Recovery of *Campylobacter* and *Salmonella* Serovars From the Spleen, Liver and Gallbladder, and Ceca of Six- and Eight-Week-Old Commercial Broilers

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**SUMMARY**

Previous studies have demonstrated that when *Campylobacter* or *Salmonella* were either orally or intraabdominally inoculated into day-old broiler chicks, within 1 h, these bacteria moved rapidly to the lymphoid organs. These bacteria were still present 1 wk after inoculation. Two different market-age (6 and 8 wk old) broilers were obtained from 2 commercial poultry operations and brought to the laboratory for analysis. Necropsy was limited to the removal of the spleen, liver and gallbladder (L-GB), and ceca using aseptic techniques. To reduce the possibility of cross-contamination between samples, the spleen and L-GB were aseptically removed before the ceca. Samples were individually bagged, and standard laboratory procedures for *Campylobacter* and *Salmonella* were carried out for all samples. Fifty-two 6-wk-old broilers were analyzed, and *Campylobacter* were found in 19 of 52 L-GB, 19 of 52 spleens, and 26 of 52 ceca. *Salmonella* were found in 5 of 52 L-GB, 8 of 52 spleen, and 4 of 52 ceca. Eighty 8-wk-old broilers were analyzed, and *Campylobacter* were found in 3 of 80 L-GB, 5 of 80 spleens, and 19 of 80 ceca. *Salmonella* were found in 41 of 80 L-GB, 38 of 80 spleens, and 52 of 80 ceca. The internal organs of the younger birds were more heavily contaminated with *Campylobacter*, whereas *Salmonella* was the predominant organism isolated in the older birds. All *Campylobacter* isolates were found to be *Campylobacter jejuni*. The predominant *Salmonella* serotype was *Salmonella* Typhimurium; however, 7 other serotypes were found. Overall, *C. jejuni* was found in 22 of 132 L-GB, 24 of 132 spleen, and 45 of 132 ceca, whereas *Salmonella* serovars were isolated from 46 of 132 L-GB, 46 of 132 spleen, and 56 of 132 ceca. There is no doubt that these bacteria are naturally present in these organs. The significance of these reservoirs in the internal organs of commercial broilers is yet to be determined but could play a role in the microbiology of the intestinal tract and hence the final food product.

**Key words:** *Campylobacter*, broiler, thymus, spleen, liver and gallbladder, ceca


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DESCRIPTION OF PROBLEM

Campylobacter and Salmonella are the leading bacterial etiological agents of acute gastroenteritis in the human population [1]. Contaminated poultry meat is considered to be an important vehicle of human infection [2, 3, 4, 5]. Due to the difficulties in controlling the spread of these bacteria in the kitchen [6, 7] and abattoir [8], control on the farm may be a more effective way of reducing the incidence of these organisms in processing plants. Before more effective intervention strategies can be implemented and achieved, a better understanding of the epidemiology of these organisms in poultry confinement rearing facilities and a better understanding of the ecology of these organisms in birds needs to be further elucidated. Numerous studies in recent years have been undertaken to determine where inside the body of the bird these organisms are located and to try to determine the significance of these reservoirs in contamination of poultry flocks [9, 10, 11, 12, 13]. From these studies, Campylobacter and Salmonella have been found not to be limited to the intestinal tract and have been recovered in significant incidences in lymphoid organs, ovarian follicles, liver and gallbladder (L-GB), and reproductive tracts of commercial broiler breeder hens [12, 13]. The objectives of this study were to determine whether Campylobacter and Salmonella spp. could be isolated from the spleen and L-GB of commercial broilers likely to be naturally exposed and determine what types of species or serovars are present in these tissues.

