

Official microbiological records of raw milk samples from self-service vending machines in seven Italian regions monitored by the regional Veterinary Authorities from 2008 to 2011 were collated from a previous survey and used for this RA model (Giacometti et al., 2013). Briefly, the seven regions, Emilia Romagna, Lazio and Tuscany (pooled data), Lombardy, Marche, Piedmont, Sicily and Veneto, account for 1,236 vending machines, i.e., 89.43% of the 1,382 vending machines registered in Italy, providing a high proportion of the true national scenario (Italian Minister of Health, 2011). The investigated regions have a total population of 39,396,008 people out of a total 59,433,744 people in Italy. Of these, 1,824,986 and 2,192,540 are children ≤ 5 or ≤ 6 years old (ISTAT, 2011).

2.2 Prevalence of Campylobacter jejuni in raw milk at vending machines and estimation of its level in raw milk

The number of vending machines in each region and the number of samples collected from each vending machine may vary from one region to another and from one year to another but all regions tested at least one sample from each vending machine for each year for the presence of *C. jejuni* in accordance with microbiological criteria stipulated in the national legal requirements of the State-Regions Agreement (Italian Ministry of Health, 2007).

All samples taken in single for each vending machine for official controls from 2008 to 2011 were considered in the study (Giacometti et al., 2013). A total of 15,282 samples were analysed for *C. jejuni* using the official International Organization for Standardization (ISO) cultural methods, ISO 10272-1:2006. All samples were processed at the Experimental Institutes for Zooprophylaxis in the different regions. All the laboratories and test procedures are accredited according to ISO 17025:2005 by ACCREDIA, the Italian accreditation body. *C. jejuni* were detected in 53 out of 15,282 (0.34%) raw milk samples. No significant differences were shown in the prevalence among regions by multivariate analysis (Giacometti et al., 2013). Four farms located in three different regions resulted positive twice in two successive months but no seasonality in the prevalence of positive samples was shown. Details of the number of samplings performed and the number of positive samples detected in the four-year monitoring in each region and the number of dairy farms authorized to produce and sell raw milk and vending machines registered by Italian Minister of Health for each region in 2011 are listed in table 1.

The beta function was used to model the variability and uncertainty introduced by sampling on the true prevalence estimation of *C. jejuni* in raw milk samples. Using Bayes' theorem the fraction of raw milk samples contaminated by *C. jejuni* was assumed to follow a Beta distribution (Vose, 1996): if we assume 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 was modelled as:

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

where S is the number of positive raw milk samples and N is the total number of raw milk samples analysed (n=15,282).

Few quantitative data on *C. jejuni* concentration in raw milk are available in the literature so it is not possible to fit a probability distribution to quantitative data (Helsel, 2005). Nevertheless, a Poisson distribution was first assumed to calculate the mean concentration of *C. jejuni* in milk sampled during official controls, and consequently the probability that no *C. jejuni* are detected during microbiological analysis in a sample volume (V_{sample}) when *C. jejuni* concentration in milk is C, is:

$P^{-} = \exp(-C * V_{sample})$ (2) (Sanaa et al., 2004)

The concentration of *C. jejuni* in the milk is therefore:

$C = -\ln(1- P^{+}) / V_{sample.}$ (3)

A distribution of C values could be obtained after a Monte Carlo simulation (with practical value of 100,000 iterations) using @Risk, version 4.5.2 (Palisade Corporation, Newfield, NY, USA).

Assuming that pathogens are normally distributed in logarithmic scale in raw milk, a normal distribution in log scale was used; to fully characterize the distribution of the *Campylobacter* concentration in raw milk, we need to estimate the mean (μ) and standard deviation (σ) of the normal distribution.

C_{milk}= Normal (μ, σ) (4).

