Campylobacter spp. and *Yersinia enterocolitica* in Growing Pigs in Iowa and North Carolina: A Pilot Study

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Summary and Implications

The prevalence of *Campylobacter* spp. and *Yersinia enterocolitica* was determined in cohorts of growing pigs on eight swine farms in Iowa and North Carolina. Approximately 60 pigs from each site were periodically sampled from the nursery to slaughter. Both all in/all out and continuous flow production systems were monitored. Overall, when results from the two states are combined, *Campylobacter coli* was found in the nursery (90%), grower (92.8%) and finisher (90.9%) stages. At slaughter, *C. coli* was detected overall on 17.1% of carcasses. For Iowa, 83% of ileocaecal lymph nodes yielded *Campylobacter*. In contrast, *Y. enterocolitica* was not found in either rectal or tonsilar swabs or in carcass swabs collected from Iowa hogs. *Y. enterocolitica* was detected in 8.8% (5 of 57) of North Carolina hogs sampled on one occasion; no isolations were made from carcass swabs at slaughter.

Introduction

Campylobacter jejuni and *C. coli* both cause human enteritis and are commonly present in livestock. *Campylobacter coli*, a normal inhabitant of the pig intestine, is found on pork products and causes human bacterial enteritis. Less frequently pigs and pork may harbor *C. jejuni*.

In contrast to the number of foodborne outbreaks attributed to consumption of undercooked poultry contaminated with *C. jejuni*, only three outbreaks of human campylobacteriosis have been attributed to pork consumption (12,6,4). Based on onset of clinical symptoms, an outbreak in school children in Japan was thought to be caused by vinegared pork contaminated by *Campylobacter* (12). In a restrospective epidemiological survey in the Netherlands, consumption of pork was identified as a risk factor for human campylobacteriosis (6). In Norway, a case control study indicated that patients in whom *Campylobacter* was cultured were more likely than controls to have consumed pork sausage (4).

In the United States, *Campylobacter jejuni* is a major cause of human foodborne enteritis. An estimated 2.1 million cases occur annually at an estimated cost of $1 billion. *Campylobacter jejuni*, *Salmonella*, *E. coli* O157:H7, and *Listeria monocytogenes* account for up to $4.3 billion in losses annually. The Centers for Disease Control and Prevention have targeted the reduction of *C. jejuni* cases (per 100,000 population) in the United States from 50 in 1987 to 25 by year 2000 (Table 1).

Traditional *Campylobacter* isolation procedures require complex media and incubation in environments with low oxygen (microaerobic). *Campylobacter jejuni* and *C. coli* are often not differentiated and are simply reported as *C. jejuni*/*C. coli*. We have developed a multiplex PCR test to detect and to differentiate *C. jejuni* and *C. coli* in livestock feces and foods (Figures 1 and 3). This assay bypasses biochemical tests to distinguish between *C. jejuni* and *C. coli*.

Healthy pigs harbor pathogenic strains of *Yersinia enterocolitica* and are regarded as a significant reservoir for human infection. The pathogen has been repeatedly isolated from tonsils, tongue, cheek meat, lymph nodes, rectal swabs, and intestinal tract of healthy pigs (1,2). *Yersinia enterocolitica* has been incriminated in foodborne outbreaks associated with consumption of pork, including chitterlings (5).

The purpose of this pilot study was to track the prevalence of *Campylobacter* spp. and *Y. enterocolitica* in pigs from the nursery to slaughter.

Materials and Methods

Production sites. Representative all in/all out as well as continuous flow production systems were selected from Iowa (total of four farms) and North Carolina (total of four farms). Approximately 60 pigs from each farm (total of 240 pigs per state) were followed from the nursery to slaughter. One loop of rectal feces (~1 gram, Jorgenson Laboratories, Loveland, CO.) and tonsilar swabs in buffered peptone water (9 ml, pH 7.4) were collected at the nursery, grower, and finisher stage, and within 48 hours of slaughter. Samples from North Carolina were shipped overnight on ice for processing. Iowa samples were processed on the day of collection or after overnight refrigeration. At slaughter for the Iowa hogs, ileocaecal lymph nodes were excised. Carcass swabs were collected after the acid wash by using a Nasco Speci-Sponge (18 ounce, Fort Atkinson, WI)

rehydrated in 20-ml buffered peptone water either the morning of sampling or the evening before and refrigerated. The three-point protocol of FSIS, which describes swabbing a 100 cm area from ham, belly and jowls, was used. In addition, tonsils were excised. For the North Carolina hogs, at slaughter, only carcass swabs were collected (as described above) before quick chilling.

