Poultry Science

Research Note: Evaluating the vertical transmission potential of *Salmonella* Reading in broiler breeders

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ABSTRACT *Salmonella* Reading (***S.* Reading**) recently emerged as a foodborne pathogen causing extensive human outbreaks in North America from consuming contaminated poultry products, mostly from turkeys. Understanding the transmission dynamics of this pathogen is crucial for preventing future outbreaks. This study investigated the ability of *S.* Reading to colonize the tissues and contaminate eggs of broiler breeders. We utilized 2 *S.* Reading strains, marked with bioluminescence gene: the outbreak strain RS330 and a reference strain RS326. We used 32 commercially sourced broiler breeder hens, 34 wk of age, randomly assigned to the 2 treatments (16 hens per strain). Each hen was intravaginally inoculated with 10^8 CFU of the respective strain on d 1 and was rechallenged on d 4. Eggs were collected daily postchallenge to recover bioluminescent *S.* Reading strains from the external eggshell surface and internal egg contents. On d 7 postchallenge, 10 hens from each treatment group were euthanized. Ovaries, oviducts, and ceca were aseptically collected to detect *S.* Reading colonization. Results showed that 70.5% (36 of 51) and 34.5% (19 of 55) of external eggshell surfaces, and 4.0% (2 of 50) and 1.8% (1 of 54) of the internal egg contents tested positive for the outbreak and nonoutbreak strains. Additionally, 40.0% of ovaries, 70.0% of oviduct, and 70.0% of ceca samples from the outbreak strain group, and 20.0% of ovaries, 70.0% of oviduct, and 80.0% of ceca samples from nonoutbreak strain group were positive. No significant difference ($P = 0.05$) was observed in all the findings among the strains except for the eggshell surface contamination. These findings suggest that *S.* Reading can effectively colonize reproductive tissues, translocate to the ceca, and contaminate the eggs of hens. Future research is needed to determine whether *S.* Reading can remain viable within the eggs throughout incubation and until hatching.

Key words: *Salmonella* Reading, foodborne pathogen, egg contamination, reproductive tissue colonization, bioluminescence imaging

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INTRODUCTION

Salmonella enterica serotype Reading is an uncommon cause of human Salmonellosis. However, recent large-scale outbreaks caused by the serotype in the United States and Canada associated with contaminated poultry products have highlighted its potential public health impact (Hassan et al., 2019). Despite advancements in sanitation, access to clean water, and stringent food safety measures, Salmonellosis remains a significant public health concern globally, affecting both developing and developed nations (Sánchez-Vargas et al., 2011; Eng et al., 2015). The Centers for disease control and prevention (**CDC**) estimated that approximately 1.4 million cases of human Salmonellosis occur annually in the United States, with a substantial proportion linked to poultry and poultry products (CDC, 2024; O'Bryan et al., 2022). The persistence, high transmissibility, and acquisition of virulence and antimicrobial resistance traits by *Salmonellae* contribute to ongoing human exposure to these pathogens (Liljebjelke et al., 2005; Miller et al., 2020).

The poultry industry operates through a structured and interconnected hierarchy, from breeder farms to hatcheries, grower farms, and ultimately, processing plants. Continuous surveillance for pathogens such as *Salmonella*, which can persist in seemingly healthy flocks

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and spread throughout the production chain, is essential to mitigate the health risks posed to consumers (Antunes et al., 2016). *Salmonella* transmission within broiler production primarily occurs through vertical transmission from broiler breeders and horizontal transmission within broiler houses (Heyndrickx et al., 2002). In many instances, *Salmonella* isolates found in broiler processing plants can be traced back to the originating breeder flock or hatchery (Bailey et al., 2002; Kim et al., 2007; Shang et al., 2021). Therefore, understanding the transmission dynamics and reducing the occurrence of *Salmonella* at the breeder and hatchery levels are critical steps in minimizing human cases of Salmonellosis.

