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Prevalence in Bulk Tank Milk and Epidemiology of *Campylobacter jejuni* in Dairy Herds in Northern Italy

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Thermotolerant *Campylobacter* spp. are frequently the cause of human gastroenteritis and have assumed more importance in Italy following the increased consumption of raw milk. Our objectives were to determine the prevalence and genotypes of *Campylobacter* spp. in dairy herds and to investigate the possible sources of bulk milk contamination. Bulk milk from dairy herds ($n = 282$) was cultured for *Campylobacter* spp. and *Enterobacteriaceae*. At three *Campylobacter jejuni*-positive farms, bovine feces, pigeon intestines, milk, and water points were also investigated. Isolates were identified by PCR and genotyped using multilocus sequence typing (MLST). *C. jejuni* was detected in 34 (12%) bulk milk samples. The strains belonged to 14 sequence types, and the most common clonal complexes were CC-21, CC-48, and CC-403. No association was demonstrated between the presence of *C. jejuni* and high levels of *Enterobacteriaceae* in bulk milk. At the three farms examined, *C. jejuni* was isolated from bovine feces (25/82 [30.5%]), pigeon intestines (13/60 [21.7%]), bulk milk (10/24 [41.7%]), and water points (4/16 [25%]). MLST revealed lineages that were common between milk and bovine feces but distinct between cattle and pigeons. In one herd, *C. jejuni* with the same genotype was isolated repeatedly from bulk milk and a cow with an udder infection. Our results showed a high prevalence of *C. jejuni* in bulk milk and suggested that udder excretion, in addition to fecal matter, may be a route of bulk milk contamination. MLST analysis indicated that pigeons are probably not relevant for the transmission of *C. jejuni* to cattle and for milk contamination.

Thermotolerant *Campylobacter* spp. colonize the intestinal tracts of a wide range of mammals and birds, usually without causing clinical disease, and are ubiquitous in the natural environment (1). *C. jejuni* and *C. coli* are the most common causes of bacterial gastroenteritis in humans (2). The European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) reported only about 500 confirmed cases per year in Italy, but this value is likely to be underestimated because notification of campylobacteriosis is based on a voluntary system (3). *Campylobacter* infections mainly originate from consumption of raw or undercooked meat products, especially poultry (3), but unpasteurized milk is also considered a possible vehicle of infection (4, 5). The importance of milk as a source of human *Campylobacter* enteritis was confirmed by the European Union summary report on food-borne disease outbreaks (3).

In Italy, contamination of milk by *C. jejuni* has assumed more importance since 2004, when the sale of raw milk for human consumption by self-service automatic vending machines began and some outbreaks were reported following raw milk consumption: two in the Emilia Romagna Region in 2008 and 2009, one in the Veneto Region and another in the Marche Region (6). In 2011, the Italian Ministry of Health reported a national prevalence of 2.3% for thermotolerant *Campylobacter* spp. in raw milk sampled from automatic vending machines (http://www.salute.gov.it/relazioneAnnuale2011/homeRA2011.jsp). In the Lombardy Region at the time of our investigations, more than 460 automatic vending machines were active, and from 2005 to 2011, official data reported a prevalence of thermotolerant *Campylobacter* spp. of <0.6% in tanks and milk dispensers (http://www.sanita.regione.lombardia.it). However, the results of diagnostic activities performed on milk samples submitted to our provincial unit of the Istituto Zooprofilattico Sperimentale and our personal observations suggest that the official data could underestimate the actual amount of contaminated bulk tank milk.

It is assumed that *Campylobacter* spp. in raw milk derive most commonly from secondary fecal contamination during the milking process (7). Poor pretreatment of the teats with disinfectant or contact of the milking cluster with the parlor floor may result in higher levels of fecal *Campylobacter* contamination (8). A few publications report *Campylobacter* contamination of milk as a result of udder infection (9, 10). We previously described two cases of dairy farms located in the Lodi Province (Lombardy Region, Northern Italy), where the source of contamination of bulk milk was a mammary infection in a single cow (11). Wild birds pecking milk-bottle tops is another reported mechanism by which milk becomes contaminated with *Campylobacter* spp. (12). Wild birds may also play a role in spreading the microorganism within the environment and to livestock (13). In an area close to that considered in our survey, Giacometti et al. (6) associated the presence of pests (mainly birds) in the herd with the detection of *Campylobacter* spp. in milk filters. In Italy, pigeons (*Columba livia*) are widespread and represent a possible transmission vehicle of pathogens to food animals because they live in permanent colonies within the herds. Environmental exposure is a described mechanism by which livestock becomes contaminated with *Campylobacter* spp.

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Humphrey and Beckett (14) demonstrated a relationship between water supply and *C. jejuni* carriage. The transmission of *Campylobacter* spp. among species within the same farm could also be a possibility as poultry, sheep, swine, and pets are known to harbor *Campylobacter* spp. in their digestive tracts (15).

