Distribution of *Campylobacter* and *Arcobacter* in Livestock

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SUMMARY AND IMPLICATIONS

We designed polymearse chain reaction (PCR) primers to distinguish *Campylobacter jejuni* from *Campylobacter coli* and to differentiate *Arcobacter* from other species of *Campylobacter*. We applied these PCR methods to estimate their prevalence in feces of healthy cattle and hogs and to identify risk factors for infection. For cattle, *C. jejuni* (23%), *Arcobacter* (11%), and *C. coli* (1.57%) were detected in healthy dairy cows (n=1,628). *Campylobacter coli* (69%), *Arcobacter* (46%) and *C. jejuni* (0.28%) were found in market weight hogs (n=1,057). This indicates the widespread distribution of these microbes livestock.

INTRODUCTION

Campylobacter and *Arcobacter* are closely related microbes of the rRNA Superfamily Vl of the Proteobacteria (9). *Campylobacter* are commensals of cattle, hogs, and poultry.

In humans, *C. jejuni* causes large outbreaks in the fall and spring due to consumption of contaminated raw milk and water. Human outbreaks may coincide with peaks of shedding in the bovine reservoirs (8). *Arcobacter* is aerotolerant and grows at 15C to 30°C, which is lower than the optimal temperature of *Campylobacter* .[9] Thus, *Arcobacter* may be better adapted than *Campylobacter* to survive in the environment. Three species have been recovered from humans, animals, and foods: *A. butzleri, A. cryaerophilus,* and *A. skirrowii* (9). Of these, *A. butzleri* is regarded as the primary human pathogen (4,10).

The goals of this study were to use polymease chain reaction (PCR) assays previously developed in our laboratory (2,3) to determine the prevalence of *C. jejuni*, *C. coli*, and *Arcobacter* spp. in healthy livestock screened during the NAHMS 1995 swine and 1996 dairy national surveys; and to identify on-farm factors for shedding of *Campylobacter* spp. and *Arcobacter* in cattle and hogs.

MATERIALS AND METHODS

Sample collection: Feces (~50 g) was collected in 50ml conical centrifuge tubes and shipped refrigerated overnight to the National Veterinary Services Laboratories, Ames, IA. The next day (within 36 h of collection) ~1 g of feces was diluted (10% weight/volume) in buffered peptone water (9 ml) and screened for *C. jejuni*, *C. coli*, and *Arcobacter* spp.

***Campylobacter identification*:** Fecal suspension (6 to 8 drops, 0.4 ml) were plated to modified CCDA plate, and incubated (42°C, 2 to 3 days, microaerobically) (5). Bacterial growth from the first quadrant was harvested with a bacteriological loop, placed in TE buffer (200 ul), and frozen (-20°C). The bacterial suspension in TE (200 ul) was boiled (5 min), centrifuged (13,000 *g*, 1 min, room temperature), and the supernatant (5 ul) was used as the template. Amplification conditions and PCR primers targeting the *fla*A gene of *C. jejuni* and *C. coli* and the *C. jejuni*-specific sequences were reported

earlier (2). PCR products were electrophoretically separated (120 V, 45 to 55 min). The appearance of a 460-bp product indicates the presence of *C. coli*; presence of both the 160- and 460-bp fragments is characteristic of *C. jejuni*.

Arcobacter identification. The fecal suspension (1 ml) was placed in EMJH-P80 (9 ml) and incubated (30°C, 3 to 5 days) aerobically. After incubation, an aliquot (200 ul) was removed, and stored frozen (-20°C). The aliquot (200 ul) was boiled (10 min), centrifuged (13,000 *g*, 1 min, room temperature), and the supernatant (5 ul) used as the template with primers targeting the 16S rRNA gene and amplification conditions detailed earlier (2). PCR products were separated by agarose gel electrophoresis (120 V, 45 to 55 min). Samples that exhibited the 1,223-bp amplicon were scored as positive; no amplicon was seen in negative samples. (3)

Herd prevalence. For *C. coli*, farms with >68% positive hogs were scored as positive in determining risk factors. For *C. jejuni*, dairy herds with >25% of infected animals were scored as positive herds. For *Arcobacter*, the presence of any positive animal scored the farm as positive.