Bacteriological culture and PCR analysis. Fecal samples were processed for *Campylobacter* as follows (Figure 1). A fecal loop sample was suspended in 9 ml of buffered peptone water at the collection site. Within 12 hours of collection or, if needed, after overnight refrigeration, an aliquot (300 ml) from the fecal suspension was placed in Tran's media (12 ml) for enrichment (7) and incubated microaerobically (2 days, 42_C, Forma model 3130 trigas incubator). An aliquot from the fecal suspension (100 ml) was also plated to Campy Cefex agar for the direct detection of *Campylobacter*. Following Tran's enrichment, an aliquot (100 _l) was plated to Campy Cefex agar and incubated microaerobically (2 days, 42_C, Forma model 3130 trigas incubator). To harvest bacteria from both direct and enrichment plating, a loopful of bacterial growth from the first quadrant was suspended in 200 ml of TE (pH 8.0). Samples were frozen (-20_C) until processed for *C. jejuni* and *C. coli* by using a previously described multiplex PCR assay (3).

For *Y. enterocolitica*, feces and tonsilar swabs were collected in peptone-buffered water (9 ml). A 1 ml aliquot was delivered to 9 ml of ITC and incubated (2 to 3 days at room temperature). DNA was extracted and multiplex PCR assay targeting the *ail* gene and the *yadA* plasmid-encoded virulence factor was performed (Figure 2).

Results and Discussion

In previous studies, *Campylobacter*, primarily *C. coli*, was easily identified in pig fecal samples via a multiplex PCR (3,11). In that earlier study, fecal samples of swine from 16 major hog-producing states, which represent 91% of the U.S. pork industry, were analyzed. Of 1,051 pig fecal samples examined, 69% were positive for *C. coli* and 0.28% were positive for *C. jejuni* (11).

In our study, as summarized in Table 2, for Iowa, *C. coli* was detected in fecal samples collected at the nursery (90.2%), grower (96%), and finisher (93.8%) stages and within 48 hours prior to the slaughter (93.8%). At slaughter, overall *C. coli* was found in 9% of carcass swabs and in 82.75% of ileocaecal lymph nodes. As summarized in Table 3, for the four North Carolina production sites, overall *C. coli* was detected at the nursery (89%), grower (89%), and finisher (88%) stages and within 48 hours prior to slaughter (90%). Similar results have been reported in the Netherlands (10). For the North Carolina hogs, *C. coli* was found on 25.2% of carcass swabs. Ileocaecal lymph nodes were not tested for the North Carolina hogs.

When data from carcass swabs collected in Iowa (9%) and North Carolina (25.2%) are averaged (17.1%), the overall prevalence of *Campylobacter* present on hog carcasses is less than the 33%, reported by FSIS in the national baseline study (9). This may be attributed to differences in carcass sampling methods, or to procedures for *Campylobacter* isolation.

As summarized in Table 3, *Y. enterocolitica* was not detected at any time in hogs in Iowa but in 8.8% in the North Carolina hogs sampled on one farm on one occasion. It was not detected on carcasses, tonsils, or lymph nodes in Iowa hogs nor on carcass swabs collected from North Carolina. In contrast, using the same techniques as in the Iowa and North Carolina portions of the study, *Y. enterocolitica* was identified in 81% of tonsils (n=31) collected in Pennsylvania.

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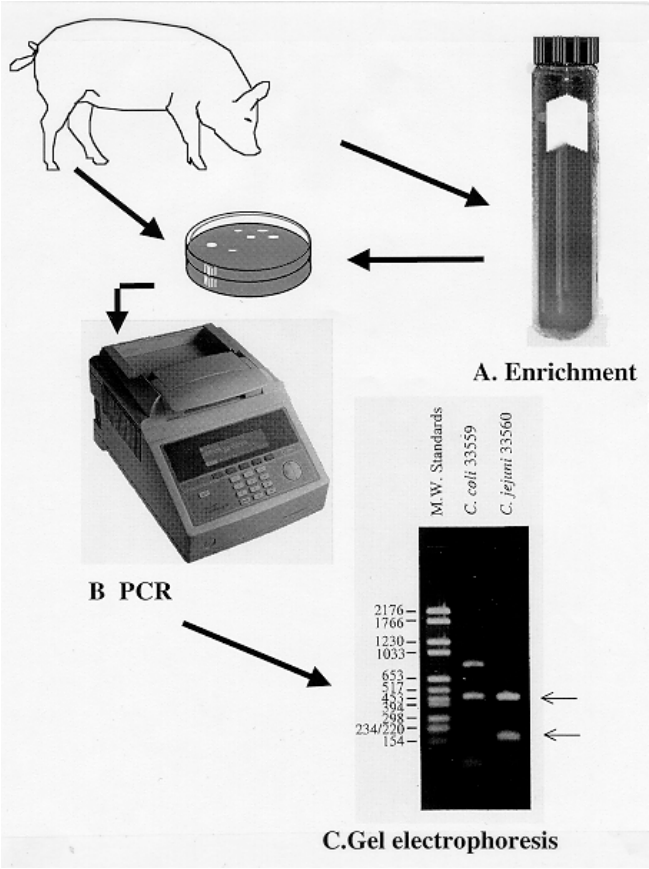

