Presence of *Campylobacter* in the Respiratory Tract of Broiler Carcasses Before and After Commercial Scalding

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**ABSTRACT** *Campylobacter* could be detected in the thoraco-abdominal cavity of broiler carcasses even if they were carefully eviscerated by hand with no evidence of intestinal rupture or leakage. If *Campylobacter* is present in the air sacs, which are unavoidably torn during evisceration, it could contaminate the thoraco-abdominal cavity of the eviscerated carcass. This study was done to determine if *Campylobacter* contamination is present in the respiratory tract of broilers prior to evisceration. Whole carcass rinses and respiratory tract washes were done on broiler carcasses collected at a commercial processing plant just before and just after scalding. Samples were cultured for presence and numbers of *Campylobacter*, *Escherichia coli*, coliforms, and total aerobic bacteria. *Campylobacter* isolates were subtyped by sequencing the short variable region of the flaA gene. The same subtypes of *Campylobacter* were detected in whole carcass rinse samples as in respiratory tract wash samples from individual broilers. Furthermore, the same numbers and subtypes of *Campylobacter* were recovered from respiratory tracts of carcasses collected before scalding and those collected after scalding. However, respiratory tracts of carcasses after scalding had higher numbers of *E. coli*, coliforms, and total aerobic bacteria than those tested before scalding. Although some bacterial counts were higher in the respiratory tracts of carcasses after scalding, *Campylobacter* counts were not. It appears that *Campylobacter* is present in the respiratory tracts of broilers as they enter processing, and contamination may be due to airborne bacteria during production or transport.

(Key words: air sac, broiler, *Campylobacter*, respiratory tract, scalding)


**INTRODUCTION**

*Campylobacter* is a human bacterial pathogen that is frequently associated with raw poultry and poultry products. This organism can be isolated from live broilers and their feces (Stern and Robach, 1995; Achen et al., 1998). It can also be found in and on broilers at the start of processing (Kotula and Pandya, 1995; Berndtson et al., 1996; Berrang et al., 2000) and after they exit the chill tank or as ready-to-cook carcasses (Izat et al., 1988; Waldroup et al., 1992; Berrang and Dickens, 2000). Much of the *Campylobacter* associated with broiler carcasses can be found on external surfaces, including the skin (Berrang et al., 2000). This fact prompted a study to examine the possibility of removing broiler skin early in processing to lower *Campylobacter* populations on broiler carcasses (Berrang et al., 2002). Removing skin did lower the numbers of *Campylobacter* on the outside of broiler carcasses. However, skin removal had no effect on the numbers of *Campylobacter* found on the internal surfaces of broiler carcasses (Berrang et al., 2002).

*Campylobacter* can be found in the thoraco-abdominal cavity of broilers despite careful aseptic evisceration with no apparent leakage from the alimentary tract (Berrang et al., 2002). *Campylobacter* is present in the environment within many growout houses. Bacteria can be found airborne in the dust in growout houses during catching and transport (Stagg and Crook, 1995; Kwon et al., 1999). It may be possible for airborne *Campylobacter* to infiltrate the respiratory tract of broilers during growout, catching, transport, or hanging. The respiratory tract of broilers includes airsacs that are large in volume relative to the lungs. Airsacs are unavoidably torn during evisceration. Therefore, the presence of *Campylobacter* in the air sacs could lead to internal contamination of the eviscerated carcass even without leakage of alimentary tract contents.

There has also been discussion about the possibility of broilers aspirating water as they enter the first scald tank in early processing (Thomson and Kotula, 1959; Tarver and May, 1963). It is possible that, if intake of scald water occurs, bacterial counts in the respiratory tract would

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**Abbreviation Key:** SVR = short variable region.
Samples

On each of three replicate sample days, broiler carcasses were collected from a commercial processing plant at approximately midshift. In each replication, 10 carcasses were removed from the shack line just prior to scald, and another 10 were removed from the shack line just after the last scald tank (prior to entering the picker). The plant had 3 counterflow scald tanks in series. Carcasses were in the first tank for 1 min and 50 s, in the second tank for 57 s, and in the third tank for 45 s. Between tanks the carcasses were out of the scald water for about 15 s. Each carcass was handled with new clean latex gloves and individually placed into a separate sterile plastic bag. The opening of each bag was sealed with a plastic cable tie, and the bagged carcasses were covered with ice during transport to the laboratory and until the samples were collected (within 2 h). One hundred milliliters of scalder water was collected on each trip to the plant. A sterile specimen cup was used to dip the water out of the turn-around end of the third scald tank. The cup was sealed, placed on ice, and cultured with the other samples.