Bioluminescence, a natural process where living organisms convert chemical energy into light via enzymatic process, has been adapted as a powerful marker in research, enabling the detection of light emitted from cells or tissues within living organisms (Badr and Tannous, 2011; Zambito et al., 2021). Bioluminescent imaging (**BLI**) has been effectively used to study the progression of infectious diseases in animal models through the tagging of pathogens with bioluminescent genes (Hutchens and Luker, 2007; Castañeda et al., 2019). The objective of this study is to evaluate the ability of both recent outbreak and reference nonoutbreak *S.* Reading strains to colonize the reproductive tract and contaminate eggs in broiler breeders, thereby gaining insights into the transmission dynamics of this serotype in poultry using bioluminescent imaging. For this purpose, 2 *S.* Reading strains, previously tagged with bioluminescence, were utilized: a recent outbreak strain (RS330) and a reference nonoutbreak strain (RS326).

MATERIALS AND METHODS

Hens Used for the Study

All procedures used in this study were approved by the Institutional Animal Care and Use Committee of Mississippi State University (IACUC 22-399). A total of 40 Ross 708 broiler breeders of 34 wk of age were obtained from a commercial farm. All hens were housed in the animal biosafety level-2 facility. Upon arrival, cloacal and vaginal swabs were taken from the hens to test for inherent *Salmonella. Salmonella* negative hens were then placed in battery cages with 1 bird in each cage. The birds were fed with a commercial layer diet, and the feed and water were provided ad libitum. The lighting schedule was set to 14 h of light and 10 h of darkness. Egg production and the apparent health of the hens were monitored throughout the experiment.

Bacterial Strains and Growth Conditions

S. Reading strains used in this experiment were donated by Dr. Timothy Johnson (Miller et al., 2020), and transformed to bioluminescent for easy tracking and studying the foodborne pathogen (Abubakar et al., unpublished). The *S.* Reading nonoutbreak strain (RS326) is an ancient isolate and a rare cause of illness in humans, whereas the *S.* Reading outbreak strain isolate (RS330) was responsible for the recent human outbreaks in North America linked to poultry consumption. Throughout the study, the bioluminescent strains were cultured at 37°C for 24h in Xylose lysine tergitol 4 (XLT4) agar (BD Difco, DF0234-17-9, Sparks, MD) and LB broth (BD Difco^{TM}, DF0414-17-1, Sparks, MD) supplemented with 10 μg/mL of chloramphenicol. Enrichment of samples was achieved using Tetrathionate (**TT**) broth (BD Difco^{TM}, DF0491-17-7, Sparks, MD).

Bacterial Inoculation

Cultures of each strain were grown overnight in Luria-Bertani broth at 37°C for 24 h. After reaching the desired concentration of 10^8 CFU/mL ($OD_{600} \sim 0.45$), cultures were pelleted and resuspended in the same volume of sterile phosphate-buffered saline (PBS). Following 4 d of acclimatization post placement, 16 hens per the *Salmonella* Reading group were experimentally infected by the intravaginal route with 1 mL of the prepared inoculum of either bioluminescent *S.* Reading outbreak strain (RS330) or the nonoutbreak strain (RS326). 6 control hens were challenged via the same route with only PBS, and 2 were not excluded for nonlaying. After 4 d of the first challenge, the hens were reinfected in a similar manner to the first challenge to ensure maximum recovery of the *Salmonella* strains carrying the bioluminescence marker and chloramphenicol resistance gene plasmid.

Recovery of Salmonella From Eggs

Eggs laid from all treatment hens were collected daily in individual sterile whirl Pak bags. Thirty mL of TT broth was added to each egg sample to rinse the eggshell surface gently. The eggs were then carefully removed, and the surface rinses were incubated as outer shell enrichment samples. The eggshell surface was then sterilized by dipping it into 70% ethanol for 5 min and then carefully cracked open. The contents of each egg were emptied into separate sterile whirl Pak bags containing 50 mL of TT broth. The egg contents were then homogenized and cultured as the egg contents enrichment samples. From each enriched sample, 100 μL was spread plated on XLT4 agar plates in duplicate and incubated at 37°C for 24h. Colonies with black centers on the plates were confirmed as the challenged *Salmonella* strains through bioluminescent imaging using the *In vivo* imaging system (IVIS). They were recorded as positive for each respective *Salmonella* strain.

