Postchill Campylobacter Prevalence on Broiler Carcasses in Relation to Slaughter Group Colonization Level and Chilling System

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ABSTRACT

Data from an ongoing national surveillance program of Campylobacter prevalence in broiler slaughter groups were related to results from a 1-year baseline study of broiler carcasses postchill. The goals were to establish the relation between Campylobacter prevalence in slaughter groups and on carcasses and to determine the effect of various chilling systems on Campylobacter prevalence. Pooled cloacal and neck skin samples from the surveillance program were analyzed after enrichment. Carcass rinse samples from the baseline study were analyzed after enrichment and by direct plating. Data from both studies were available for 614 carcasses. Direct-plating analyses indicated that the percentages of carcasses positive for Campylobacter jejuni and other Campylobacter spp. in slaughter groups with negative cloacal samples were 2% and 10%, respectively, whereas enrichment analyses indicated prevalences of 2% in both cases. Campylobacter prevalence in slaughter groups with a high degree of intestinal colonization (more than half of the pooled cloacal samples positive) was significantly higher than in slaughter groups with a low degree of colonization (76 to 85% and 30 to 50%, respectively, depending on Campylobacter spp. and analytical method). The prevalence of Campylobacter-positive carcasses postchill was at the same level as the prevalence of carcasses that originated from slaughter groups with positive neck skin samples at four of the six slaughterhouses. Only at one slaughterhouse, with an air-chilling system, was the postchill prevalence (13%) lower than that expected from slaughter group data (23%). The postchill prevalence (43%) was higher than that expected from slaughter group data (33%) at one slaughterhouse with immersion chilling.

Campylobacteriosis is one of the most common bacterial intestinal disorders of humans in many industrial countries. Two Campylobacter spp., C. jejuni and C. coli, account for most human infections (27). Poultry and poultry products are considered important sources of human infections (9). Poultry meat can become contaminated with Campylobacter during slaughter if the live chickens are intestinal carriers of the organism. During the slaughter process, bacterial levels may either increase or decrease at the various process steps. Bacterial numbers often decrease after scalding and increase after evisceration and defeathering (16, 28). Most studies indicate that bacterial numbers decrease after the chilling of carcasses (6, 8, 16, 26, 34, 37), but there is also a risk of cross-contamination during the chilling process (22, 36).

Three major systems are currently used for chilling: (i) cold water (counterflow immersion chilling), (ii) cold dry air, and (iii) a combination of air chilling and water spraying (evaporative chilling). Chlorine could be added to the water used for chilling, but current European Union regulations do not allow the use of chlorinated water, and only potable water without additives is used at Swedish slaughterhouses. Differences between the effects of various chilling systems on bacterial numbers have been investigated in a number of studies, but the results vary and are, to some extent, contradictory. Sanchez et al. (33) found that the prevalence of Campylobacter and Salmonella was lower in broilers from an air-chilling facility than in broilers from a slaughterhouse with immersion chilling. Oosterom et al. (28) reported that the reduction of Campylobacter numbers after air chilling was more variable than after immersion chilling. Allen et al. (1) showed that chilling in chlorinated water resulted in decreased levels of aerobic microorganisms, coliforms, and Pseudomonas spp. Dry air chilling did not affect skin contamination, but it did reduce bacterial numbers in the body cavity. Water spraying, especially with nonchlorinated water, was associated with increased bacterial numbers in the body cavity.

Many broiler production facilities with programs that monitor Campylobacter target flock prevalence (2) rather than percentage of contaminated carcasses, and establishing the relation between flock prevalence and carcass prevalence would be useful for risk assessment purposes. For example, Rosenquist et al. (32) used flock prevalence as an estimate of Campylobacter prevalence on individual carcasses. This was based on the assumption that the probability that a flock will contain both positive and negative birds is low. An alternative approach is to model within flock prevalence over time as a function of parameters, such as flock size and the estimated time of first exposure to Campylobacter (15).

In this article, we combined data from a 1-year baseline study of Campylobacter prevalence on broiler carcasses.
postchill with data for the same time period from an ongoing Campylobacter surveillance program for broilers in Sweden. The goals were to determine the relation between the degree of intestinal colonization in a slaughter group, as estimated by the surveillance program and the prevalence and numbers of Campylobacter on carcasses postchill. Additionally, our goal was to estimate the effect of various chilling systems used at Swedish slaughterhouses on Campylobacter prevalence postchill.

