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Fecal Shedding of *Campylobacter* and *Arcobacter* spp. in Dairy Cattle

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***Campylobacter jejuni*, *Campylobacter coli*, and *Arcobacter* spp. were detected in feces of healthy dairy cows by highly specific multiplex-PCR assays. For *C. jejuni*, at this one-time sampling, cows from 80.6% of farm operations ($n = 31$) and 37.7% of individual dairy cattle fecal samples ($n = 2{,}085$) were positive. Farm management factors were correlated with prevalence in herds in which >25% of cows were positive for *C. jejuni*. Statistical significance was set at a P of 0.20. Using these criteria, application of manure with broadcast spreaders ($P = 0.17$), feeding of whole cottonseed or hulls ($P = 0.17$) or alfalfa ($P = 0.15$), and accessibility of feed to birds ($P = 0.17$) were identified as possible risk factors for *C. jejuni* infection. *C. coli* was detected in at least one animal in 19.4% of operations and 1.8% of individual cows ($n = 2{,}085$). At the herd level, use of broadcaster spreaders was not a risk factor for *C. coli* infection. For *Arcobacter*, cows from 71% of dairy operations ($n = 31$) and 14.3% of individual dairy cattle fecal samples ($n = 1{,}682$) were positive. At the herd level, for *Arcobacter* spp., feeding of alfalfa ($P = 0.11$) and use of individual waterers ($P = 0.19$) were protective. This is the first description of *Arcobacter* spp. in clinically healthy dairy cattle and the first attempt to correlate their presence with *C. jejuni*.**

Campylobacter jejuni and *Campylobacter coli* cause human enteritis and are commensals of livestock. In the United States, *C. jejuni* is the most frequent cause of food-borne diseases in humans, with incidence estimated at 30 to 60 cases per 100,000 people. The frequency of human infection may be due to the low infectious dose for *C. jejuni*, which, based on human volunteer studies, ranges from 100 to 500 organisms (45).

In human populations, *C. jejuni* infections may occur as sporadic infections or as epidemics. Approximately 70% of sporadic cases involve consumption of contaminated poultry, up to 80% of which may harbor *Campylobacter*. Sporadic cases also result from drinking contaminated water, raw milk, or milk contaminated after pasteurization (5%) and exposure during foreign travel (9%) (49).

Although the majority of cases are sporadic, outbreaks involving consumption of contaminated raw milk and untreated water occur (4, 21, 35). In the United States, from 1978 to 1986, of the 57 food-borne outbreaks caused by *C. jejuni*, 26 were traced to raw milk and 11 were traced to consumption of contaminated water (55). *Campylobacter* may enter the supply of water or raw milk from bovine feces (54). Waterborne outbreaks of *Campylobacter*, involving up to 3,500 individuals, have been related to drinking untreated or inadequately chlorinated water (62).

Healthy cattle may be reservoirs for a number of *Campylobacter* species, including *C. jejuni* (1, 14, 15, 26, 42, 53, 64). The prevalence of *C. jejuni* and *C. coli* in dairy cattle ranges from 5 to 53%, depending on methods of isolation (direct plating or enrichment), age of animal (calf or adult), season, and sample analyzed (feces or intestinal contents). The recovery of *C. jejuni* of identical biotypes from ground water and dairy cattle suggests transmission between water and livestock (53). In the environment, *Campylobacter* remains viable at 4°C for up to 3 weeks in feces, 4 weeks in water, and 5 weeks in urine (5).

The incidence of *C. jejuni* in cattle may be seasonal, with peak shedding occurring in either the winter or the summer (6, 53, 46). A bimodal trend with fecal shedding occurring in spring and autumn has also been observed (53). Human campylobacteriosis outbreaks associated with consumption of contaminated milk or water occur in the fall and spring (50). This seasonal trend may reflect peaks in either fecal shedding in the bovine reservoir or exposure to a common source of contamination (53, 55).