The distribution of C values obtained in (3) after a Monte Carlo simulation (see above) was used as mean (μ) of the Normal distribution of *C. jejuni* in raw milk samples (4). To take into account the qualitative results obtained during the official control survey (Giacometti et al., 2013) and lacking quantitative data, the raw milk concentration of *C. jejuni* was modelled considering the detection limit of the microbiological method applied (usually considered 0.04 CFU, -1.398 log CFU/ml) as follows: given the (μ) of the Normal distribution for the concentration of the pathogen in milk, the equation was resolved for (σ) obtaining a fraction of modelled raw milk samples above the detection limit comparable to the prevalence observed during the official control survey.

2.3 Time-temperature history of raw milk from vending machine to consumption and growth model

Data reported in a previous study (Giacometti et al., 2012c) pertaining to vending machine storage and consumer habits in terms of home transportation (use of insulated bags for transportation of raw milk and the journey time from the vending machine to home) and the time to consumption after purchase were used to build time-temperature conditions. The worst time-temperature conditions (WS), defined as the sum of all the thermal abuses detected in the investigations, and the best theoretical storage conditions (BS) set at 4°C as fixed by law throughout all the phases, were used to perform a challenge test simulating the behaviour of *C. jejuni* in WS and BS.

The WS simulated in this study were 11°C±0.5 for 22.5 h (maximum temperature recorded in vending machines and maximum storage time established by law for raw milk), 30°C±0.5 for 30 min (air temperature during the simulation of transport of raw milk from vending machines to home in summer) and 12°C±0.5 for 68 h (home storage) (Giacometti et al., 2012c). *C. jejuni* counts decreased during both experimental storage conditions in vending machines, so the decimal reduction time (DRT) *C. jejuni* was calculated and expressed in hours (624.31±399.22 h and 132.65±19.3 h in the BS and WS respectively). A triangular distribution was chosen with the most likely value fixed as equal to the experimental mean DRT observed, and minimum and maximum parameters in the triangular distribution were calculated considering the standard deviation observed experimentally (Giacometti et al., 2012c):

$DRT_{(C.\ jejuni\ bsT)}$ = Triangular (225.1; 624.31; 1023.53) h

$DRT_{(C.\ jejuni\ wsT)}$ = Triangular (113.35; 132.65; 151.95) h

By law, raw milk has a shelf-life of three days, but according to consumers milk was consumed up to five days after purchase (Giacometti et al., 2012c). For this reason, the time to consumption T (h) was modelled by the triangular distribution:

T (h) = Triangular (0.5, 24, 120) h.

Therefore the pathogen number reduction (rn) after storage in vending machines, home transportation and home storage is:

rn = T (h) / *DRT*

and the concentration of the pathogen in raw milk after home storage for both the best and worst time-temperature scenario was modelled as:

$C_{afterstorage} = 10^{\text{C-rn}}$

Data obtained from experiments in which the milk was boiled were used to model the survival of *C. jejuni* in milk after a homemade heat treatment (Giacometti et al., 2012c). No viable pathogenic bacteria were recovered from boiled milk in experimental boiling and a > 10^8 log reduction was shown, but to consider possible under-treatment of milk (microwave treatment or insufficient heating) the log reduction count was modelled using the triangular distribution:

$D_{\text{boil } C.\ jejuni}$ = Triangular (6, 7, 8) log reduction.

On this basis, the potential concentration of *C. jejuni* in milk after boiling was calculated for the best and worst time-temperature raw milk scenarios:

$C_{after\ boiling} = 10^{(\log C_{afterstorage} - \text{Dboil})}$.

The distribution of raw milk serving size (S_i) was characterized by a previous survey (Giacometti et al., 2012a); S_i was modelled by the triangular distribution as:

S_i = Triangular (100, 250, 1,000) ml.

2.4 Pathogen dose per serving size

Considering very different proportions of consumers boiling milk before drinking were observed in the three available consumer investigations (Anonymous, 2012, Giacometti et al., 2012c and 2013), two separate RA models were developed: 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 calculated for the best and worst time-temperature milk storage scenarios as:

Dose = Poisson ($C_{\text{after storage (after boiling)}} \times S_i$.)