MATERIALS AND METHODS

Cloacal and neck skin samples. Cloacal and neck skin samples have been collected since 2001 in Sweden as part of the Campylobacter Surveillance Program (14). For that project, all slaughter groups sent for slaughter were sampled at the slaughterhouses to estimate the slaughter group prevalence of thermophilic Campylobacter spp. Cloacal swabs were collected after stunning and bleeding but before scalding. From each slaughter group, 40 individual cloacal swabs were collected. Each individual cotton swab contained about 0.5 g of feces, and 10 swabs were pooled to form one sample. From each slaughter group, 10 individual neck skin samples, each measuring about 2 cm², were collected from the carcasses before chilling and pooled to form one sample. The samples were pooled in capped plastic tubes containing 10 ml of Cary-Blair transport medium (7) with 0.16% agar (21).

Most samples were shipped on the day of sampling, together with an identification report, and arrived the following day at the National Veterinary Institute. However, samples collected on a Friday were stored at +4°C and sent to the laboratory the following Sunday or Monday.

Culturing was performed within 24 h of sampling, except for the samples collected on Fridays. Before 1 July 2003, cloacal samples were cultured by placing two sterile cotton swabs in each tube, along with the 10 pooled cloacal swabs, in Cary-Blair transport medium (Difco, Becton Dickinson, Sparks, Md.; Merck, Darmstadt, Germany). The two cotton swabs were then immersed in 5 ml of Preston Campylobacter selective enrichment broth (PEB; Oxoid, Basingstoke, UK; Difco, Becton Dickinson; and Merck). To facilitate the reallocation of limited resources within the surveillance program, the pooling protocol was changed on 1 July 2003. Since then, four pooled cloacal samples from the 40 cloacal swabs have been pooled into two samples in the enrichment broth at the laboratory. For the culturing of neck skin samples, about 10 g from each sample is transferred to PEB in a ratio of 1:9. The samples in PEB are incubated microaerobically (Campygen, Oxoid) at 42°C for 48 h. About 20 μl (two loopfuls) of the PEB culture is streaked on Preston Campylobacter selective agar (Oxoid; Difco, Becton Dickinson; and Merck) and incubated microaerobically (Campygen, Oxoid) at 42°C for 48 h. Identification of Campylobacter is based on colony morphology, microscopic appearance, and the following phenotypic characteristics: motility, production of oxidase and catalase, and results of the hippurate hydrolysis reaction (23).

Carcass rinse samples. A 1-year microbiological baseline study was performed from September 2002 to August 2003 (20). In total, 636 broiler carcasses were analyzed in the postchill group. The numbers of carcasses sampled from each slaughterhouse were proportional to the annual numbers of slaughtered broilers at each site. Each sample consisted of a randomly selected carcass and was placed in double plastic bags and shipped overnight in an insulated box with refrigerant gel packs to the National Food Administration. The temperature and weight of each carcass were registered on arrival at the laboratory. Samples with a temperature of 10°C or less were accepted.

Carcass rinse samples were analyzed to estimate the prevalence and levels of a number of indicator organisms and pathogenic bacteria. The carcasses were rinsed in 400 ml of buffered peptone water, and analyses were performed on aliquots of the rinse fluid. Thermophilic Campylobacter were analyzed qualitatively by enrichment of 10 ml of rinse fluid in 100 ml of PEB for 24 h at 42°C and then isolation on Campylobacter blood-free selective medium (CBFS; Oxoid). Quantitative analyses were performed by direct plating serially diluted rinse fluid on CBFS agar. All CBFS agar plates were incubated microaerobically for 48 h at 42°C. One isolate (enrichment) or up to five isolates (direct plating) were incubated microaerobically overnight at 42°C on blood agar. For confirmation of Campylobacter, one isolate from each analytical method was examined microscopically for characteristic morphology and motility. Data from samples in which isolates failed to grow on blood agar were excluded. One colony from the enrichment procedure and up to five colonies from the direct-plating procedure were frozen in brain heart infusion broth with 20% glycerol and stored at −70°C until the autumn of 2003 for species identification by PCR assays. First, a real-time PCR assay (25) was used to identify C. jejuni. Then, negative samples from the real-time PCR were analyzed by a PCR assay that detected thermophilic Campylobacter (10). Isolates positive by this analysis were regarded as thermophilic Campylobacter other than C. jejuni. Isolates negative by both methods were regarded as unidentified Campylobacter spp. Later on, in the spring of 2005, a real-time PCR assay (19) was used to determine whether thermophilic Campylobacter other than C. jejuni could be identified as C. coli.

Merging data from the two studies. Campylobacter data from the baseline study were combined with slaughter group data from the surveillance program. Data from both studies were available from 611 carcass rinse samples analyzed after enrichment and 594 samples analyzed by direct plating (for a total of 614 samples analyzed by either method). For each individual carcass, the numbers of positive pooled cloacal and neck skin samples in the slaughter group from which it originated were indicated.