The genus *Arcobacter* includes bacteria formerly designated *Campylobacter cryaerophila* (59). *Arcobacter* spp. grow in the presence of atmospheric oxygen (aerotolerant) and at 15 to 30°C, which is lower than the temperature range used for incubation of *Campylobacter* (36, 38). These features adapt *Arcobacter* to survive in the environment.

Arcobacter spp. were first isolated from aborted bovine fetuses by using protocols originally designed for *Leptospira* spp. (10, 39). With cattle, subsequent isolations have been made from preputial swabs of a healthy bull and cases of enteritis, mastitis, and abortion (11, 16, 22, 33, 38, 39, 66). The prevalence of *Arcobacter* in healthy beef or dairy cattle is unknown.

Three species have been recovered from humans and animals, including cattle: *Arcobacter butzleri*, *Arcobacter cryaerophilus*, and *Arcobacter skirrowii* (61). In humans, *A. butzleri* causes a reportable disease which is manifested as enteritis and occasionally septicemia (24, 29, 41, 56, 57; J. A. Kiehlbauch, R. V. Tauxe, and I. K. Wachsmuth, Microb. Ecol. Health Dis. S92, abstr. C14-2, 1991). Risk factors for human infection include consumption of contaminated poultry (2, 8, 28, 30, 32, 34, 44, 67), travel to developing countries, and consumption of contaminated water (52; Kiehlbauch et al., Microb. Ecol. Health Dis. S92). *A. butzleri* has been reported in drinking water res-

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TABLE 1. Description of *Campylobacter* study operations by herd size and region[a]

Region of United States	No. of operations sampled with milk cows numbering:		Total	No. of operations sampled with cull cows from:	
	<100	>100		Farms with >100 cows	Market operations
South	1	9	10	6	11
North	10	11	21	7	25
Total	11	20	31	13	36

[a] Milk cows from the south were from the states of California, Florida, Tennessee, and Texas. Milk cows from the north were from Idaho, Illinois, Iowa, Michigan, Minnesota, New York, Oregon, Pennsylvania, Vermont, Washington, and Wisconsin. Cull cows from the south were from California, New Mexico, Tennessee, and Texas. Cull cows from the north were from New York, Oregon, Vermont, and Wisconsin. Cull cows from market operations in the south were from California, Florida, New Mexico, Tennessee, and Texas. Cull cows from market operations in the north were from Iowa, Michigan, Minnesota, Missouri, New York, Ohio, Pennsylvania, Vermont, and Wisconsin.

ervoirs in Germany, in water treatment plants, rivers, the canals of Bangkok, water samples obtained from oil fields, and well water serving an Idaho youth camp following an outbreak of waterborne enteritis (9, 27, 43, 51, 63, 67).

Traditional methods of recovering *C. jejuni*, *C. coli*, and *Arcobacter* restrict the screening of a large number of samples. We have developed a multiplex-PCR assay for the simultaneous identification of *C. jejuni* and *C. coli* (18). The assay targets the *flaA* genes of *C. jejuni* and *C. coli* and yields a 460-bp product. A second set of primers specifically amplifies a nucleic acid sequence unique to *C. jejuni* and yields a 160-bp amplicon. Thus, the appearance of only the 460-bp product indicates *C. coli*; the presence of both the 160- and 460-bp fragments is characteristic of *C. jejuni* (18).

In addition, we have developed a PCR assay which targets the 16S rRNA gene of *Arcobacter*, yields a specific 1,223-bp amplicon, and clearly distinguishes *Arcobacter* from *Campylobacter* and *Helicobacter* species (19). The assay is rapid, bypasses the ambiguities of phenotypic testing, and is adaptable to screening large numbers of hogs, poultry, and meat products (66, 67).

