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Fate and Dissemination of Salmonella Reading in Market-age Turkeys at Processing using Oral Gavage Challenge Model

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Fate and Dissemination of *Salmonella* Reading in Market-age Turkeys at Processing using Oral Gavage Challenge Model

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Abstract

This study aimed to evaluate the fate and dissemination of *Salmonella* Reading (SR) in market-age 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 10^8 cfu SR by oral gavage on days 4 and 7 post-placement. Subsets were subjected to simulated commercial processing on days 14 (n=40), 21 (n=40) and 28 (n=32) post-placement (corresponding to 10, 11, and 12 weeks of age). After scald and feather picking, samples of stifle joint, skin, trachea, crop, lung, liver, and spleen (L.S.), and ceca were aseptically sampled, enriched in tetrathionate broth, and streaked on XLT-4 agar for recovery and serotyping of SR colonies. SR could not be recovered from stifle joint 14 days P.I. Skin samples showed the highest incidence of SR recovery (80 %) 14 PI, followed by crop (75 %); LS (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 days P.I., ceca samples showed the highest rate of positive samples followed by the crop, suggesting a fecal-oral infection that allows the colonization and systemic organ invasion of SR that persisted at 28 days P.I. While cecal samples were consistently positive for SR at all time points, recovery of SR from skin and trachea declined rapidly. While interventions to reduce foodborne pathogens such as *Salmonella* should target all parts of the supply chain, including slaughter and processing facilities, and upstream farm sources, public health agencies, and industry must take steps to provide more consumer education about food safety. The present work suggests that pulmonary tissue may be an unexpected source of turkey carcass and ground turkey contamination with this

serovar at processing. If confirmed, new intervention steps to reduce cross contamination from lung tissue during evisceration may be needed.

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Chapter 1

Introduction

There are several serovars of *Salmonella enterica* which, in contrast to most pathogens, lack specific host-adaptations resulting in their ability to colonize the gastrointestinal (GI) tract of a wide variety of vertebrate animals (Scherer and Miller, 2001). Colonization of humans by these serovars produces the pathological condition known as salmonellosis, which may present with a broad range of symptoms; the most notable and severe being acute gastroenteritis associated with diarrhea. Incidences of human salmonellosis are typically attributed to improperly prepared or handled meats including pork, beef, and, most commonly, poultry (Hsi *et al*., 2015). However, recent studies have revealed that *Salmonella* infections may also originate from products comprised solely of vegetables and cereal grains such as vegetarian snack foods and dry puffed breakfast cereal (Andrews-Polymenis *et al*., 2010). Contamination of these products is believed to occur through the large-scale application of manure as fertilizer during crop production and is supported by research indicating that manure commonly harbors enteric pathogens including *Campylobacter jejuni, Escherichia coli*, and *Salmonella* (Kyakuwaire *et al*., 2019; Venglovsky *et al*., 2009). While it is well recognized that *Salmonella* are frequently present throughout animal feeding operations, their presence in waste used as fertilizer appears to indicate that *Salmonella* are quite resilient and able to persist outside of the host for extended periods of time. Their ubiquitous presence would also suggest that salmonellae are not easily eradicated from the production environments and, as a result, may continue to proliferate in the absence of an animal host and survive to infect subsequent flocks placed within the same facility.

With this understanding, we have suggested a mechanism by which *Salmonella* bacteria spread and infect new hosts via the generation and inhalation of bioaerosols which are produced as a direct result of modern poultry production practices (Téllez *et al*., 2014). While the fecal-oral route has been the presumed predominant method by which transmission occurs in poultry, studies from our laboratory and others have suggested that the respiratory tract plays a much larger role in the establishment of initial infections, as well as subsequent reinfections, than has been previously recognized (Kallapura *et al*., 2014[a]; Téllez *et al*., 2014; Dungan, 2010). Therefore, the following discussion includes several mechanisms believed responsible for the spread of *Salmonella* throughout poultry production facilities, as well as the generation of bioaerosols capable of supporting *Salmonella*, followed by potentially exacerbating practices of modern poultry production throughout various time points. Lastly, a previously theorized mechanism by which *Salmonella* may gain access into systemic circulation via the respiratory tract is presented in conjunction with supporting evidence from our recent research attempts will be discussed.

