*Salmonella* Environmental Persistence Informs Management Relevant to Avian and Public Health

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# 1. **Summary/Abstract**

Globally, *Salmonella* is a significant public health threat. We evaluated the persistence of *Salmonella* on various surface materials (e.g., picnic tables and bird feeders). Our studies suggest that *Salmonella* can have prolonged persistence on shared spaces. However, persistence depends on surface material and precautionary measures should be adopted to reduce exposure.

# 2. **Introduction (Why *Salmonella*?)**

*Salmonella enterica* infections are a significant public health threat, responsible for over 93 million annual cases of human illness worldwide (Majowicz et al. 2010). In the United States alone, 1.35 million cases of salmonellosis and 420 deaths are reported annually (Centers for Disease Control and Prevention 2021a). Most cases of human salmonellosis are caused by food-borne *Salmonella* strains associated with produce or undercooked meat. However, a rising subset of human infections are often associated with unidentified environmental exposures or contact with animals (Centers for Disease Control and Prevention 2016).

The American white ibis (Eudocimus albus), forms large nesting colonies in natural wetlands. However, due to habitat destruction in Everglades National Park, this gregarious member of the Pelecaniformes, has begun to urbanize parks with abundant anthropgenic food and water resources throughout South Florida. Infection with *Salmonella* spp. in American white ibis is well documented. It has been found that ibis can harbor diverse *Salmonella* strains and shed it at a higher prevalence than their natural counterparts (Hernandez et al. 2016). Studies have genetically matched the *Salmonella* shed by ibis to human salmonellosis cases in South Florida during the same time period ibis were sampled (Hernandez et al. 2016).

Similarly, in 2021, following an epidemiological investigation of 29 human cases of *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium), the Centers for Disease Control and Prevention (CDC) documented a relationship between human cases and a concurrent avian salmonellosis outbreak. Of the 29 cases, 14 individuals were hospitalized and the outbreak was likely due to contact with bird feeders, sick or dead wild birds, or pets that had contact with wild birds (Centers for Disease Control and Prevention 2021b).

Persistence of *Salmonella* can be affected by ambient temperature, presence/absence of a biofilm, and environmental nutrient conditions (Will, Diesch, and Pomeroy 1973; Davies and Wray 1995; Barker and Bloomfield 2000; Renner 2002). Under ideal conditions (e.g. optimal temperature 35-43°C, pH 7-7.5, etc.), *Salmonella* is known to persist in the environment for extended periods of time. While *Salmonella* persistence has been examined on soil and poultry litter, there is a lack of understanding of the persistence of *Salmonella* on various surfaces including wood, plastic, and surfaces lined with antimicrobial coatings.

## 2.1 **Data acquisition**

The data from the Picnic Table Trial in South Florida was collected by an undergraduate student of Dr. Sonia M. Hernandez and provided to me by Dr. Hernandez. All data from the Feeder Trial was collected by me, Kimberly Perez under the guidance of Dr. Sonia Hernandez.

### 2.1.1 **Description of data and data source**

The data selected for this project is persistence and prevalence data from two trial studies. The first trial was conducted in South Florida where picnic tables, under the same pavilion and exposed to conditions known to affect *Salmonella*, were selected, swabbed daily for a week and shipped back to the University of Georgia for *Salmonella* isolation. The second trial was conducted in Athens, Georgia, at Whitehall Forest in a modified shade house. Bird feeders of different materials (e.g., cedar, plastic, plastic coated, and wood coated) were hung in triplicate and seeded with an isolate of *S.* Typhimurium isolated from a songbird.

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The Picnic Table Trial data is composed of several worksheets embedded in one excel file. After viewing the file, 192 observations were returned. Feeder Trial data was collected and noted upon confirmation of *Salmonella* from processed samples. The Feeder Trial data returned 144 observations for the nearly two months of sample collection. Both trials were designed to determine the persistence of *Salmonella*. The Picnic Table Trial also collected data in an attempt to determine prevalence.

## 2.2 **Questions/Hypotheses to be addressed**

Given the uncertainty of *Salmonella’s* persistence on various surface materials, questions such as:

* What is the persistence of *Salmonella* over time?
* How long does *Salmonella* persist on different surface materials (e.g., wood, plastic, and those with an antimicrobial coating)?
* Is there a greater risk of acquiring *Salmonella* from objects that both humans and animals use (e.g. picnic tables in parks)?