Whole Carcass Rinse

Upon arrival at the laboratory, feathered carcasses were unbagged, feet and head were removed, and a sanitized plastic cable tie was tightened around the neck. Cable ties effectively occluded the trachea and prevented respiratory tract contamination during the whole carcass rinse procedure. Each feathered carcass was placed into a new bag with 500 mL of PBS and subjected to a 60-s rinse procedure whereby the bag was repeatedly inverted by hand. Following the rinse, each feathered carcass was removed from its bag and placed on a table while the rinsate was collected. Rinsate was poured into a sterile 500 mL bottle and held at 4°C until cultured (within 2 h).

Bacterial Culture Methods

Serial dilutions of the respiratory tract and carcass rinsates as well as scald water were made in PBS. *Campylobacter* were enumerated by plating in duplicate onto the surface of Campy-Cefex agar (Stern et al., 1992). One-tenth of a milliliter was spread on the surface of each plate with a sterile plastic inoculating loop, after which plates were incubated at 42°C for 48 h in a microaerophilic environment (5% O₂, 10% CO₂, 85% N₂). Colony-forming units characteristic of *Campylobacter* were counted. Each colony type counted as *Campylobacter* from each sample was confirmed as a member of the genus by examination of cellular morphology and motility on a wet mount under phase contrast microscopy. Each colony type was further characterized by a positive reaction on a latex agglutination test kit. Ten milliliters from each sample was plated into 90 mL *Campylobacter* enrichment broth. Enrichment broth was incubated for 4 h at 35°C and then 20 h at 42°C. Total aerobic bacterial populations were enumerated on plate count agar. One-tenth of a milliliter from a serial dilution of the rinsate sample was plated in duplicate on the surface of the agar, spread and incubated at 35°C for 18 to 24 h prior to counting the resulting colony-forming units. Coliform and *Escherichia coli* counts were made by plating 1 mL from a serial dilution of the sample onto duplicate Petrifilm *E. coli*/coliform count plates. Petrifilm plates were incubated at 35°C for 18 to 24 h, and colony types characteristic of coliforms and *E. coli* were counted.

*Campylobacter* Subtyping

In replications 2 and 3, *Campylobacter* isolates recovered by direct plating and from enrichment of whole carcass

MATERIALS AND METHODS

**Respiratory Tract Rinse**

Eight centimeter lengths of autoclaved clear plastic tubing (o.d. 4.8 mm, i.d. 3.2 mm) were used to introduce rinse into the respiratory tract. With the carcass lying on its back, the trachea was aseptically exposed in the neck below the killing cut. The trachea was held with sterile forceps and cut partially through at an angle using sterile scissors. Sterile tubing was inserted into the trachea and advanced until resistance was met at the bifurcation of the trachea into primary bronchi at the syrinx. A sanitized plastic cable tie was fixed around the outside of the trachea to secure the tubing in place. A sterile 60-cc syringe loaded with sterile PBS was attached to the exposed end of the plastic tubing. Sixty milliliters of PBS was slowly forced into the trachea. Preliminary experiments showed that this volume allowed airsacs to become partially filled with liquid but did not cause rupture. Holding the carcass gently with the syringe against the breast, the carcass was repeatedly inverted for 30 s to wash the inside of the respiratory tract. PBS rinsate was withdrawn from the trachea with a syringe, placed into a sterile specimen cup, and held on ice until cultured (within 2 h). All carcasses were examined for gross pathology including air sacculitis.

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1Microgen Bioproducts Limited, Camberley, U.K.
2Accumedia Inc., Baltimore, MD.
3Becton Dickinson and Co., Sparks, MD.
4Becton Dickinson and Co., Sparks, MD.
53M Health Care, St. Paul, MN.
rinses and respiratory tract washes were subjected to sub-
typing by DNA sequencing of the short variable region
(SVR) of the flaA gene (Meinersmann et al., 1997). Up to
4 colonies were chosen from each positive sample for
subtyping. PCR was used to amplify the SVR of the flaA
gene. Fifty microliter PCR reactions were carried out in
a Mastercycler Gradient thermocycler containing 2 µL
of a boiled lysate, 0.2 µM of each primer, 200 µM of each
deoxyribonucleotide, 5 µM of each deoxyribonucleotide, 5 µM
of 10× buffer, and 1.5 units of Taq DNA polymerase.7 PCR reactions were run for a total of
30 cycles with times and temperatures of 94°C for 30 s,
56°C for 30 s, and 72°C for 30 s. To obtain sequence
information for the flaA SVR, PCR products were purified
using the Qiaquick PCR purification system.8 Thirty na-

nograms of PCR product was sequenced using Big Dye
Terminator 2.09 and 0.3 µM of primer. Data from the
sequencing reactions were obtained using an ABI Prism
3700 sequencer and analyzed using Sequencher v3.1.2
for Power Macintosh.10 The following primers were used
for the PCR and sequencing reactions: flaAF 5′-CTAT- 

GAGGAATTWAAAAT-3′, flaAR 5′-CAAGWCCCTG-

TTCCWACTGAAG-3′. Different subtypes were defined
due to one or more base differences in the SVR of the flaA
gene.