The primary goal of this study was to use PCR assays to estimate the prevalence of *C. jejuni*, *C. coli*, and *Arcobacter* spp. in healthy U.S. dairy cows. Since this study was of a subsampling of the National Animal Health Monitoring System (NAHMS) 1996 national survey and included interviews with dairy farmers, it was anticipated that on-farm risk factors for the shedding of *C. jejuni* and *Arcobacter* spp. would also be identified.

MATERIALS AND METHODS

NAHMS Dairy '96 study. All samples were obtained during the NAHMS Dairy '96 study conducted by the U.S. Veterinary Services division of the Animal and Plant Health Inspection Service, U.S. Department of Agriculture (37, 65). For *Campylobacter*, the numbers of herds, types of cows analyzed, and their geographic distributions are summarized in Table 1. Fecal samples (n = 2,085) from a combination of 31 milk cow dairy operations, 13 farms on which lactating cows were to be culled within 7 days, and 36 market operations in 23 states were included in the study. For *Arcobacter* spp., the total number of farm operations examined, their geographic locations, and a description of animals surveyed (milk cows, on-farm cull cows, and culled market cows) are summarized in Table 2. Fecal samples (n = 1,682) from 31 milk cow dairy operations, 13 farms on which lactating cows were to be culled within 7 days, and 17 market operations in 19 states were included in the study.

Fecal samples (50 g) were obtained from lactating milk cows and from on-farm cull milk cows by direct rectal retrieval. Fresh fecal material was obtained from pen floors at cull cow market operations. Biologic samples were collected from 20 February through 10 July 1996.

Fecal samples. Fecal samples (~50 g) were collected in 50-ml conical centrifuge tubes and shipped refrigerated overnight to the National Veterinary Services Laboratories, Ames, Iowa. The next day (within 36 h of collection) approximately 1 g of feces was diluted (10% wt/vol) in buffered peptone-water (9 ml).

***Campylobacter* identification.** An aliquot of the fecal suspension (6 to 8 drops, 0.4 ml) was plated to the surface of modified blood-free charcoal, cefoperazone deoxycholate agar (CM 739; Oxoid Ogdensburg, N.Y.) as described previously (42). After incubation (42°C, 2 to 3 days, microaerobically), bacterial growth from the first quadrant was harvested with a bacteriological loop, placed in Tris-EDTA buffer (pH 7.4, 200 μl), and frozen (−20°C) for PCR analysis, as described previously (18). The bacterial suspension in Tris-EDTA (200 μl) was boiled (5 min) and centrifuged (13,000 × g, 1 min, room temperature), and a 5-μl aliquot of the supernatant was used as the PCR template. Sequences and specificities of PCR primers targeting the *flaA* genes of *C. jejuni* and *C. coli* and the sequences unique to *C. jejuni* have been reported previously (18). Samples were subjected to an initial denaturation step (94°C for 4 min), followed by 25 amplification cycles. Each amplification cycle consisted of denaturation (1 min at 94°C), primer annealing (1 min at 45°C), and primer extension (1 min at 72°C). Final primer extension (72°C for 7 min) followed the last amplification cycle. PCR products were electrophoretically separated (120 V, 45 to 55 min). *C. coli* was identified by the appearance of a 460-bp product. *C. jejuni* exhibited both the 160- and 460-bp fragments (18).

