

Research Note: Fate and dissemination of *Salmonella enterica* serovar reading in turkeys at processing using an oral gavage challenge model

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ABSTRACT This study aimed to evaluate the fate and dissemination of *Salmonella* Reading (SR) in turkeys using an oral gavage challenge model. One hundred twenty-eight-week-old commercial turkey hens were moved from commercial production to research facilities. Upon arrival, a combination of enrofloxacin, 10 mg/kg, and florfenicol, 20 mg/kg, were orally administered sequentially before comingled placement on fresh pine shavings. Turkeys were challenged with 10⁸ cfu SR by oral gavage on d 4 and 7 postplacement. Subsets were subjected to simulated commercial processing on d 14 (n = 40), 21 (n = 40) and 28 (n = 32) postplacement (corresponding to 10, 11, and 12 wk of age). Stifle joint, skin, trachea, crop, lung, liver + spleen (LS), and ceca were

aseptically sampled and cultured for *Salmonella* recovery and serotyping. SR could not be recovered from stifle joint 14 d post inoculation (PI). However, at 14 d PI, recovery of SR were: Skin 80%; crop 75%; LS 67.5%; lungs 60%; and ceca 57.5%. ($P < 0.01$). Interestingly, the lowest recovery of SR was observed from trachea (40%). At 21 d PI, the highest rate of positive samples to SR were observed in ceca (87.5%) and crop (67.5%). By 28 d PI, SR was only recovered from ceca (75%); crop (43.8%); lung (34.4%); and LS (21.9%). The results of this study confirms that SR is an emerging problem for the turkey industry and immediate measurements to reduce foodborne pathogens such as *Salmonella* should target all parts of the supply chain and consumer education about food safety.

Key words: salmonella reading, turkeys, tissue recovery, processing

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INTRODUCTION

Food-borne or water-borne microbial pathogens are associated with diarrheal disorders killing an estimated two million people annually at the global level (Schlundt et al., 2004). Just in the United States of America, it has been estimated that nontyphoidal *Salmonella* causes over one million foodborne infections every year (Scallan et al., 2011). Contamination of poultry carcasses with *Salmonella* has been linked to flock infection during rearing and transportation to slaughter. However, risk factors for poultry colonization by *Salmonella* include season, hatchery of origin, feed mills, and various hygienic measures (Arsenault et al., 2007). These outbreaks highlight the need to focus efforts on strategies to decrease and prevent human illness

associated with live poultry contact through comprehensive interventions from “farm-to-fork” levels.

Several multistate outbreaks of human *Salmonella* infections have been associated with the consumption of poultry products (Loharikar et al., 2012). In 2011, the Centers for Disease Control and Prevention (CDC) identified a multistate cluster of *Salmonella* Heidelberg infections and two multidrug-resistant isolates from raw ground turkey retail samples (Routh et al., 2015). Even though *Salmonella enterica* serovar Reading (SR) is a serotype that is uncommonly associated with human illness, during 2018–2019, CDC, the U.S. Department of Agriculture (USDA), and the Food and Drug Administration (FDA) investigated a multistate outbreak of 356 *Salmonella* Reading infections from 42 states associated with turkey products. The outbreak strain was isolated from raw ground turkey meat and live turkeys (Hassan et al., 2019). During this time, four recalls of turkey meat were published, suggesting that *Salmonella* Reading was an emerging problem for the turkey industry. The report published four *Salmonella* Reading infections with indistinguishable pulsed-field gel electrophoresis (PFGE) pattern, suggesting the outbreak had a common source. Hence, immediate interventions

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encompassed all parts of the supply chain, including slaughter and processing facilities and upstream farm sources. The purpose of the present research note was to preliminarily evaluate potential areas of appropriate focus for interventions at processing using an experimental challenge model in turkeys.

MATERIALS AND METHODS

Animal Source and Diet

A total of 120 eight-week-old commercial turkey hens were obtained from nearby commercial facilities. They were transported to the University of Arkansas Poultry Health Laboratory (PHL), where they were housed for the experiment's duration. All animal handling procedures complied with the Institutional Animal Care and Use Committee (IACUC protocol #20004) of the University of Arkansas. A corn-soy-based grower feed that met or exceeded age-appropriate nutrient requirements recommended for Nicholas hens, and water, were provided *ad libitum* for the experiment's entire duration.