**Statistical Analysis**

Bacterial counts from direct plating were transformed
to log10 colony-forming units per milliliter of rinse recov-
ered. An analysis of variance was conducted using the
general linear models module of Statistica.11 A random-
ized complete block design was used with replication as
the block and sample site (pre- or postscald) as the main
effect. Prevalence data were examined using chi-squared
test for independence. In all cases significance was as-
signed at \( P < 0.05 \).

**RESULTS AND DISCUSSION**

The numbers of bacteria recovered per milliliter of
whole broiler carcass rinse before and after commercial
scald are shown in Table 1. After scalding, carcasses had
significantly lower numbers of all bacterial populations
measured than those examined before scalding. Carcasses
after scald also had a significantly lower prevalence of
Campylobacter than those before scalding. All carcasses
had detectable numbers of Campylobacter prior to scald;
after scald roughly two-thirds had levels of Campylobacter
below the limit of detection by direct plating (approximately
10 cells/mL of rinse). This finding supports earlier
reports that scalding lowers bacterial recovery from
broiler carcasses (Izat et al., 1988; Berrang and Dickens,
2000). Scalding not only loosens feathers, making de-
feathering more practical, but also lowers the numbers
of bacteria and the prevalence of Campylobacter on the
external surfaces of broiler carcasses.

The numbers of bacteria recovered per milliliter of
respiratory tract rinse are shown in Table 2. Campylobacter
was detected in respiratory tracts of about half of car-
casses tested before and after scald. This finding suggests
that prior to processing broilers may have some level of
Campylobacter colonization of the respiratory tracts. Nei-
ther the level of Campylobacter nor the prevalence was
affected by passage through the scald tank. Relative to
Campylobacter, respiratory tract rinses from scalded car-
casses were not different than those of prescald carcasses.
When the number of Campylobacter detected per milliliter
of rinse is transformed to reflect the total number likely
present within the entire 60-mL rinse, the overall mean
number of Campylobacter detected was log10 2.6 cfu/rinse.
This number is close to the log10 3.0 cfu Campylobacter
detected by sponge sampling the thoraco-abdominal cav-
ity of aseptically eviscerated broilers in a previous study
(Berrang et al., 2002). It is often assumed that contamina-
tion of the internal surfaces of eviscerated broiler car-
casses is due to alimentary tract leakage during eviscera-
tion. However, it is possible that some Campylobacter con-
tamination of the internal surfaces of eviscerated broiler
carcasses is due to contamination of the respiratory tract.

_E. coli_ was detected in 10 out of 30 prescald respiratory
tract rinses (Table 2); none of these carcasses had any
gross pathological signs consistent with air sacculitis. One

<table>
<thead>
<tr>
<th>Sample site</th>
<th>Campylobacter</th>
<th>Escherichia coli</th>
<th><em>T. coli</em></th>
<th>Total aerobic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prescald</td>
<td>5.4 ± 0.3 (30)</td>
<td>4.6 ± 0.1 (30)</td>
<td>3.0 ± 0.1 (27)</td>
<td>7.7 ± 0.1 (30)</td>
</tr>
<tr>
<td>Postscald</td>
<td>1.3 ± 0.4 (11)</td>
<td>2.0 ± 0.3 (27)</td>
<td>2.4 ± 0.3 (30)</td>
<td>5.4 ± 0.2 (30)</td>
</tr>
</tbody>
</table>

1These counts are mean values from only those samples found to be positive. Numbers in parenthesis are
   the number of samples positive out of 30.
2Means plus or minus 95% confidence interval.