***Arcobacter* identification.** An aliquot (1 ml) of the fecal suspension was placed in Ellinghausen McCullough, Johnson, and Harris-polysorbate 80 (9 ml) and incubated (30°C, 3 to 5 days) aerobically. After incubation, an aliquot (200 μl) was removed and stored frozen (−20°C) for PCR analysis (19). The aliquot (200 μl) was boiled (10 min) in a heat block and centrifuged (13,000 × g, 1 min, room temperature). A 5-μl aliquot of the supernatant was used as the template for PCR analysis using primers targeting the 16S rRNA gene detailed previously (19). Samples were subjected to an initial denaturation step (94°C for 4 min), followed by 25 amplification cycles. Each amplification cycle consisted of 1 min at 94°C, 1 min at 56°C, and 1 min at 72°C. Primer extension (72°C for 7 min) followed the final amplification cycle. PCR products were examined by agarose gel electrophoresis (120 V, 45 to 55 min). Samples exhibiting the specific 1,223-bp amplicon were scored as positive; no amplicon was observed in negative samples (19). A small number of *Arcobacter*-positive samples (n = 33) were then rescreened using the newly developed multiplex PCR for the identification of *A. butzleri* (20). In addition to amplifying the 16S rRNA gene of *Arcobacter* spp., the multiplex assay also targets the 23S rRNA gene of *A. butzleri* (3). Thus, the 1,223-bp amplicon indicates the presence of *Arcobacter* spp.; the smaller 686-bp product is specific for the 23S rRNA gene of the species *butzleri* (20). Because the multiplex assay was developed while this study was in progress, only 33 samples which were originally identified as *Arcobacter* (n = 240) were available for subsequent *A. butzleri* screening.

Statistical analysis. Herd- and cow-level prevalence data were analyzed using the statistical software program SAS, release 6.12 (47). All operations with milk cows harbored at least one *C. jejuni*-positive cow. Therefore, to identify trends in herd risk factors, herds in which *C. jejuni* was found in >25% of cows were scored as positive. Herds in which either *C. coli* or *Arcobacter* was found in one cow were scored as positive for that species. Herd-level associations were tested for significance at the 80% probability level ($P < 0.2$). All associations with *C. jejuni* and *Arcobacter* carrier status were determined via chi-square test. Fisher's exact test was utilized in cases where the number of samples was <5.

Because large sample sizes are the basis for statistical analysis and resultant levels of significance, individual cow data were also reviewed to identify addi-

TABLE 2. Description of *Arcobacter* study operations by herd size and region[a]

Region of United States	No. of operations sampled with milk cows numbering:		Total	No. of operations sampled with cull cows from:	
	<100	>100		Farms with >100 cows	Market operations
South	1	9	10	6	4
North	10	11	21	7	13
Total	11	20	31	13	17

[a] Milk cows from the south were from California, Florida, Tennessee, and Texas. Milk cows from the north were from Idaho, Illinois, Iowa, Michigan, Minnesota, New York, Oregon, Pennsylvania, Vermont, Washington, and Wisconsin. Cull cows from the south were from California, New Mexico, Tennessee, and Texas. Cull cows from the north were from New York, Oregon, Vermont, and Wisconsin. Cull cows from market operations in the south were from New Mexico, Tennessee, and Texas. Cull cows from market operations in the north were from Iowa, Michigan, Minnesota, Missouri, New York, and Pennsylvania.

tional risk factors, which could be masked in the herd analysis because of the small number of herds examined. For individual cow analysis, the level of statistical significance was set at a P of 0.10.

RESULTS

***Campylobacter*.** At least one cow positive for *C. jejuni* was detected on all farm operations with milk cows, 13 farm operations (84.6%) with cull cows, and 33 of 36 market operations (91.2%) with cull cows.

***Campylobacter* herd risk factors.** To identify trends in risk factors, herds in which *C. jejuni* was found in >25% of cows were scored as positive; statistical significance was set at a P of 0.2. Using these criteria (Table 3), *C. jejuni* herd prevalence was correlated with use of broadcast spreaders to dispose of manure ($P = 0.17$); type of feed, including alfalfa ($P = 0.15$) and whole cottonseed or hulls ($P = 0.17$); and accessibility of feed to birds ($P = 0.17$).

C. coli was detected in herds from 19.4% of farm operations (Table 3). For *C. coli*, herds were scored as positive if they included a single positive cow. Use of broadcast spreaders to dispose of manure emerged as a protective factor (Table 3) ($P = 0.17$).

Cow-level risk factors. As shown in Table 4, *C. jejuni* was found in 37.7% of dairy cattle fecal samples. Significantly more isolations ($P < 0.01$) were made from lactating milk cows (42.9%) than from cull market cows (30.3%).

