



Survey of prevalence and seasonal variability of *Listeria monocytogenes* in raw cow milk from Northern Italy



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ABSTRACT

Listeria monocytogenes is an important food-borne pathogen causing meningitis, meningo-encephalitis and abortion. Both sporadic and epidemic human listeriosis cases are associated with the consumption of contaminated foods. To assess the potential risk to consumer health, the presence of *L. monocytogenes* was investigated using qualitative and quantitative methods in raw milk (bulk tank milk and milk for vending machine) collected from 2010 to 2013 in Northern Italy (Lombardy and Emilia-Romagna regions). Overall, *L. monocytogenes* was detected in 145 on 8716 of raw milk samples, with a prevalence of 1.66% (95% C.I. 1.4%–1.7%). The prevalence ranged from 0.52% (95% C.I. 0.3%–0.9%) in 2012 to 2.7% (95% C.I. 2.0%–3.8%) in 2013, but no trend of increase was observed in four-years of investigation. The pathogen was detected from 2.2% (95% C.I. 1.9%–2.6%) of bulk tank milk and from 0.5% (95% C.I. 0.3%–0.8%) of milk for vending machine. A significant difference ($p < 0.05$) of the prevalence data was observed between data collected in two different regions of Northern Italy with an higher prevalence in Lombardy. In addition to the geographical area, the *L. monocytogenes* presence was influenced also by the seasonal period of collection samples, with peaks in spring and autumn. These results confirm the raw milk can be a source of foodborne illness outbreaks if consumed without sanitizing treatments, but the low prevalence and the low contamination levels (more than 80% of the contaminated samples contained <10 cfu ml⁻¹ of *L. monocytogenes*) proving the hygienic quality of the milk produced in Northern Italy.

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1. Introduction

The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks, reported that the majority of strong evidence food-borne outbreaks were associated with foodstuffs of animal origin including dairy product. In 2013, 2.14% of strong-evidence food-borne outbreaks (18 on 839 outbreaks) were attributed to the consumption of cheese and dairy products (11 and 7 outbreaks respectively) in Europe (EFSA/ECDC, 2015). From 1982 to 2010, sixty-four reported human cases and outbreaks in Europe, the United States and Canada related to the consumption of dairy products were reported in a non-exhaustive

list by Verraes et al. (2015). The presence of foodborne pathogens in raw milk and bulk tank milk has been widely reported (D'Amico and Donnelly, 2010; De Reu, Grijspeerd, & Herman, 2004; Gaya, Saralegui, Medina, & Nunez, 1996; Hassan, Mohammed, McDonough, & Gonzalez, 2000; Jayarao & Henning, 2001; Kousta, Mataragas, Skandamis, & Drosinos, 2010; Moshtaghi & Mohamadpour, 2007; Van Kessel, Karns, Gorski, McCluskey, & Perdue, 2004; Waak, Tham, & Danielsson-Tham, 2002). The prevalence of pathogens in milk is influenced by numerous factors such as farm size, number of animals on the farm, hygienic conditions, farm management practices, variation in sampling and types of samples evaluated, differences in detection methodologies used, geographical location, and season. However, in spite of the variation, all of these surveys clearly demonstrated that milk can be a major source of foodborne pathogens of human health significance (Oliver, Jayarao, & Almeida, 2005).

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In this contest, *Listeria monocytogenes*, responsible of listeriosis, represents one of the most serious food safety concerns. It has been isolated from many foods (Gianfranceschi, Gattuso, Tartaro, & Aureli, 2003), among which milk and cheeses (Bernini et al., 2013; Dalmasso & Jordan, 2014; Manfreda, De Cesare, Stella, Cozzi, & Cantoni, 2005; Pintado, Oliveira, Pampulha, & Ferreira, 2005; Torres-Vitela et al., 2012) and it has been involved in numerous outbreaks occurring after consumption of contaminated milk and milk products throughout the world (Donnelly, 2001; Linnan et al., 1988; Lundén, Tolvanen, & Korkeala, 2004; Lyytikäinen et al., 2000). In particular, dairy products contaminated with *L. monocytogenes* have been implicated at almost the half of the reported listeriosis outbreaks in Europe (Lundén et al., 2004). The pathogen is widespread in nature and lives naturally in plants and soil environments. Its ability to survive and grow over a wide range of environmental conditions, including refrigeration temperatures, high salt concentration and low pH, makes it a potential hazard in foods (Ryser, 2007). Moreover, *L. monocytogenes* into food processing plants results in reservoirs that are difficult to eradicate: this is the case of biofilms that are a constant issue in food processing environments (Oliver et al., 2005). So, in addition to the risk associated to direct consumption, the introduction of raw contaminated milk into dairy processing plants represents a risk to human health if milk is used unpasteurized for cheese making or in case of cross contamination (Kousta et al., 2010).

Considering the threat represented by the pathogen in raw milk, a survey was conducted from January 2010 to September 2013, involving a large number of samples collected in different geographical area in the North of Italy. This research aimed to give a considerable overview of *L. monocytogenes* presence in raw cow milk, intended both for cheese making and for direct consumption, by evaluating the prevalence of the pathogen at farm level also in relation to the seasonality.