From these questions, we hope to better inform the public and public health practitioners on safer feeder handling methods and precautions to take when frequenting public locations where humans and wildlife (e.g., ibis) interact.

# 3. **Methods**

## 3.1 **Ibis Picnic Table Trial Experimental Design and Sample Collection Methodology**

The ibis picnic table trial was conducted at Dreher Park, a public park in Palm Beach County frequented by white ibis who are known to carry *Salmonella*. Because humans and ibis both utilize the wooden picnic tables at the park, three tables under a single pavilion with similar environmental conditions (e.g., exposure to sunlight, rainfall, etc.) were selected. All tables were divided into four quadrants (e.g., tabletop plus bench) and ibis were encouraged to feed on the picnic tables until they deficated. Pictures were taken of every quadrant daily to facilitate the counting of new fecal piles. If feces were not deposited in a quadrant, fresh feces from the concrete pad was collected and placed onto the quadrant. To determine persistence, one large fecal pile from each quadrant was selected as the “persistence pile” and monitored and sampled daily for 8 days. To determine table prevalence, a pooled sample was taken daily from every quadrant by swabbing the picnic table surface with a sampling sponge, avoiding the designated “persistence pile”. To maintain cleanliness for other park patrons, new piles were not smeared with the sampling sponge. Samples were placed into 20 mL of dulcitol selenite and maintained at room temperature prior to being shipped to the University of Georgia (UGA), every 2-4 days, for processing.

## 3.2 **Feeder Trial Experimental Design and Sample Collection Methodology**

Our Feeder trial aimed to assess the persistence of *Salmonella* on four types of feeders (cedar, plastic, cedar + antimicrobial coating, and plastic + antimicrobial coating) in Georgia. 18 g of fresh chicken feces was collected, homogenized, 1 g was aliquoted into individual 15 mL test tubes, then sterilized through autoclaving. The individual alioquots were then inoculated with 1.5x10^7 mL of a *Salmonella* Typhimurium isolate responsible for a previous avian salmonellosis outbreak. Samples were then transported to a modified shade house at Whitehall Forest where four feeder types, represented in triplicate and randomized, were seeded with 1 g of inoculated feces and 0.5 mL of sterile physiologic saline. Feeders were then swabbed daily for one week, every other day for one week, and weekly for an additional week. Swabs were embedded into 10 mL of dulcitol selenite broth and transported back to UGA for processing.

## 3.3 **Sample Processing Methodology**

After collection, dulcitol selenite was incubated overnight at 37°c, before 100 uL of the sample vortexed in dulcitol selentite was inoculated into 10 mL of Rappaport Vassiliadis (RV) broth. The broth was incubated for 24 hours at 37°c. The RV broth was then streaked onto XLT-4 agar plates and incubated at 37°c for 24 hours, then an additional 24 hours on the lab bench at room temperature. If colonies morphometrically consistent with *Salmonella* were present, one colony per plate was chosen and a stab in Luria Broth (LB) agar was created. To confirm the presence of *Salmonella*, patch plates were made on ChromAgar using the stabbed colonies. If the patches turned magenta, that indicated positive *Salmonella* samples. A blue color indicated something other than *Salmonella* (Maurer et al. 2015).

## 3.4 **Data Processing Methodology**

Before attention can be focused on the data analysis portion, removal of unnecessary variables and visualizing data will provide a clearer picture of the data. This section will focus on data cleaning and wrangling. First, both datasets must be loaded. Prior to loading any data into RStudio, ensure that a new Quarto or RMarkdown File is established and linked to the preferred working directory. Basic packages need to be loaded to read in the data. Such packages to download include read\_csv(), read\_excel(), and here(), each of which can be loaded by utilizing the library() function (e.g., library(here)). Next, the datasets from both the Ibis and Feeder trials are loaded into RStudio utilizing the appropriate function (e.g., read\_csv() for .csv files and read\_excel() for .xlsx files). After data is loaded into R, it is best to get acquainted with the data by utilizing basic functions such as glimpse(). Glimpse provides users with insight into the data such as column names, number of rows and how each row is classified (e.g., character or numeric). After viewing the data, cleaning the data by removing unnecessary variables can occur.

Ensuring the data is uniform by removing unnecessary variables and renaming columns will assist in the analysis process downstream. To begin, “Yes” responses should be coded to produce a 1, while *NAs* would produce a 0. Given the variability in the column names, standardizing or renaming them a preferred name I will streamline future coding or analysis. With all of that, it is finally time to visualize the data by making some tables and/or graphs.

