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# Research Article
# Persistence of *Campylobacter* and *Salmonella* in the Ceca, Spleen and Liver/gallbladder of Inoculated Broilers

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## Abstract

**Background and Objective:** Poultry is a major source of *Salmonella* and *Campylobacter* involving human illness. Several body openings of the young chick are exposed to bacteria. The objective of this study was to determine how long artificially inoculated *Salmonella* or *Campylobacter* persist in the ceca, spleen and/or liver/gallbladder of broilers. **Materials and Methods:** Day old chicks (10/isolation unit and 5/floor pen) were orally gavaged with $10^3$ cells of either a marker *Salmonella* Typhimurium or a marker *Campylobacter coli* and ceca, spleen and liver/gallbladder were aseptically sampled. **Results:** All organs from all birds were positive for *Salmonella* Typhimurium and *C. coli* at 1 week, most were still positive at 2 and 3 weeks. By 6 weeks, no *Salmonella* Typhimurium was detected in any tested organs. By 6 weeks all ceca and spleens were positive for *Campylobacter coli* but none found in liver/gallbladder samples. **Conclusion:** Translocation of *Campylobacter* and *Salmonella* to internal organs and their persistence in these organs are important because these bacteria will not be detected by the currently used methods. Current methods sample the intestinal tract only with drag swabs or fecal samples. Research is needed to understand translocation and persistence of both *Campylobacter* and *Salmonella* in poultry.

Key words: *Campylobacter*, *Salmonella*, poultry meat, *Salmonella* typhimurium, *Campylobacter coli*, inoculated

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

*Campylobacter* and *Salmonella* are the leading bacterial agents of acute gastroenteritis in humans[1]. Poultry meat contaminated by these two organisms has long been considered to be an important vehicle of human illness[2,3]. Because of the difficulty in controlling the spread of these bacteria in broiler slaughter and processing facilities, control on the farm may be an effective means of reducing prevalence of these human pathogens on broiler meat products. A complete understanding of the epidemiology and microbial ecology of *Salmonella* and *Campylobacter* associated with poultry rearing facilities will greatly increase our chances of designing effective intervention strategies. Recently, numerous studies have been done to determine what location in the bird's body do microorganisms reside after being introduced via different body openings[4-8]. Those studies show that *Campylobacter* and *Salmonella* are not limited to the intestinal tract but have been recovered frequently in lymphoid organs, liver and gallbladder, spleen, ovarian follicles and all segments of the reproductive tract of commercial broiler breeder hens[5,7]. The objective of the current study was to determine translocation and persistence of artificially inoculated *Campylobacter* and *Salmonella* in broilers housed on either a wire floor or solid floor covered with pine shavings.

## MATERIALS AND METHODS

Day old broiler chicks were obtained from a commercial hatchery and placed in either wire floor isolation units (0.3×0.48 m, IU) or floor pens (4×5 ft) on fresh pine shavings. Each chick was orally gavaged with 0.1 mL of phosphate buffered saline (PBS) containing $10^3$ cells of either a nalidixic acid resistant *Salmonella* Typhimurium ($ST^{NR}$) or a gentamicin resistant *Campylobacter coli* ($CC^{GR}$). At 1 and 2 weeks of age, 10 broilers per IU were humanely euthanized by cervical dislocation and the ceca, spleen and liver/gallbladder (LG) aseptically sampled for $ST^{NR}$ or $CC^{GR}$. After 3 and 6 weeks 20 broilers per floor pen on litter were humanely sacrificed by cervical dislocation and the same organs were aseptically sampled 10 birds for $ST^{NR}$ or 10 birds for $CC^{GR}$. For $ST^{NR}$, the tissues to be sampled were individually placed in sterile plastic bags, weighed and macerated using a rubber mallet to expose the contents. Next buffered peptone water (BPW, Neogen, Acumedia, Lansing, MI) was added to the sample bags at a ratio of 3:1 volume to weight and subjected to 60 s in a paddle blender (Interscience Laboratories Inc., Woburn, MA). Samples were incubated for 24 h at 37°C. A 0.1 mL aliquot of incubated BPW was plated onto BG Sulfa (BGS, Becton Dickinson and Co., Sparks, MD) plates containing 200 ppm nalidixic acid (Sigma Chemicals Inc., St. Louis, MO) incubated 24 h at 37°C. Following incubation, plates were observed for presumptive *Salmonella* colonies. Characteristic colonies were confirmed as *Salmonella* using Latex agglutination to *Salmonella* Poly H flagellar antigens (Microgen Bioproducts, Surrey, UK).

For $CC^{GR}$, sample preparation was the same as that for $ST^{NR}$ except that for *Campylobacter* enrichment broth (CEB, Oxoid, Ogdensburg, NY) was used instead of BPW. After incubation of 24 h at 42°C in a microaerobic environment (5% $O_2$, 10% $CO_2$, 85% N) a 0.1 mL aliquot of incubated CEB was plated onto Campy Cefex agar (Acumedia Manufacturers Inc., Lansing, MI) containing 200 ppm gentamicin (Sigma Chemicals Inc., St. Louis, MO)[9]. Plates were incubated in a microaerobic atmosphere at 42°C for 48 h. Presumptive $CC^{GR}$ colonies were confirmed by microscopic observation of characteristic spiral shaped cells, darting motility in wet mount preparations and further confirmed through latex agglutination test (Integrated Diagnostics Inc., Baltimore, MD).

