Research Note

Quantification of Campylobacter in Swine before, during, and after the Slaughter Process

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ABSTRACT

Campylobacter has been implicated as a major cause of foodborne illness worldwide. Pigs can be subclinically infected, and fecal contamination of meat during slaughter is a food safety risk. The objective of this study was to determine the association between the concentration of Campylobacter pre- and periharvest with postharvest contamination in swine. Samples were collected from 100 individually identified swine during the pre-, peri-, and postharvest periods. For each animal, the following phases were sampled: on farm (fecal sample), in lairage (hide swab), post-stunning and exsanguination (rectal contents), prechilling (carcass swab), and final product (rib meat) sample. The proportions of samples that were Campylobacter positive were 90, 95, 76, 100, and 49% for fecal, rectal content, hide, carcass, and rib meat samples, respectively. The mean Campylobacter concentrations for each sample were fecal sample, 1.7 \times 10^6 CFU/g; rectal content, 1.2 \times 10^7 CFU/g; hide swab, 1.4 CFU/cm²; carcass swab, 1.7 \times 10^3 CFU per half carcass; and rib meat, 18 CFU/g. There was a positive correlation between Campylobacter concentrations in fecal samples (R = 0.20, P = 0.065) and concentration of Campylobacter on rib meat, and between rectal content sample concentration (R = 0.20, P = 0.068) and the concentration on rib meat. There was no association between the isolation of Campylobacter on rib meat and the isolation of Campylobacter at any pre- or periharvest stage. This could indicate that the risk of a meat product being contaminated is associated with pigs that shed higher concentrations of Campylobacter before slaughter.

Campylobacter spp. are among the most common causes of bacterial diarrhea worldwide (27) and are estimated to cause approximately 845,000 illnesses annually in the United States, and most of these illnesses are food-related (31). Pigs can be subclinically infected with these pathogens; fecal contamination of meat during slaughter is a food safety risk (11, 14, 18, 32). For preharvest control, it is unclear whether intervention strategies should focus more on elimination of pathogens or on decreasing the concentration of pathogens shed by animals, which has significant implications for both the cost and efficiency for preharvest control programs. Although C. jejuni is considered the most important Campylobacter spp. associated with disease in humans, recent studies have highlighted the importance of C. coli (the most commonly isolated species in swine) in causing foodborne illness, with an added challenge of being more frequently resistant to antimicrobial agents (4, 9, 14, 30, 32). The objective of this study was to determine the association between the concentration of Campylobacter in fecal, rectal content, hide, and carcass samples collected before or during the slaughter process, with concentrations in rib meat. The hypothesis was that there would be a positive association between Campylobacter detection and concentration between samples taken before and during harvest when compared with detection and concentration on rib meat.

MATERIALS AND METHODS

Animals. Samples from 100 crossbred (Landrace × Berkshire and the reciprocal cross) market pigs weighing between 95 and 120 kg (4.5 to 6 months of age), raised at The Ohio State University’s Western Agricultural Research Station (South Charleston), were utilized for the study. Pigs were raised in one deep, straw-bedded finishing facility composed of two pens that allowed nose-to-nose contact between pigs. The floor of the facility was a solid concrete base. Pigs were provided a 1.30-m² space allocation per pig, with 90 pigs per pen. The pigs had ad libitum access to feed and water throughout the finishing period. Pigs were transported at an average of 102.2 kg live weight, placed in lairage, and slaughtered after an 18-h fast at The Ohio State University Meat Science Laboratory. Pigs were rendered unconscious by electrical stun, exsanguinated, and placed in a scald tank for 5 min at 61.5°C, and then dehaired, flamed, and rinsed prior to evisceration. The numbers of pigs and the dates of harvest were as follows: 10 on 12 October 2006, 10 on 2 November 2006, 16 on 1 February 2007, 16 on 8 February 2007, 16 on 15 February 2007, 16 on 22 February 2007, and 16 on 1 March 2007.

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Sample collection. Within 18 h of transport to the slaughter facility, a fresh fecal sample (~20 g) was obtained from each individually identified pig. The area of the hide that was most visibly contaminated with mud and feces was swabbed while the pigs were in lairage (~625 cm²), with a sterile sponge. A rectal content sample (~20 g) was obtained from the individually identified pigs immediately postmortem. The entire hot carcass was swabbed after washing and prior to chilling by using three sponges for an entire half carcass. One week after slaughter, ~1 lb (0.453 kg) of rib meat (meat and bone) was obtained from each carcass. All sponges were sterile and premoistened in 10 ml of buffered peptone water (BD, Franklin Lakes, NJ). Three dilutions were made, taking 1 ml from the initial 10⁻¹ dilution and adding to 9 ml of buffered peptone water, which was repeated to a maximum dilution of 10⁻⁴. One hundred microliters from each dilution was plated in duplicate on Campy-Cefex plates (26) and incubated under microaerobic conditions (6 to 16% O₂ and 2 to 10% CO₂) with the use of the GasPak EZ Campy Container System and GasPak EZ Campy Container Sachets (BD) for 48 h at 42°C. Campylobacter suspect colonies (pink colonies of typical morphology) were then counted.