Individual cow data were also reviewed to identify additional risk factors, which could be overlooked by herd analysis. Associations were tested for significance at the 90% probability level ($P = 0.10$). For *C. jejuni*, cows from large herds ($P = 0.01$) as well as cows fed brewers' by-products ($P = 0.01$) were more likely to be infected.

C. coli was detected in 1.8% of fecal samples (Table 4). Infections were lower in cows fed brewers' by-products ($P = 0.01$).

As shown in Table 5, *C. jejuni* prevalence was higher in cull cows at markets ($n = 849$) sampled before May 1 (38.9%) than those taken later (28%; $P = 0.01$). *C. coli* was detected more frequently in samples collected from southern (4%) than from the northern (1.8%; $P = 0.06$) markets.

***Arcobacter* spp.** For milk cows, 71% of operations ($n = 31$) had at least one animal positive for *Arcobacter* spp. For on-farm cows to be culled within 7 days, *Arcobacter* spp. were detected in at least one animal in 46% of farm operations ($n = 13$). For cull cows at market, *Arcobacter* spp. were recovered at least once in 76.5% of markets ($n = 17$).

Overall, *Arcobacter* spp. were identified in 14.3% of cows ($n = 1{,}682$ cows). *Arcobacter* spp. were found more frequently ($P < .01$) in cull market cows (22.3%) than in either lactating cows (12%) or on-farm cull cows scheduled to be removed within 7 days (8.7%). Analysis via the multiplex PCR of 33 samples positive for *Arcobacter* indicated that 17 fecal samples were positive for *A. butzleri*.

***Arcobacter* spp. herd risk factors.** As summarized in Table 3, herd prevalence was lower (protective effects) in lactating cows fed alfalfa ($P = 0.11$) and on premises where individual waterers were used ($P = 0.19$).

Cow-level risk factors. As shown in Table 4, cows in the southern region of the country were more likely to harbor *Arcobacter* (18.6%) than cows in the northern region (12.4%; $P < 0.001$). When only lactating cows, including farm cows to be culled within 7 days, were analyzed ($n = 1{,}282$), cows from large herds (14.7%) were more likely to be infected than cows from small herds (5.1%; $P < 0.001$). *Arcobacter* spp. were more frequently identified in cows on premises where alley flushing was the method of cleaning ($P < 0.001$). Infection was lower in lactating cows fed brewers' by-products ($P = 0.03$).

As shown in Table 5, when cull cows at markets ($n = 400$) were analyzed, prevalence was higher for samples collected after 1 May (26.7%) than for those taken earlier (16.6%; $P = 0.02$).

DISCUSSION

The primary objective of this study was to estimate the prevalence of *C. jejuni*, *C. coli*, and *Arcobacter* spp. in healthy dairy cows by using PCR assays for which the specificities have been previously established (18, 19).

Overall, *C. jejuni* was detected in 37.7% of dairy cattle fecal samples. *C. coli* was detected in 1.8% of fecal samples. Previous estimates of *C. jejuni* in dairy cows range from 0.8 to 100%, depending on season, age of animal analyzed, number of animals surveyed, and isolation methods.

Arcobacter spp. were detected in fecal samples of 14.3% of dairy cows. The multiplex assay for the simultaneous detection of *Arcobacter* spp. and *A. butzleri*, which was developed during the course of this study (20), identified *A. butzleri* in 17 of 33 *Arcobacter*-positive samples available for analysis. Thus, *Arcobacter* spp., including *A. butzleri*, like *C. jejuni*, exist in the guts of healthy dairy cows.

A second goal of this study was to attempt to identify herd characteristics, which were likely to be associated with *C. jejuni* infections, and determine if they paralleled those identified in herds with *Arcobacter* carriers. Because of the relatively small number of herds analyzed, individual cow sample data were also examined in order to identify potential trends in risk factors. At the herd level, geographic location in the southern or northern region of the United States was not a risk factor for infection with either *C. jejuni* or *Arcobacter* spp. Yet at the sample level, *Arcobacter* was detected more frequently in cows in southern dairy herds than in those in the north, which may reflect ease of transmission in warmer climates (Kiehlbauch et al., Microb. Ecol. Health Dis.).

