Genotyping of *Campylobacter jejuni* from Broiler Carcasses and Slaughterhouse Environment by Amplified Fragment Length Polymorphism

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**ABSTRACT**

We examined the occurrence and diversity of *Campylobacter jejuni* on broiler carcasses during slaughter of an infected flock and in the slaughterhouse environment during slaughter and postdisinfection before a new production run. During the slaughter of a known *C. jejuni* infected broiler flock, samples were taken from broiler carcasses at 7 different stages during the process. Thirty-seven sites in the slaughterhouse environment were sampled both during process and postdisinfection. The samples were analyzed for *C. jejuni*, and genetic fingerprinting was performed using amplified fragment length polymorphism. All carcass samples were positive. Of the environmental samples collected during slaughter, 89% were positive; 100% of those from the arrival, stunning, scalding, defeathering, and evisceration facilities and 67% of those from the cooling and sorting facilities. Postdisinfection, 41% of the samples were positive; 71% of those from the arrival and stunning area, 60% of those from the scalding and defeathering area, and 20% of those from the evisceration, cooling, and sorting area. The *C. jejuni* isolates (n = 60) recovered were grouped into 4 different amplified fragment length polymorphism clones with a similarity index of 95% or greater. All isolates obtained from the flock and 94% of the isolates obtained from the environment during slaughtering belonged to clone A, whereas 1 environmental isolate belonged to each of the clones B and C. Isolates from clones A, B, and D were present postdisinfection. Only clone B was detected on flocks slaughtered during the previous week. The high level and continuous presence of *Campylobacter* in the environment constitutes a risk for transmission to negative carcasses. In Norway, where above 96% of the broiler flocks are *Campylobacter*-negative, this aspect is of special importance. The ability of *Campylobacter* to remain in the slaughterhouse environment through washing and disinfection is associated with constructional conditions of equipment and buildings, complicating cleaning and providing sufficient moisture. To reduce the probability of the workers acquiring campylobacteriosis, precautions should be taken when slaughtering *Campylobacter*-positive flocks.

**Key words:** *Campylobacter jejuni*, broiler, slaughterhouse, genotyping, amplified fragment length polymorphism

©2006 Poultry Science Association Inc.
Received March 18, 2006.
Accepted August 4, 2006.
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**INTRODUCTION**

Campylobacteriosis is currently the most frequently reported bacterial enteric infection in humans in Norway, as well as in many other developed countries (Blaser et al., 1983; Altekruse et al., 1999; Friedman et al., 2000). There was a doubling in the reported incidence rate in Norway from 1997 to 2001 (Nygård and Kapperud, 2005). Similar trends have been reported from other developed countries (Friedman et al., 2000). Two case-control studies conducted in Norway during 1989 and 1990 and 1999 and 2000 identified consumption of poultry meat purchased raw as a significant risk factor in regard to campylobacteriosis (Kapperud et al., 1992, 2003). Minimizing consumers’ exposure to *Campylobacter* from broilers is an important measure for preventing campylobacteriosis in humans.

In Norway, an action plan against *Campylobacter* in broilers was implemented in 2001 to reduce the consumers’ exposure to *Campylobacter* through domestically produced broiler meat (Hofshagen and Kruse, 2005). The action plan consists of 3 parts: 1) a surveillance program including on farm and at slaughter sampling of all Norwegian broiler flocks slaughtered before 50 d of age, 2) a follow-up advisory service on farms with *Campylobacter*-positive flocks, and 3) surveys of broiler meat products at a retail level. The meat from *Campylobacter*-positive flocks detected at farm level is either frozen or heat-treated. After implementation of the action plan, there has been a steady reduction in the frequency of *Campylobacter*-positive broiler flocks in Norway, with a reduction from 6.3% in 2002 to 3.3% in 2004 (Hofshagen and Kruse, 2005). The proportion of broiler flocks colonized with *Campylobacter* in Norway must be regarded as low, compared with other countries with proportions varying from about 10 to 90% (Newell and Fearnley, 2003). This may be due to a
great extent be due to the production system of broilers in Norway with a limited number of small-scale farms with high biosecurity levels (Hofshagen and Kruse, 2005; Johnsen et al., 2006). *Campylobacter*-positive broilers entering the slaughter line can cause extensive cross-contamination to noninfected carcasses and to the slaughterhouse environment. Cross-contamination from *Campylobacter*-positive flocks to negative birds during slaughter and processing and to the environment represents a significant challenge for the poultry industry (Corry and Atabay, 2001; Newell et al., 2001; Garnett et al., 2004). Some studies indicate that certain *Campylobacter* clones can survive in the slaughterhouse environment (Newell et al., 2001; Alter et al., 2002), but data are too limited to support the theory that surviving clones represent a significant public health risk. More studies are needed to investigate this.