Literature Review

Salmonella, Disease, and Poultry

Salmonellae serovars of the species *Salmonella enterica*, belonging to the family Enterobacteriaceae, are a genus of rod-shaped, gram-negative, non-spore forming bacteria which derive energy from the oxidation and reduction of organic sources readily available throughout their environments. Presently, more than 2,600 serovars of *Salmonella* have been identified, with over 200 of these known to be zoonotic. These *Salmonella* serovars are classified into two distinct groups known as paratyphoid (i.e., non-typhoidal) and typhoidal (Gal-Mor *et al*., 2014). The typhoidal serovars are those which are host-adapted and typically unable to spread and infect other animal species. In regard to poultry, typhoidal strains include both *S. gallinarum* and *S. pullorum* which cause serious morbidity and mortality, and are host adapted for gallinaceous birds. In contrast, the paratyphoid *Salmonella* are mostly considered potentially zoonotic, and typically only pathogenic in poultry when coupled with stress or immunosuppressive disease (Gomes *et al*., 2014; Hoerr, 2010; Humphrey, 2004). The clinical signs and lesions caused by infection with paratyphoid serovars are very diverse and may range from subclinical or asymptomatic, to indistinguishable from typhoidal *Salmonella*. As a result, these species may remain completely undetected in poultry (Barrow, 2000).

Human infection by the paratyphoid serovars is routinely the result of improper handling or preparation of poultry and egg products. While human infections are typically limited to the mucosa of the small intestine, systemic disease is known to occur and can be quite severe (Roth, 2013) As stated previously, cattle, swine, and poultry operations are known to act as reservoirs for such enteric, zoonotic pathogens, suggesting that preventing the introduction of *Salmonella* into poultry flocks, in addition to swift and accurate detection, is vital to successfully preventing

human disease, as are interventions during poultry processing (Loharikar *et al*., 2012; Hafez, 1999). Prevention of the introduction of Salmonella to poultry is quite difficult and costly, as common sources include rodents, wild birds, and sometimes insects that can migrate between other poultry or livestock sources into otherwise uncontaminated poultry premises (Backhans and Fellström, 2012; Lapuz *et al*., 2012; Jones, 2011). Introduction of Salmonella by way of contaminated feed is another potential, but less common, source of Salmonella infections in poultry (Jones, 2011).

Fecal – Oral Transmission of *Salmonella*

The fecal-oral route is well recognized as the primary method by which *Salmonella* are spread among poultry and humans. Infection through this path involves the uptake and ingestion of feces or fecally contaminated material, leading to eventual colonization of the ceca in birds, followed by shedding of additional *Salmonella* into the environment (Shivaprashad, 1997; Byrd *et al*., 1998). Therefore, discussion in this section focuses primarily on the introduction and fecal-oral spread of *Salmonella* at various time points across production. Consideration is given to the various barriers typically encountered by *Salmonella* at each of these points, and current knowledge regarding the mechanisms used to overcome these barriers is presented. Finally, current practices used by the industry and their potential effects on susceptibility of the flock are introduced.

Entry of *Salmonella* at Various Stages of Poultry Production

To enable salmonellae to infect a host, these pathogens must be introduced and situated at a point within the host's environment which facilitates their interaction and ability to infect. Somewhat predictably, known points of interaction between *Salmonella* and poultry appear highly dependent upon bird age, and seem to be relatively conserved across both chickens and turkeys. Curiously, however, attempts to identify the most frequent introduction point(s) of *Salmonella* has yielded a wide variety of answers, implying that introduction can occur at any age or time across the entire live production process (Heyndrickx *et al*., 2002; Crabb *et al*., 2018). As such, the commonly recognized routes of introduction tend to involve rodents and humans, as well as contamination introduced through substances including feed, water, and eggshell contamination prior to entry into a hatchery (Gantois *et al*., 2009; Braden, 2006; Shivaprashad, 1997). Conceptualization of the proceeding topics requires the thorough understanding that entry of *Salmonella* can occur at any time during production, and through any one of a multitude of vectors.