Prechallenge Administration of Antibiotics

Previous research published by our laboratories, have shown that prophylactic or therapeutic administration of antibiotics increase the susceptibility to *Salmonella* infections in poultry (Manning et al., 1992; Morales-Barrera et al., 2016). Immediately upon arrival, individual hen body weights were obtained, and a combination of enrofloxacin, 10 mg/kg, and florfenicol; 20 mg/kg was orally administered sequentially before comingled placement on fresh pine shavings. These broad-spectrum antibiotics were used to potentially perturb and reduce the hens established microbiota, to increase the probability of successful infection with reasonable doses of *Salmonella*, and to potentially reduce or eliminate detectable pre-existing salmonellae infections. Using a direct selective enrichment method described below, fecal samples gathered from nonoverlapping areas of the delivery vehicle were screened for the presence of *Salmonella*. Recovery results were compared to samples collected from individual hens at 3 d postadministration of antibiotics. Of the delivery vehicle samples, *Salmonella* was recovered from 100% (5/5) of samples. Conversely, *Salmonella* was recovered from 0% (0/10) of samples gathered 3 d postadministration of antibiotics, suggesting that this prechallenge administration eliminated detectable levels of *Salmonella* spp.

Salmonella Strain, Culture Conditions, and Challenge Model

Conventional methodologies were used to enrich, enumerate and serotype a contemporary, wild-type *S. enterica* serovar Reading isolate obtained from commercial turkeys. This strain has been identified as PHL-SR-2019. In the present study, 100 μ L of SR from a frozen

aliquot was added to 10 mL of tryptic soy broth (TSB, Catalog No. 22092, Sigma, St. Louis, MO), incubated at 37 °C for 8 h, and passed three times every 8 h to ensure that all bacteria were in log phase. Postincubation, bacteria were washed three times with sterile 0.9% saline by centrifugation at 1864 g for 10 min, reconstituted in saline, quantified by densitometry with a spectrophotometer (Spectronic 20DC, Spectronic Instruments Thermo Scientific, Rochester, NY) and finally diluted to an approximate concentration of 10⁸ cfu/mL. Levels of SR were further verified by serial dilutions and plated on brilliant green agar (BGA, Catalog No. 70134, Sigma, St. Louis, MO) for enumeration of actual cfu used in the experiment. Turkeys were challenged with 10⁸ cfu of SR by oral gavage on both d 4 and 7 postplacement, with care being taken to ensure each hen received both full doses.

Sampling Methods

Subsets were subjected to simulated commercial processing at the University of Arkansas Pilot Processing Plant on d 14 (n = 40), 21 (n = 40), and 28 (n = 32) postplacement (corresponding to 10, 11, and 12 wk of age). Following scald and feather picking, 2 cm from crop, lung, liver + spleen, and ceca were aseptically sampled at all three ages (10, 11, and 12 wk of age). Additional aseptic samples of synovial fluid from the hock joint (10 wk) were obtained from seared and aseptically opened joint by sterile swab, skin from the thoracic inlet region (~ 4 cm²; at 10 and 11 wk), and tracheal swabs, accessing the trachea from a seared and opened incision about 2 cm caudal to the larynx, and with the swab inserted to the depth of the syrinx (10 and 11 wk) were collected and immediately placed in swab bacteriological transport medium. All samples were collected using flamed, sterilized instruments, and immediately placed into sealed Whirl-Pak bags or transport medium tubes before being stored on ice for shipment.

Sample Enrichment and Recovery

Following collection, samples were promptly delivered to NWA Vet Services (Springdale, AR) for *Salmonella* recovery and serotyping. Samples were physically stomached, enriched in tetrathionate broth with iodine overnight at 40°C, and streaked on to XLT-4 agar for recovery. To verify the results from the colonies on XLT-4 agar and confirm the identity of the recovered *Salmonella* as *S. Reading*, *Salmonella* recovered from samples collected at 10 and 12 wk of age were serotyped to verify the identity of the recovered *Salmonella* as *S. Reading*. For serotyping, the four-tube panel of polyvalent antisera method was used as described by the American Society for Microbiology (<https://aem.asm.org/content/aem/24/5/846.full.pdf>).