Recovery of Salmonella Reading From Reproductive Tissues and Ceca

On d 7 postinoculation, 10 hens from each treatment group were euthanized to recover *Salmonella* from their tissues. The ovary, upper oviduct (centered on the infundibulum/magnum junction), lower oviduct (centered on

the isthmus/uterus junction), and ceca were collected in separate sterile whirl Pak bags. Samples were weighed, 10-fold diluted in TT broth and incubated for enrichment at 42°C for 24-h. From each enriched sample, 100 μL were spread plated on XLT4 agar plates supplemented chloramphenicol in duplicates to recover *Salmonella* and incubated at 37°C for 24-h. Plates with black-centered colonies were confirmed by bioluminescent imaging using IVIS and recorded as positive for each treatment sample.

Statistical Analysis

The percentage prevalence of each of the 2 *Salmonella* Reading strains recovered from the eggshell surface, egg contents, reproductive tissues, and ceca was calculated, and the differences in the ability to contaminate eggs and colonize tissues between the 2 strains were compared using chi-squared test using SAS 9.4 with $P = 0.05$ (Tables 1 and 2).

RESULTS AND DISCUSSION

Investigations into the recent outbreak of *S.* Reading identified various poultry product brands as sources, rather than a common supplier (CDC, 2019; Public Health Agency of Canada, 2020). This has led to speculation that the outbreak strain may have spread from a common breeder source rather than being confined to a single farm or processing plant (Miller et al., 2020). The spread of certain *Salmonella* serotypes from infected breeders through fertile eggs to the chicks has been previously reported (Cui et al., 2023; Dórea et al., 2010).

Table 1. Recovery of *S.* Reading from eggshell surfaces and egg contents.

		Number of positive samples in each treatment	
Treatment Group	Total eggs	Egg shell surfaces	Egg contents
SRO	51	36/51 (70.5%[a])	2/50* (4%[a])
SRN	55	19/55 (34.5%[b])	1/55 (1.8%[a])

[a,b]Column not sharing a common superscript were different ($P < 0.05$).
[1]Total eggs, total number of eggs laid by hens in each treatment group after inoculation.
[2]Numerators, number of positive samples.
[3]Denominators, total number of samples.
[4*], one missing egg content due to broken shell at the time of collection.
Abbreviations: SRO, *Salmonella* Reading outbreak strain; SRN, *Salmonella* Reading nonoutbreak strain.

Similarities between *Salmonella* isolates from hatchlings and those from farms where they were sourced have also been observed (Jibril et al., 2023). This pattern of spread makes *Salmonella* control more challenging within the poultry industry.

This study demonstrates that both the outbreak and a nonoutbreak *S.* Reading strains can colonize the reproductive tract and contaminate eggshell surface and egg content in broiler breeder hens when experimentally infected intravaginally. Notably, the outbreak *S.* Reading strain exhibited a higher contamination rate of eggshell surface and egg contents compared to nonoutbreak strain, although the difference was statistically significant only in eggshell surface contamination ($P < 0.05$). Specifically, 70.1% of eggshell surface samples from the outbreak strain tested positive, compared to 34.5% from the nonoutbreak group, indicating the high contamination potential of the outbreak strain.

Previous studies, such as those by Okamura et al. (2001a, b), have shown that S. Enteritidis, S. Infantis, S. Heidelberg, and S. Montevideo can contaminate eggshell surfaces when hens are challenged intravaginally, while S. Typhimurium and S. Mbandaka were isolated from eggshell surfaces following oral infection (Pande et al., 2016). The cecal translocation observed in the present study is a clear sign that fecal shedding of the bacteria occurs. Hence, the eggshell surface contamination ability of *S.* Reading serotype observed could be due to either fecal contamination, reproductive tissue colonization, and the ability of the serotype to remain viable and attached to the eggshell surface after the eggs are laid.