Questionnaire data. Data regarding herd size and management-related factors were collected during several phases of the Dairy 1996 and Swine 1995 studies.[1]

Statistical analysis. The PCR data were analyzed with the statistical software program SAS, release 6.12 (SAS Institute Inc., Cary, NC). Data were analyzed at the herd level for association with several animal husbandry-related factors. All associations were determined via X^2 test. Fisher's test was used when expected sample size was <5. Because of the relatively small numbers of herds surveyed, statistical significance was set at P =0.2 to recognize on-farm risk factor trends. Sample-level data were analyzed to identify potential factors that may have been obscured in herd level analysis. For this analysis, statistical significance was set at P=0.1.

RESULTS AND DISCUSSION

<u>CATTLE.</u> *Campylobacter jejuni* (23.5%), *C. coli* (1.8%), and *Arcobacter* (14.3%) were detected in dairy cattle. *Campylobacter* . *C. jejuni* was detected on all farms with milk cows (n=31 farms), 84.6% of premises with on-farm cull cows (n=13 farms), and in 91.2% of cull-cow markets (n=36).

Herds with >25% of positive animals were scored as positive for recognizing on-farm risk factors (P=.2, Table 2). Possible herd risk factors included use of broadcast spreading of manure (P=0.169), feeding of whole cotton seed or hulls (P=0.174), and feed accessible to birds (P =0.172). Feeding alfalfa was protective (P=0.151). Neither season nor chlorinated drinking water were associated with *C. jejuni* herd prevalence (P>.2), as reported earlier (6,7),

At the sample-level (Table 3), *C. jejuni* was more likely to be found in cows in large herds (> 100 cows, p =0.014) and if fed brewers' by-products (p =0.013).

For *C. coli* at the herd level (Table 2), broadcast spreading of manure was a risk factor (Table 3, P =0.169). At the sample-level (Table 3), prevalence was higher in cows on premises where alleys were flushed (P=0.125), and lower in lactating cows fed brewers' by products (P=0.013).

Arcobacter spp. were identified in 14.3% of cows (n=1,682). It was present in dairy milk cows (12%), lactating on-farm cows to be culled within 7 days (8.7%), and in cull cows at market (22.3%).

Herd prevalence (Table 2) was determined for milk cows (71%), on-farm cows to be culled within 7 days for (46% of 13 herds), and cull cows at market (76.5% of 17 operations). Herd prevalence (Table 3) was lower in lactating cows fed alfalfa

(*P*=0.106) and on premises with individual waterers (*P*=0.185). Herd prevalence was significantly higher in cull cows at market (76.5%) than in on-farm cull cows (46%, *P*=.088). Herd prevalence was marginally higher in larger (80%) versus small (54.5%) herds (*P*= .217), in northern (61.9%) *versus* southern (10%) herds (*p* =.205), and on premises where manure was applied as a slurry (*P*=.210).

At the sample-level (Table 3), *Arcobacter* was more likely to be found in cows in the southern region (*p*<.001), in cows of large herds, and on premises where alleys were flushed (all *P*<.001). Feeding brewers' by-products was protective (*P*=.034). For lactating cows, no association was found between *Arcobacter* spp. infection and season (sampled before or after May 1, 1996, *P*=.961). However, for cull cows at market (n=400), prevalence was higher for samples collected after May 1 than those taken earlier (*p*=.016).

Pigs. *C. coli* (69%), *C. jejuni* (0.28%), and *Arcobacter* spp. (46%) were detected in healthy hogs 1 month prior to market (n=1,057).

Campylobacter . On-farm risk factors for *C. coli* infection included antibiotics in feed of hogs (*P*=0.126), absence of *Salmonella* in the grower/finisher pigs (*P*=0.126), absence of *Actinobacillus pleuropneumonia* (APP, *P*=.194) in the nursery pigs, and vaccination of herds for PRRS virus (*P*=.134). Protective factors included frequency of cleaning feeders (*P*=0.054) and antibiotic injections of market weight hogs (*P*=0.059).