Data and Statistical Analysis

Enrichment data were expressed as positive/total turkeys (%), and the percentage of *Salmonella* Reading positive samples were compared by a chi-square test of independence, testing all possible combinations to determine the significance ($P < 0.01$).

RESULTS AND DISCUSSION

In the present study, SR could not be recovered from stifle joint 14 d P.I and was not determined at 21- and 28-d P.I (Table 1). Skin samples showed the highest incidence of SR recovery (80%) 14 PI, followed by crop (75%); liver and spleen (67.5%); lungs (60%); and ceca (57.5%). The organ with the lowest percentage of SR recovery was the trachea with 40% of positive samples ($P < 0.01$). At 21 d PI, ceca samples showed the highest rate of positive samples followed by the crop, suggesting a fecal-oral infection that allows the persistent colonization and systemic organ invasion of SR that persisted at 28 d PI. While cecal samples were consistently positive for SR at alltime points, recovery of SR from skin and trachea declined rapidly. By four wk postchallenge (12 wk of age), SR was recovered from 22% of liver + spleen and 34% of lung samples (Table 1).

The first report of paratyphoid infection in turkey poults due to *Salmonella* Reading was published in 1956, involving 150 turkey poults with a 66% mortality, with the probable egg-borne transmission of infection (Mitrovic, 1956). Currently, at turkey processing, the anatomical source of *Salmonella* contamination in products, especially ground turkey, is largely unreported. To provide a preliminary evaluation of potential anatomical sites of potential contamination, we developed a model for infection of older turkeys with a contemporary wild-type *S. enterica* serovar Reading (SR). The results of this work, in combination with previous works completed by our laboratory, appears to indicate that the pulmonary tissue of turkeys may play a much larger role

in *Salmonella* contamination during processing than was previously known (Kallapura et al., 2014). Furthermore, the high incidence of SR in crop and ceca at 28 d PI suggest that SR can persist infecting the birds by oral-fecal infection (Table 1). While interventions to reduce food-borne pathogens such as *Salmonella* should target all parts of the supply chain, including slaughter, processing facilities, and upstream farm sources, public health agencies, and industry must take steps to provide more consumer education about food safety. Hence, the importance of science and education programs is required to reduce this zoonotic pathogen at relevant points of the 'farm-to-fork' food production chain. The results of this study confirm that SR is an emerging problem for the turkey industry and provides producers with useful microbiological information to make informed decisions during processing. The relatively high frequency of recovery from lung tissue was unexpected and may have implications for potential interventions at processing as well as preventing infections in the field.

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DISCLOSURES

Brian Wooming is employed by Cargill Turkeys LLC. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships construed as a potential conflict of interest.

REFERENCES

- Arsenault, J., A. Letellier, S. Quessy, V. Normand, and M. Boulianne. 2007. Prevalence and risk factors for *Salmonella* spp. and *Campylobacter* spp. caecal colonization in broiler chicken and turkey flocks slaughtered in Quebec, Canada. *Prev. Vet. Med.* 81:250–264.
- Hassan, R., S. Buuck, D. Noveroske, C. Medus, A. Sorenson, J. Laurent, D. Rotstein, L. Schlater, J. Freiman, and A. Douris. 2019. Multistate outbreak of salmonella infections linked to raw Turkey products—United States, 2017–2019. *Morb. Mortal. Wkly. Rep.* 68:1045.
- Kallapura, G., A. Botero, S. Layton, L. Bielke, J. Latorre, A. Menconi, X. Hernández-Velasco, D. Bueno, B. Hargis, and G. Téllez. 2014. Evaluation of recovery of *Salmonella* from trachea and ceca in commercial poultry. *J. Appl. Poult. Res.* 23:132–136.
- Loharikar, A., E. Briere, C. Schwensohn, S. Weninger, J. Wagendorf, J. Scheffel, A. Garvey, K. Warren, E. Villamil, J. A. Rudroff, K. Kurkjian, S. Levine, K. Colby, B. Morrison, A. May, S. Anderson, E. Daly, N. Marsden-Haug, M. M. Erdman, T. Gomez, A. Rhorer, J. Castleman, J. K. Adams, L. Theobald, P. Lafon, E. Trees, J. Mitchell, M. J. Sotir, and C. B. Behraves. 2012. Four multistate outbreaks of human *Salmonella* infections associated with live poultry contact, United States, 2009. *Zoonoses Public Health* 59:347–354.