Statistical analysis. Differences in postchill prevalences of Campylobacter-positive carcasses between slaughter groups with different degrees of intestinal colonization were examined by the χ² test. Analysis of variance was used to test for differences in mean log numbers of Campylobacter on positive carcasses. The within-group prevalence was considered low in slaughter groups with postchill test results from one fourth or one half of the cloacal samples (one or two of four pooled samples from September 2002 to June 2003 and one of two pooled samples from July to August 2003). The within-group prevalence was considered high for slaughter groups when more than half all or four of the cloacal samples were positive. Analyses of variance and χ² tests were performed with Minitab version 12.22 software (Minitab Inc., State College, Pa.).

The effect of chilling on the prevalence of Campylobacter spp. was assessed by comparing the percentage of carcasses originating from positive slaughter groups prechill (i.e., groups with positive neck skin results) with the percentage of carcasses positive postchill (as estimated by analyses after enrichment) at the six largest slaughterhouses in Sweden. Slaughterhouses A, B, and C use dry air-chilling systems, D uses an evaporative system, E uses immersion chilling, and F uses immersion chilling or a combination of immersion and dry air chilling. The numbers of samples per slaughterhouse (A through F) were 55, 61, 221, 99, 49, and 82. McNemar’s test for dependent proportions was used to test the null hypothesis that the percentage of carcasses originating...
from positive slaughter groups did not differ from the percentage of positive carcasses postchill at each slaughterhouse. Differences between enrichment techniques in the surveillance program and the baseline study were assumed to be negligible, because the same enrichment broth was used in both studies, and isolation was carried out on two media (Preston Campylobacter selective agar and CBFS) with similar performance profiles (29).

**RESULTS**

**Number of positive samples.** Of the 614 carcass samples included in the study, 103 (17%) originated from slaughter groups with at least one positive pooled cloacal sample, and 107 (17%) originated from slaughter groups with positive pooled neck skin samples. Eighty-five (14%) of the 614 carcass rinse samples analyzed by enrichment were positive for *Campylobacter* spp. (confirmed microscopically), and 82 (14%) were positive for *C. jejuni* (positive by real-time PCR). Of the 594 carcasses rinse samples analyzed by direct plating, 122 (21%) were positive for *Campylobacter* spp., and 75 (13%) were positive for *C. jejuni* (at least one isolate per sample tested positive by real-time PCR).

In total, 109 of 468 isolates confirmed microscopically as *Campylobacter* were negative in real-time PCR for *C. jejuni*. Further PCR analyses of these 109 isolates showed that 74 were positive and 35 were negative in the PCR assay for thermophilic *Campylobacter*. Thirty-eight of the 74 positive isolates could be identified as *C. coli* by real-time PCR.

**Relation between slaughter group and postchill *Campylobacter* prevalence.** The percentage of *C. jejuni*-positive carcasses from slaughter groups in which no cloacal samples tested positive was low (2%), irrespective of the analytical method (Table 1). About half of the *C. jejuni*-positive samples from slaughter groups with negative cloacal samples (6 of the 10 samples positive after enrichment and 5 of the 11 samples positive by direct plating) originated from slaughter groups with positive neck skin samples, indicating a contamination of the slaughter group after arrival at the slaughterhouse. Enrichment analyses also resulted in a low estimate (2%) of the prevalence of *Campylobacter* spp. in slaughter groups with negative cloacal samples. However, direct-plating analyses resulted in a higher percentage (10%) of *Campylobacter* spp.–positive carcasses (Table 1).

The percentages of *Campylobacter* spp.–positive or *C. jejuni*–positive carcasses were significantly higher for slaughter groups with a high degree of intestinal colonization (all or more than half of the cloacal samples tested positive) than for slaughter groups with a low degree of colonization (one fourth or one half of the cloacal samples tested positive) (χ² test, enrichment P < 0.01 and direct plating P < 0.001). Between 76 and 85% (depending on *Campylobacter* spp. and analytical method) of the carcasses originating from the high-colonized groups tested positive, whereas 30 to 50% of the carcasses from the low-colonized slaughter groups tested positive (Table 1).

The mean log numbers of *Campylobacter* spp. on carcasses that tested positive by direct-plating analyses differed significantly among slaughter groups with differing degrees of colonization (analysis of variance, P < 0.001). The numbers of *Campylobacter* spp. on positive carcasses from slaughter groups with a high level of intestinal colonization were about 10 times as high as the numbers on positive carcasses from slaughter groups with no positive cloacal samples (Table 2).

**Campylobacter prevalence pre- and postchill at different slaughterhouses.** At four of the six slaughterhouses, postchill prevalences of *Campylobacter* were similar to those expected from prechill neck skin test results (Fig. 1). Various chilling systems (dry air, evaporation, or a combination of immersion and dry air chilling) were used at these four establishments. Only at one slaughterhouse (C), with a dry air–chilling system, was the postchill prevalence...
FIGURE 1. Observed percentages of broiler carcasses testing positive for Campylobacter spp. postchill in relation to expected percentages (under the assumption that all carcasses from slaughter groups with positive prechill neck skin samples will be positive postchill). Slaughterhouses A, B, and C with dry air-chilling systems; D, an evaporative system; E, immersion chilling; and F, immersion chilling or a combination of immersion and dry air chilling. Error bars indicate 95% confidence intervals.