Salmonella Ingestion, Survival, Adhesion, and Invasion

As an enteric pathogen, the primary habitat of *Salmonella* is the intestinal tract of humans and animals. Establishing this intestinal infection requires *Salmonella* to endure and overcome several barriers of the host GI tract, which have previously been explained in detail by Téllez *et al* (2014). Briefly summarized, ingestion carries *Salmonella* to the proventriculus where, upon entrance, the expression of a variety of acid shock tolerance regulatory factors by *Salmonella* aids in survival of the low pH environment. Subsequent transit into the intestinal lumen exposes

the surviving *Salmonella* to bile, which plays a well-recognized role in the modulation of *Salmonella* pathogenesis. Regarding protective function, the highly impermeable nature of the Gram-negative outer membrane, in combination with the bile excreting multidrug efflux pumps contained there, enables *Salmonella* to endure such harsh conditions. In birds, the greatest frequency of colonization and the primary site of amplification of Salmonellae is primarily at the distal ileum and the ceca; a process which requires *Salmonella* to first penetrate through the mucus layer found shielding the epithelium. Bacterial adhesion to host epithelial cells then occurs and is a crucial first step in the intestinal infection process, with *Salmonella* capable of initiating adhesion to a variety of cell types. Host-cell invasion follows, allowing *Salmonella* to evade recognition by the immune system by residing intracellularly.

As reviewed by Téllez *et al*., 2014, intracellular invasion triggers a cascade of inflammatory processes, guiding the expression and release of various proinflammatory cytokines and chemokines. In response, heterophils and macrophages are recruited to the site of intestinal infection. Interestingly, this intestinal inflammation actually causes leakage of tetrathionate from the mucosa and submucosa to the luminal epithelial surface. Salmonellae are among the relatively few bacteria that are able to use tetrathionate as an energy source, providing these organisms a selective advantage over most other enteric competitive microflora (Winter *et al*., 2010).

Bioaerosol Generation and Poultry Practices

Data from earlier works completed by our lab appears to suggest that the avian respiratory tract contributes significantly towards the harborage, transmission, and eventual systemic spread of

Salmonella within birds and throughout poultry houses (Téllez *et al*., 2014; Kallapura *et al*., 2014[abc]). More precisely, we have proposed that bioaerosols may act as a micro-environment capable of briefly sustaining the highly adaptive *Salmonella* species afflicting poultry, ultimately facilitating systemic infections by way of inspiration. As discussed below, it is proposed that salmonellae entering the respiratory tract as particles less than 4um in diameter are inspired into the lower respiratory tract which is non-ciliated (Hayter and Besch, 1974). At this point, salmonellae and other particles are primarily removed by the monocyte-macrophage system, and salmonellae have the well-documented ability to evade destructive intracellular systems within these cells (Bijlsma and Groisman, 2005; Cirillo *et al*., 1998) Traveling within these disabled macrophages, S*almonella* are able to colonize the gallbladder by first accessing the liver by way of portal blood (Gonzalez-Escobedo *et al*., 2011). This presents the possibility of enterohepatic circulation of *Salmonella*; a route which has previously been described for bile acids. Discussion related to the current understanding of bioaerosols and their generation throughout various production time points, as well as factors enabling their airborne spread during hatch, grow-out, and processing, is offered below.

Bioaerosols at Hatch

The presence of airborne *Salmonella* in hatcheries has been proven utilizing a variety of sampling techniques and identification methods, indicating that bioaerosols are produced from eggshell fragments (Gast *et al*., 1998). It is believed that circulating air is responsible for the movement of these bioaerosol particles which settle throughout the incubator and on to hatchlings and uninfected eggs. Following this, infection of chicks and poults is believed to

occur through the previously described fecal-oral route, as the natural, curious pecking behaviors expressed by hatchlings results in the ingestion of eggshell fragments.

Furthermore, hatchlings are regularly held at the hatchery facility for a period of time ranging from 12-24 hours following hatch. During this time vaccinations are administered, and hatchlings typically do not receive food or water, potentially inducing stress and adversely affecting maturation of the gut microbiome while further increasing susceptibility to pathogens such as *Salmonella*. In addition to this, transport to production facilities for placement is highly variable, and further delays access to feed and water while increasing overall exposure time to pathogen rich environments. In total, it is not uncommon for placement to occur as late as 36 to 48 hours after hatch.