Arcobacter. On-farm risk factors for *Arcobacter* spp. infection included low frequency (<5%) of nursery scours (*P*=.052), *E. coli* vaccination (*P*=0.042), all in/all out farrowing (*P*=.196), absence of PRRS virus in breeders (*P*=029) and in grower/finisher pigs (*P*=0.165) and, antibiotics in feed (*P*=0.196). Yet antibiotics in water (*P*=0.020) and oral antibiotic treatment (*P*=0.087) were protective. Infection of adult hogs was correlated with absence of PRRS (*P*=.110), dysentery (*P*=.102), and Pseudorabies virus (PRV) (*P*=.102) in nursery pigs.

CONCLUSION

Using highly specific PCR assays, *C. jejuni* (23%), *Arcobacter* (13%) and *C. coli* (1.57%) were detected in feces of healthy cattle; *C. coli* (69 %), *Arcobacter* (46%) and *C. jejuni* (0.28%) in were in feces of healthy hogs. This is the first report to describe the widespread distribution of *Arcobacter* in healthy livestock and to delineate on-farm risk factors for *Arcobacter*. Like *Campylobacter*, *Arcobacter* is found in dairy cattle and market weight hogs.

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TABLE 1. Factors possibly associated with herd prevalence.

	C. jejuni*	***P***	***C. coli***	***P***	***Arcobacter***	***P***
Overall herds	25/31 (80.6%)		6/31 (19.4%)		22/31 (71.0%)	
Herd Size		.638		1.00		.217
<100 Cows	8/11 (72.7%)		2/11 (18.2%)		6/11 (54.5%)	
100 or more	17/20 (85.0%)		4/20 (20.0%)		16/20 (80.0%)	
Region		1.000		0.634		**.205***
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%)	
Broadcast/ solid spreader for manure		**.169***		**.169***		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		0.637		.210
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%)	

	C. jejuni*	***P***	***C. coli***	***P***	***Arcobacter***	***P***
Feed Alfalfa		**.151***		.638		**106***
Yes	18/20 (90.0%)		3/20 (15.0%)		12/20 (60.0%)	
No	7/11		3/11		10/11	

	(63.6%)	(27.3%)	(90.9%)	
Feed Whole Cotton Seed or Hulls	**.174***	1.000		.418
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	.634	1.000		**.185***
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	**.172***	1.000		.704
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%)	

**P* values underscored, in bold, with an asterisk are significant or marginally significant ($p < 0.2$).

TABLE 2. Risk factors at the cow level.

	C. jejuni	*Chi-sq/P*	*C. coli*	*Chi-sq/P*	*Arcobacter*	*Chi-sq/P*
Total	786/2085 (23.5%)		38/2085 (1.8%)		240/1682 (14.3%)	
Region						
North	562/1447 (38.8%)	2.62 0.105	24/1447 (1.7%)	0.71 0.399	147/1183 (12.4%)	11.07 **<.001***
South	224/638 (35.1%)		14/638 (2.2%)		93/499 (18.6%)	
Season						
Before May 1	214/542 (39.5%)	0.99 0.319	8/542 (1.5%)	0.49 0.483	81/570 (14.2%)	0.00 0.961
May 1 or later	572/1543 (37.1%)		30/1543 (1.9%)		159/1112 (14.3%)	
Herdsize						
<100 Cows	147/390 (37.7%)	6.07 0.014	3/390 (0.8%)	1.54 0.214	20/390 (5.1%)	23.86 **<.001***
>100 Cows	382/846 (45.2%)		14/846 (1.7%)		131/892 (14.7%)	
Alley flushing						
Yes	103/246 (41.9%)	0.11 0.742	6/246 (2.4%)	2.56 0.125*	62/256 (24.2%)	47.64 **<.001***
No	426/990 (43.0%)		11/990 (1.1%)		89/1026 (8.7%)	
Lactating cows fed brewers' by-Products						
Yes	246/525 (46.9%)	6.14 0.013	2/525 (0.4%)	6.65 0.010	55/570 (9.6%)	4.48 **.034***
No	283/711 (39.8%)		15/711 (2.1%)		96/712 (13.5%)	

P values underscored in bold with an asterisk are significant or marginally significant ($P<0.2$).