Both the enormous diversity of genotypes and the potential for genetic instability in *Campylobacter* presents challenges in the choice of typing methods (Wassenaar and Newell, 2000). Reports of genetic instability have been anecdotal but also based on observations (Wassenaar and Newell, 2000). Studies describe recombination events in the flagellin locus and suggest rearrangements on a genomic scale (Wassenaar and Newell, 2000). A number of methods have been developed for investigation of genetic diversity among *Campylobacter*. The methods differ in their taxonomic range, discriminatory power, reproducibility, and ease of interpretation and standardization. Amplified fragment length polymorphism (AFLP) genotyping is a comparatively rapid method based on PCR and capillary electrophoresis that combines universal applicability with high discriminatory power (Lindstedt et al., 2000). This technique is regarded less sensitive to genetic rearrangements (Wassenaar and Newell, 2000).

The aim of the current study was to examine the occurrence and diversity of thermotolerant *Campylobacter* on broiler carcasses during slaughter of an infected flock and in the slaughterhouse environment during slaughter and postslaughter and disinfection before a new production run.

**MATERIALS AND METHODS**

**Sampling**

One poultry slaughterhouse in the southwestern part of Norway was studied during 2 d in the middle of August 2004. The slaughterhouse is 1 of the 5 largest poultry slaughterhouses in Norway. The slaughterhouse was visited twice; during the slaughter of 1 specific known infected broiler flock, as well as on the following morning after washing and disinfection before the subsequent days’ slaughter commenced.

The slaughterhouse was divided into 7 areas according to the ongoing activity: arrival, stunning, scalding, defeathering, evisceration, meat control, and sorting-packing. In each area, 1 to 4 samples were taken from broilers (n = 14) and from 5 to 6 environmental sites both in direct and indirect contact with the carcasses (n = 37). The same environmental sites were also sampled the following day. The samples were collected during the sampler’s 3-h walk through the slaughterhouse, from arrival and onwards. All samples from d 1 were collected during the slaughter of the *Campylobacter*-infected flock under study. All samples from d 2 were collected postdisinfection, before any process was started up. The sampling areas and the number of samples per area are presented in Table 1.

The sampling of specimens from broilers from the infected flock under examination was performed as follows: An area of at least 100 cm² of 10 dirty transport containers was swabbed, transferred to Buffered Peptone Water (BPW; CM0509, Oxoid Ltd., Basingstoke, UK), and pooled to 1 sample in the laboratory. For the stunning, scalding, and defeathering stages, an area of at least 100 cm² of the surface of each of 10 carcasses per stage was swabbed, transferred to BPW, and pooled by tens in the laboratory. For the evisceration and sorting stages, 30 samples per stage, each of at least 100 g of neck skin, were pooled by tens into sterile sample containers. In addition, at evisceration, 40 whole ceca were collected from the start, middle, and end of the flock under study, transferred to sterile sample containers, and the contents were pooled by tens in the laboratory.

The sampling of specimen from the environment was performed as follows: An area of at least 100 cm² of each of 10 cleaned transport containers, various equipment, floor, and drain was swabbed and transferred to BPW. Air was sampled with a specialized air sampler (MicroBio Air Sampler MB2, F. W. Parrett Ltd., London, UK) directly onto modified CCDA agar (CM0739 and SR0155, Oxoid Ltd.) according to the manufacturer’s instructions. In addition, 50 mL of the processing water used and 50 g of material was collected from the drains for analysis.