Bioaerosol during Grow-out and during Pre-processing holding

Bioaerosols encountered during grow-out are primarily comprised of mucus secretions, fecal material, feed particles, dander, and litter fragments (Just *et al*., 2009). Depending upon particle size and environmental moisture, these bioaerosols may provide a suitable environment upon which viable salmonellae will persist (Just *et al*., 2009; Lighthart, 2000). Airflow is controlled by large fans located at one end of a poultry house and function to control and maintain various factors including litter moisture, temperature, and ammonia levels. Similar to the spread of bioaerosols in hatchery incubators, it is hypothesized that this airflow may disturb and distribute *Salmonella*-containing bioaerosols throughout the house, leading to their eventual inhalation or ingestion after settling throughout the facility.

In addition to this, catching and load-out often results in the dispersal of large quantities of dust and bioaerosols. During this process, which includes physical catching and loading of birds, bird stress is known to be increased and may potentially increase the susceptibility of poultry to infection (de Lima *et al*., 2019). Moreover, in an attempt to decrease the volume of intestinal contents, feed is removed approximately 8 to 12 hours prior to catch and transport. This process contributes to elevated stress, increasing susceptibility to infection (Durant *et al*., 1999), and leads to increased coprophagy of feces, cecal droppings, and contaminated litter (Corrier *et al*., 1999).

Furthermore, upon arrival to processing facilities, poultry are not usually unloaded immediately. Instead, covered, open-sided sheds lined with fans are utilized for temporary holding. During warmer months, these fans are used to keep the birds comfortable but have also been implicated in the airborne spread of *Salmonella* after as a little as two hours of exposure as cooling fans and water are placed horizontal to trailers with relatively high air velocity, sometimes with trailers directly "down wind" of other trailers (Harbaugh *et al*., 2006).

Mucociliary Escalator

Structure and function of the avian respiratory tract has been previously reviewed by Téllez *et al* (2014). Particles or aerosols less than 4-6 um may reach the lower respiratory tract of birds, and areas lower than the initiation of the secondary bronchi are devoid of cilia. Thus, those particles reaching the lower respiratory tract must be cleared by the monocyte-macrophage system (Hayter and Besch, 1974; Mutua *et al*., 2016; Yamasaki and van Eeden, 2018)

Formed by a combination of ciliated epithelial cells and mucus produced by the trachea mucosa, defense of the URT against pathogens and, by extension, the lower respiratory tract (LRT), is primarily carried out by the mucocilliary escalator (MCE). The MCE plays a central role as a clearance mechanism in the prompt removal of inhaled foreign particles from the URT. Removal is facilitated by the coordinated sweeping motion of the aforementioned ciliated cells lining the URT structures, propelling mucus and entrapped foreign particles cranially towards the epiglottis for either expulsion or ingestion into the acidic environment of the proventriculus. Interestingly, the MCE becomes absent shortly after the bifurcation of primary bronchi into secondary bronchi, suggesting that defense of distal pulmonary structures is highly dependent upon the phagocytic immune cells found there (Mutua *et al*., 2016). Furthermore, research has implicated formaldehyde, which is typically used to control the presence of pathogenic bacteria in hatcheries, as a potential agent responsible for the decreased function of the MCE (Johnson, 2018). Therefore, even slight inhibition of MCE function may drastically increase the susceptibility of poultry to infections of the URT, as well as the LRT, as inhaled bioaerosols freely pass into deeper respiratory structures and become deposited there.

Larger particles are excluded from the LRT by additional divisions of the secondary bronchi into smaller diameter parabronchi; a system referred to as aerodynamic filtration (Mutua *et al*., 2016). As mentioned above and discussed below, particles entering the non-ciliated portions of the respiratory tract are removed by the monocyte-macrophage system which can allow systemic distribution of the salmonellae.

Respiratory Cellular Defense

Functional clearance of pathogens and debris from the respiratory system is largely dependent upon the phagocytic activity of resident and free macrophages. However, various studies have shown that only a very low number of macrophages are present here, potentially providing an environment where pathogens may easily avoid detection by the acquired immune system. Moreover, it is also possible that pathogens are promptly recognized by immune cells here, but that these cells are not present in adequate numbers required to induce an appropriate response, which is proportional to the release of phagocytic chemotactic activating factors, resulting in impaired function and aiding in systemic distribution (Kiama *et al*., 2008).