MATERIALS AND METHODS

Six-week-old broilers were obtained from a commercial processing facility on 3 separate visits from 3 different flocks for a total of 52 birds, and 8-wk-old broilers were obtained from a separate commercial processing facility on 4 separate visits from 4 different flocks for a total of 80 birds. The samples were collected over the fall and winter seasons. For all visits, the carcasses were removed from the processing line following defeathering (rehang table), placed in coolers, and transported back to the laboratory for necropsy. Each carcass was aseptically opened, and the spleen, L-GB, and ceca were aseptically removed. Individual samples were placed in sterile bags, packed on ice, and evaluated. Standard laboratory methods for the recovery of Campylobacter spp. were performed utilizing Bolton’s enrichment broth (containing lysed horse blood) and Cefex agar [12]. For recovery of Salmonella spp., standard laboratory methods were performed utilizing buffered peptone water, tetraphionate broth (Hajna), Rappaport Vassiliadis, and brilliant green sulfur and modified Lys FE agar for plating media [13]. For Campylobacter speciation, isolates were obtained from the −80°C freezer and placed onto Campylobacter Cefex agar and incubated for 48 h at 42°C in a microaerophilic condition. The isolates were then picked and placed onto blood agar plates and incubated at 42°C for 24 h. Template DNA was prepared by picking 3 to 4 colonies from a plate using a sterile disposable plastic loop, and a polymerase chain reaction system [14] was utilized for differentiation of Campylobacter jejuni and Campylobacter coli. For Salmonella speciation, all saved triple sugar agar slants containing confirmed Salmonella isolates were shipped to the USDA National Veterinary Services Laboratory in Ames, Iowa, for serovar identification. All Campylobacter and Salmonella data from the experiments are expressed as number of positive isolates from each sample site over the number of sample sites tested from each carcass or as a percentage of the aforementioned.

RESULTS AND DISCUSSION

In experiment 1, from 6-wk-old broilers obtained from a commercial processing facility, Campylobacter were found in 37, 37, and 50% of the L-GB, spleens, and ceca, respectively (Table 1). Campylobacter were recovered in the spleen of 1 bird but not in the ceca, and this was observed in 2 birds in regards to L-GB sample. In 1 bird, Campylobacter were recovered from the spleen and L-GB but not the ceca. Campylobacter were recovered from the spleen and ceca of 4 birds but not the L-GB, and this was observed in regards to recovery from the L-GB in 2 birds. In 5 birds, Campylobacter were recovered from all sample sites. Furthermore, Campylobacter were isolated from 6 ceca samples but not from the other 2 sample sites. Salmonella were found in 10, 15, and 8% of the L-GB, spleens, and ceca, respectively (Table 1). Salmonella were recovered from the spleen of 6 birds but not in the ceca, and this was also observed in 1 bird in regard to the L-GB sample.
In 1 bird, *Salmonella* were recovered from the spleen and L-GB but not the ceca. In another bird, *Salmonella* were recovered from the spleen and ceca but not the L-GB, and this was observed in 3 birds in regards to the L-GB. *Salmonella* were only recovered in the ceca of 1 bird and not one of the other sample sites. *Salmonella* were never recovered from all 3 sample sites within a bird. *Campylobacter* and *Salmonella* were recovered from sample sites in 1 repetition. From that repetition, *Campylobacter* and *Salmonella* were isolated simultaneously from 1 of 52 L-GB, 4 of 52 spleens, and 3 of 52 ceca.

In experiment 2, from 8-wk-old broilers obtained from a separate commercial processing facility, *Campylobacter* were found in 4, 6, and 24% of the L-GB, spleens, and ceca, respectively (Table 2). In 3 birds, *Campylobacter* were recovered from the spleen and ceca but not the L-GB, and this was also observed in regards to the L-GB. In 1 bird, *Campylobacter* were isolated from all sample sites. In another bird, *Campylobacter* were isolated from the spleen and not the ceca. In 12 birds, *Campylobacter* were only isolated from the ceca.

