Seasonal variability of thermophilic *Campylobacter* spp. in raw milk sold by automatic vending machines in Lombardy Region

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Abstract

In temperate climates, a seasonal trend was observed in the incidence of human campylobacteriosis cases, with peaks reported in spring and autumn in some countries, or in summer in others; a similar trend was observed in *Campylobacter* spp. dairy cattle faecal shedding, suggesting that cattle may play a role in the seasonal peak of human infection. The objectives of this study were to assess if a seasonal trend in thermophilic *Campylobacter* spp. contamination of raw milk exists and to evaluate a possible relation between this and the increase of human campylobacteriosis incidence in summer months. The results showed a mean prevalence of 1.6% of milk samples positive for thermophilic *Campylobacter* spp. with a wide range (0.0-3.1%) in different months during the three years considered. The statistical analysis showed a significant difference ($P<0.01$) of the prevalence of positive samples for thermophilic *Campylobacter* spp. between warmer and cooler months (2.3 *vs* 0.6%). The evidence of a seasonal trend in thermophilic *Campylobacter* spp. contamination of raw milk sold for direct consumption, with an increase of the prevalence in warmer months, may represent one of the possible links between seasonal trend in cattle faecal shedding and seasonal trend in human campylobacteriosis.

Introduction

Thermophilic *Campylobacter* spp. strains are some of the most frequent etiological agents of bacterial gastroenteritis in humans in many developed countries. *C. jejuni*, *C. coli*, *C. lari*, and *C. upsaliensis* are considered to be thermophilic because of their ability to grow at 42°C. Most detection methods are designed for the human pathogens *C. jejuni* and *C. coli*, and the majority of the studies deal with *C. jejuni*, a common commensal of the gastrointestinal tracts of wild and farm animals that is ubiquitous in the natural environment (Kwan *et al*., 2008). Thermophilic *Campylobacter* can cause severe post-infection neuropathies, including Guillan-Barré syndrome and Miller Fisher syndrome (Rapp *et al*., 2012). Campylobacteriosis is a commonly reported zoonosis in the European Union (EU) since 2005 and the number of reported confirmed cases of human campylobacteriosis in the EU in 2014 was 236,851, which registered an increase of 22,067 cases compared with 2013. The EU notification rate was 71.0 per 100,000 population in 2014, which showed an increase by 9.6% compared with 2013 (64.8 per 100,000 population) (EFSA, 2015). In temperate climates, a seasonal trend was observed in the incidence of human campylobacteriosis cases, with peaks reported in spring and autumn in some countries, or in summer in other countries (Stanley *et al*., 1998). A similar trend was observed in *Campylobacter* spp. dairy cattle faecal shedding (Stanley and Jones, 2003), suggesting that cattle may play a role in the seasonal peak of human infection (Kwan *et al*., 2008).

The significance of cattle as reservoir of human campylobacteriosis is related to food products contamination (meat and milk), to environmental and water contamination, and to a direct transmission to humans. Several studies have identified poultry meat as the main food vehicle associated with sporadic cases of campylobacteriosis, but there is also strong evidence that raw milk consumption represents a risk factor for campylobacteriosis. Raw cow's milk has been reported to be a vehicle of human campylobacteriosis in outbreaks both in the United States and Europe (Claeys *et al*., 2013; Hauri *et al*., 2013; Longenberger *et al*., 2013; Taylor *et al*., 2013; EFSA, 2015). In the United States the expert consultation on the global view of campylobacteriosis reported that raw unpasteurised milk is the most common food vehicle for campylobacteriosis outbreaks, 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, 2015).

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*., 2012, 2013; Bianchini *et al*., 2014). In addition, two outbreaks of *C. jejuni* linked to the consumption of raw milk have been reported in Italy, one in Veneto Region and the other in Marche Region (Amato *et al*., 2007).

The objective of this study was to assess if a seasonal trend in thermophilic *Campylobacter* spp. contamination of raw milk exists and to evaluate a possible link with the increase of human campylobacteriosis cases in summer months.

Materials and Methods

Data were collected from official microbiological records monitoring raw milk samples from self-service vending machines in Lombardy Region. All samples were analysed at the Institute for Experimental Veterinary Medicine of Lombardy and Emilia-Romagna, Brescia, following the ISO 10272-1:2006 method (ISO, 2006). Test procedures are accredited according to the International Organization for Standardization 17025:2005 (ISO, 2005) by ACCREDIA, the Italian accreditation body.

In this study a total of 1629 raw milk samples analysed during 2012-2014 was considered; statistical analysis was performed using

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SPSS software (SPSS Statistics ver. 21; IBM Corp., Chicago, IL, USA) by χ^2 test comparing the prevalence of thermophilic *Campylobacter* contamination during warmer months (April-September) *vs* cooler months (October-March).