**Cultivation of Samples**

Fecal and cecal material were plated onto modified CCDA and incubated microaerobically (CampyGen, CN0025, Oxoid Ltd.) for 40 to 48 h at 41.5 ± 0.1°C. Nonfecal material was enriched in Bolton broth (CM0983, SR0183, and SR0048, Oxoid Ltd.) microaerobically for 20 to 24 h at 41.5 ± 1°C and then 10 µL was plated on modified CCDA for *Campylobacter* isolation. To obtain a pure culture, 1 typical *Campylobacter* colony from each positive agar plate, verified by phase-contrast microscopy, was suspended in BPW and applied to a sterile 0.45-µm membrane filter placed on a blood agar plate (CM 0331, Oxoid Ltd.), the filter was removed after 15 to 30 min, and the plates were incubated as described above. Isolates were identified to species level using a multiplex PCR method described previously (Wang et al., 2002).

**AFLP Genotyping**

DNA was extracted in a biorobot (EZ-1; Qiagen Instruments AG, Hilden, Germany) according to the manufac-
RESULTS

Occurrence of Thermotolerant Campylobacter

Thermotolerant Campylobacter was isolated from 62 of the 88 samples, from all 14 samples from broilers (100%) and from 33 (89%) and 15 (41%) of the 37 environmental sites during process and the postdisinfection samplings, respectively. In total, 62 isolates were obtained for further analyses. All these isolates were identified as C. jejuni.

During the slaughter of the positive flock, all environmental samples (swabs, process water, material, and air) from the facilities where birds arrive, are stalled, stunned and bled, from the scalding and defeathering room and evisceration hall were C. jejuni-positive. Only 4 out of 12 samples taken in the sorting and packing hall and cooling tunnel were Campylobacter-negative (Table 1). In whole, 6 of the 8 air samples and 13 of the 14 samples from drain and floor were positive for C. jejuni when sampled during slaughter of the Campylobacter-positive flock.

Postdisinfection before any process had started on day two, 41% of the 37 environmental samples were C. jejuni-positive. The C. jejuni-positive samples were collected from moist surfaces and materials. In total, 71% of samples from the facilities where birds arrive, are stalled, stunned and bled, from the scalding and defeathering room and evisceration hall were positive for C. jejuni, as opposed to 20% of the samples from the evisceration hall, the sorting and packing hall, and the cooling tunnel (Table 1). Half of the samples from drain and floor were positive for C. jejuni postdisinfection. The organism was also detected from the air samples collected at the lairage area, but was not processed further due to loss of viability on freezing.

From the Norwegian action plan against Campylobacter in broilers, 5 C. jejuni isolates were obtained: 1 from the studied flock and 3 and 1 isolates from each of 2 different broiler flocks slaughtered 1 and 5 d earlier, respectively. These additional isolates collected outside the study period were from the only Campylobacter-positive broiler flocks slaughtered during the week before. These additional isolates were included to obtain optimal explanation of the results.

Table 1. Occurrence of Campylobacter jejuni on carcasses from a known infected broiler flock, in the slaughterhouse environment during slaughtering of this flock and postdisinfection, according to sampling site

<table>
<thead>
<tr>
<th>Sampling site</th>
<th>During slaughter</th>
<th>Postdisinfection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item</td>
<td>No. of positive samples/ sampling sites</td>
<td>AFLP clone type</td>
</tr>
<tr>
<td>Broiler carcasses</td>
<td>14/14</td>
<td>A (n = 14)</td>
</tr>
<tr>
<td>Studied flock</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Environment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arrival</td>
<td>4/4</td>
<td>A (n = 4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scalding</td>
<td>4/4</td>
<td>A (n = 4)</td>
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<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Defeathering</td>
<td>2/2</td>
<td>A (n = 2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evisceration</td>
<td>5/5</td>
<td>A (n = 5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meat inspection</td>
<td>5/5</td>
<td>A (n = 5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sorting and packing</td>
<td>8/12</td>
<td>A (n = 8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>47/51</td>
<td>A, B, C</td>
</tr>
</tbody>
</table>

1The amplified fragment length polymorphism (AFLP) clone types of the isolates are indicated.
2Two isolates were not processed further due to loss of viability on freezing.
Characterization of Isolates