Table 1. Percent recovery of *Salmonella* reading from different tissues in turkeys evaluated in an oral gavage challenge model.¹

Tissue	Culture at 14 d PI	Culture at 21 d PI	Culture at 28 d PI
Stifle Joint	0/40 (0%)	ND	ND
Skin	32/40 (80.0%) ^{a,x}	5/40 (12.5%) ^{b,z}	ND
Trachea	16/40 (40.0%) ^{a,z}	3/40 (7.5%) ^{b,z}	ND
Crop	30/40 (75.0%) ^{a,x,y}	27/40 (67.5%) ^{a,x}	14/32 (43.8%) ^{b,z}
L/S	27/40 (67.5%) ^{a,y}	18/40 (45.0%) ^{b,y}	7/32 (21.9%) ^{c,z}
Lung	24/40 (60.0%) ^{a,y}	21/40 (52.5%) ^{a,y}	11/32 (34.4%) ^{b,z}
Ceca	23/40 (57.5%) ^{b,y}	35/40 (87.5%) ^{a,w}	24/32 (75.0%) ^{ab,y}

¹Turkeys were challenged with 10⁸ colony-forming units (cfu) of *S. Reading* (SR) by oral gavage on d 4 and 7 postplacement. Subsets were subjected to simulated commercial processing at the U. Arkansas Pilot Processing Plant on d 14 (n = 40), 21 (n = 40) and 28 (n = 32) postplacement (corresponding to 10, 11, and 12 wk of age). Data expressed as positive samples of *S. Reading*/total number of samples (%).

^{a,c}Values within sample rows, or ^{x,z} values within culture time of evaluation column with different superscripts differ significantly ($P < 0.01$). ND, nondetermined.

- Manning, J., B. Hargis, A. Hinton Jr, D. Corrier, J. DeLoach, and C. Creger. 1992. Effect of nitrofurazone or novobiocin on *Salmonella enteritidis* cecal colonization and organ invasion in leghorn hens. *Avian Dis.* 334–340.
- Mitrovic, M. 1956. First report of paratyphoid infection in turkey poults due to *Salmonella* reading. *Poult. Sci.* 35:171–174.
- Morales-Barrera, E., N. Calhoun, J. L. Lobato-Tapia, V. Lucca, O. Prado-Rebolledo, X. Hernandez-Velasco, R. Merino-Guzman, V. M. Petrone-Garcia, J. D. Latorre, B. D. Mahaffey, K. D. Teague, L. E. Graham, A. D. Wolfenden, M. F. Baxter, B. M. Hargis, and G. Tellez. 2016. Risks involved in the use of enrofloxacin for *Salmonella enteritidis* or *Salmonella heidelberg* in commercial poultry. *Front. Vet. Sci.* 3:72.
- Routh, J., J. Pringle, M. Mohr, S. Bidol, K. Arends, M. Adams-Cameron, W. Hancock, B. Kissler, R. Rickert, and J. Folster. 2015. Nationwide outbreak of multidrug-resistant *Salmonella* Heidelberg infections associated with ground turkey: United States, 2011. *Epidemiol. Infect.* 143:3227–3234.
- Scallan, E., P. M. Griffin, F. J. Angulo, R. V. Tauxe, and R. M. Hoekstra. 2011. Foodborne illness acquired in the United States—unspecified agents. *Emerg. Infect. Dis.* 17:16.
- Schlundt, J., H. Toyofuku, J. Jansen, and S. Herbst. 2004. Emerging food-borne zoonoses. *Rev. Sci. Tech.* 23:513–534.