Campylobacter enumeration. Fecal and rectal content samples were processed by mixing the sponge with 30 ml of Bolton broth (Oxoid, Ltd., Hampshire, UK). Ten milliliters of the mixture was added to three tubes, and 1 ml was taken from the 10⁻¹ dilution and added to 9 ml of Bolton broth to make the next dilution and repeated to complete the three-tube, four-dilution most probable number (MPN). The tubes were incubated under microaerobic conditions for 48 h at 42°C. One hundred microliters from each dilution was plated onto Campy-Cefex plates and incubated as previously described. The plates were read “yes” or “no” for Campylobacter suspect colonies.

The three sponges from the carcass swab were pooled and mixed with 90 ml of Bolton broth. A 30-ml aliquot of the pooled carcass swab sample was added to each of three tubes, and 3 ml from the 10⁻¹ dilution was added to 27 ml of Bolton broth to make a three-tube, four-dilution MPN. The samples then were prepared according to the protocol for the hide swabs, as previously described.

One pound (0.453 kg) of meat was mixed with 500 ml of Bolton broth, and 10 ml of this mixture was added to 90 ml of Bolton broth to conduct the three-tube, four-dilution MPN. The samples then were prepared according to the protocol for the hide swab and carcass swabs. Suspect Campylobacter isolates were stored at −80°C in brucella broth (BD) and 10% glycerol for further characterization.

For all samples, Campylobacter MPN was calculated based on the spreadsheet and recommendations by Garthright and Blodgett (12).

Campylobacter confirmation and speciation. Frozen Campylobacter suspect colonies were revived onto Mueller-Hinton (BD) plates for 48 h, under microaerobic conditions at 42°C. Samples that did not grow initially on Mueller-Hinton plates were enriched in Bolton broth for 48 h under microaerobic conditions at 42°C and then plated onto Mueller-Hinton agar and incubated under the same conditions. Catalase (BD) and oxidase (BD) tests were performed, and all colonies that were positive for both in addition to colonies that were positive for oxidase only were grown in brucella broth (BD) for 72 h at 42°C for DNA extraction by using the DNeasy Tissue kit (QIAGEN, Valencia, CA). PCR was performed on the extracted DNA, targeting the hipO gene for C. jejuni and the glyA gene for C. coli (19). C. jejuni subsp. jejuni ATCC 33560 and C. coli ATCC 49941 were used as positive controls for the PCR.

Statistical analysis. Descriptive statistics were performed for prevalence, mean, and median concentration of Campylobacter for each sample type. Spearman’s rank correlation coefficient was calculated to ascertain correlations between meat samples and (i) fecal samples collected on-farm, (ii) rectal contents, (iii) hide swab, and (iv) carcass swab samples. A Spearman’s R is interpreted as a value of 0, indicating no association; 0 to 0.5, indicating a weak positive correlation; 0.5 to <1, indicating a strong positive correlation; and 1, indicating a perfect positive correlation. Univariate odds ratios were calculated to determine the odds of a meat sample being positive when fecal samples, rectal samples, hide swab, or carcass swab samples were positive. All statistics were performed with STATA statistical software (Intercooled STATA 9, StataCorp, College Station, TX).

RESULTS

The proportions of samples that were Campylobacter positive was 90, 95, 76, 100, and 49% for fecal, rectal content, hide swab, carcass swab, and rib samples, respectively. The mean (range, standard deviation) Campylobacter concentration were as follows: feces, 1.7 × 10⁶ CFU per g (0–2.7 × 10⁷, 4.5 × 10⁶); rectal contents, 1.2 × 10⁷ CFU/g (0–1.7 × 10⁸, 3.1 × 10⁷); hide swab, 1.4 CFU/cm² (0–3.7 × 10³, 1.45 × 10³); carcass swab, 1.7 × 10³ CFU per half carcass (1.19–3.7 × 10³, 1.69 × 10³); and ribs, 18 CFU/g (0–5.4 × 10⁴, 1.9 × 10⁴). The median Campylobacter concentrations were as follows: fecal, 1.2 × 10⁵ CFU/g; rectal contents, 1.1 × 10⁶ CFU/g; hide swab, 1.4 × 10⁵ CFU/cm²; carcass swab, 1.2 × 10⁵ CFU per half carcass; and ribs, 1.4 × 10⁴ CFU/g.

A total of 403 putative Campylobacter colonies were frozen and 309 (76.7%) samples were successfully recovered from frozen samples. From the 309 samples, a total of 301 (97.4%) were PCR confirmed as C. coli, and one sample was also PCR positive for C. jejuni. The remaining eight samples were not further tested.

We observed a weak positive correlation (R = 0.20, P = 0.065) between Campylobacter concentrations in feces with Campylobacter concentrations on rib meat as well as a weak positive correlation (R = 0.20, P = 0.068) between the concentration in rectal contents and concentrations on rib meat. The univariate analysis found no associations between the isolation of Campylobacter pre- or periharvest and isolation on meat.

DISCUSSION

The Campylobacter prevalence we observed in fecal samples on farm was within the range of prevalence...
reported by previous authors. The prevalence of *Campylobacter* recovered from the feces of pigs from other studies ranged between 53 and 100% (22–25, 28, 33, 34).