Our work was aimed at establishing the prevalence of thermotolerant *Campylobacter* spp. in bulk tank milk of dairy herds in the Lodi Province. Furthermore, in some *C. jejuni*-positive farms, we investigated the presence of the microorganism in different sources and their role in milk contamination.

MATERIALS AND METHODS

Sampling. (i) Bulk milk sampling. Milk samples were collected between September 2010 and February 2012 from the bulk milk tanks of 282 farms, representing 80% of the 351 dairy herds of Lodi Province. In this area, the average herd size and milk production were 150 milking cows and 9,000 kg per year. Local authorities submitted the milk as a part of their routine monitoring programs. The samples were chilled and transported to the laboratory, and bacteriological analyses began within 24 h of milk collection.

(ii) Sampling in *C. jejuni*-positive farms. To investigate the potential source of *C. jejuni* contamination of the bulk milk, three farms (indicated as farms A, B, and C) were arbitrarily selected for further analysis among the herds that were determined to be positive during bulk milk sampling. The three farms rear Italian Friesian cattle, and farm C has a herd with mixed production (dairy cattle and fattening pigs). Farms A, B, and C had 165, 120, and 50 milking cows with average milk productions of 7,000, 10,000, and 7,600 kg per year per cow, respectively. At all of the farms, several dozen pigeons were present, and these pigeons had free access to farm buildings, feeders, and water points.

A total of 194 samples were analyzed, including bovine feces, pigeon intestines, bulk milk, water points, and the feces of other animals. A minimum of 17 cattle per herd were randomly selected with a systematic sampling, and their feces ($n = 82$) were obtained by direct rectal retrieval. The minimum sample size of 17 was calculated by using Win Episcope 2.0 based on 95% confidence and 15% expected prevalence (16, 17; M. Luini, unpublished data) to detect at least one excreting cow, accounting for a total population of 165 heads (largest herd). When possible, the sampling fraction was increased (up to 35) in order to better estimate the prevalence. Pigeon intestines were collected during postmortem examination of 20 birds per herd provided by licensed shooters according to Law 26/1993 of the Lombardy Region. Bulk milk was repeatedly tested ($n = 24$), collecting four samples each from farms B and C. At farm A, bulk milk samples were collected on several occasions ($n = 16$) and, in order to identify potentially infected cows, the milk of the total number of cows ($n = 165$) was collected in 15 pools, and then the milk of individual cows and finally the milk of single quarters were consecutively taken. Water samples ($n = 16$) were taken from the available water points present in each farm (ranging from 2 to 10). Dog fecal material from farm A ($n = 4$) and pooled swine feces from farm C ($n = 8$) were also analyzed. At farm A, cattle feces, pigeons, and bulk milk samples were collected about once per month between January 2010 and July 2010. For herd B, bulk milk and feces sampling took place from March to August 2011, and pigeon sampling took place in October 2011. For herd C, bulk milk and fecal samples were collected from February to July 2012 and from pigeons in July 2012. Samples were transported chilled to the laboratory and immediately processed for *Campylobacter* culture.

Microbiological analysis. (i) *Campylobacter* culture. Samples were put into Bolton selective enrichment broth (Oxoid, Ltd., Basingstoke, United Kingdom) at a ratio of 1:10, followed by incubation at 42°C in an microaerophilic atmosphere (GENbox; bioMérieux, Marcy l'Etoile, France). After 48 h of incubation, a 100-μl aliquot of the enrichment broth was streaked onto modified charcoal cefoperazone deoxycholate (mCCD) agar and Skirrow selective medium (Oxoid) after filtration through a 0.45-μm-pore-size membrane (GN-6 Metricel membrane disc filters; Pall Life Sciences, Ann Arbor, MI). The plates were incubated at 42°C for 48 h under the same conditions, and colonies with typical morphology identified as oxidase-positive, catalase-producing, curved Gram-negative rods were reported as *Campylobacter* species.

(ii) Indicator of milk hygiene. In order to investigate bulk milk hygiene quality, samples were analyzed for *Enterobacteriaceae* contamination. Decimal dilution series of milk samples were prepared in sterile 0.1% peptone water and inoculated on Violet Red Bile Glucose (VRBG) agar (Oxoid). The plates were incubated at 37°C for 24 h, the number of typical colonies was determined, and the CFU per ml were calculated as the weighted average of two serial dilutions. *Enterobacteriaceae* counts were subdivided into five classes, and the percentage of *C. jejuni*-positive samples was calculated for each group. In addition, the *Enterobacteriaceae* counts in *C. jejuni*-positive and -negative samples were compared.

Template preparation and PCR. Colonies presumptive for *Campylobacter* spp. were subjected to DNA extraction using a DNeasy tissue kit (QIAgen, Dusseldorf, Germany). Species identification was performed using the PCR method described by Persson and Olsen (18) with the following modification: only primers targeting the *hipO* and *asp* genes of *C. jejuni* and *C. coli* were used in the duplex. To identify other *Campylobacter* spp., a second, *Campylobacter*-specific PCR assay (19) was carried out on colonies suspected of being negative for *C. jejuni* and *C. coli*.