2.5 Dose response

Two different dose-response (D-R) relationships were used: D-R-I, the most frequently used dose-response model for *Campylobacter,* that is the classic Beta-Poisson model based on the data of a volunteer study (Black et al., 1988 Medema et al., 1996; Teunis et al., 1999), and D-R-II updated by Nauta et al. (2009) on the basis of two similar outbreaks among school children drinking raw milk at farms in the UK and the Netherlands (Teunis et al., 2005).

According to the Beta-Poisson model the probability of human infection can be defined by:

D-R-I = $P_{inf} = 1 - (1+\text{Dose} / \beta)^{-\alpha}$ (Furomoto and Mickey, 1967)

expressing 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 D-R-I, the parameters of the Beta-Poisson model estimated are α = 0.145 and β = 7.589, where α and β are the parameters of the distribution of microorganism-host survival probabilities (beta distribution with parameters of α and β).

In the D-R-II model proposed by Nauta et al. (2009) *C. jejuni* was supposed to be more infectious than previously concluded and α = 0.024 and β = 0.011 were proposed. With this estimation of α and β the Beta-Poisson model as approximation of the exact single-hit dose-response model was not acceptable. In the present study the pathogen dose per serving size is assumed to follow a Poisson distribution. If the dose is a discrete number of organisms the probability of infection given by the hypergeometric Beta-Poisson dose-response model (exact single-hit dose-response model) is simplified to the "conditional dose-response function" (Hass, 2002), also named Betabinomial model, using the same parameters:

D-R-II = $P_{inf} = 1 - \frac{\Gamma(\alpha+\beta)\,\Gamma(n+\beta)}{\Gamma(\alpha+\beta+n)\,\Gamma(\beta)}$

where, $\Gamma(.)$ is Euler's gamma function, n is the discrete dose and α = 0.024 and β = 0.011 (Nauta et al., 2009).

The infection status linked to consumption of raw milk (presence or absence of infection) was simulated as:

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

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

Regarding estimation of the probability of campylobacteriosis given the infection, the approach used by Nauta et al. (2007) was followed, assuming a constant probability of illness equal to 0.33. Consequently, consumer illness can be calculated as:

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

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

2.6 Model outputs

The models were implemented with software for Monte Carlo simulation @Risk, version 4.5.2, and 1,000,000 iterations were done for each simulation. The first output of the model was the log-normal distribution of the milk concentration of *C. jejuni* estimated on the basis of official control results on vending machines. Four different simulation models were run, two for the best and worst time-temperature scenarios in raw milk storage considering all milk servings correctly boiled or not boiled by consumers (see figure 1). In addition, the models considered two different D-R relationships. 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 total amount of raw milk sold at vending machines was estimated from economic considerations: about 50 litres/day to be economically sustainable for the farmers.

The number of expected cases was calculated for the different time-temperature scenarios and for the two D-R relationships on the basis of two variables, namely the percentage of consumers boiling milk before consumption and the sensitive population (children). These data were extracted from three previous investigations (Anonymous, 2012, Giacometti et al., 2012c and 2013) in which the percentages of consumers boiling milk were 13.9, 23 and 43 respectively, whereas the percentages of the sensitive population drinking raw milk were 3.57, 5.57 and 7.87 respectively.

The expected number of cases was calculated for each combination of the observed percentage of consumers not boiling milk and of the sensitive population and weighted to the total population in order to calculate the number of cases/100,000 persons a year. This combination was calculated both in the best and worst time-temperature scenarios. In addition, the two different D-R relationships were also considered (see figure 1 and table 2).