More packages were loaded that would assist in the visualization process before beginning this process. Before visualization began, the glimpse() function was utilized to view the recently cleaned data. Given the uncertainty of *Salmonella’s* persistence on various surface materials and the information these trials hoped to provide, the summary() function was utilized to provide a quick glimpse of basic result summaries for positive *Salmonella* samples and if the pile present was the original pile or not. Exploration continued on these two variables of interest by plotting each using a stacked bar graph via ggplot().

The same workflow for the Feeder Data was utilized, however, this dataset required less wrangling and did not provide as many exploratory options as the main focus was on feeder type, date, and the presence/absence of *Salmonella* for each day the feeders were sampled.

# 4. **Results**

## 4.1 **Exploratory/Descriptive Analysis**

Our samples persisted for an average of 2 days (feeders), 3 days (picnic tables) with 4 (picnic tables) and 5 (feeders) being the maximum number of days persisted. The varied persistence during both trials could be a result of a lack of moisture, the fluctuation in ambient temperature during sampling, and water activity. Our studies suggest that *Salmonella* can have prolonged persistence on shared spaces, persistence depends on surface material, and precautionary measures (i.e., increased hygiene) should be adopted to reduce human exposure.

### 4.1.1 **Picnic Table Data: Exploration and Visualization**

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| Figure. 1 Positive Salmonella Samples by Sample Type and Date |

Figure 1 depicts the the number of positive *Sallmonella* samples by sample type, persistence or pooled. Pooled samples were collected from every quadrant daily, by swabbing a sampling sponge along the picnic table surface. Persistence piles, on the other hand were, selected based on two criteria, quadrant and size of fecal pile. The persistence pile was sampled everyday for 8 days. While the information presented in Figure 1 depicts count data, it is a useful visual to convey numbers of positive samples for each sample type based on day.

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| Figure. 2 Positive Salmonella Samples by Quadrant and Date |

Figure 2 depicts the the number of positive *Sallmonella* samples by quadrant. While the information presented in Figure 2 depicts count data, it is a useful visual to convey which quadrants may have been “hot spots” for the presence of *Salmonella*.

**Persistence** On Day 1 of the study, four fecal piles per table (one per quadrant, n = 12) were designated for tracking *Salmonella* persistence. **Salmonella** was isolated from three of the 12 designated persistence study piles on Day 1, allowing the persistence to be tracked on these three piles. Persistence, expressed as the number of days that a positive fecal pile on Day 1 remained positive, varied with an average persistence of 3 days.

**Prevalence** Of 96 deposited feces, we obtained 27% positive (26/96). *Salmonella* persisted for an average of 3 days, and maximum of 4 days on wooden picnic tables. *Salmonella* was isolated from all tables, at least once.

### 4.1.2 **Bird Feeder Data Exploration and Visualization**

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| Figure. 3 Positive Salmonella Samples by Feeder Type and Date |

Figure 3 depicts the the number of bird feeders that remained *Salmonella* positive throughout the trial. Stacked bars in various colors indicate the number of feeders (plastic, plastic + antimicrobial coating, wood, and wood + antimicrobial coating) from which we isolated *Salmonella*. We confirmed successful inoculation of all feeders with Salmonella (presence on Day Post Inoculation (DPI) 0- 2/22/2022). Further, results indicate that Salmonella may persist on some feeders up to five DPI and current analysis indicates that Salmonella persisted on both coated and uncoated feeders. With persistence occurring with both plastic and plastic coated feeders.

# 5. **Statistical Analysis**

Statistical analysis was conducted on the Picnic Table Data and Feeder Data, utilizing RStudio “Cherry Blossom” (2023-03-09). Analysis for both datasets were evaluated utilizing several models including Linear Regression (LR) and Generalized Linear Model (GLM). Given that two datasets were utilized for this analysis, a holistic approach, considering various limitations of both trials, such as sample size, was taken into account when selecting a model. Because the majority of data is binary in nature (e.g., presence v. absence) and few variables exist for comparison, a LR was the first model selected to run on both datasets, followed by a GLM. Several predictors were selected to include in this the analysis including ‘Salmonella\_Positive~Date’ , ‘Salmonella\_Positive~Sample\_Type’ (e.g., pooled v. persistent), and ‘Salmonella\_Positive~.’ with all predictors for the Picnic Table Trial and ‘Absence\_0\_Presence\_1~Feeder\_number’, ‘Absence\_0\_Presence\_1~Feeder\_number+Feeder\_Type’, and ‘Absence\_0\_Presence\_1~.’ and all predictors for the Feeder Trial.