Genotyping. *C. jejuni* strains were genotyped by multilocus sequence typing (MLST) as described by Dingle et al. (20). Sequence types (STs) and clonal complexes (CCs) were assigned using the *C. jejuni* PubMLST database (http://pubmlst.org/campylobacter/). MLST was performed on a part of the isolates because some of them were lost during subculturing or freeze storage.

Statistical analysis. Statistical analysis was carried out using GraphPad Prism v5.0 (GraphPad Software, Inc., La Jolla, CA). Differences in the levels of *Enterobacteriaceae* contamination in *C. jejuni*-positive and -negative samples were compared by using an unpaired *t* test. A *P* value of <0.05 was considered statistically significant.

TABLE 1 Genotyping of 28 *C. jejuni* isolates from bulk tank milk by MLST[a]

CC ($n = 10$)	ST ($n = 14$)
CC-21 (9)	ST-21 (6); ST-19 (2); ST-883 (1)
CC-48 (5)	ST-38 (5)
CC-403 (4)	ST-933 (4)
CC-42 (2)	ST-42 (1); ST-604 (1)
CC-61 (2)	ST-61 (2)
CC-206 (2)	ST-122 (1); ST-572 (1)
CC-22 (1)	ST-22 (1)
CC-45 (1)	ST-45 (1)
CC-658 (1)	ST-658 (1)
CC UA (1)	ST-441 (1)

[a] Numbers in parentheses represent the numbers of isolates. UA, unassigned to any CC.

RESULTS

Bulk milk samples. Thermotolerant *Campylobacter* spp. were isolated from 34 of the 282 bulk tank milk samples (12%; 95% confidence interval = 8.3 to 15.9%). All of the isolates were identified as *C. jejuni* by specific PCR. The results of MLST, available for 28 of the 34 isolates, are summarized in Table 1. A total of 14 different STs were identified, and ST-21 ($n = 6$), ST-38 ($n = 5$), and ST-933 ($n = 4$) were the most frequently observed. Except for one isolate that could not be assigned to any CC, the others belonged to 9 CCs. The three most common clonal complexes were CC-21 ($n = 9$), CC-48 ($n = 5$), and CC-403 ($n = 4$), followed by CC-42, CC-61, and CC-206 ($n = 2$). CC-22, CC-45, and CC-658 were each represented by only one strain.

TABLE 2 Distribution of *C. jejuni*-positive samples per *Enterobacteriaceae* count performed on bulk milk

Count (CFU/ml)	No. of samples	No. (%) of positive samples
<10	32	4 (12.5)
$10–10^2$	63	7 (11.1)
$10^2–10^3$	107	15 (14.0)
$10^3–10^4$	44	5 (11.4)
$>10^4$	24	2 (8.3)
ND[a]	12	1 (8.3)
Total	282	34 (12.0)

[a] ND, not determined.

Indicator of milk hygiene. *Enterobacteriaceae* counts in bulk tank milk samples were extremely variable, with values ranging from <10 to $>10^4$ CFU/ml. Within the five classes of *Enterobacteriaceae* contamination, the rate of *C. jejuni* isolation ranged from 8.3 to 14.0% (Table 2). The possible association between the contamination of milk by *C. jejuni* and a high count of *Enterobacteriaceae* was also investigated, but the increase of *Enterobacteriaceae* did not correspond to a higher number of *C. jejuni*-positive samples. No significant difference could be demonstrated between counts in positive samples and those in negative ones ($P = 0.968$, unpaired *t* test) as highlighted in the box plot (Fig. 1).

***C. jejuni*-positive farms.** Within the three farms selected, we investigated the presence of *C. jejuni* in different sources. A total of 52 strains were isolated from bovine feces (25/82 [30.5%]), pigeon intestines (13/60 [21.7%], milk (10/24 [41.7%]), and water points (4/16 [25%]). *C. jejuni* was not isolated from feces of other animals existing in the herd (0/8). None of the animals (cows and pigeons) that tested positive for *C. jejuni* showed any obvious external symptoms of disease. Of the 52 *C. jejuni* isolates, 44 were characterized by MLST. An overview of the typing results is shown in Table 3.

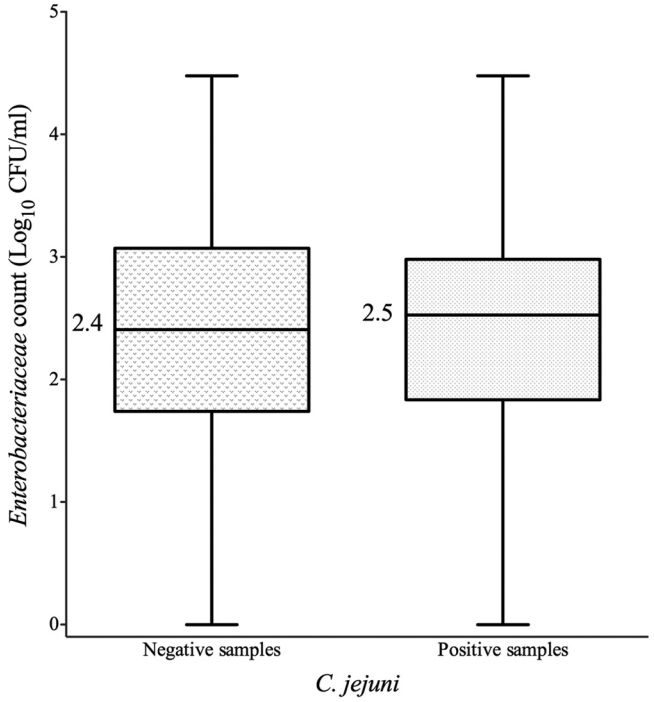

FIG 1 Box plot of the *Enterobacteriaceae* count in *C. jejuni*-positive and -negative milk samples.