At the herd level, neither *C. jejuni* nor *Arcobacter* prevalence could be correlated with herd size. However, at the sample level, both *C. jejuni* and *Arcobacter* spp. were recovered more frequently from fecal samples obtained from large versus small herds. Comingling of a large number of susceptible hosts with carrier animals facilitates transmission of enteric pathogens. For *Escherichia coli* O157:H7, grouping of calves was identified as a risk factor for fecal shedding (12). In humans, identical strains of *A. butzleri* were recovered from a nursery school outbreak, which was facilitated by crowding (60).

At the herd level, use of broadcast spreaders was correlated with *C. jejuni* but not with *Arcobacter* infection status. Type of manure disposal was earlier indicated as a risk factor for *E. coli* O157 infection in dairy cows (13, 17).

At the herd level, use of individual waters appeared to protect against *Arcobacter* infection but had no impact on *C. jejuni* herd prevalence. Chlorinated drinking water was not a protective factor for either *C. jejuni* or *Arcobacter* infection in dairy herds. Humphrey and Beckett reported that rectal shedding of *C. jejuni* occurred only in cows with access to drinking river water; *C. jejuni* was not detected in cows which drank chlorinated water (26). However, no association was found between *E. coli* O157 in dairy cattle and access to chlorinated water (13). Human *Arcobacter* spp. infections have been linked with travel to developing countries and subsequent exposure to untreated water (Kiehlbauch et al., Microb. Ecol. Health Dis.).

Garber et al. (13) correlated use of alley flushing to remove manure and *E. coli* O157 herd prevalence. In this study, at the

TABLE 3. Summary of distributions of *Campylobacter* and *Arcobacter* in dairy cattle correlated with herd factors[a]

