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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

Federica Giacometti[a], Paolo Bonilauri[b], Simonetta Amatiste[c], Norma Arrigoni[d], Manila Bianchi[e], Marina Nadia Losio[f], Stefano Bilei[c], Giuseppe Cascone[g], Damiano Comin[h], Paolo Daminelli[f], Lucia Decastelli[e], Giuseppe Merialdi[i], Renzo Mioni[h], Angelo Peli[a], Annalisa Petruzzelli[k], Franco Tonucci[k], Silvia Piva[a], Andrea Serraino[a,*]

[a] University of Bologna, Department of Veterinary Medical Sciences, Via Tolara di Sopra, 50, Bologna, 40064 Ozzano Emilia, Italy
[b] Experimental Institute for Zooprophylaxis in Lombardy and Emilia Romagna, Via Pitagora 2, 42100 Reggio Emilia, Italy
[c] Experimental Institute for Zooprophylaxis in Lazio and Tuscany, Via Appia Nuova 1411, 00187 Rome, Italy
[d] Experimental Institute for Zooprophylaxis in Lombardy and Emilia Romagna, Strada della Faggiola 1, 29027 Gariga di Podenzano, Italy
[e] Experimental Institute for Zooprophylaxis in Piedmont, Liguria and Valle D'Aosta, Via Bologna, 148, 10154 Turin, Italy
[f] Experimental Institute for Zooprophylaxis in Lombardy and Emilia Romagna, Via Bianchi 7/9, 25124 Brescia, Italy
[g] Experimental Institute for Zooprophylaxis in Sicily, Via G. Marinuzzi, 3, 90129, Palermo, Italy
[h] Experimental Institute for Zooprophylaxis in Veneto, Viale dell'Università, 10, Padua,35020 Legnaro, Italy
[i] Experimental Institute for Zooprophylaxis in Lombardy and Emilia Romagna, Via P. Fiorini, 5, 40127 Bologna, Italy
[k] Experimental Institute for Zooprophylaxis in Umbria and Marche, Via G. Salvemini, 1, 06162, Perugia, Italy

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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.

1. Introduction

Campylobacteriosis is a diarrhoeal disease caused by bacteria of the genus *Campylobacter* and represents one of the leading causes

* Corresponding author. Fax: +39 51 2097346.
E-mail address: andrea.serraino@unibo.it (A. Serraino).

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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 neighbouring 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 1236 vending machines, i.e., 89.43% of the 1382 vending machines registered in Italy, providing a high proportion of the true national scenario (Italian Ministry 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 $\leq$5 or $\leq$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.