3. Results

3.1 Concentration of C. jejuni in raw milk

The model provided a mean concentration of *C. jejuni* per ml of milk equal to $1.42*10^{-4}$ CFU/ml and standard deviation (SD) $1.93*10^{-5}$ CFU/ml. Assuming that the pathogen in milk was normally distributed on a logarithmic scale and considering that the fraction of positive samples observed during official controls was concentrated above the detection limit of 0.04 CFU/ml, a standard deviation of 0.91 log was calculated and the concentration of *C. jejuni* was modelled as normal [log(normal($1.42*10^{-4}$; $1.93*10^{-5}$); 0.91]. The curve modelled admitted 99.65% of milk samples with a *C. jejuni* concentration below the detection limit of microbiological methods, as observed on average during the official controls conducted on raw milk in Italy from 2008 to 2011.

3.2 Risk characterization

In simulations where all consumers boil raw milk before drinking, no dose above 0 CFU was calculated for both the best and worst time-temperature scenarios. In simulations where no consumer boils raw milk before drinking, a mean dose of 0.814 CFU (50^{th} percentile 0 CFU; 90^{th} percentile 1 CFU; 95^{th} percentile 3 CFU; maximum simulated 1,789 CFU) and a mean dose of 0.467 CFU (50^{th} percentile 0 CFU; 90^{th} percentile 0 CFU; 95^{th} percentile 2 CFU; maximum simulated 1,459 CFU) were obtained from the model for the best and worst time-temperature scenarios, respectively. In 1 million (M) iterations the model predicts 8,500 infections and 6,500 infections applying D-R-I relationships for the best and worst time-temperature scenarios, respectively

(Black et al. 1988). Considering a total of 1,236 vending machines in Italy and that each vending machine works 365 days a year selling at least 50 litres per 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. Considering the probability of campylobacteriosis given the infection fixed at 0.33, the numbers of expected cases of campylobacteriosis in the total population (39,396,008) of the investigated regions were 301,785 and 230,776 cases for the best and worst time-temperature scenarios, respectively.

Applying the D-R-II relationship, the number of infections predicted by the model in 1M iterations was 148,000 and 105,000 that, applying the same considerations on the number of servings (1.08×10^8) and the probability of campylobacteriosis (0.33 of infections) in the total population (39,396,008) of the investigated regions, becomes 5,254,603 and 3,727,928 cases of campylobacteriosis per year for the best and worst time-temperature scenarios, respectively (Nauta et al. 2009).

The overall number of expected campylobacteriosis cases predicted by the different simulation models was stratified into several scenarios in relation to consumer behaviour (percentages of consumers that do not boil milk) and age of consumers. The findings are summarised in table 2.

4. Discussion

The RA of campylobacteriosis associated with the consumption of raw milk described in the present study is based on results of official controls of raw milk samples collected at vending machines in seven Italian regions from 2008 to 2011 and on consumer habits investigated in the different sampling areas, providing a good insight into the nationwide raw milk scenario. Some sources of uncertainty may be identified in the data used for the RA, as also emphasized in previous RAs performed for other microorganisms or previously (Giacometti et al., 2012a; Giacometti et al., 2015): 1) given the lack of data, the estimated amount of raw milk sold at vending machines was based on economic considerations; 2) the raw milk serving size was estimated, rather than measured, on the basis of the weekly purchase data declared by consumers (1 to 2 litres/week); 3) 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.