Parameter	No. of herds positive for *C. jejuni*/no. of herds tested (%)	*P*	No. of herds positive for *C. coli*/no. of herds tested (%)	*P*	No. of herds positive for *Arcobacter*/no. of herds tested (%)	*P*
Overall	25/31 (80.6)		6/31 (19.4)		22/31 (71.0)	
Herd size		0.64		1.00		0.22
<100 cows	8/11 (72.7)		2/11 (18.2)		6/11 (54.5)	
>100 cows	17/20 (85.0)		4/20 (20.0)		16/20 (80.0)	
Season		0.36		1.00		1.00
Before 1 May	7/10 (70.0)		2/10 (20.0)		7/10 (70.0)	
1 May or later	18/21 (85.7)		4/21 (19.0)		15/21 (71.4)	
Region		1.00		0.63		0.21
North	17/21 (81.0)		5/21 (23.8)		13/21 (61.9)	
South	8/10 (80.0)		9/10 (90.0)		1/10 (10.0)	
Flush alleys		1.00		1.00		0.64
Yes	6/7 (85.7)		1/7 (14.3)		6/7 (85.7)	
No	19/24 (79.2)		5/24 (20.8)		16/24 (66.7)	
Chlorinated drinking water		0.24		1.00		1.00
Yes	3/5 (60.0)		1/5 (20.0)		4/5 (80.0)	
No	22/26 (84.6)		5/26 (19.2)		18/26 (69.2)	
Use of irrigation to dispose of manure		0.65		0.30		1.00
Yes	6/8 (75.0)		3/8 (37.5)		6/8 (75.0)	
No	18/22 (81.8)		3/22 (13.6)		15/22 (68.2)	
Use of broadcast or solid spreader to dispose of manure		0.17		0.17		1.00
Yes	22/26 (84.6)		4/26 (15.4)		18/26 (69.2)	
No	2/4 (50.0)		2/4 (50.0)		3/4 (75.0)	
Slurry, surface application		1.00		0.64		0.21
Yes	7/9 (77.8)		1/9 (11.1)		8/9 (88.9)	
No	17/21 (81.0)		5/21 (23.8)		13/21 (61.9)	
% of cows not born on farm		0.60		0.60		0.39
0	5/7 (71.4)		2/7 (28.6)		6/7 (85.7)	
1 or more	19/23 (82.6)		4/23 (17.4)		15/23 (65.2)	
Feeding of alfalfa		0.15		0.64		0.11
Yes	18/20 (90.0)		3/20 (15.0)		12/20 (60.0)	
No	7/11 (63.6)		3/11 (27.3)		10/11 (90.9)	
Feeding of corn silage		0.32		0.64		0.69
Yes	19/22 (86.4)		5/22 (22.7)		15/22 (68.2)	
No	6/9 (66.7)		1/9 (11.1)		7/9 (77.8)	
Feeding of whole cottonseed or hulls		0.17		1.00		0.42
Yes	17/19 (89.5)		4/19 (21.1)		12/19 (63.2)	
No	8/12 (66.7)		2/12 (16.7)		10/12 (83.3)	
Individual waterer		0.63		1.00		0.19
Yes	6/8 (75.0)		1/8 (12.5)		4/8 (50.0)	
No	19/23 (82.6)		5/23 (21.7)		18/23 (78.3)	
Feed accessible to birds		0.17		1.00		0.70
Yes	14/15 (93.3)		3/15 (20.0)		10/15 (66.7)	
No	11/16 (68.8)		3/16 (18.8)		12/16 (75.0)	
Feed accessible to rodents		0.36		0.36		0.70
Yes	13/18 (72.2)		5/18 (27.8)		12/18 (66.7)	
No	12/13 (92.3)		1/13 (7.7)		10/13 (76.9)	

[a] Herd-level associations were tested at the 80% probability level ($P = 0.2$).

herd level, no correlation could be made between flushing alleys and either *C. jejuni* or *Arcobacter* herd prevalence. However, at the sample level, a disproportionately higher number of *Arcobacter* infections occurred in cows on premises where alleys were flushed. Rice et al. reported that *A. butzleri* decreased by only 0.5 $\log_{10}$ unit when it was held in ground water at 5°C for 14 days (43). In contrast, *C. jejuni* declined by 6 to 7 $\log_{10}$ units when it was held for a similar interval. Thus, *Arcobacter* spp. may be better adapted to survive in recycled water used for flushing alleys than *Campylobacter*.

TABLE 4. Correlation of distribution of *C. jejuni*, *C. coli*, and *Arcobacter* with individual animal characteristics[a]

Parameter	No. of cows positive for *C. jejuni*/no. of cows tested (%)	*P*	No. of cows positive for *C. coli*/no. of cows tested (%)	*P*	No. of cows positive for *Arcobacter*/no. of cows tested (%)	*P*
Total[b]	786/2,085 (37.7)		38/2,085 (1.8)		240/1,682 (14.3)	
Region		0.12		0.40		<0.001
North	562/1,447 (38.8)		24/1,447 (1.7)		147/1,183 (12.4)	
South	224/638 (35.1)		14/638 (2.2)		93/499 (18.6)	
Season		0.32		0.48		0.96
Before 1 May	214/542 (39.5)		8/542 (1.5)		81/570 (14.2)	
1 May or later	572/1,543 (37.1)		30/1,543 (1.9)		159/1,112 (14.3)	
Herd size[c]		0.01		0.21		<0.001
<100 cows	147/390 (37.7)		3/390 (0.8)		20/390 (5.1)	
100 or more cows	382/846 (45.2)		14/846 (1.7)		131/892 (14.7)	
Alley flushing		0.74		0.13		<0.001
Yes	103/246 (41.9)		6/246 (2.4)		62/256 (24.2)	
No	426/990 (43.0)		11/990 (1.1)		89/1,026 (8.7)	
Lactating cows fed brewers' by-products		0.01		0.01		0.03
Yes	246/525 (46.9)		2/525 (0.4)		55/570 (9.6)	
No	283/711 (39.8)		15/711 (2.1)		96/712 (13.5)	

[a] Cow-level associations were tested at the 90% probability level ($P = 0.1$).
[b] Cull cows at market operations are included only for total region and season.
[c] All on-farm culls were from farms with 100 or more cows.