## RESULTS

*Salmonella* recovery data from internal organs are shown in Table 1. For $ST^{NR}$, all (10/10) ceca, spleen and LG samples were positive from birds housed for 1 week on wire in IU's. At 2 weeks, $ST^{NR}$ were recovered from 29/30 organ samples. At 3 weeks of age, broilers grown on pine shavings had 5/5, 4/5 and 5/5 samples positive for $ST^{NR}$ from ceca, spleen and LG, respectively. At 6 weeks no $ST^{NR}$ was detected from any sample collected from broilers on pine shavings.

*Campylobacter* recovery data are shown in Table 2. $CC^{GR}$ was recovered from shavings 10 out of 10 samples of ceca, spleen and LG for broilers grown for one week in an IU (Table 1). After 2 weeks in the IU 100%, 40% and 70% of the ceca, spleen and LG were positive, respectively. For broilers grown on pine shavings after 3 weeks 100%, 80% and 100% of the ceca, spleen and LG were positive for $CC^{GR}$, respectively. At 6 weeks, 100%, 100% and zero were positive in the ceca, spleen and LG, respectively. This study was done with 1 and 2 week old birds in the IC units and 3 and 6 week old birds in floor pens. This is because it is not practical for birds older than 2 weeks to be kept in IC units, unless the number is very small.

Table 1: Detection of *Salmonella* in the ceca, spleen and LG of orally gavaged broilers

| Age (weeks) | Housing | Ceca (%) | Spleen (%) | LG (%) |
|---|---|---|---|---|
| 1 | IU[1] | 100 | 100 | 100 |
| 2 | IU | 100 | 90 | 100 |
| 3 | FP[2] | 100 | 80 | 100 |
| 6 | FP | 0 | 0 | 0 |

[1]Wire floor isolation unit, [2]Litter covered floor pen

Table 2: Detection of *Campylobacter* in the ceca, spleen and LG of orally gavaged broilers

| Age (weeks) | Housing | Ceca (%) | Spleen (%) | LG (%) |
|---|---|---|---|---|
| 1 | IU[1] | 100 | 100 | 100 |
| 2 | IU | 100 | 40 | 70 |
| 3 | FP[2] | 100 | 80 | 100 |
| 6 | FP | 100 | 100 | 0 |

[1]Wire floor isolation unit, [2]Litter covered floor pen

## DISCUSSION

Both $ST^{NR}$ and $CC^{GR}$ translocated from an oral inoculation to various internal organs and persisted therein for 1 and 2 weeks in an IU. $ST^{NR}$ showed slightly greater persistence than $CC^{GR}$ at 2 weeks. After 3 weeks in broilers raised on pine shavings, both organisms had translocated and persisted in 80-100% of the samples. On litter after 6 weeks $CC^{GR}$ was recovered from all ceca and spleen samples but not from any LG samples and $ST^{NR}$ was not detected in any of the ceca, spleen or LG samples. This indicates that translocation and persistence can differ by organism, age, floor surface or housing type. In other published studies, *Campylobacter* have been isolated from bile in 19 of 87 (21.8%) bovine bile samples[10] and have been shown to readily colonize the liver[11]. In the current study both *Campylobacter* and *Salmonella* were not detected in the LG samples of 6 weeks old broilers raised on pine shavings.

Back in 2005, in two separate studies[4,6], both *Salmonella* and *Campylobacter* had been shown to translocate to internal organs and to persist for 1 week. Persistence was only followed for one week and translocation was shown to very rapidly occur in the *Campylobacter* study[6]. In the present study, we decided to determine persistence in various organs of both organisms for up to 6 weeks. In other studies with Japanese quail[12,13], *Campylobacter* was shown to translocate into the spleen, liver and lungs after oral inoculation and persisted in these tissues for up to 17 days postinoculation and in that study, the liver samples were shown to be positive for 19 and 20 days after oral inoculation.

Determining how, when and for how long *Campylobacter* and *Salmonella* invades and resides in internal tissues could determine the best intervention strategies to reduce their presence in broiler and broiler breeder flocks. Also maintaining long term persistence will be necessary to study if these reservoirs ultimately contribute to contamination of the intestinal and reproductive tracts later in the life of a breeder bird.

Naturally occurring *Salmonella* and *Campylobacter* have been isolated from all segments of the reproductive tract[14], including the mature and immature ovarian follicles[5]. Therefore it's important to determine the mechanism for how these organisms were able to contaminate these tissues. Clearly this demonstrates that this contamination is not limited to the digestive tract. Determining if reservoirs established in lymphoid and non-lymphoid organs play a role has yet to be conclusively shown. This study is the first step in the process of doing so.

## CONCLUSION

Translocation of *Campylobacter* and *Salmonella* to internal organs and their persistence in these organs are important for several reasons. First of all, any reservoirs of these bacteria in the internal organs will not be detected by the currently used methods of sampling flocks of birds. Current methods of environmental sampling focuses on contamination from the intestinal tract which is assessed by drag swabs or fecal samples. Long term persistence is necessary to determine if reservoirs in the internal organs of broilers and breeders can later recontaminate the intestinal and/or reproductive tracts. Therefore, much research is needed to fully define and understand the variables which affect translocation and persistence of both *Campylobacter* and *Salmonella* in poultry.

## SIGNIFICANCE STATEMENT

*Campylobacter* and *Salmonella* translocates and persists to internal organs. Current methods cannot detect

microorganisms that are not in the intestinal tract. This study is the first step in this process of how they differ in their abilities to contaminate different organs and what factors contribute to persistence.

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