There are few reports of *Campylobacter* detection or concentration in rectal contents from pigs postmortem. A study conducted in 1985 in England from samples taken postmortem found a prevalence of 66% (21), which is somewhat lower than our findings (94.6%). One potential explanation is in sample handling. In our study, samples were kept on ice and cultured within 4 h, while Manser et al. (21) held samples at ambient temperature for up to 48 h.

To our knowledge, this study is the first to sample porcine hides for *Campylobacter*. The high hide prevalence we observed is likely a reflection of the high fecal prevalence, since fecal contamination of hides, whether from the individual pig or as a result of contamination from other pigs in the group, is common. Further, we sampled the most visibly contaminated area of the hide, increasing the probability of *Campylobacter* isolation.

The prevalence of *Campylobacter* we observed on carcasses is much greater than previously reported. A Danish study that included 600 pigs from 152 herds found the prevalence of *Campylobacter* to be 66% on carcasses measured before chilling (5). Another study found the prevalence of *Campylobacter* to be 9 and 0% on the carcasses of pigs before and after chilling, respectively (24). A study in Belgium found a prevalence of 17% on the carcass (600 cm$^2$) (13). The study conducted by Thakur and Gebreyes (33) compared isolation rates of *Campylobacter* on pig carcasses between samples collected previsceralization (about 25%) and samples collected postvisceralization (about 50%) and reported a significant difference between prevalence at these two points in processing. One explanation for the greater proportion of positive carcasses in the present study could be a function of the greater surface area sampled. In this study, an entire carcass side was swabbed, whereas in other studies, only a predefined section was sampled. Time from stun to final wash at the slaughter plant in this study was approximately 40 min, which would be a greater period than observed in some packing plants and which could have contributed to variation in prevalence among trials.

The proportion of positive rib samples contaminated with *Campylobacter* was greater in the present study when compared with other studies of raw pork. Previous reports indicated prevalence ranging from 1.3 to 18.4%, most commonly measured in pork chops (8, 10, 13, 15–17, 23, 35, 37, 38). Several studies have been unable to isolate *Campylobacter* from pork (3, 20, 23, 24). One main difference between the methodologies in the previous studies and in this work was that 1-lb (0.453-kg) samples of rib were cultured. This is a greater weight and different cut of meat (most studies used pork chops) than other studies have utilized. Rib meat as a sample might also help explain the greater isolation rate because of a greater likelihood of contamination from intestinal contents during evisceration as compared with the loin area. Ribs were chosen over pork chops for this study because of the perceived greater likelihood of contamination during evisceration, and because they are the second most consumed fresh pork product in the United States after pork chops (6).

The average *Campylobacter* concentrations reported in the present study were higher than reports in the literature for some sample types. For carcass contamination concentrations, one report observed between 100 and 1,000 times lower concentration than reported in the present study (24). In two studies evaluating pork meat, the concentrations reported were 10 times less than the concentration observed in this study (36). The disparity among reports might be because of the larger surface area sample of carcass swabbed and greater sample weight of meat evaluated in this study, resulting in an increased sensitivity in detecting *Campylobacter*. To the best of our knowledge, this is the only study quantifying *Campylobacter* in feces and on the hide of pigs. We recently completed a similar study in cattle (1) and observed a twofold greater fecal concentration of *Campylobacter* in swine feces when compared with fecal samples from cattle. We also observed an increase in *Campylobacter* concentration in postmortem samples compared with those obtained on the farm when evaluated in cattle at slaughter (1).

The weak positive correlation between the concentration of *Campylobacter* on rib meat with the concentration of *Campylobacter* in fecal samples and rectal contents suggests that fecal concentration might explain at least a small component of the factors that contribute to the risk of carcass contamination. Although the $P$ value was greater than 0.05, we consider that the 7% probability of a type I error in this study, given its design and limitations, does not represent a substantially greater type I error risk than this traditional cutoff. This weak correlation does suggest that other factors contribute to this risk, such as processing techniques. The possibility of cross-contamination might also explain why all carcasses were *Campylobacter* positive.

The lack of an association between detection of *Campylobacter* in any pre- or periharvest sample with the presence of *Campylobacter* on rib meat of the same animal could be a result of the high prevalence of *Campylobacter* in animals in this study, limiting the population of negative animals for risk analysis. Cross-contamination during processing is also a feasible explanation. Group or farm level studies to evaluate the effect of *Campylobacter* status are warranted to further understand *Campylobacter* contamination of swine carcasses. The association found between fecal and meat concentration suggests that interventions which target the reduction of fecal concentration or identification of “high shedders” should be investigated.

Only 500 bacterial cells of *C. jejuni* have been found to cause clinical signs of campylobacteriosis in humans (2, 7, 29). A low infectious dose highlights the importance of reducing the prevalence of *Campylobacter* in retail pork samples. Cross-contamination of *Campylobacter* from raw meat to other surfaces during food preparation is also possible and could result in human illness. Further research on the contribution of raw pork for cross-contamination of food preparation surfaces is warranted.
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