Figure 1. PCR-based detection of thermotolerant *Campylobacter* in poultry. (A) Samples are enriched in Tran's media and then plated to Campy Cefex agar. (B) Bacterial colonies are harvested and processed for PCR. (C) The amplicons are detected by gel electrophoresis. The presence of a 460 bp PCR amplicon) is exhibited by both *C. jejuni* and *C. coli Campylobacter jejuni* exhibits both the 450 bp product and a specific smaller 160 bp amplicon.

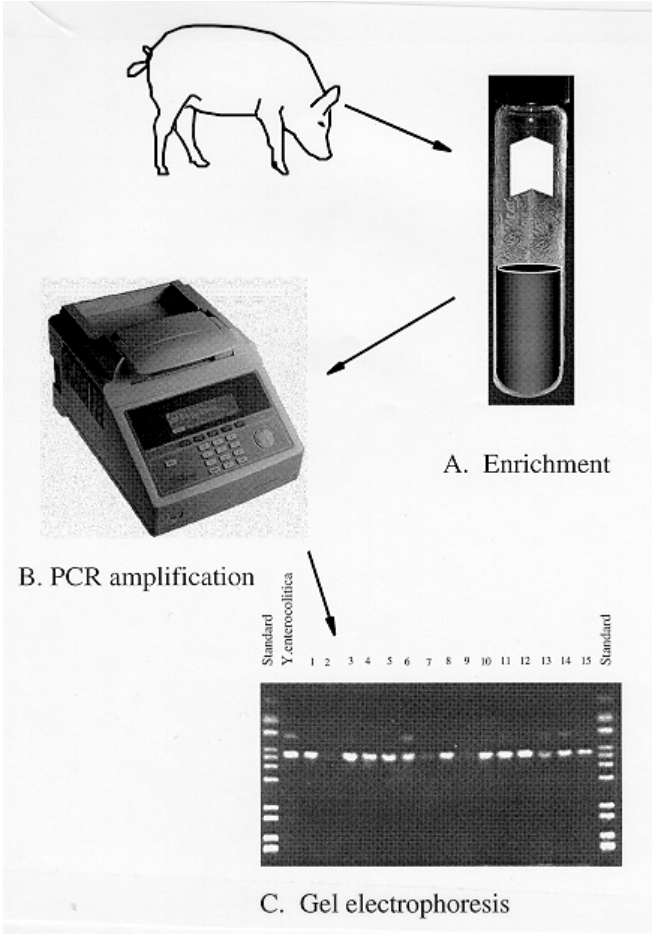

Figure 2. PCR-based detection of *Yersinia enterocolitica* in pigs. (A) Samples are enriched in ITC media (2 or 3 days at room temperature). (B) DNA is extracted and screened by a PCR assay that is specific for the virulent *ail* gene (425 bp) and a plasmid-encoded virulence factor, *yadA* (~300 bp). (C) The amplicons are detected by gel electrophoresis. The PCR amplicons are seen only in pathogenic *Y. enterocolitica* strains.

Table 1. U.S. Public Health Service targeted reductions in major foodborne pathogens. Data are in cases per 100,000 US population (8).

Cases (per 100,000)	1987	2000
*Campylobacter jejun*i	50	25
*Salmonell*a spp.	18	16
*E. col*i 0157:H7	8	4
*L. monocytogene*s	0.7	0.5

Table 2. Distribution (%) of *Campylobacter* in pigs (Iowa).

	Nursery	Grower	Finisher	Preslaughter	Carcass	Lymph nodes	
Farm 1	78	98	98	100	7		70 (16/23)
Farm 2	90	96	85	90	0		88 (23/26)
Farm 3	100	98	94	~95	9		85 (29/34)
Farm 4	93	92	98	90	20		88 (35/40)
AVERAGE	90.2	96	93.8	93.8	9		82.75

Table 3. Distribution (%) of *Campylobacter* in pigs (North Carolina).

	Nursery	Grower	Finisher	Preslaughter	Carcass
Farm 1	77	71	95	97	0
Farm 2	100	97	92	98	21
Farm 3	100	93	83	83	80
Farm 4	80	97	82	82	0
AVERAGE	89.2	89.5	88	90	25.2

Table 4. Summary of Recovery of *Y. enterocolitic*a from pigs

State	Number of Pigs	% Positive
Iowa	240	0
North Carolina	240	2.1
Pennsylvania	31	84