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# Prevalence, antimicrobial resistance, and molecular characterization of *Campylobacter* spp. in bulk tank milk and milk filters from US dairies

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## ABSTRACT

*Campylobacter* spp. are frequently isolated from dairy cows as commensal organisms. Sporadic *Campylobacter* infections in humans in the United States are generally attributed to poultry, but outbreaks are also commonly associated with dairy products, particularly unpasteurized or raw milk. Bulk tank milk samples and milk filters from US dairy operations were collected during the National Animal Health Monitoring System Dairy 2014 study and analyzed using real-time PCR and traditional culture techniques for the presence of thermophilic *Campylobacter* species. The weighted prevalence of operations from which we detected *Campylobacter* spp. in either bulk tank milk or milk filters was 24.9%. We detected *Campylobacter* spp. in a higher percentage of operations with 100–499 cows (42.8%) and 500 or more cows (47.5%) than in operations with 30–99 cows (6.5%). *Campylobacter* spp. were also more frequently detected in operations in the west than the east (45.9 and 22.6%, respectively). We isolated *Campylobacter* spp. from approximately half of PCR-positive samples, representing 12.5% (weighted prevalence) of operations. The majority (91.8%) of isolates were *C. jejuni*, but *C. lari* and *C. coli* were also isolated. We detected resistance to tetracycline in 68.4% of *C. jejuni* isolates, and resistance to ciprofloxacin and nalidixic acid in 13.2% of *C. jejuni* isolates. Based on pulsed-field gel electrophoresis, we found that dairy-associated *C. jejuni* were genotypically diverse, although clonal strains were isolated from different geographic regions. These results suggest that bulk tank milk can be contaminated with pathogenic *Campylobacter* spp., and that the consumption of unpasteurized or raw milk presents a potential human health risk.

**Key words:** *Campylobacter*, bulk tank milk, milk filter

## INTRODUCTION

*Campylobacter* spp. are microaerophilic, spiral-shaped, gram-negative bacteria that are a leading cause of bacterial diarrhea in humans in the United States, accounting for an estimated 1.3 million cases and 120 deaths annually (CDC, 2016). Campylobacteriosis is usually self-limiting, with symptoms such as mild diarrhea and cramping; however, severe cases may require antimicrobial treatment. Post-infection sequelae such as Guillain-Barré syndrome may cause lifelong health problems. According to preliminary 2015 data published by the Centers for Disease Control and Prevention (**CDC**), *Campylobacter* spp. infections in humans increased by 9% from 2006 to 2015 (CDC, 2016). The most common cause of campylobacteriosis in humans is *Campylobacter jejuni*, but other thermophilic species such as *Campylobacter coli* and *Campylobacter lari* have also been known to cause disease (Taylor et al., 2013; CDC, 2016).

Although sporadic *Campylobacter* spp. infections are most commonly associated with poultry, outbreaks have also been attributed to the consumption of other foods, particularly unpasteurized or raw milk. Based on a review of CDC data from 1997 to 2008, 28% of foodborne *Campylobacter* outbreaks (defined as 2 or more affected people) were due to the consumption of contaminated raw milk products, compared with 11% due to the consumption of poultry (Taylor et al., 2013). Langer et al. (2012) and Mungai et al. (2015) analyzed milk-related outbreak data from 1993 to 2006 and 2007 to 2012, respectively, and found that the majority of outbreaks associated with unpasteurized milk were due to *Campylobacter* contamination. Furthermore, in a recent analysis of *Campylobacter* spp. isolated from human and animal sources, Tyson et al. (2016) discovered that isolates from human clinical samples more frequently matched isolates from dairy cattle (65.0%) than isolates from poultry sources (49.5%). Therefore,

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dairy cattle and dairy products, particularly unpasteurized milk, should be considered an important source of *Campylobacter* spp. infection.