TABLE 3 Frequency of *C. jejuni* isolation and distribution of clonal complexes among farms and sources

Location and source	No. of samples	No. of isolates	No. of typed strains	Genotype(s)[a]
Farm A				
Bovine feces	35	10	8	ST-19 CC-21 (5); ST-21 CC-21 (1); ST-38 CC-48 (1); ST-61 CC-61 (1)
Pigeon intestine	20	3	2	ST-45 CC-45 (1); ST-2209 CC-179 (1)
Bulk tank milk	16	5	4	ST-19 CC-21 (2); ST-38 CC-48 (2)
Water point	10	4	2	ST-21 CC-21 (1); ST-4447 CC-179 (1)
Other animals[b]	4	0		
Farm B				
Bovine feces	17	1	1	ST-572 CC-206 (1)
Pigeon intestine	20	3	3	ST-220 CC-179 (1); ST-4447 CC-179 (2)
Bulk tank milk	4	4	4	ST-21 CC-21 (4)
Water point	4	0		
Other animals	0			
Farm C				
Bovine feces	30	14	12	ST-19 CC-21 (11); ST-42 CC-42 (1)
Pigeon intestine	20	7	7	ST-220 CC-179 (3); ST-2209 CC-179 (4)
Bulk tank milk	4	1	1	ST-933 CC-403 (1)
Water point	2	0		
Other animals[c]	8	0		

[a] The numbers in parentheses represent the numbers of strains belonging to the reported genotype.
[b] Dog.
[c] Swine.

(i) Farm A. From the farm A herd, 35 milking cows were sampled, 10 of which (28.6%) shed *C. jejuni* in their feces. MLST performed on eight strains showed the following STs: ST-19 ($n = 5$), ST-21 ($n = 1$), ST-38 ($n = 1$), and ST-61 ($n = 1$). ST-19 and ST-21 were included in the same CC (CC-21), whereas the remaining two STs belonged to CC-48 and CC-61, respectively. Three *C. jejuni* strains were isolated from the intestinal tracts of pigeons (15%). Genotyping the strains indicated the presence of ST-45 and ST-2209, a member of CC-179.

C. jejuni was detected repeatedly in bulk tank milk ($n = 5$, 31.3%), and the profile of the strains initially isolated was ST-38 (CC-48). In order to verify whether udder infection was responsible for the persistent bulk milk contamination, serial sampling was used. First, the cattle were subdivided into 11 pools of 15 cows, and only one pool showed contamination by *C. jejuni*. The milk of individual cows from this positive group was subsequently analyzed, and only one positive cow was identified. The milk of single quarters of this cow was then cultured twice at intervals of 10 days, revealing that the same quarter was repeatedly positive for *C. jejuni*, without evident clinical signs of mastitis. The MLST profiling

of the strains isolated from bulk tank milk and the infected quarter was the same, and the segregation of the infected cow resulted in an undetectable level of the microorganism in bulk milk. In the subsequent 11 samplings, bulk tank milk tested positive occasionally ($n = 2$), but in these cases genotyping of the strains revealed a different MLST profile: ST-19 (CC-21).

Four *C. jejuni* strains were isolated from water samples (40%). MLST was applied to two isolates: one was identified as ST-21 (CC-21), and the other was identified as ST-4447 (CC-179). The feces of dogs circulating at this farm ($n = 4$) were always negative for *Campylobacter* species.

(ii) Farm B. Among the 17 milking cattle sampled at farm B, only one excreted *C. jejuni* in the feces (5.9%), and the genotype of the strain was ST-572 (CC-206). Isolates from pigeons ($n = 3$, 15%) were genotyped as ST-220 (CC-179) ($n = 1$) and ST-4447 (CC-179) ($n = 2$). Bulk tank milk was tested repeatedly ($n = 4$) over 6 months, and results were always positive (100%) for *C. jejuni* belonging to ST-21 (CC-21). No water points were contaminated by *Campylobacter* species.