Proposed *Salmonella* Infection Process via the Avian Respiratory Route

Similar to the commercial production of broilers, the turkey industry has relied heavily upon genetic selection, in combination with modern husbandry and management practice, for increased production efficiency. While remarkably successful in improving animal welfare while reducing costs and overall time required for flock production, several undesirable side effects, such as metabolic disease and growth-related disorders, have arisen as a direct result of such selection programs (Julian, 2005). Therefore, it is plausible that the apparent vulnerability of poultry to respiratory disease is also an undesirable side effect of genetic selection, as well as other various human interventions including improved management practices, which have aided in the ability to rear birds in higher densities (Kiama *et al*., 2008).

Survival in the Upper Respiratory Tract

As discussed previously, even slight disfunction of the respiratory defense mechanisms results in the unobstructed passage of *Salmonella*-containing bioaerosols into deep respiratory structures. By transiting through the respiratory tract, *Salmonella* do not encounter any of the harsh conditions found in the GI tract. Combined with both the physiological defects discussed above, as well as the inherent lack of immune cells in respiratory organs, to indicate that turkeys and chickens are highly susceptible to bacterial respiratory infections (Kiama *et al*., 2008). Coincidentally, the respiratory route has been previously described as a potential mechanism by which *Salmonella* are able to establish systemic infections in poultry. Potentially, this, in combination with the known ability of salmonellae to incapacitate the macrophage system from destroying these pathogens, and the necessity of macrophage removal of particles below the ciliated portion of the respiratory tract, provides additional evidence that the respiratory tract could be a portal of entry for Salmonella infections and could provide at least a temporary site of infection by salmonellae, leading to an additional anatomical consideration for interventions within commercial processing plants.

The manuscript below describes an effort to identify the most common anatomical sites harboring *Salmonella* Reading within processed commercial turkeys at market age following intentional inoculation. For the reasons above, respiratory tissue was included in the study below, in addition to other known or suspects sources of Salmonella Reading residual contamination at commercial processing.

Chapter 2

Fate and Dissemination of *Salmonella* Reading in Market-age Turkeys at Processing using Oral Gavage Challenge Model

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Abstract

This study aimed to evaluate the fate and dissemination of *Salmonella* Reading (SR) in market-age 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 10^8 cfu SR by oral gavage on days 4 and 7 post-placement. Subsets were subjected to simulated commercial processing on days 14 (n=40), 21 (n=40) and 28 (n=32) post-placement (corresponding to 10, 11, and 12 weeks of age). After scald and feather picking, samples of stifle joint, skin, trachea, crop, lung, liver, and spleen (L.S.), and ceca were aseptically sampled, enriched in tetrathionate broth, and streaked on XLT-4 agar for recovery and serotyping of SR colonies. SR could not be recovered from stifle joint 14 days P.I. Skin samples showed the highest incidence of SR recovery (80 %) 14 PI, followed by crop (75 %); LS (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 days P.I., ceca samples showed the highest rate of positive samples followed by the crop, suggesting a fecal-oral infection that allows the colonization and systemic organ invasion of SR that persisted at 28 days P.I. While cecal samples were consistently positive for SR at all time points, recovery of SR from skin and trachea declined rapidly. While interventions to reduce foodborne pathogens such as *Salmonella* should target all parts of the supply chain, including slaughter and processing facilities, and upstream farm sources, public health agencies, and industry must take steps to provide more consumer education about food safety.

Keywords: *Salmonella* Reading, turkeys, ceca, lungs, processing

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). 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* Reading 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 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 marked age 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.

Pre challenge Administration of Antibiotics

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 non-overlapping areas of the delivery vehicle were screened for the presence of *Salmonella*. Recovery results were compared to samples collected from individual hens at three days post-administration 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 three days post-administration of antibiotics,

suggesting that this pre-challenge administration successfully perturbs the established microbiota.