Dairy cows are known reservoirs of *Campylobacter* spp., and several surveys have determined the prevalence of *Campylobacter* spp. on dairy farms. According to National Animal Health Monitoring System (**NAHMS**) studies in 1996, 2002, and 2007, *Campylobacter* spp., primarily *C. jejuni*, were detected in the feces of cows on 90–100% of US dairy operations (USDA-APHIS, 2011). The within-herd prevalence of most operations was greater than 10%. Dairy cows have been described as intermittent shedders; however, cows may also shed *C. jejuni* consistently or sporadically in high concentrations (Häkkinen and Hänninen, 2009; Rapp et al., 2012). *Campylobacter* spp. have also been detected in bulk tank milk (**BTM**; Halbert et al., 2006). Although *Campylobacter* spp. have occasionally been implicated in cases of mastitis (Morgan et al., 1985; Orr et al., 1995; Bianchini et al., 2014) and can be excreted in the milk of infected cows, this is an uncommon occurrence, and the presence of *Campylobacter* spp. in BTM is usually due to fecal contamination (Oliver et al., 2005). Therefore, even with good animal hygiene and strict milking protocols, some level of contamination in the bulk tank by asymptomatic animals is inevitable.

Pasteurization is a highly effective tool for managing BTM contamination; however, raw milk can be legally distributed in some manner in more than half of US states (AAP, 2014; NCSL, 2016). Although dairy animals are common carriers of *Campylobacter* spp. and fecal shedding can lead to BTM contamination, no national surveys have been conducted on the prevalence of *Campylobacter* spp. in BTM in the United States. Sampling milk filters in addition to BTM can also improve the ability to detect pathogens in BTM (Van Kessel et al., 2008; Latorre et al., 2011; Van Kessel et al., 2011). The objective of this study was to determine the prevalence of *Campylobacter* spp. in BTM and milk filters from US dairy operations from samples collected during the NAHMS Dairy 2014 study.

## MATERIALS AND METHODS

To identify operations that would participate in the NAHMS Dairy 2014 study, a stratified random sample of dairy operations was selected from the USDA National Agricultural Statistics Service list frame. The survey design was a stratified random sample with unequal selection probabilities to ensure the inclusion of dairy operations with 500 or more cows and organic operations. Operations were chosen from each of 17 dairy states in 2 regions of the country (west: California, Colorado, Idaho, Texas, and Washington; east: Indiana, Iowa, Kentucky, Michigan, Minnesota, Missouri, New York, Ohio, Pennsylvania, Vermont, Virginia, and Wisconsin). These states represent 80.5% of dairy herds and 81.3% of dairy cows in the United States. Operations were classified into 3 herd size categories based on the number of cows: small (30–99 cows), medium (100–499 cows), and large (500 or more cows).

Producers (n = 3,500) that reported 1 or more milk cows in their inventory on January 1, 2014, were selected for phase I of the NAHMS Dairy 2014 study. During this phase, National Agricultural Statistics Service enumerators administered a general management questionnaire to participating producers (n = 1,191). In phase II, federal and state veterinary medical officers or animal health technicians administered an additional questionnaire to operations with 30 or more milk cows on January 1, 2014, that had participated in phase I and agreed to continue participating in the study (n = 265).

Samples from BTM and milk filters were collected from March to July 2014. Trained staff aseptically collected BTM (50–150 mL) and milk filters from participating operations. Sample collectors were instructed to ensure that the BTM and milk filter samples represented at least 1 complete milking cycle of the herd. Thus, if the herd had more than 1 bulk tank, then each bulk tank was sampled, and all milk filters used during a milking cycle were collected. Samples were shipped overnight on ice to the USDA-Agricultural Research Service in Beltsville, Maryland.

For enrichment of *Campylobacter* spp. from BTM, multiple tubes of milk from an operation were first combined and mixed, and then a 25 mL aliquot was centrifuged at 20,000 × *g* for 35 min at 8°C (Figure 1). The supernatant was discarded, and the pellet was resuspended in 45 mL Bolton Broth (Oxoid, Basingstoke, UK) with 5% Laked Horse Blood (Lampire, Pipersville, PA) and a *Campylobacter* selective supplement that contained cefoperazone, cycloheximide, trimethoprim, and vancomycin (Dalynn, Calgary, AB, Canada). For enrichment of *Campylobacter* spp. from milk filters, the filters were cut into small pieces and placed in filtered stomacher bags, to which 1% buffered peptone water (1:1, wt/wt) was added, as previously described by Van Kessel et al. (2011). The bags were pummeled in an automatic bag mixer for 2 min, repositioned, and then pummeled for an additional 2 min. Then, 5 mL of filtrate was added to 40 mL of Bolton Broth with 5% Laked Horse Blood and *Campylobacter* selective supplement.

For all samples, enrichment tubes were incubated at 37°C for 48 h in a 10% $CO_2$ incubator with loosened caps. After incubation, 2 mL of enrichment broth were centrifuged (12,000 × *g*). The supernatants were