Results

Twenty-six out of 1629 raw milk samples examined resulted positive. All isolates were identified as *C. jejuni*. Results showed a mean prevalence of 1.6% of samples positive for thermophilic *Campylobacter* spp. with a range of 0.0-3.1% in different months during the three years considered. Mean prevalence per month of samples positive for thermophilic *Campylobacter* spp. is reported in Figure 1.

Most of the samples were analysed by the official veterinary services in the periods between May and November. One month (January) showed a noticeable lower number of samples tested by official authorities and in one month (August) no sample was performed.

The statistical analysis showed a significant difference ($P<0.01$) of the prevalence of samples positive for thermophilic *Campylobacter* spp. between warmer and cooler months (2.3 *vs* 0.6%).

Discussion

A total of 1629 milk samples collected during 2012-2014 in Lombardy Region were analysed for the presence of thermophilic *Campylobacter* spp.; twenty-six out of 1629 examined (1.6%) resulted positive. The overall prevalence of milk samples positive for thermophilic *Campylobacter* spp. is in agreement with previous studies performed in Italy on raw milk sold for direct consumption, which ranged between 0 and 2.2% in different regions (Giacometti *et al*., 2012, 2013). The observed prevalence is also in agreement with other investigations performed in different countries (Desmasures *et al*., 1997; Dontorou *et al*., 2003; Meyer-Broseta *et al*., 2003; Oliver *et al*., 2005, 2009; Wysok *et al*., 2011).

Most of the samples were analysed by official veterinary services in warmer months; this is in agreement with the principle that sampling should be planned according to the microbiological risk and should follow the regulatory guidelines of the Italian Ministry of Health (2007) on raw milk. Regional rules specify that every year at least two samplings must be performed for each farm authorised to sell raw milk and that at least one sampling must be performed during summer. In the 3-year-period of this study, a mean of 230 farms was authorised to sell raw milk; the total number of samples tested (1629) shows that the mean value of milk samples collected from each farm was 2.3/year.

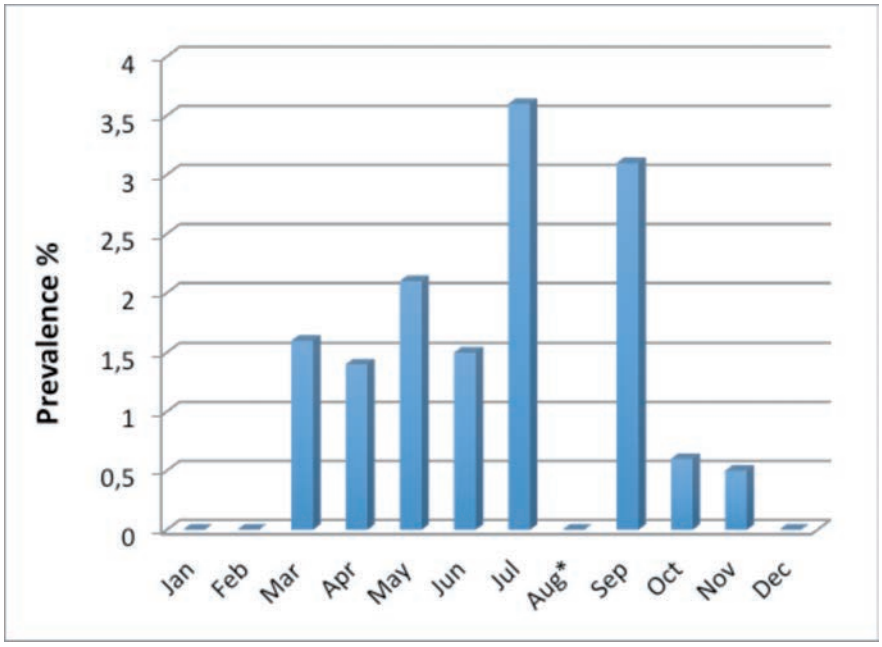

Figure 1. Monthly distribution of the prevalence of raw milk samples positive for thermophilic *Campylobacter* spp. during 2012-2014. *No samples were tested in August.