(iii) Farm C. A total of 14 *C. jejuni* strains (46.7%) and 1 *C. hyointestinalis* subsp. *hyointestinalis* (3.3%) strain were isolated from the bovine feces tested ($n = 30$). All but one strain of *C. jejuni* genotyped belonged to ST-21 (CC-21; $n = 11$), the remaining isolate was ST-42 (CC-42). Moreover, PCR performed directly on Bolton enrichment broth revealed that in two cases there was a mixed colonization of *C. jejuni* and *C. coli*, but *C. coli* was not isolated. At this farm a high prevalence of *C. jejuni* in pigeons ($n = 7$, 35%) was observed. Isolates were distributed between ST-220 (CC-179) ($n = 3$) and ST-2209 (CC-179) ($n = 4$). Bulk tank milk was contaminated by *C. jejuni* (ST-933 CC-403) in only one sample. No *Campylobacter* spp. was isolated from water points. Since this farm had mixed production, we collected eight pooled feces of swine reared near cattle stables. Species identification by PCR carried out on enrichment broth detected *C. coli* in five samples and the concurrent presence of *C. jejuni* and *C. coli* in one case. However, despite the fact that the isolation was attempted in all of the PCR-positive samples, no isolate for either species was obtained.

DISCUSSION

In our study we considered 282 dairy herds, representative of a high-density dairy farming area of northern Italy, and *C. jejuni* was detected in 12% of the examined bulk tank milk samples. This prevalence is higher than those reported in 5 of 7 studies conducted in other countries cited by Oliver et al. (7), where the isolation rates ranged from 0.4% to 1.5%. Stanley and Jones (21) described an incidence between 3.8 and 8.1% in the United Kingdom and Yang et al. (22) recovered *C. jejuni* from 27.3% of the bulk tank milk in China. The prevalence reported here is in line with a study conducted in an area close to that considered in our survey, in which *C. jejuni* was detected in 14.8% (4/27) of the bulk milk filters from farms authorized to produce and sell raw milk (6). However, the isolation rate observed here was higher than that reported in the same period during official controls of raw milk at vending machines in Lombardy (<0.6%) (http://www.sanita.regione.lombardia.it). This could be mainly due to transport conditions and the time elapsed in official controls between sampling and culturing (samples are stored at 4°C for at least 24 to 48 h), which could significantly reduce the viability of *C. jejuni* (23; M. Luini, unpublished data). The lower prevalence found during official controls performed in Lombardy could also be attributed to the fact that they are restricted only to farms licensed to produce and sell raw milk, which must implement higher standards of hygiene practices according to regional regulations. In contrast, the present study involved farms producing milk for different dairy products (e.g., raw or pasteurized milk, soft or hard cheeses).

MLST of 28 *C. jejuni* isolates from bulk tank milk identified 14 different STs, grouped into 9 CCs. CC-21, CC-48, and CC-403 were the three most common CCs found. To the best of our knowledge, this is the first time that MLST has been applied to characterize *C. jejuni* isolated from milk in Italy and, generally, there are few published data available concerning MLST genotyping of bovine milk isolates. In Canada, Lévesque et al. (24) reported that CC-21, CC-45, and CC-61 were overrepresented in isolates from milk and that CC-61 was also associated with milk-borne infection in humans (25). Some CCs recovered in the present study from milk samples (CC-21, CC-42, CC-48, and CC-61) were commonly described among *C. jejuni* isolates from bovine feces (18, 26).

At all three *C. jejuni*-positive farms, the microorganism was isolated from fecal samples of milking cows, with in-herd prevalences of carrier animals of 28.6, 5.9, and 46.7% at farms A, B, and C, respectively (overall prevalence, 30.5%). These data agree with the contamination rates found in other European countries and in Italy: 12.3% in Luxembourg (27), 67.1% in Northern Spain (28), and 53.9 and 38.6% in Italy (16, 17). Up to four different genotypes were found at each farm. The most prevalent MLST profile was ST-19 (CC-21; $n = 16$), and each of the five remaining STs (ST-21, ST-38, ST-42, ST-61, and ST-572) was represented by one isolate. All of the clonal lineages have already been described as associated with cattle, in particular from Europe (26, 29, 30; A. Parisi, unpublished data).

Among the milk and cattle feces isolates, five of the CCs detected more frequently in human disease in Europe were recovered: CC-21, CC-45, CC-48, CC-61, and CC-206 (2, 20, 31). This emphasizes the role of cattle as a major source of food contamination and human infection.

Since feces are considered the primary source of milk contamination during or after the milking process (7, 8), a relationship between the presence of *C. jejuni* in bulk milk and a high load of *Enterobacteriaceae* was expected. However, the *Enterobacteriaceae* counts of the samples from the positive farms did not differ significantly from those from negative herds, indicating that fecal contamination could not be the only mechanism responsible for the presence of the pathogen in milk. An alternative method of milk contamination is udder infection, and in this case the microorganism can be directly excreted into the milk (9, 10, 11). This was demonstrated at farm A, where, at weekly intervals, the bulk milk was repeatedly positive for *C. jejuni*, and a strain with the same genotype (ST-38 CC-48) was isolated from a single quarter of a cow, without evident clinical signs of mastitis. The segregation of the infected animal resulted in an undetectable level of *C. jejuni* in bulk milk, confirming that udder infection was responsible for the milk contamination. A similar situation was suspected at farm B, where *C. jejuni* with the same genotype (ST-21) was repeatedly isolated from bulk tank milk, but not from any examined cow feces. Unfortunately, at this farm it was not possible to perform further investigations on the suspected presence of a *C. jejuni* udder infection.