The main source of uncertainty is the concentration of pathogens in raw milk. According to the national legal requirements of the State-Regions Agreement (Italian Minister of Health, 2007), official control of raw milk sold by self-service vending machines is based on a qualitative (presence/absence) method for *C. jejuni*: when a positive sample is observed, no quantification of *Campylobacter* in milk samples is required by law. Therefore, no quantitative data are available to fit the concentration of the pathogens in milk. The method we used for fitting the lognormal distribution of the pathogens was based on the sensitivity of the presence/absence method applied by the reference laboratories for official controls in Italy and on estimation of the mean concentration of the pathogens in milk officially sampled across the country in four years of official surveys. The way in which the parameters of the lognormal distribution were estimated supposes that all dairy farms share the same mechanism of raw milk contamination and this is a major drawback of this model. However, lacking quantitative data and with an apparent prevalence of 0.34% (Giacometti et al., 2013), no unbiased fitting methods could be applied to the available data, even considering recently proposed Markov chain Monte Carlo fitting methods (Williams et al., 2013). Nevertheless, the comparison of the Dose modelled in this study with the scarce literature available on quantitative *C. jejuni* milk contamination seems to support the results obtained by the developed curve. In fact, Humprey and Beckett's (1987) repetitive sampling for enumeration of *C. jejuni* in bulk tank milk of one farm disclosed an intermittent contamination (5/11 samples positive) of 1-5 CFU/100 ml in four out of five positive samples and a contamination of 100 CFU/100ml in the other sample. These values are in the range of the mean and maximum levels of contamination predicted by the model in simulation, namely a mean of 0.81 CFU and a maximum of 1,789 CFU per serving (210 ml). Bianchini et al. (2014) observed that, although rare, a cow with *C. jejuni*-induced mastitis causes a more constant positivity of bulk milk for *C. jejuni*. Here too quantitative data on milk shedding by cows with *C. jejuni*-induced mastitis are few, but two Italian studies reported milk contamination by direct milk excretion from a single animal with a single quarter with *C. jejuni*-induced mastitis (Cammi et al., 2009 and Luini et al., 2009). These studies enumerated the *C. jejuni* in the quarter of the sick cow as 275 MPN/ml and 1,700 CFU/ml (Cammi et al., 2009 and Luini et al., 2009). Considering the number of cows and milk yield reported in the two papers, it is easy to estimate a Dose ranging from 64 to 1,750 CFU in a 250 ml serving, that is also in the range of 0.81 - 1,789 CFU per serving estimated by our model.

Therefore, although our estimates may have been biased by some factors, the proposed *C. jejuni* RA model reflects current trends in raw milk consumption in Italy and uses specific national data and consumer habits as model inputs, addressing storage conditions in vending machines, sale and consumption patterns, domestic storage, consumer habits of boiling milk and age of consumers in an attempt to reproduce real-life scenarios.

As expected, this RA model predicted no human campylobacteriosis cases per year due to raw milk consumption if all consumers boil milk before consumption in all the simulated storage conditions of milk during its shelf life and consequently the probability of illness could be considered negligible in this specific scenario. In case of consumption without boiling milk, the annual number of predicted campylobacteriosis cases varies widely depending by the D-R relationships used in the model, the storage conditions of milk during its shelf life and even the age of consumers, meaning the percentage of young versus adult consumers, as detailed in table 2. The lower predicted cases in the worst time-temperature storage conditions reflects the higher DRT detected in the challenge test at higher storage temperatures. However, this finding should not affect the fact that optimal storage of raw milk by farmers and also by consumers is a simple measure that must be implemented to reduce the risk of *C. jejuni* or other infections due to raw milk consumption. Results confirm that raw milk may be an important vehicle of *C. jejuni* exposure, even if broiler meat remains the main source of strong-evidence *Campylobacter* outbreaks (EFSA, 2014).

In all of these cases, the total predicted number of campylobacteriosis cases due to raw milk consumption does not concur with the overall confirmed cases reported in the period considered in Italy which accounted for between 265 and 531, corresponding to an apparent incidence of 0.44-0.89 cases/100,000 inhabitants per year (EFSA, 2014). On one hand, the true incidence of campylobacteriosis in Italy is probably greatly underestimated by official data reported given that *Campylobacter* notification rates in 2013 in Europe versus Italy were 55.49 cases/100,000 inhabitants versus 1.27 cases/100,000 inhabitants (EFSA, 2014). The cause of this underestimation is due to the deficiency of the reporting system applied in Italy since it is well known that cases from passive routine surveillance programmes are frequently under-reported (Scavia et al., 2012). Estimates derived from outbreak data may be combined with estimates of under-reporting to determine the total burden of outbreak-associated disease (WHO, 2013). A recent study estimated the under-reporting (number of cases/number of reported cases) factor for campylobacteriosisis in Italy at 662 (Havelaar et al., 2013). Applying this under-reporting factor to the annual average of 430 campylobacteriosis cases reported in Italy by EFSA in the period 2008-2011, a total of 284,660 (430 cases x 662 times) cases of campylobacteriosis can be estimated in the general population each year.