A seasonal trend in thermophilic *Campylobacter* spp. contamination of raw milk samples was observed in this study, with higher prevalence in warmer months (April-September), corresponding to the higher prevalence rates of faecal shedding observed in cattle (Grove-White *et al*., 2010) even in Italy (Merialdi *et al*., 2015) and in humans (Stampi *et al*., 1992). Different reasons for seasonal increase of human campylobacteriosis were proposed, including increase shedding of animal reservoirs and higher levels of the food chain contamination, changes in human behaviour and the abundance of invertebrate vectors. However, the driver of this trend is still unknown (Spencer *et al*., 2012). Despite the most important risk factors for campylobacteriosis are recognised to be handling and consumption of poultry meat and cross-contamination of uncooked products, there is now a growing evidence, based on molecular typing of isolates, that non-poultry sources of human infection has been underestimated. Epidemiological data suggest that the vehicles of common sources outbreaks are different from sporadic infections (Stanley and Jones, 2003): in fact the most frequent food vehicle reported in campylobacteriosis outbreaks is represented by raw unpasteurised milk, whereas for sporadic cases poultry represents the main risk factor (Taylor *et al*., 2013). Also, a rural-urban association of strains was demonstrated, with the majority of children infections caused by cattle strains in rural areas and by poultry strains in urban areas. Cattle products, the environment contamination, and direct contact between cattle and humans are well-recognised sources of infection of pathogenic agents (Ellis-Iversen *et al*., 2009). The transmission pathways of thermophilic *Campylobacter* spp., and in particular *C. jejuni*, from dairy cows to humans may include direct contact with dairy animals, consumption of untreated water contaminated with animal effluents and consumption of contaminated milk (Gilpin *et al*., 2013; Taylor *et al*., 2013).

High rates of campylobacteriosis have been reported in high-density dairying areas (Gilpin *et al*., 2013) and in particular rural residence was identified as a recognised risk factor for children (Bessel *et al*., 2010). Recently, in Italy, multi locus sequence typing of *C. jejuni* isolates showed a correspondence between milk and cattle isolates and five of the most frequently detected clinical human CCs isolates (CC-21, CC-45, CC-48, CC-61, CC206) in Europe, emphasising the role of cattle as a major source of food contamination and human infection (Bianchini *et al*., 2014).

Conclusions

Common sources of campylobacteriosis outbreaks are rare but show a marked seasonality trend and in particular contaminated water, milk or cattle represent the most commonly implicated vehicle or risk factor (Stanley *et al*., 1998; Kwan *et al*., 2008). In their study, Gilpin *et al*. (2013) attributed the significant higher number of campylobacteriosis cases in summer months to an increase of food-related cases. The evidence of a seasonal trend in thermophilic *Campylobacter* spp. contamination of raw milk sold for direct consumption with an increase of the prevalence in warmer months may represent one of the possible links between seasonal trend in cattle faecal shedding and seasonal trend in human campylobacteriosis. Bacteriological surveillance of raw milk samples may help to understand the role of *Campylobacter* spp. in foodborne and/or zoonotic infections transmitted to humans.

References

Amato S, Maragno M, Mosele P, Sforzi M, Mioni R, Barco L, Dalla Pozza MC, Antonello K, Ricci A, 2007. An outbreak of Campylobacter jejuni linked to the consumption of raw milk in Italy. Zoonoses Public Health 54(Suppl.1):23.

Bessell PR, Matthews L, Smith-Palmer A, Rotariu O, Norval JC, Forbes KJ, Cowden JM, Reid SWJ, Innocent GT, 2010. Geographic determinants of reported human Campylobacter infections in Scotland. BMC Publ Health 10:423.

Bianchini V, Borrella L, Benedetti V, Parisi A, Miccolupo A, Santoro E, Recordati C, Luini M, 2014. Prevalence in bulk tank milk and epidemiology of Campylobacter jejuni in dairy herds in Northern Italy. Appl Environ Microb 80:1832-7.

Claeys WL, Cardoen S, Daube G, Block JD, Dewettinck K, Dierick K, De Zutter L, Huyghebaert A, Imberechts H, Thiange P, Vandenplas Y, Herman L, 2013. Raw or heated cow milk consumption: review of risks and benefits. Food Control 31:251-62.

Desmasures N, Bazin F, Gueguen M, 1997. Microbiological composition of raw milk from selected farms in the Camembert region of Normandy. J Appl Microbiol 83:53-8.

Dontorou C, Papadopoulou C, Filioussis G, Economou V, Apostolou I, Zakkas G, Salamoura A, Kansouzidou A, Levidiotou S, 2003. Isolation of Escherichia coli O157:H7 from foods in Greece. Int J Food Microbiol 82:273-9.

EFSA, 2015. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2014. EFSA J 13:4329.

Ellis-Iversen J, Pritchard GC, Wooldridge M, Nielen M, 2009. Risk factors for Campylobacter jejuni and Campylobacter coli in young cattle on English and Welsh farms. Prev Vet Med 88:42-8.

Giacometti F, Bonilauri P, Serraino A, Peli A, Amatiste S, Arrigoni N, Bianchi M, Bilei S, Cascone G, Comin D, Daminelli P, Decastelli L, Fustini M, Mion R, Petruzzelli A, Rosmini R, Rugna G, Tamba M, Tonucci F, Bolzoni G, 2013. Four-year monitoring of foodborne pathogens in raw milk sold by vending machines in Italy. J Food Protect 76:1902-7.