Our data suggest that the contribution of chronic udder infections to bulk milk contamination is probably underestimated and

should be suspected especially when *C. jejuni* is demonstrated repeatedly. The presence of *C. jejuni* in milk can also be due to direct contact with contaminated sources in the dairy farm environment and some studies identified wild birds as a possible reservoir for *Campylobacter* transmission to livestock (6, 13).

The prevalence of contaminated pigeons in the three farms considered here ranged from 15 to 35% and overall was 21.7%. Few data exist on the occurrence of *Campylobacter* colonization in pigeons and none regarding Italy specifically. Ogden et al. (32) and Hughes et al. (33) reported prevalencec of 27.8% in Scotland and 4.3% in Northern England.

Pigeons showed a very low MLST diversity, since 11 of 12 pigeon isolates were typed as ST-220, ST-2209, and ST-4447, all grouped in the CC-179. The strains of this CC were already found in bathing beach sand and in wild birds (20, 32). In particular, ST-220 and ST-2209, detected in our study four and five times, respectively, have been associated with pigeons (32). Only one strain belonged to a different MLST profile: ST-45 (CC-45), a genotype distributed widely in terms of hosts and ecologic niche (34) and already described in wild birds (23, 33).

Data presented here are consistent with the view that certain CCs tend to be preferentially associated with particular hosts (20): CC-21 appeared to be overrepresented in isolates from cattle, in both milk and feces, whereas CC-179 was predominant among pigeons. This led us to suppose that these birds are not a significant source of *C. jejuni* for cattle, and they are probably not responsible for milk contamination. However, since ST-45 (CC-45) was found in pigeons, a strain also found in one bulk milk sample and frequently associated with human disease (2), it has to be considered that these birds may play a role in cattle and human epidemiology.

C. jejuni was only isolated from water points at farm A, recovering genotypes shared by cattle and pigeons: ST-21 (CC-21) and ST-4447 (CC-179), respectively. This suggests that water points could be contaminated by fecal material of cattle and pigeons and could represent a possible source of infection in cows.

No *Campylobacter* spp. were isolated from the feces of the other animals tested. However, PCR performed directly on the enrichment broth of swine feces revealed the presence of *C. coli*. This finding was expected since *C. coli* is the most common *Campylobacter* species recovered from swine (15).

Except with farm A, where both fecal contamination and udder infection can be considered responsible for bulk milk contamination, it was not possible to clearly identify the source of bulk milk contamination in the two other farms examined (farms B and C), since genotypes of *C. jejuni* isolated from bulk milk were different from those isolated from bovine feces or pigeon intestines. The lack of identification of the source of bulk milk contamination is likely attributable to the low numbers of isolates that were genotyped at these farms or to the presence of another source of contamination (e.g., wild animals) that was not investigated in the present study.

Conclusion. Our survey indicated that a high number of bulk tank milk samples obtained from an intensive dairy area of northern Italy was contaminated by *C. jejuni*, and the most common STs found were grouped in CC-21, CC-48, and CC-403. In *C. jejuni*-positive farms, fecal excretion was common among milking cows, and CC-21 and CC-48 were also very common. These data suggest that bovine feces could be responsible for the presence of the pathogen in milk. However, the current work underlines the need to also consider udder infections as a possible source of *C. jejuni*, especially when bulk milk contamination occurs persistently. On the other hand, it was concluded that pigeons probably do not play a significant role in the spread of *C. jejuni* among cattle. Finally, our results highlight the importance of cattle and/or dairy products, especially raw milk, as a potential source of human *Campylobacter* gastroenteritis.