Of the 62 isolates, 2 isolates from the sampling postdisinfection on d 2 were lost during freeze storage. Five additional broiler isolates were obtained from the national action plan against Campylobacter in broilers, leaving 65 isolates for inclusion in the AFLP analysis. A total of 12 distinct AFLP profiles were identified among the 65 C. jejuni isolates. Isolates with 95 to 100% similarity were regarded as belonging to the same AFLP clone, resulting in 2 different clusters (A and B) and 3 single isolates with <95% similarity with any of the other isolates (clones C, D, and E), shown in the dendrogram (Figure 1). All isolates from the studied broiler flock belonged to cluster A, together with 31 of the 33 environmental isolates obtained during slaughter and 9 of the 13 environmental isolates obtained postdisinfection. Cluster B (n = 7) consisted of isolates from drains in the stunning room and 65 isolates from drains in the stunning room during slaughter and 9 of the 13 environmental isolates obtained during slaughter and 9 of the 13 environmental isolates obtained postdisinfection. Cluster B (n = 7) consisted of isolates from drains in the stunning room during process, from cleaned transport containers, and from blood drain in the stunning room postdisinfection. Isolates from a broiler flock slaughtered 1 d before the studied flock also belonged to cluster B. The isolate belonging to clone C was obtained from the stabbing device during processing of the infected flock under study. Campylobacter was not detected on the stabbing device postdisinfection. The isolate belonging to clone D was obtained postdisinfection from the floor in the arrival hall. During slaughter, the samples from broiler droppings on transport containers (DNA no. G200 in Figure 1), blood drain, and floor in the arrival hall contained Campylobacter belonging to cluster A. Clones C and D were not detected on any broiler flocks slaughtered during the week before the flock of interest. Clone E, an isolate obtained from the action plan, was not found during the 2 d of sampling, but was obtained from a broiler flock slaughtered 5 d before this.

DISCUSSION

Samples obtained from the slaughterhouse environment during slaughtering of a Campylobacter-positive broiler flock were found to be contaminated with C. jejuni. This is reinforced by others and is likely due to high numbers of Campylobacter in the poultry intestines, with levels up to 10^6 cfu/g of intestinal content and also the fact that close to 100% of the birds in an infected flock are infected (Corry and Atabay, 2001; Sahin et al., 2002; Newell and Fearnley, 2003). In contrast to the slaughter of larger animals, the nature of poultry slaughter is highly automated, involving certain production steps, making cross-contamination unavoidable (Corry and Atabay, 2001). The presence of Campylobacter in the air samples, especially in the stalling, stunning, and defeathering facilities where the air is especially dusty and humid, has also been found by others (Corry and Atabay, 2001; Whyte et al., 2001) and may be a contributing factor in campylobacteriosis of poultry workers (Wilson, 2004). Due to the low numbers of positive broiler flocks, the Norwegian poultry workers are not constantly being exposed to Campylobacter, making them more vulnerable for infection due to a low degree of acquired immunity (Wilson, 2004).

The high number of sites contaminated with C. jejuni postdisinfection the morning after slaughter of an infected broiler flock is probably due to insufficient cleaning and disinfection. Campylobacter jejuni was most often found in humid and wet places in the slaughterhouse. This is in accordance with reports that Campylobacter survives best in wet environments (Park et al., 1991; Corry and Atabay, 2001). The survival of Campylobacter in the environment through washing and disinfection may be due to Campylobacter remaining in biofilm layers. Campylobacter has been shown to survive for more than 1 wk and is reported to have an increased resistance to disinfection agents when present in biofilms (Buswell et al., 1998; Trachoo et al., 2002) and when internalized by protozoa (Axelsson-Olsson et al., 2005; Snelling et al., 2005, 2006). Contaminated process water and equipment coming in direct contact with carcasses may pose a risk for Campylobacter being transmitted to uncontaminated carcasses. In addition, cross-contamination of separate days production run has also been reported (Corry and Atabay, 2001).