Salmonella strain, culture conditions, and challenge model

Conventional methodologies were used to enrich and enumerate a contemporary, wild-type *S. enterica* Serovar Reading (SR) isolate previously obtained from the field. In the present study, 100 µL of SR from a frozen aliquot was added to 10 mL of tryptic soy broth (TSB, Catalog No. 22092, Sigma, St. Louis, MO, USA), incubated at 37 °C for eight hours, and passed three times every eight hours to ensure that all bacteria were in log phase. Post-incubation, 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, USA) and finally diluted to an approximate concentration of 10^8 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, USA) for enumeration of actual cfu used in the experiment. Turkeys were challenged with 10^8 cfu of SR by oral gavage on both days 4 and 7 post-placement, 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 days 14 (n=40), 21 (n=40), and 28 (n=32) post-placement (corresponding to 10, 11, and 12 weeks of age). Following scald and feather picking, samples of crop, lung, liver +

spleen, and ceca were aseptically sampled at all three ages (10, 11, and 12 weeks of age). Additional aseptic samples of hock joint (10 weeks), skin from the thoracic inlet region (10 and 11 weeks), and trachea (10 and 11 weeks) were collected. All samples were collected using flamed, sterilized instruments, and immediately placed into sealed Whirl-Pak bags before being stored on ice.

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 weeks of age were serotyped to verify the identity of the recovered *Salmonella* as *S.* Reading.

Data and statistical analysis

Enrichment data were expressed as positive/total chickens (%), and the percentage of *Salmonella* Reading positive samples were compared by a chi-square test of independence (Zar, 1984), testing all possible combinations to determine the significance ($p < 0.01$).

Results and Discussion

Salmonella is one of the most important foodborne zoonotic pathogens, and there is evidence that poultry products play an important role in this zoonosis (Schlundt et al., 2004). 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. In the present study, SR could not be recovered from stifle joint 14 days P.I and was not determined at 21- and 28-days P.I. 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 days P.I., 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 days P.I. While cecal samples were consistently positive for SR at all time points, recovery of SR from skin and trachea declined rapidly. By four weeks post-challenge (12 weeks of age), SR was recovered from 22% of liver + spleen and 34% of lung samples (Table 1).

Conclusion

The first report of paratyphoid infection in turkey poults due to *Salmonella* Reading was published in 1956, involving 150 turkey poults with a 66 percent 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 strain's environmental presence can persist infecting the birds by oral-fecal infection (Table 1). While interventions to reduce foodborne pathogens such as *Salmonella* should target all parts of the supply chain, including slaughter and 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 required to reduce this zoonotic pathogen at relevant points of the 'farm-to-fork' food production chain.

Conflict of Interest

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.

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Appendix

UNIVERSITY OF ARKANSAS

Office of Research Compliance

To:	Billy Hargis
Fr:	Craig Coon
Date:	July 22nd, 2019
Subject:	IACUC Approval Expiration Date: July 18th, 2022

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your protocol # **20004:** *Identification of Salmonella contamination sources during turkey processing*.

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond July 18th, 2022 you must submit a newly drafted protocol prior to that date to avoid any interruption. By policy the IACUC cannot approve a study for more than 3 years at a time.

The following individuals are approved to work on this study: Billy Hargis, Guillermo Tellez, Christine Vuong, Callie McCreery, Danielle Graham, Cheryl Lester, Aaron Ashcraft, Makenly Coles, and Casey Owens. Please submit personnel additions to this protocol via the modification form prior to their start of work.

The IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

CNC/tmp

Table 1. Percent recovery of *Salmonella* Reading from different tissues in market-age turkeys evaluated in an oral gavage challenge model [1]

	Culture at 14 days P.I.	Culture at 21 days P.I.	Culture at 28 days P.I.
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, xy]	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]

[1] Turkeys were challenged with 10^8 colony-forming units (cfu) of *S.* Reading (SR) by oral gavage on days 4 and 7 post-placement. Subsets were subjected to simulated commercial processing at the U. Arkansas Pilot Processing Plant on days 14 (n=40), 21 (n=40) and 28 (n=32) post-placement (corresponding to 10, 11, and 12 weeks of age).

Data expressed as positive samples of *S.* Reading / total number of samples (%).

[a–b] Values within sample rows, or [x-z] values within culture time of evaluation column with different superscripts differ significantly ($P < 0.01$). ND= Non determined