## 5.1 **Regression Models**

### 5.1.1 **Picnic Table Trial**

Small sample adjusted Akaike’s Information Criteria (AICc) model weights and performance scores for linear and generalized linear models predicting Salmonella Persistance on Picnic Tables.

| Name | Model | AICc\_wt | Performance\_Score |
| --- | --- | --- | --- |
| lmfit\_PTglm1 | \_glm | 0.5242322 | 1.0000000 |
| lmfit\_PT1 | \_lm | 0.2543028 | 0.4850958 |
| lmfit\_PT | \_lm | 0.2107812 | 0.4020760 |
| lmfit\_PT\_glm | \_glm | 0.0078935 | 0.0150573 |
| lmfit\_PTglm2 | \_glm | 0.0027903 | 0.0053226 |
| lmall\_PT | \_lm | 0.0000000 | 0.0000000 |
| lmall\_PTGLM | \_glm | 0.0000000 | 0.0000000 |

Of the models evaluated, the highest performing of all contained the predictors original persistence pile [Original\_Persistence\_Pile], table, and date and the outcome of *Salmonella* [Salmonella\_Positive]. Given the AICc\_wt along with performance score, a GLM was selected for the Picnic Table Trial data, as depicted in Table 1. When evaluating a model using AICc, we would ideally like to select a model with a low AICc and a high performance score. Out of all of the models run, the model labeled “lmfit\_PTglm1” was selected. In this model, original persistence pile along with table and date was associated with a higher likelihood of Salmonella persistence.

### 5.1.2 **Feeder Trial**

Small sample adjusted Akaike’s Information Criteria (AICc) model weights and performance scores for linear and generalized linear models predicting Salmonella Persistance on Various Feeder Surfaces.

| Name | Model | AICc\_wt | Performance\_Score |
| --- | --- | --- | --- |
| lmall\_FD | \_lm | 0.4999988 | 1.0e+00 |
| lmall\_FDGLM\_all1 | \_glm | 0.4999988 | 1.0e+00 |
| lmfit\_FD | \_lm | 0.0000010 | 1.7e-06 |
| lmfit\_FD\_glm | \_glm | 0.0000010 | 1.7e-06 |
| lmfit\_FD2 | \_lm | 0.0000002 | 0.0e+00 |
| lmfit\_FD\_GLM | \_glm | 0.0000002 | 0.0e+00 |

Of the models evaluated, the highest performing of all contained all predictors. Given the AICc\_wt along with performance score, a LM was selected for the Feeder Trial data, as depicted in Table 2. When evaluating a model using AICc, we would ideally like to select a model with a low AICc and a high performance score, as mentioned above. However, because this model can be considered “global” as it includes all predictors, it is not the most ideal as it may overfit the data. Despite this, the model labeled “lmall\_FD” was selected. In this model, feeder number, collection data, and feeder type was associated with a higher likelihood of Salmonella persistence on feeders.

# 6. **Discussion**

As cases of environmental salmonellosis increase throughout the United States, environs that individuals come into contact with on a regular basis need to be evaluated to fill knowledge gaps relating to microbial transmission (Hernandez et al. 2016). With these trials we will research the factors that influence *Salmonella* persistence, which will allow us to better inform public health agencies and mitigate seasonal outbreaks in humans and wildlife.

## 6.1 **Summary and Interpretation**

Based on the modeling conducted above, there seems to be positive effects of the various predictors on *Salmonella* absence/presence. The Picnic Table Data showed that the original pile, date, and table all had statistically significant effects on *Salmonella* presence on picnic tables in South Florida. Persistence of *Salmonella* varied, however, the maximum number of days it persisted was four days (12 positive persistence piles/32 piles total).

Feeder data also indicate that *Salmonella* can persist on various surfaces for several days. *Salmonella* persisted for an average of ~2 days, (5 days max). Surprisingly, however, *Salmonella* persisted on plastic and plastic coated feeders longer than wooden and wooden coated feeders and had a prevalence of 15% on plastic coated feeders.