During slaughtering, the slaughterhouse environment was dominated by C. jejuni belonging to the same cluster (A) as those from the infected broiler flock, whereas a relatively higher genetic diversity was detected among the C. jejuni isolated postdisinfection, considering the number of isolates. This finding is probably due to a masking during slaughter of those Campylobacter strains being present in the slaughterhouse environment in low numbers by the dominating broiler cluster. Such a masking is not surprising, taking into consideration the high levels of Campylobacter in the slaughtered birds (Corry and Atabay, 2001).

The 2 clones found in the stunning area during processing (C) and on the floor in the arrival hall postdisinfection (D) may originate from the processed flock, assuming this flock was infected with more than 1 clone. Both clones were isolated from unclean and humid areas where adequate cleanliness is difficult to achieve. A more plausible explanation is that clones C and D originated from earlier slaughtered flocks, and evidence suggests that these may be associated with laying hens slaughtered 6 d before the study being carried out. It is known that adult hens carry a diverse flora of Campylobacter (Jacobs-Reitsma et al., 1995). Isolates of these 2 clones may have remained in the slaughterhouse environment, protected in biofilm layers. Some Campylobacter subtypes are reported to survive in the slaughterhouse environment better than others (Newell et al., 2001; Alter et al., 2002). The clone detected on the floor of the arrival hall (D) may have been introduced by trailers bringing poultry from farms.

The genetic diversity of the Campylobacter population in young birds is discussed. The low genetic diversity obtained among Campylobacter from broilers in the same flock is supported by others (Newell and Fearnley, 2003; Johnsen et al., 2006). On the other hand, more heterogene-
Figure 1. Dendrogram based on amplified fragment length polymorphism (AFLP) fragment patterns of 65 *Campylobacter jejuni* isolates from broiler carcasses and slaughterhouse environment during slaughtering of an infected broiler flock and the next morning postdisinfection, with sampling time, sampling place and site, strain identity, and AFLP clone.
city has also been reported (Corry and Atabay, 2001; Sahin et al., 2002; Newell and Fearnley, 2003; Johnsen et al., 2006). Due to different typing methods applied, our results are not directly comparable with other work, although the AFLP typing method is generally applied in Norway both when genotyping human isolates and in other poultry studies (Lindstedt et al., 2000; Johnsen et al., 2006). The present methods for isolation of thermotolerant Campylobacter selected for C. jejuni and Campylobacter coli may explain why no other Campylobacter spp. was detected (Corry et al., 1995). Selective enrichment was used for all samples except for fecal material, and this may contribute to skewed results (Corry and Atabay, 2001). It must also be taken into consideration that only 1 colony per positive sample was isolated and typed, a limitation allowing the most predominant clones to be isolated.

The high level of Campylobacter-positive environmental sites constitutes a risk for transmittance of Campylobacter from the slaughterhouse environment to Campylobacter-negative carcasses. In Norway, where above 96% of the broiler flocks are Campylobacter-negative, this aspect is of special importance (Hofshagen and Kruse, 2005). The ability of Campylobacter to remain in the slaughterhouse environment through washing and disinfection is associated with constructional conditions of equipment and buildings, complicating cleaning and providing sufficient moisture. The significance of the continuous presence of Campylobacter in the slaughterhouse environment will influence the extent of transmission to Campylobacter-negative carcasses. More knowledge can be obtained by studying a slaughterhouse over a certain time period and by quantifying the Campylobacter level on broiler carcasses. To reduce the probability of the workers acquiring campylobacteriosis, precautions should be taken when slaughtering Campylobacter-positive flocks.

ACKNOWLEDGMENTS

This work was supported by a grant from the Norwegian Ministry of Agriculture and Food, administered via the Fund of Research Duty, The Centre for Poultry Science (Oslo, Norway), the Norwegian Agricultural Purchasing and Marketing Co-Op in Rogaland and Agder, and The Norwegian Zoonosis Centre. Thanks to Traute Vardund at the Norwegian Institute of Public Health (Oslo) for helpful assistance during the AFLP analysis and to Prior Norge for assistance and making the slaughterhouse available for sampling. The cultivation of samples was conducted at M-Lab in Stavanger, Norway. The AFLP analyses were performed at the Norwegian Institute of Public Health. Thanks to Georg Kapperud and Eystein Skjerve for critically reviewing the paper.

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