Giacometti F, Serraino A, Finazzi G, Daminelli P, Losio MN, Arrigoni N, Piva S, Florio D, Riu R, Zanoni RG, 2012. Sale of raw milk in northern Italy: food safety implications and comparison of different analytical methodologies for detection of foodborne pathogens. Foodborne Pathog Dis 9:293-7.

Gilpin BJ, Walshe G, On SL, Smith D, Marshall JC, French NP, 2013. Application of molecular epidemiology to understanding campylobacteriosis in the Canterbury region of New Zealand. Epidemiol Infect 141:1253-66.

Grove-White DH, Leatherbarrow AJH, Cripps PJ, Diggle PJ, French NP, 2010. Temporal and farm-management associated variation in the faecal-pat prevalence of Campylobacter jejuni in ruminants. Epidemiol Infect 138:549-58.

Hauri AM, Just M, McFarland S, Schweigmann A, Schlez K, Krahn J, 2013. Campylobacteriosis outbreaks in the state of Hesse, Germany, 2005-2011: raw milk yet again. Deut Med Wochenschr 138:357-61.

ISO, 2005. General requirements for the competence of testing and calibration laboratories. ISO Norm 17025:2005. International Standardization Organization ed., Geneva, Switzerland.

ISO, 2006. Microbiology of food and animal feeding stuffs. Horizontal method for detection and enumeration of Campylobacter spp. Part 1. Detection method. ISO Norm 10272-1:2006. International Standardization Organization ed., Geneva, Switzerland.

Italian Ministry of Health. 2007. [Intesa Stato Regioni e Province Autonome di Trento e Bolzano e ISR. 25 January 2007]. [Regulation in Italian]. Italian Ministry of Health ed., Rome, Italy.

Kwan PSL, Birtless A, Bolton FJ, French NP, Robinson SE, Newbold LS, Upton M, Fox AJ, 2008. Longitudinal study of the molecular epidemiology of Campylobacter jejuni in cattle on dairy farms. Appl Environ Microb 374:3626-33.

Longenberger AH, Palumbo AJ, Chu AK, Moll ME, Weltman A, Ostroff SM, 2013. Campylobacter jejuni infections associated with unpasteurized milk-multiple States, 2012. Clin Infect Dis 57:263-6.

Merialdi G, Giacometti F, Bardasi L, Stancampiano L, Taddei R, Serratore P, Serraino A, 2015. Fecal shedding of thermophilic Campylobacter in a dairy herd producing raw milk for direct human consumption. J Food Protect 78:579-84.

Meyer-Broseta S, Diot A, Bastian S, Rivière J, Cerf O, 2003. Estimation of low bacterial concentration: Listeria monocytogenes in raw milk. Int J Food Microbiol 80:1-15.

Oliver SP, Boor KJ, Murphy SC, Murinda SE, 2009. Food safety hazards associated with consumption of raw milk. Foodborne Pathog Dis 6:793-806.

Oliver SP, Jayarao BM, Almeida RA, 2005. Foodborne pathogens in milk and dairy farm environment: food safety and public health implications. Foodborne Pathog Dis 2:115-29.

Rapp D, Ross CM, Pleydell EJ, Muirhead RW, 2012. Differences in the fecal concentrations and genetic diversities of Campylobacter jejuni populations among individual cows in two dairy herds. Appl Environ Microb 78:7564-71.

Spencer SE, Marshall J, Pirie R, Campbell D, Baker MG, French NP, 2012. The spatial and temporal determinants of campylobacteriosis notifications in New Zealand, 2001-2007. Epidemiol Infect 140:1663-77.

Stampi S, Varoli O, de Luca G, Zanetti F, 1992. Occurrence, removal and seasonal variation of "thermophilic" campylobacters in a sewage treatment plant in Italy. Zbl Hyg Umweltmed 193:199-210.

Stanley K, Jones K, 2003. Cattle and sheep farms as reservoirs of Campylobacter. J Appl Microbiol 94:104-13.

Stanley KN, Wallace JS, Currie JE, Diggle PJ, Jones K, 1998. The seasonal variation of thermophilic campylobacters in beef cattle, dairy cattle and calves. J Appl Microbiol 85:472-80.

Taylor EV, Herman KM, Ailes EC, Fitzgerald C, Yoder JS, Mahon BE, Tauxe RV, 2013. Common source outbreaks of Campylobacter infection in the USA, 1997-2008. Epidemiol Infect 141:987-96.

WHO, 2013. The global view of campylobacteriosis. Report of an expert consultation. World Health Organization ed., Geneva, Switzerland.

Wysok B, Wiszniewska-Łaszczych A, Uradziński J, Szteyn J, 2011. Prevalence and antimicrobial resistance of Campylobacter in raw milk in the selected areas of Poland. Pol J Vet Sci 14:473-7.