Incidence of Campylobacter jejuni/coli on Pork Carcasses in the Northeast Georgia Area

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ABSTRACT

One hundred and twelve freshly slaughtered pork carcasses from three packing plants were sampled before and after chilling for the presence of Campylobacter jejuni/coli by the use of two isolation methods (Preston enrichment and Skirrow direct plating). Preston enrichment media gave the highest isolation rate, 12.5%, on freshly slaughtered carcasses. No isolations were obtained from chilled carcasses. More isolates were obtained from the ham skin area compared with the jowl area. All isolates were confirmed as Campylobacter coli.

Campylobacter jejuni was isolated from swine as early as 1965 (18). Researchers have found contamination levels of 11% from swine cecal contents (14), 43% from pork livers (16) and 59% from feces of healthy pigs (20). Isolation rates from pork carcasses have also yielded various results. Bolton et al. (2) reported 56% of pork carcasses at an abattoir were contaminated while Hudson (10) reported only a 40% incidence. Hudson and Roberts (11) and Stern (19) also reported various incidences of 59% and 28%, respectively. Blaser (1) attributed these different isolation rates to variations in location, variance in specific population characteristics such as age, sex and breed, and also to cultural methodology.

Gill and Harris (7) reported a decreased recovery rate from chilled carcasses of calves, sheep and lambs as compared to freshly slaughtered carcasses. Similar results were reported previously by Hudson (10) when he noted a 29% decrease on pork carcasses after chilling. Bolton et al. (2) attributed the decrease in isolation to the oxygen-sensitive nature of the organism. Hudson and Roberts (11) suggested that drying of the carcass surface could affect recovery.

Various methods have been used in the isolation of Campylobacter from numerous animal species. Differences in rates of recovery may in part be attributed to this factor (1). Patton et al. (13) compared three selective media, Skirrow's, Bultzer's and Modified Bultzer's, for primary isolation of C. jejuni from canines and felines and found Skirrow and Bultzer media comparable. Bolton and Robertson (3) developed a new medium, Preston medium, that was shown to be more selective than Skirrow's medium and better suited for animal and environmental specimens. The results indicated this medium was superior for isolation of C. jejuni from swine. One major advantage appeared to be the improved inhibition of competing microorganisms. Bolton et al. (4) compared Skirrow's, Bultzer's, Campy-BAP and Preston media for isolation of Campylobacter species from human, animal and environmental specimens. Preston medium yielded the highest isolation rate due to its greater selectivity.

Although extensive research has been conducted to determine the incidence of C. jejuni on various animal carcasses, little work has been reported in the United States. The purpose of this study was to determine the incidence of C. jejuni/coli on pork carcasses in the Northeast Georgia area and evaluate the effectiveness of Preston enrichment medium as compared to direct plating on Skirrow's medium for isolation of the organism from pork carcasses. The effect of chilling on recovery of the organism was also examined.

MATERIALS AND METHODS

One hundred and twelve pork carcasses were sampled over a 3-month period. Approximately 36 carcasses each were selected at random from a large commercial processor, a small local processor, and the university meat laboratory for this study. The carcasses were sampled immediately after slaughter at both ham and shoulder locations for a total of 224 samples. Adjacent areas of the same carcasses were used to compare direct isolation and enrichment techniques for detection.

Direct isolation (Skirrow medium)

Samples for direct isolation were obtained by pre-moistening a sterile nylon swab in the transport medium, swabbing a 100-cm² area of skin on either the ham or shoulder region of carcasses and placing the swab into the medium for transport to the laboratory. The transport medium was Brucella broth (Difco) supplemented with ferrous sulfate, sodium pyruvate and sodium bisulfite (FPB), as described by George et al. (6), and dispensed in 2-ml portions in test tubes. Samples were taken from the right side of freshly slaughtered carcasses before chilling. Carcasses were again sampled, on the left side, at both locations following approximately 20 h of chilling at 4°C. Samples were transported in ice to the laboratory and analyzed immediately upon arrival.

Sample analysis

Each specimen tube was mixed with a vortex-type mixer and
0.25 to 0.30 ml of inoculum was streaked for isolation onto Skirrow agar prepared from Brucella agar (Difco) to which 10% defibrinated sheep blood, Skirrob antibiotics and FBP supplements were added. Following inoculation, streak plates were incubated at 42°C for 48 h in a reduced (5%) oxygen atmosphere. Suspect colonies of typical morphology were transferred to Brucella broth (Difco) plus FBP and 0.16% agar and incubated at 37°C in a reduced oxygen atmosphere for 24 h. Each isolate was checked for proper, morphological characteristics, purity, and later was identified by biochemical testing.

**Enrichment isolation (Preston medium) and identification**

Swab samples were obtained as previously described and deposited in Preston enrichment broth (3) dispensed in 2.5-ml portions in sterile tubes. Following sampling, tubes were placed in ice, transported to the laboratory, then incubated 24 h at 42°C in an atmosphere containing 5% O₂, 10% CO₂ and 85% N₂. Samples from enrichment cultures were streaked on Skirrow agar for isolation.