On the other hand, the expected cases resulting from the RA model estimate are greatly influenced by two main factors. The first is related to the two different D-R relationships used in the model. The D-R model used most is the Beta-Poisson model based on data obtained in the human adult volunteer study by Black et al. (1988) that applies all currently available data and is biologically the most plausible model (FAO/WHO, 2003). However, this does not imply that the *C. jejuni* D-R relationship obtained in Black et al.'s study (1988) should always be considered applicable, and therefore the 'classic' D-R relationship for *C. jejuni* has been updated based on two surprisingly similar outbreaks among school children drinking raw milk at farms in the UK and the Netherlands (Teunis et al., 2005; Nauta et al., 2009). Although the ingested doses of *C. jejuni* were not known in these outbreaks, the relative amounts of milk consumed could be retrieved. Application of the hazard function for the probability of illness given infection yielded new estimates for the D-R relationship for the probability of illness, for both a 'sensitive' population (like young children) and others (Teunis et al., 1999; Nauta et al., 2009). Children may have an higher susceptibility to enteric pathogens even at low infectious doses due to their lower levels of immunological protection (Scavia et al., 2012). *C. jejuni* RAs do not usually split consumers into "sensitive" and "normal" populations as is routinely done in *Listeria monocytogenes* RA studies, and most of the studies use a constant probability of illness given the infection. However, considering that the infection rate in children under five years is 1.7–2.6 times higher than the rates in other age groups (ECDC, 2013), future RA studies can consider the Nauta et al. (2009) D-R relationship to model the probability of *C. jejuni* infection in a "sensitive" population, while the D-R relationship reported by Black et al. (1988) can be used for older populations. In this hypothesis, applying a different D-R relationship to adult and young consumers, the number of expected cases can be assessed in adult consumers as between 79.4/100,000 population/year (92.13% of adult consumers in WS conditions, 13.9% of consumers not boiling milk, see table 2) and 333.1/100,000 population/year (96.43% of adult consumers in BS conditions, 43% of consumers not boiling milk, see Table 2) and for young consumers between 1013.7/100,000 population/year (3.57% of young consumers in WS conditions, 13.9% consumers not boiling milk) and 8110.3/100,000 population/year (7.87%

of young consumers in BS conditions, 43% of consumers not boiling milk, see Table 2). In this scenario and on the basis of the under-reporting factor reported by Havelaar et al. (2013), the estimated cases can be considered more compatible with the total estimated cases (284,660) of campylobacteriosis in the total Italian population (59,433,744) that corresponds to 478.9 cases/100.000 population/year.

The second factor is related to consumer habits reported in previous studies and used in the present RA: different percentages of young consumers (from 3.57 to 7.87%, see Table 2) and different percentages of consumers that do not boil milk before consumption (13.9 to 43%, see Table 2) roughly double the number of estimated cases, showing how consumer habits affect the probability and number of campylobacteriosis cases emphasizing yet again that, in countries where the sale of raw milk is allowed, consumer educational programmes are key to protecting consumers against the risks of illness inherent in the consumption of raw milk (Anonymous, 2012; Giacometti et al., 2012c, 2013).

The reasons for the differences in consumer habits reported by the three studies are difficult to define, but can be attributed to differences in the geographical area of investigation or to the time of the investigation. As different consumer habits in terms of boiling milk and giving raw milk to children strongly influence RA results, consumer behaviour should be monitored to determine a profile of risky habits which can be used by risk managers for RAs in different countries and in different epidemiological situations. The proposed RA model provides a good indication of the estimated burden of raw milk-borne campylobacteriosis and may be a useful and flexible guidance tool (freely available on request) to devise strategic raw milk safety policies.

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