Diet, feeding regimens, dietary supplements, and use of probiotic bacteria or yeast influence the population dynamics of enteric pathogens, including *E. coli* O157:H7, *Salmonella enterica*, and *Campylobacter* (23, 31, 40, 48, 69). In the present study, at the herd level, feeding of alfalfa was a protective factor for *Arcobacter* infection; feeding of either cottonseed or hulls had no effect. In contrast, *C. jejuni* was more frequently recovered in herds where alfalfa or whole cottonseed or hulls were fed. Previously, *E. coli* O157 infections were observed more frequently in herds fed cottonseed (13), although an earlier study had shown no such association (17). Sample-level data suggested that *Arcobacter* spp. prevalence was lower in lactating cows fed brewers' by-products than in cows not given this supplement. Thus, feed and dietary supplements may alter gut homeostatis and influence colonization of *Campylobacter* and *Arcobacter*.

At the herd level, no seasonal differences were noted in the detection of *C. jejuni* and *Arcobacter* spp. However, at the sample level, *C. jejuni* was detected more frequently in feces of cull market cows collected prior to 1 May. Yet *Arcobacter* was detected more frequently in samples taken after 1 May. A seasonal trend in fecal shedding of *C. jejuni* is known for dairy cattle (47, 54). Human epidemics caused by water- and raw-milk-borne *C. jejuni* have been temporally associated with the peak in seasonal shedding in cattle (56).

The accessibility of feed to birds was positively correlated with herd prevalence of *C. jejuni* but not *Arcobacter*. That birds harbor and disseminate *C. jejuni* via postpasteurization contamination of bottled milk has been documented (25).

The food safety risk of the cull market cow is unknown (58). In this study, *C. jejuni* prevalence was lower in the lactating cow than in the cull cow. Yet, the reverse was true for *Arcobacter*. *C. jejuni* and *Arcobacter* spp. have been detected in clinically healthy pigs and poultry, as well as in ground pork, beef, and turkey products (2, 7, 8, 28, 30, 34, 44, 67, 68, 69). This is the first report that *Arcobacter* spp., like *C. jejuni*, exist in the guts of healthy cattle and thus that they may contaminate the environment and the human food chain. Results of this survey indicate that factors associated with *C. jejuni* herd prevalence may not predict *Arcobacter* spp. infection status. Nevertheless,

TABLE 5. *Campylobacter* and *Arcobacter* in cull cows at markets[a]

Parameter	No. of cows positive for *C. jejuni*/no. of cows tested (%)	*P*	No. of cows positive for *C. coli*/no. of cows tested (%)	*P*	No. of cows positive for *Arcobacter*/no. of cows tested (%)	*P*
Total	257/849 (30.3)		21/849 (2.5)		89/400 (22.3)	
Region		0.41		0.06		0.78*
North	187/601 (31.1)		11/601 (1.8)		68/301 (22.6)	
South	70/248 (28.2)		10/248 (4.0)		21/99 (21.2)	
Season		0.01*		0.28*		0.02*
Before 1 May	68/175 (38.9)		2/175 (1.1)		29/175 (16.6)	
1 May or later	189/674 (28.0)		19/674 (2.8)		60/225 (26.7)	

[a] Cow-level associations were tested at the 90% probability level ($P = 0.1$). * indicates use of two-tailed Fisher's exact test.

this study suggests potential on-farm risk factors for further evaluation.

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