*Salmonella* were found in 51, 48, and 65% of the L-GB, spleens, and ceca, respectively (Table 2). In 25 of the birds sampled, *Salmonella* were recovered from all 3 sample sites. In 11 birds, *Salmonella* were recovered from only the L-GB and ceca. This was observed in 5 other birds in which *Salmonella* were recovered from the spleen and ceca. In 4 birds, *Salmonella* were recovered from the spleen only, and from an additional 4 birds, *Salmonella* were recovered only from the spleen and L-GB. In 2 birds, *Salmonella* were only recovered from the L-GB and from only the ceca of 11 birds. Both *Campylobacter* and *Salmonella* were recovered from sample sites in 1 repetition. From that repetition, *Campylobacter* and *Salmonella* were isolated simultaneously from 3 of 80 L-GB, 4 of 80 spleens, and 19 of 80 ceca.

The internal organs of the younger birds were more heavily contaminated with *Campylobacter*, whereas *Salmonella* were the predominant organism isolated in the older birds in this study. Isolations of *Campylobacter* or *Salmonella* were not limited to a particular sample site, and recovery from sample sites varied significantly from bird to bird. All *Campylobacter* isolates were found to be *C. jejuni*. However, the methodology procedure utilized in this study has been shown to select for this type of species and excluded other species from being detected [15]. The predominant *Salmonella* serotype was *Salmonella Typhi-murium*, but 7 other serotypes were found. *Salmonella Typhi-murium* accounted for 68%, whereas

**Table 1.** Number of positive *Campylobacter* and *Salmonella* samples from tissues of 6-wk-old commercial broilers

<table>
<thead>
<tr>
<th>Repetitions</th>
<th>Spleen</th>
<th>Liver and gallbladder</th>
<th>Ceca</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Campylobacter</td>
<td>Salmonella</td>
<td>Campylobacter</td>
</tr>
<tr>
<td>1</td>
<td>5/16(^1)</td>
<td>0/16</td>
<td>4/16</td>
</tr>
<tr>
<td>2</td>
<td>0/16</td>
<td>2/16</td>
<td>0/16</td>
</tr>
<tr>
<td>3</td>
<td>14/20</td>
<td>6/20</td>
<td>15/20</td>
</tr>
<tr>
<td>Total</td>
<td>19/52</td>
<td>8/52</td>
<td>19/52</td>
</tr>
</tbody>
</table>

\(^1\)Represents the number of positive samples over the number of total samples.

**Table 2.** Number of positive *Campylobacter* and *Salmonella* samples from tissues of 8-wk-old commercial broilers

<table>
<thead>
<tr>
<th>Repetitions</th>
<th>Spleen</th>
<th>Liver and gallbladder</th>
<th>Ceca</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Campylobacter</td>
<td>Salmonella</td>
<td>Campylobacter</td>
</tr>
<tr>
<td>1</td>
<td>0/20(^1)</td>
<td>7/20</td>
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<td>0/20</td>
</tr>
<tr>
<td>4</td>
<td>0/20</td>
<td>15/20</td>
<td>0/20</td>
</tr>
<tr>
<td>Total</td>
<td>5/80</td>
<td>38/80</td>
<td>3/80</td>
</tr>
</tbody>
</table>

\(^1\)Represents the number of positive samples over the number of total samples.
Salmonella Muenster and Salmonella Kentucky accounted for 9% of the isolates. Salmonella Montevideo and Salmonella Thompson each accounted for 5% of the isolates obtained, but Salmonella London, Salmonella Berta, and Salmonella Schwarzengrund were also obtained.

CONCLUSIONS AND APPLICATIONS

1. Campylobacter and Salmonella are naturally present in the internal tissues of commercial broilers.
2. Campylobacter jejuni was naturally present in 17% of the L-GB, 18% of the spleens, and 34% of the ceca.
3. Salmonella were isolated from 35% of the L-GB and spleens and from 42% of the ceca.
4. Significance of these reservoirs in the internal tissues of commercial broilers is yet to be determined but may have an effect on the microbiology of the intestinal tract and hence the final food product.

REFERENCES AND NOTES

14. BAX polymerase chain reaction system, Dupont Inc., Wilmington, DE.