While some *Salmonella* serotypes did not show the potential to contaminate the inner contents of eggs, *S.* Reading in this study has shown the potential to contaminate egg contents with the outbreak strain showing a prevalence of 4%. These results suggested that genetic differences among the *Salmonella* serotypes play a role. In previous studies, *S.* Typhimurium, *S.* Enteritidis, and *S.* Heidelberg have been recovered from egg contents with *S.* Enteritidis serotype showing higher potential to penetrate and contaminate egg contents (Gast et al., 2004; Pande et al., 2016; Okamura et al., 2001b; Gast et al., 2013a,b). Okamura et al., (2001a) has found that *S.* Enteritidis can have recovery rates of up to 7.5% from egg content of infected hens.

The ability of *Salmonella* to colonize reproductive tissues and gastrointestinal tract of hens is the main reason for egg contamination (Miyamoto et al., 1998). This study highlights the capacity of *S.* Reading to

Table 2. Recovery of *S.* Reading from reproductive tissues and ceca of hens.

	Number of positive samples in each treatment				
Treatment group	Ovariess	Upper oviduct	Lower oviduct	Total Oviduct	Ceca
SRO	4/10 (40%[a])	2/10 (20%)	6/10 (60%)	7/10 (70%[a])	7/10 (70%[a])
SRN	2/10 (20%[a])	0/10 (0%)	7/10 (70%)	7/10 (70%[a])	8/10 (80%[a])

[a,b]Column not sharing a common superscript were different ($P < 0.05$).
[1]Numerators, number of positive samples.
[2]Denominators, total number of samples.
[3]Total oviduct, total number of hens with either or both of upper and lower segments of oviducts positive.
Abbreviations: SRO, *Salmonella* Reading outbreak strain; SRN, *Salmonella* Reading Nonoutbreak strain.

translocate to the ceca in hens intravaginally. Other studies have also found ceca as one of the preferential site of colonization of *Salmonella* in infected hens possibly because ceca provide a favorable condition for *Salmonella* survival (Okamura et al., 2001b; Gast et al., 2013). In the present study, both *S.* Reading strains were recovered from the ovaries, oviducts, and ceca of hens with 40% of ovary samples from the outbreak group and 20% samples from the nonoutbreak strain group testing positive. Also, 20% of upper oviduct samples from outbreak strain group and no sample from nonoutbreak group was positive. For the lower oviduct samples, 60% from outbreak strain group and 70% from nonoutbreak group were positive. Okamura et al., (2001a) recovered *S.* Enteritidis, *S.* Hadar, and *S.* Heidelberg from oviducts of hens intravaginally infected with *S.* Enteritidis colonizing up to the ovaries. *S.* Typhimurium and *S.* Mbandaka were similarly found colonizing reproductive tissues in orally challenged hens (Pande et al., 2016). Significant difference has been observed in the ability to colonize reproductive tract among different *S.* Enteritidis strains. This shows that different isolate or strains of the same *Salmonella* serotype can have different colonization ability (Guard et al., 2010). However, in this study, no statistical difference in reproductive tissue colonization was observed between the 2 *Salmonella* Reading strains. The *Salmonella* Reading behaviors observed in this study could be the way the pathogen spread and caused the recent outbreak in North America. The virulence genes and antimicrobial resistance gained by the outbreak strain as observed by Miller et al., (2020) increases its chance of causing diseases in humans.

These findings indicate that *S.* Reading can successfully colonize reproductive tissues, leading to egg contamination and cecal translocation. Future research is necessary to determine whether *S.* Reading can remain viable within the eggs throughout the incubation and until hatching.

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DISCLOSURES

The authors have declared no conflict of interest.

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