The results from both trials indicate that *Salmonella* can persist on various surfaces. As human and wildlife interactions increase due to urbanization, precautions should be taken to avoid potential pathogen transmission from the wildlife directly or through interactions with anthropogenic environs that may harbor wildlife excrement. While the data collected was mainly absence/presence, through data visualization, statistical analysis, and performance evaluations, gaps in knowledge surrounding *Salmonella* persistence outside of the poultry industry can be closed.

## 6.2 **Strengths and Limitations**

While information on *Salmonella* persistence outside of the commercial poultry industry is relatively minimal, our work with the Picnic Table and Feeder Trials, provides some interesting insight into the persistence of this pathogen and possible sources of transmission during avian outbreaks (e.g., feeders). Because both experiments were trials, limited funding was allocated to each and a small sample size was a result of such. Small sample sizes limit data interpretation. Despite the small sample size, all models were analyzed with the model\_check() function and most produced the expected results. However, some residuals in certain models were not normally distributed which can lead to over fitting.

Despite the limitations of these trials, steps were taken to close knowledge gaps. Trials, such as the ones conducted, can lay the groundwork for future studies that improve upon our sampling and collection methodology, as well as statistical analysis. Through the information garnered from these trials as well as future studies, more insight into *Salmonella* persistence and ways to mitigate it in our communities will inevitably be developed and enacted.

## 6.3 **Conclusions**

Globally, salmonellosis outbreaks are common in aquatic and songbird populations and Salmonella continues to be a significant pathogen for public health. Notably, in 2021 a Salmonella Typhimurium outbreak in people was associated with the use and handling of bird feeders. Research is needed to understand the interaction between humans, birds, and their interface. Key to preventing outbreaks is understanding transmission dynamics, specifically, how long Salmonella persists on various materials. Persistence has been studied in food systems and commercial poultry operations—neither are relevant to free-living birds or people in contact with shared areas. Through further research and investment some of these pressing questions may be answered. Saving lives of various species globally.

# 7. **References**

Barker, J, and S. F. Bloomfield. 2000. “Survival of Salmonella in bathrooms and toilets in domestic homes following salmonellosis.” *Journal of Applied Microbiology* 89 (1): 137–44. <https://doi.org/10.1046/j.1365-2672.2000.01091.x>.

Centers for Disease Control and Prevention. 2016. “Surveillance for Foodborne Disease Outbreaks United States, 2016: Annual Report.” <https://www.cdc.gov/fdoss/pdf/2016_FoodBorneOutbreaks_508.pdf>.

———. 2021a. “Salmonella.” <https://www.cdc.gov/salmonella/index.html>.

———. 2021b. “Salmonella Investigation Details.” <https://www.cdc.gov/salmonella/typhimurium-04-21/details.html>.

Davies, R, and C Wray. 1995. “Observations on disinfection regimens used on Salmonella enteritidis infected poultry units.” *Poultry Science* 74 (4): 638–47. https://doi.org/<https://doi.org/10.3382/ps.0740638>.

Hernandez, Sonia M., Catharine N. Welch, Valerie E. Peters, Erin K. Lipp, Shannon Curry, Michael J. Yabsley, Susan Sanchez, et al. 2016. “Urbanized White Ibises (Eudocimus albus) as carriers of Salmonella enterica of significance to public health and wildlife.” *PLoS ONE* 11 (10). <https://doi.org/10.1371/journal.pone.0164402>.

Majowicz, SE, Musto J, Scallan E, Angulo FJ, Kirk M, O’brien SJ, Jones TF, Fazil A, and Hoekstra RM. 2010. “The global burden of nontyphoidal Salmonella gastroenteritis.” *Clinical Infectious Diseases*, 882–889.

Maurer, John J., Gordon Martin, Sonia Hernandez, Ying Cheng, Peter Gerner-Smidt, Kelley B. Hise, Melissa Tobin D’Angelo, et al. 2015. “Diversity and persistence of Salmonella enterica strains in rural landscapes in the southeastern United States.” *PLoS ONE* 10 (7): 1–19. <https://doi.org/10.1371/journal.pone.0128937>.

Renner, Rebecca. 2002. “From flush to farm. Sewage is a great fertilizer, but is it a health hazard?” *Scientific American*.

Will, Loren, Stanley Diesch, and B Pomeroy. 1973. “Survival of Salmonella Typhimurium in animal manure disposal in a model oxidation ditch.” *American Journal of Public Health* 63 (4): 322–26. <https://doi.org/10.2105/ajph.63.4.322>.