All isolates suspected of being *Campylobacter* were subjected to biochemical tests to identify *C. jejuni* and *C. coli* (8). Sensitivity to triphenyltetrazolium chloride (TTC) (15) and hippurate tests (12) were used to discriminate between *C. jejuni* and *C. coli*. All carcasses from which *C. jejuni /coli* were isolated were recorded as positive.

**RESULTS AND DISCUSSION**

Contamination of pork carcasses by *Campylobacter* has been reported by other researchers (3,10,11,16,19), but the incidence was found to be significantly lower than results of this study. Efforts were not made to distinguish *C. jejuni* from *C. coli*. Only *C. coli* was isolated from carcasses sampled in this report. Of the 112 carcasses sampled immediately after slaughter, 10 (9.8%) were positive when direct isolation was employed, whereas 14 (12.5%) were positive after enrichment in Preston medium followed by isolation on Skirrow's medium (Table 1). Of the 224 samples analyzed by each method, 13 positives (5.8%) were isolated by the direct method, as compared to 17 (7.6%) by the enrichment method (Table 2).

Preston enrichment medium was confirmed to be superior to direct plating on Skirrow agar. Similar results had been found by Bolton and Robertson (3). Fewer colony types were observed on Skirrow plates that were streaked from enriched cultures compared to samples plated directly onto the Skirrow medium. Greater numbers of *Campylobacter* colonies that were more easily distinguishable by colony morphology were also seen on the Skirrow plates streaked from the Preston medium. This can be attributed, in part, to the greater inhibition of competitive microorganisms by the more selective antibiotics present in the Preston enrichment broth (4).

Two locations, ham and shoulder, were sampled per side of carcass. Of the 30 *Campylobacter* isolates, 20 (66.7%) were found on the ham (Table 2). Carpenter et al. (5) studied *Salmonella* on pork carcasses and suggested that sampling near the anal region would result in a higher isolation rate due to the possibility of fecal contamination. These results are contrary to those of Weissman and Carpenter (21) which revealed no particular part of the carcass was likely to be contaminated more than any other.

Following chilling, no *C. jejuni /coli* were isolated from the paired sides of the carcasses by either direct plating or enrichment procedures. The colonies present on direct isolation appeared to be primarily bacilli and yeasts. Essentially no growth of any organisms was observed on plates streaked from enriched samples. Previous experiments indicate that the swab sampling technique employed was capable of detecting 1 cell/cm² (unpublished data), therefore these results suggest that *Campylobacter* cannot easily survive chilling conditions. Reduced rates of isolation from red meat carcasses following chilling have also been reported by Bolton et al. (2), Gill and Harris (7), Hudson (10), Hudson and Roberts (11) and Rosef (16). The death of the organism was attributed to oxygen-sensitivity, as well as drying of the carcass skin. Further research will be reported that elucidates the factors affecting death of *Campylobacter* on pork carcasses under chilling.

Of the presumptive *Campylobacter* isolated by both direct and enrichment methods, all were confirmed to be *C. coli* by the inability to hydrolyze hippurate (9). These

**TABLE 1. Recovery of Campylobacter coli from pork carcasses before chilling by Preston broth enrichment and Skirrow agar direct plating.**

<table>
<thead>
<tr>
<th>Plant</th>
<th>Carcasses examined (no.)</th>
<th>Incidence</th>
<th>Preston</th>
<th>Skirrow agar</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>36</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>36</td>
<td>14</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>112</td>
<td>14 (12.5%)</td>
<td>10 (9.8%)</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 2. Incidence of Campylobacter coli from ham and shoulder sampling locations on freshly slaughtered pork carcasses as determined by direct plating (Skirrow agar) and enrichment (Preston broth).**

<table>
<thead>
<tr>
<th>Plant</th>
<th>No. samples</th>
<th>Incidence</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoulder</td>
<td>Ham</td>
<td>Shoulder</td>
<td>Ham</td>
</tr>
<tr>
<td>A</td>
<td>40</td>
<td>40</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>36</td>
<td>36</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>36</td>
<td>36</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>112</td>
<td>112</td>
<td>5 (4.46%)</td>
<td>12 (10.71%)</td>
</tr>
</tbody>
</table>

224 17 (7.6%) 13 (5.8%)
results concur with other studies where C. coli was most frequently associated with swine (17).

The results of this study indicate that the contamination levels of pork carcasses by Campylobacter in the Northeast area of Georgia were much lower than those reported by researchers in other locations. These differences may be attributed to differences in herd characteristics, geographical location, as well as slaughter methods. Preston enrichment media was confirmed to be superior for the isolation of Campylobacter from pork carcasses. Chilling reduced C. jejuni/coli numbers to below detectable levels. All Campylobacter isolates were confirmed to be C. coli. These results suggest pork carcasses processed by conventional methods are probably not a potential problem in food-associated campylobacteriosis.

REFERENCES