Understanding and predicting food safety risks posed by wild birds

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Summary

Co-existence of fresh produce with animal agriculture is a significant problem for produce growers. There is a need to understand how pathogens move from animals and their environments to fresh produce. To understand food safety risks posed by wild birds, we collected 223 fecal samples from produce farms across the southeast in 2021 and determined the presence of viable *Salmonella* and *Campylobacter*. Mapping and modeling is being used to assess the influence of proximal animal agriculture to the presence of pathogens in wild bird feces to develop risk profiles. Genomic and molecular tools are being used to characterize *Salmonella* and *Campylobacter* to identify potential sources.

Objectives

1. Assess the risk posed by wild bird feces on fresh produce plants, and the influence of proximal animal agriculture on pathogen presence in wild bird feces.
2. Determine the diversity of *Campylobacter* and *Salmonella* in wild bird feces and perform fine-scale tracking and source attribution using whole genome sequencing.

Methods

A total of 36 commercial produce farms were visited between Spring and Fall 2021 (eight farms were visited at least twice) within four hours of dawn and before harvest (**Figure 1**). Fecal samples ($n = 223$) were collected and scored (dry vs. moist), along with 223 swabs from produce and 223 swabs from neighboring plants, and viable *Salmonella* and *Campylobacter* were isolated by culture enrichment. The presence of proximal animal agriculture was surveyed on site and using U.S. Geological Survey (USGS) information. Bird counts were performed on site at four caudal points, and bird species was determined from feces by sequencing the *COI* gene. *Salmonella*-positive samples were analyzed by CRISPR-SeroSeq to determine the presence of multiple serovars. *Salmonella* and *Campylobacter* isolates are being analyzed by whole genome sequencing.

Results to Date

The incidence of viable *Salmonella* was 6.7% (15/223) and incidence of viable *Campylobacter* was 2.2% (5/223) (**Figure 2**), which is lower than for previous similar studies in the west.

Two thirds (146/223) of fecal samples were moist, and all feces that contained either *Salmonella* or *Campylobacter* were moist (20/223) (**Figure 3**). Neither pathogen was cultured from produce or neighboring plants, suggesting that transmission does not frequently occur.

For the *Salmonella*-positive feces, 10/15 contained multiple serovars (average 2.5 serovars/fecal sample; range 1–7) (**Figure 4**). The most common serovars were Hadar and Saintpaul (each found in six samples); other serovars included Newport and Enteritidis (each in four samples). One sample contained seven serovars and this sample was attributed to a chipping sparrow.

Benefits to the Industry

There are over 15,000 produce farms in the southeast U.S., and data from this work will provide region-specific data to facilitate decision-making when contamination events occur through the growing season and during preharvest risk assessments. Thus far, our finding that viable *Salmonella* and *Campylobacter* were not detected in dried feces and that they do not spread onto produce below fecally contaminated leaves or onto neighboring plants is encouraging; we hope to see this same trend during sampling in 2022. The data gleaned through this work will address the relevance of current GAPs associated with wild bird presence in produce fields. The tools and data we generate will be made available to producers via UGA Extension, Produce Safety Rule Grower trainings, and research publications and presentations.

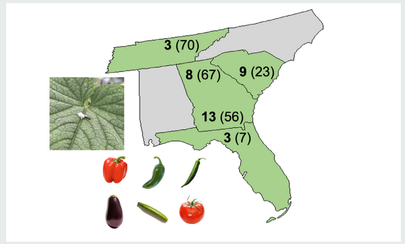

Figure 1. Map showing the number of farms in each region (bold) and the number of bird fecal samples collected in each region (in parentheses). In 2021, 36 individual farms were visited 46 times, yielding a total of 223 fecal samples. Produce farms included those growing tomatoes, cucumbers, okra, peppers, and eggplant.

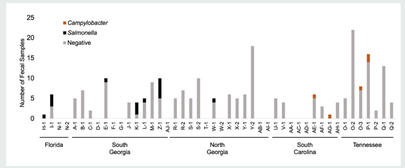

Figure 2. Incidence of viable *Salmonella* (black) and *Campylobacter* (orange) found in fecal samples. In 223 samples across 46 sample collections, 6.7% and 2.2% of samples were positive for *Salmonella* and *Campylobacter*, respectively. Letters on the X-axis represent a non-descriptive farm identifier, where N-2 represents a second sampling at the location, N. Where there are no values, fields were visited but no feces found.

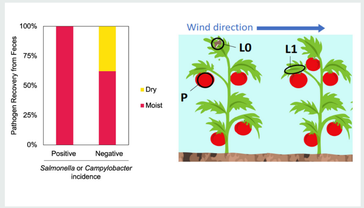

Figure 3. Viable pathogens were found only in moist feces and none were found on produce below feces (P) or on leaves of a neighboring plant (L1). Of the 223 fecal samples collected, all samples in which viable *Salmonella* or *Campylobacter* were detected were fresh and moist; no pathogens were found in dried fecal samples. For each fecal sample collected, surface swabs of the produce underneath the leaf with the feces and of a leaf on a neighboring plant downwind were collected and analyzed; these provided no evidence of viable contamination from the original fecal sample.

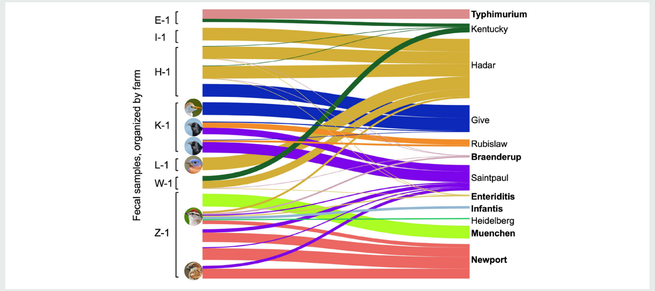

Figure 4. *Salmonella* population analyses showed presence of multiple serovars in individual fecal samples. The 15 *Salmonella*-positive samples were analyzed using CRISPR-SeroSeq to determine the relative frequency of different serovars within a single sample. In total, 66% (10/15) of samples contained two or more serovars. Serovars found among the CDC's "top 10" are shown in bold. Sequence analysis of the *COI* gene was used to identify the bird species from the fecal DNA. An image of the identified bird species is shown for samples where analysis yielded >96% genetic similarity with at least a 665bp *COI* gene sequence. Species shown in descending order: Cattle Egret (*Bubulcus ibis*; K-1), Fishing Crow (*Corvus ossifragus*; K-1), Eastern Bluebird (*Sialia sialis*; L-1), Chipping Sparrow (*Spizella passerine*; Z-1), and House Sparrow (*Passer domesticus*; Z-1).