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REFERENCES

1. **Humphrey T, O'Brien S, Madsen M.** 2007. Campylobacters as zoonotic pathogens: a food production perspective. Int. J. Food Microbiol. **117:** 237–257. http://dx.doi.org/10.1016/j.ijfoodmicro.2007.01.006.
2. **Sopwith W, Birtles A, Matthews M, Fox A, Gee S, Painter M, Regan M, Syed Q, Bolton E.** 2006. *Campylobacter jejuni* multilocus sequence types in humans, Northwest England, 2003-2004. Emerg. Infect. Dis. **12:**1500–1507. http://dx.doi.org/10.3201/eid1210.060048.
3. **European Food Safety Authority.** 2013. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2011. EFSA J. **11:**3129. http://dx.doi.org/10.2903/j.efsa.2013.3129.
4. **Schildt M, Savolainen S, Hänninen ML.** 2006. Long-lasting *Campylobacter jejuni* contamination of milk associated with gastrointestinal illness in a farming family. Epidemiol. Infect. **134:**401–405. http://dx.doi.org/10.1017/S0950268805005029.
5. **Heuvelink AE, van Heerwaarden C, Zwartkruis-Nahuis A, Tilburg JJ, Bos MH, Heilmann FG, Hofhuis A, Hoekstra T, de Boer E.** 2009. Two outbreaks of campylobacteriosis associated with the consumption of raw cows' milk. Int. J. Food Microbiol. **34:**70–74. http://dx.doi.org/10.1016/j.ijfoodmicro.2008.12.026.
6. **Giacometti F, Serraino A, Finazzi G, Daminelli P, Losio MN, Bonilauri P, Arrigoni N, Garigliani A, Mattioli R, Alonso S, Piva S, Florio D, Riu R, Zanoni RG.** 2012. Foodborne pathogens in in-line milk filters and associated on-farm risk factors in dairy farms authorized to produce and sell raw milk in Northern Italy. J. Food Prot. **75:**1263–1269. http://dx.doi.org/10.4315/0362-028X.JFP-12-028.
7. **Oliver SP, Jayarao BM, Almeida RA.** 2005. Foodborne pathogens in milk and the dairy farm environment: food safety and public health implications. Foodborne Pathog. Dis. **2:**115–129. http://dx.doi.org/10.1089/fpd.2005.2.115.
8. **Beumer RR, Cruysen JJ, Birtantie IR.** 1988. The occurrence of *Campylobacter jejuni* in raw cows' milk. J. Appl. Bacteriol. **65:**93–96. http://dx.doi.org/10.1111/j.1365-2672.1988.tb01496.x.
9. **Hutchinson DN, Bolton FJ, Hinchliffe PM, Dawkins HC, Horsley SD, Jessop EG, Robertshaw PA, Counter DE.** 1985. Evidence of udder excretion of *Campylobacter jejuni* as the cause of milk-borne campylobacter outbreak. J. Hyg. (Lond.) **94:**205–215. http://dx.doi.org/10.1017/S0022172400061416.
10. **Gudmundson J, Chirino-Trejo JM.** 1993. A case of bovine mastitis caused by *Campylobacter jejuni*. Zentralbl. Veterinarmed. B **40:**326–328.
11. **Luini M, Benedetti V, Piccinini R, Vezzoli F.** 2009. Casi di infezione mammaria da *Campylobacter jejuni* nel bovino. Large Animal Rev. **15:**51–54.
12. **Neal KR, Slack RC.** 1997. Diabetes mellitus, anti-secretory drugs and other risk factors for campylobacter gastro-enteritis in adults: a case-control study. Epidemiol. Infect. **119:**307–311. http://dx.doi.org/10.1017/S0950268897008224.
13. **Sippy R, Sandoval-Green CM, Sahin O, Plummer P, Fairbanks WS, Zhang Q, Blanchong JA.** 2012. Occurrence and molecular analysis of *Campylobacter* in wildlife on livestock farms. Vet. Microbiol. **157:**369–375. http://dx.doi.org/10.1016/j.vetmic.2011.12.026.
14. **Humphrey TJ, Beckett P.** 1987. *Campylobacter jejuni* in dairy cows and raw milk. Epidemiol. Infect. **98:**263–269. http://dx.doi.org/10.1017/S0950268800062014.
15. **Horrocks SM, Anderson RC, Nisbet DJ, Ricke SC.** 2009. Incidence and ecology of *Campylobacter jejuni* and *coli* in animals. Anaerobe **15:**18–25. http://dx.doi.org/10.1016/j.anaerobe.2008.09.001.