\( ^* \)Statsoft, Tulsa, OK.
\( ^7 \)Promega, Madison, WI.
\( ^8 \)Qiagen, Valencia, CA.
\( ^9 \)Applied Biosystems, Foster City, CA.
\( ^10 \)Gene Codes Corp., Ann Arbor, MI.
of the carcasses examined postscald did have signs of air sacculitis. However, the bacterial counts associated with the respiratory tract of that carcass were not different than the other postscald carcasses examined that day. *E. coli*, coliform, and total aerobic bacterial populations were significantly higher in the respiratory tracts of carcasses following scalding than in those sampled prescald. It appears that bacteria are added to the respiratory tract some-
time in the immersion scalding process. It is unclear how bacteria are entering the respiratory tract. Previously published reports suggest that broiler carcasses entering the scald tank may take up contaminated scald water (Thomson and Kotula, 1959; Tarver and May, 1963). Bacteria were detected in the scald water, albeit in relatively low numbers (Table 3). However, because the scald water tested came from the third tank of a counterflow scalding

<table>
<thead>
<tr>
<th>Replication</th>
<th>Campylobacter</th>
<th><em>Escherichia coli</em></th>
<th>Coliform</th>
<th>Total aerobic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ND</td>
<td>0.7</td>
<td>1.5</td>
<td>4.2</td>
</tr>
<tr>
<td>2</td>
<td>ND</td>
<td>ND</td>
<td>1.3</td>
<td>4.0</td>
</tr>
<tr>
<td>3</td>
<td>ND</td>
<td>ND</td>
<td>−0.3</td>
<td>3.3</td>
</tr>
<tr>
<td>Mean</td>
<td>ND</td>
<td>0.7</td>
<td>0.83</td>
<td>3.8</td>
</tr>
</tbody>
</table>

1None detected.
2Mean of positive samples.

### TABLE 4. *Campylobacter* isolate subtype groups detected in whole carcass rinse (WCR) and respiratory tract wash (RTW) samples as defined by *flaA* short variable region (SVR) sequence subtyping

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Prescald</th>
<th>Postscald</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WCR</td>
<td>RTW</td>
</tr>
<tr>
<td>1</td>
<td>B, K</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>A, B</td>
<td>C</td>
</tr>
<tr>
<td>3</td>
<td>A, B</td>
<td>B</td>
</tr>
<tr>
<td>4</td>
<td>B, J</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>6</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>7</td>
<td>B, G, L</td>
<td>—</td>
</tr>
<tr>
<td>8</td>
<td>B, D, I</td>
<td>—</td>
</tr>
<tr>
<td>9</td>
<td>B, D</td>
<td>D</td>
</tr>
<tr>
<td>10</td>
<td>B, C, F</td>
<td>—</td>
</tr>
<tr>
<td>11</td>
<td>A</td>
<td>—</td>
</tr>
<tr>
<td>12</td>
<td>A</td>
<td>—</td>
</tr>
<tr>
<td>13</td>
<td>A</td>
<td>—</td>
</tr>
<tr>
<td>14</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>15</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>16</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>17</td>
<td>A, E</td>
<td>A</td>
</tr>
<tr>
<td>18</td>
<td>A</td>
<td>—</td>
</tr>
<tr>
<td>19</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>20</td>
<td>A</td>
<td>—</td>
</tr>
</tbody>
</table>

1Pre- and postscald samples were different carcasses from the same flock.
2Numbers 1 to 10 are samples from replication 2, numbers 11 to 20 are samples from replication 3.
3Different subtypes are indicated by letters A to M.
4Sample tested negative for *Campylobacter* by direct plating and enrichment.
5Frozen isolate failed to grow at time of subtyping; subtype unavailable.
system, it represented the cleanest water in the scalding system. If carcass muscular action caused aspiration of a significant volume of water, it could conceivably contribute toward an increase in bacterial numbers in the respiratory tract. There may, however, be a more passive contributor of bacteria as well. Air in the scalding and picking areas of poultry processing plants has been shown to carry high numbers of bacteria (Ellerbroek, 1997; Whyte et al., 2001). As water pressure on the outer surface of carcasses is relieved upon exiting the scald tank, slight negative pressure may form within the airsacs causing contaminated air near the scaldner to be pulled into the respiratory tract.

Subtyping results from Campylobacter isolates detected in the last two replications are shown in Table 4. In replication 2, subtype B was detected in whole carcass rinse samples from every prescald carcass. The same subtype was detected in some respiratory tracts of carcasses prior to scald. Furthermore, subtype B was recovered from whole carcass rinse samples and respiratory tract samples from carcasses that had been scalded. Similar results with subtype A were observed in replication 3. Relatively few isolates were detected in either replication that were unique or different than the predominant type for that replication. In general, the same subtypes of Campylobacter were detected in the respiratory tracts of broilers very early during their time in the processing plant. That contamination may present itself as thoraco-abdominal cavity contamination following evisceration. Therefore, even if evisceration is done flawlessly, there may be some Campylobacter present on the internal surfaces of ready to cook whole processed broiler carcasses.

ACKNOWLEDGMENTS

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REFERENCES