DISCUSSION

Using data from two independent studies, we examined the relation between Campylobacter slaughter group prevalence prescalding or prechill and the percentage of Campylobacter-positive carcasses postchill. In general, the results of the surveillance program were corroborated by the results of the baseline study. The prevalence of contaminated carcasses postchill increased with increasing degrees of intestinal colonization in the slaughter groups. Comparing results that were based on similar analytical methods in both studies (enrichment before plating) showed that only 2% of the carcasses that originated from slaughter groups testing negative prescalding were positive for Campylobacter spp. postchill. About half of these originated from groups with negative cloacal samples prescalding but positive neck skin samples prechill, which indicated that they had been contaminated during the slaughter process.

Enrichment is supposed to be the optimal method for the recovery of Campylobacter under stress conditions, such as from poultry carcasses. However, direct-plating analyses indicated that 10% of broiler carcasses from slaughter groups with negative cloacal samples actually carried Campylobacter spp. postchill. Because the percentage of carcasses positive for C. jejuni in direct-plating analyses was lower (2%), the results indicate that most of these carcasses were contaminated with Campylobacter spp. other than C. jejuni. In PCR analyses, 74 of the 468 strains isolated from broiler carcasses were confirmed as thermophilic Campylobacter other than C. jejuni, and about half of these could be identified as C. coli. This species is the second most common species found on broiler chickens (3, 17, 35), and certain strains of C. coli are known to be sensitive to polymyxine B (4, 11), a component of PEB. This may explain the difference between the results of the surveillance program (by enrichment analyses) and the direct-plating results from the baseline study. C. jejuni is the species most commonly involved in human campylobacteriosis (24, 31), and if only this species is considered, there were few positive carcasses originating from slaughter groups with negative surveillance results. It is also noteworthy that the average number of Campylobacter spp. on positive carcasses from slaughter groups with negative cloacal samples was relatively low, about 1,000 CFU per carcass, compared to about 10,000 CFU per carcass for positive carcasses from slaughter groups with positive cloacal samples.

Because the spread of Campylobacter within a flock may occur within a few days once it has become colonized, it is often assumed that all birds in a Campylobacter-positive flock are contaminated at the time of slaughter (18, 32). This is corroborated by several studies that indicate prevalence figures reaching 100% (5, 12, 30). However, our results show that this is not always the case. Low- and high-colonized slaughter groups yielded maximum levels of 50 and 85%, respectively, of postchill carcasses that were Campylobacter positive. The reason for the lower percentage of contaminated carcasses in slaughter groups with a low degree of intestinal colonization prescalding may be the result of the relatively recent introduction of Campylobacter into the flock. The late introduction of Campylobacter into a flock decreases the probability that an individual bird is Campylobacter positive at slaughter (15). Alternatively, a low number of positive cloacal samples may indicate that part of a Campylobacter-free slaughter group has become contaminated during transportation from the producer to the slaughterhouse.

Our results indicate that changes in Campylobacter prevalence during chilling were insignificant at four of six slaughterhouses, although the low prevalence at some of the establishments makes it difficult to assess any minor chilling effects. The postchill prevalence was higher than expected on the basis of prechill data at one of the two slaughterhouses with immersion chilling. In this case, chill water may be thought to be a possible source of cross-contamination. Conversely, the percentage of contaminated carcasses postchill was lower than expected at one slaughterhouse with dry air for chilling (C in Fig. 1). A possible explanation is that air chilling may reduce levels of Campylobacter by drying the carcass skin. No obvious effects on Campylobacter prevalence were noted at the other slaughterhouses with air-chilling systems. However, a study of the transmission of Campylobacter to chickens during transport to slaughter (13) showed that Campylobacters were found in 85% of transport crates from slaughterhouse C and in 28% from slaughterhouse E. Contamination of chickens from transport crates could result in low levels of Campylobacter in cloacae and on neck skins. Hence, the reduction of Campylobacter prevalence postchill at this slaughterhouse may reflect a relatively low level of contamination on carcasses before chilling.

In conclusion, our results show that surveillance data at the flock level are correlated with Campylobacter carcass...
prevalence, although a within-flock prevalence lower than 100% can be expected, especially in low-colonized slaughter groups. No consistent effects of chilling on Campylobacter prevalence postchill were observed. Rather, it seems likely that possible chilling effects should be evaluated at various slaughterhouses individually.

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REFERENCES

20. Lindblad, M. Unpublished data.