16. **Pezzotti G, Serafin A, Luzzi I, Mioni R, Milan M, Perin R.** 2002. Occurrence and resistance to antibiotics of *Campylobacter jejuni* and *Campylobacter coli* in animals and meat in North-Eastern Italy. Int. J. Food Microbiol. **82:**281–287.
17. **Parisi A, Lanzilotta SG, Addante N, Normanno G, Di Modugno G, D'Ambrosio A, Montagna CO.** 2007. Prevalence, molecular characterization and antimicrobial resistance of thermophilic campylobacter isolates from cattle, hens, broilers and broiler meat in south-eastern Italy. Vet. Res. Commun. **31:**113–123. http://dx.doi.org/10.1007/s11259-006-3404-3.
18. **Persson S, Olsen KE.** 2005. Multiplex PCR for identification of *Campylobacter coli* and *Campylobacter jejuni* from pure cultures and directly on stool samples. J. Med. Microbiol. **54:**1043–1047. http://dx.doi.org/10.1099/jmm.0.46203-0.
19. **Yamazaki-Matsune W, Taguchi M, Seto K, Kawahara R, Kawatsu K, Kumeda Y, Kitazato M, Nukina M, Misawa N, Tsukamoto T.** 2007. Development of a multiplex PCR assay for identification of *Campylobacter coli*, *Campylobacter fetus*, *Campylobacter hyointestinalis* subsp. *hyointestinalis*, *Campylobacter jejuni*, *Campylobacter lari* and *Campylobacter upsaliensis*. J. Med. Microbiol. **56:**1467–1473. http://dx.doi.org/10.1099/jmm.0.47363-0.
20. **Dingle KE, Colles FM, Ure R, Wagenaar JA, Duim B, Bolton FJ, Fox AJ, Wareing DR, Maiden MC.** 2002. Molecular characterization of *Campylobacter jejuni* clones: a basis for epidemiologic investigation. Emerg. Infect. Dis. **8:**949–955. http://dx.doi.org/10.3201/eid0809.020122.
21. **Stanley K, Jones K.** 2003. Cattle and sheep farms as reservoirs of *Campylobacter*. J. Appl. Microbiol. **94:**104S–113S. http://dx.doi.org/10.1046/j.1365-2672.94.s1.12.x.
22. **Yang C, Jiang Y, Huang K, Zhu C, Yin Y.** 2003. Application of real-time PCR for quantitative detection of *Campylobacter jejuni* in poultry, milk and environmental water. FEMS Immunol. Med. Microbiol. **38:**265–271. http://dx.doi.org/10.1016/S0928-8244(03)00168-8.
23. **Doyle MP, Roman DJ.** 1982. Prevalence and survival of *Campylobacter jejuni* in unpasteurized milk. Appl. Environ. Microbiol. **44:**1154–1158.
24. **Lévesque S, Frost E, Arbeit RD, Michaud S.** 2008. Multilocus sequence typing of *Campylobacter jejuni* isolates from humans, chickens, raw milk, and environmental water in Quebec, Canada. J. Clin. Microbiol. **46:**3404–3411. http://dx.doi.org/10.1128/JCM.00042-08.
25. **French N, Barrigas M, Brown P, Ribiero P, Williams N, Leatherbarrow H, Birtles R, Bolton E, Fearnhead P, Fox A.** 2005. Spatial epidemiology and natural population structure of *Campylobacter jejuni* colonizing a farmland ecosystem. Environ. Microbiol. **7:**1116–1126. http://dx.doi.org/10.1111/j.1462-2920.2005.00782.x.
26. **Kwan PS, Birtles 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. Microbiol. **74:**3626–3633. http://dx.doi.org/10.1128/AEM.01669-07.
27. **Ragimbeau C, Schneider F, Losch S, Even J, Mossong J.** 2008. Multilocus sequence typing, pulsed-field gel electrophoresis, and *fla* short variable region typing of clonal complexes of *Campylobacter jejuni* strains of human, bovine, and poultry origins in Luxembourg. Appl. Environ. Microbiol. **74:**7715–7722. http://dx.doi.org/10.1128/AEM.00865-08.
28. **Oporto B, Esteban JI, Aduriz G, Juste RA, Hurtado A.** 2006. Prevalence and strain diversity of thermophilic campylobacters in cattle, sheep and swine farms. J. Appl. Microbiol. **103:**977–984. http://dx.doi.org/10.1111/j.1365-2672.2007.03328.x.
29. **Colles FM, Jones K, Harding RM, Maiden MC.** 2003. Genetic diversity of *Campylobacter jejuni* isolates from farm animals and the farm environment. Appl. Environ. Microbiol. **69:**7409–7413. http://dx.doi.org/10.1128/AEM.69.12.7409-7413.2003.
30. **Manning G, Dowson CG, Bagnall MC, Ahmed IH, West M, Newell DG.** 2003. Multilocus sequence typing for comparison of veterinary and human isolates of *Campylobacter jejuni*. Appl. Environ. Microbiol. **69:**6370–6379. http://dx.doi.org/10.1128/AEM.69.11.6370-6379.2003.
31. **Cody AJ, McCarthy NM, Wimalarathna HL, Colles FM, Clark L, Bowler IC, Maiden MC, Dingle KE.** 2012. A longitudinal 6-year study of the molecular epidemiology of clinical campylobacter isolates in Oxfordshire, United Kingdom. J. Clin. Microbiol. **50:**3193–3201. http://dx.doi.org/10.1128/JCM.01086-12.
32. **Ogden ID, Dallas JF, MacRae M, Rotariu O, Reay KW, Leitch M, Thomson AP, Sheppard SK, Maiden M, Forbes KJ, Strachan NJ.** 2009. *Campylobacter* excreted into the environment by animal sources: prevalence, concentration shed, and host association. Foodborne Pathog. Dis. **6:**1161–1170. http://dx.doi.org/10.1089/fpd.2009.0327.
33. **Hughes LA, Bennett M, Coffey P, Elliott J, Jones TR, Jones RC, Lahuerta-Marin A, Leatherbarrow AH, McNiffe K, Norman D, Williams NJ, Chantrey J.** 2009. Molecular epidemiology and characterization of *Campylobacter* spp. isolated from wild bird populations in northern England. Appl. Environ. Microbiol. **75:**3007–3015. http://dx.doi.org/10.1128/AEM.02458-08.
34. **Sopwith W, Birtles A, Matthews M, Fox A, Gee S, Painter M, Regan M, Syed Q, Bolton E.** 2008. Identification of potential environmentally adapted *Campylobacter jejuni* strain, United Kingdom. Emerg. Infect. Dis. **14:**1769–1773. http://dx.doi.org/10.3201/eid1411.071678.