2. Materials and methods

2.1. Samples collection

A total of 8716 raw cow milk samples were collected from January 2010 to September 2013 in 942 farms located in Lombardy and Emilia-Romagna regions, Northern Italy. All the samples, consisting in 5897 samples of bulk tank milk intended for cheese making and 2819 samples of milk intended for sale in automatic vending machines, were collected into sterile containers, kept below 4 °C during transportation and analyzed within 2 h after receipt. Samples were collected in the frame of Food Business Operator's self-control programs or in the frame of monitoring surveys officers of the Regional Veterinary Authority.

2.2. Detection and enumeration of *L. monocytogenes*

All samples were tested for the presence of *L. monocytogenes* on 25 ml of raw cow milk by means of qualitative methods.

The samples collected from 2010 to 2011 were examined qualitatively according to ISO 11290-1 (ISO, 1996). The samples collected from 2012 to 2013 were examined by a biomolecular method (real-time PCR) (Biorad AFNOR BRD 07/10-04/05) (AFNOR, 2004) to detect *L. monocytogenes* DNA. Samples testing positive were retested under a microbiological protocol according to ISO 11290-1 (ISO, 1996). Typical colonies (n = 5) presumed to be *Listeria* spp. were streaked from Agar *Listeria* acc. to Ottaviani & Agosti (ALOA) (Biolife Italiana, Teramo, Italy) supplemented with ALOA enrichment-selective supplements (Biolife Italiana) onto Tryptone Soya Yeast Extract Agar, TSYEA (Oxoid, Basingstoke, UK) and plates were incubated at 37 °C for 24 h. By following the Gram's staining,

catalase reaction and tumbling motility were performed using the pure cultures obtained from TSYEA. The isolates resulted positive to phenotypic tests were inoculated on 5% sheep blood agar (Oxoid) to determine the Beta haemolytic reaction. For following confirmation, carbohydrate utilization and CAMP tests were performed.

On samples found to be positive, the enumeration of *L. monocytogenes* was carried out according to the method described by ISO 11290-2 (ISO, 1998) to evaluate the prevalence of contamination level.

2.3. Data analyses

The prevalence of *L. monocytogenes* in raw milk, calculated as proportion between positive samples on total sample, was expressed in percentage values. Statistical analysis was performed by Epi tools (<http://epitools.ausvet.com.au>): the confidence intervals (C.I.) of proportions were calculated with using the binomial exact method and the statistical significance of differences between proportions was evaluated by Chi-square (χ^2) test.

3. Results and discussion

From 2010 to 2013, 8716 raw milk samples, intended both for cheese making and for vending machine, were collected in Northern Italy. Samples were taken from local farms within self-control sampling programs and by the official veterinarians within state surveillance programs and were investigated for the presence of *L. monocytogenes*.

The results are summarized in Table 1. The prevalence values in raw milk ranged from 0.52% (95% C.I. 0.3%–0.9%) in 2012 to 2.73% (95% C.I. 2.0%–3.8%) in 2013, but no trend of increase was observed in four-year investigation. Overall, *L. monocytogenes* was detected in 145 raw milk samples out of 8716, with a prevalence of 1.66% (95% C.I. 1.4%–2.0%). This result is mainly due ($p > 0.05$) to bulk tank milk contamination rather than to raw milk intended for vending machine. In fact, concerning bulk tank milk, *L. monocytogenes* was found in 2.22% (95% C.I. 1.9%–2.6%) of samples (131/5897) in four years of survey (Table 2). These findings were in agreement with those reported in several studies carried out internationally and recently published, in which the prevalence of *L. monocytogenes* in raw milk has ranged from 'not detected' to 7.1%. In particular, the pathogen was "not detected" in Norway in 2011 (Jakobsen, Heggebø, Sunde, & Skjervheim, 2011), and to the extent of 0.68% in New Zealand in 2012 (Hill, Smythe, Lindsay, & Shepherd, 2012), 2.12% in Turkey in 2006 (Aygun & Pehlivanlar, 2006), 2.61% in Algeria in 2007 (Hamdi, Naïm, Martin, & Jacquet, 2007), 5.5% in Finnish in 2013 (Ruusunen et al., 2013), 6.1% in North-West Spain in 2007 (Vilar, Yus, Sanjuán, Diéguez, & Rodríguez-Otero, 2007) and 7.1% in USA in 2011 (Van Kessel, Karns, Lombard, & Kopral, 2011). Previously, in 2005, the prevalence of *L. monocytogenes* in bulk tank milk has been reported to range from 1 to 12% (Oliver et al., 2005), therefore, a reduction of contamination samples seems to have been monitored in the last years. Anyway, the above data collected in different world areas underline the wide variability of the

Table 1
Detection and prevalence of *L. monocytogenes* in raw milk collected in Northern Italy according to the year of sampling.

Year	Samples	Positive for <i>L. monocytogenes</i> (%)
2010	1728	20 (1.16%)
2011	3150	76 (2.41%)
2012	2519	13 (0.52%)
2013	1319	36 (2.73%)
Total	8716	145 (1.66%)

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