Salmonella and Campylobacter Prevalence in Adult Vermont Backyard Poultry

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Summary

Across the United States, backyard poultry has been increasing in popularity. With this comes an increase in the risk of transmission of the zoonotic pathogens which poultry can carry. Common backyard birds such as chickens, turkeys, and geese can be carriers of *Salmonella enterica* and *Campylobacter*, zoonotic pathogens which can be spread to people through contact with birds and their fecal matter or through consumption of their eggs or meat. This study sought to identify the prevalence of *S. enterica* and *Campylobacter* spp. in adult Vermont backyard poultry (BYP). Other studies performed in this area outside of the state of Vermont have identified *S. enterica* prevalence ranging from 2-4%, while *Campylobacter* prevalence has ranged from 10-35%. Studies also identified *S.enterica* more commonly among younger or multiage flocks. Previous work performed in the Etter lab found that eight of 42 sampled flocks (19%) tested positive for *S. enterica* between 2019-2021, with the majority of those positive samples being identified in 2021 (seven of 13 flocks sampled in 2021 (53.8%)). The Etter lab also tested four flocks for *Campylobacter* in 2021 and found one flock (25%) to be positive. Based on these past studies, we hypothesized that *Campylobacter* would be more prevalent compared to *S. enterica*, and that *S. enterica* would be more prevalent among younger or multiage flocks. Samples were collected from across Vermont between September and November of 2022, and analyzed for both *S. enterica* and *Campylobacter*. While seven of 58 flocks (11.11%) were positive for *S. enterica*, only one of 62 tested flocks (1.6%) was positive for *Campylobacter*. This sample was plated on R&F Products *C. jejuni/C. Coli* Chromogenic media in an effort to grow *Campylobacter*, which proved unsuccessful.

<u>Key Words</u>

Public health, *S. enterica*, *Campylobacter* spp., zoonoses, backyard poultry (BYP)

<u>Impacts</u>

- The rate of *Campylobacter* in backyard poultry was lower than expected based on previous studies.
- The incidence of *Salmonella enterica* was lower than that of 2021 research conducted by the Etter lab.
- *S. enterica* was more prevalent among flocks in rural areas and among flocks which were free-ranged or penned over those which were indoors, although these trends did not prove to be statistically significant. Younger and multiage flocks were positive at a higher rate compared to single-age adult flocks, though this was also not statistically significant.

<u>Introduction</u>

Chickens and other backyard poultry can be carriers of *Salmonella enterica* and *Campylobacter*, which, if shed in their feces, can be transmitted to people through consumption of eggs or meat, or contact with poultry and their fecal matter (Rukambile et al., 2019; Beam et al., 2013). Ownership of backyard poultry (BYP) has recently been rising in popularity in the United States (Basler et al., 2016), and as more people interact with backyard birds, the risk of transmitting these zoonotic pathogens increases. In the U.S., the number of reported *S. enterica* cases associated with live poultry has been increasing along with BYP ownership (Basler et al., 2016), with 1,230 BYP-associated cases being reported nationwide in 2022 (CDC, 2022). Although birds are recognized as the main carriers of *Campylobacter* spp. (Rukambile et al., 2019), a Pennsylvania study found that only around 20% of backyard poultry owners were aware that this bacterium could be passed from their birds to them (Nicholson et al., 2020). Owners were more aware of *S. enterica* risk, with a rate (60%) consistent with or higher than *S. enterica* awareness in other U.S. cities (Beam et al., 2013). Finally, about 15% of BYP owners were unaware that their birds might carry zoonoses (Nicholson et al., 2020).

The risk for zoonotic pathogens also depends on owner biosecurity practices. Many view their birds as pets and have reported cuddling or kissing their birds (Rukambile et al., 2019; Basler et al., 2016; Brochu et al., 2019). Others do not wash their hands after handling birds or eggs (Beam et al., 2013), or will let visitors interact with poultry without discussing the risk of disease exposure (Nicholson et al., 2020). Such practices increase the chance that people will be exposed to *S. enterica* or *Campylobacter*.

A study investigating *S. enterica* in layer chicks found that infected chickens were no longer shedding by laying age, suggesting that *S. enterica* is more prevalent in younger birds (Berchieri et al., 2001). Meanwhile, *Campylobacter* is more common after two to three weeks of age and is obtained from environmental sources (Lee & Newell, 2006). If one bird contracts *Campylobacter*, it often spreads rapidly through the flock, and birds remain carriers until death (Lee & Newell, 2006).

Limited research has been performed regarding *S. enterica* and *Campylobacter* in North American adult BYP populations. A study conducted in the greater Boston area tested 53 backyard chicken flocks for *S. enterica* and found only one (2%) to be positive. The positive flock contained 3-month-old pullets (McDonagh et al., 2019). An Ontario study tested 159 backyard chicken flocks of which five (3%) were positive. This study also investigated

Campylobacter prevalence and identified a 35% positivity rate (56 positive flocks out of a total 158 sampled) (Brochu et al., 2019). Studies have been performed in other countries where BYP ownership is becoming increasingly common, such as Australia. One Australian study found three samples out of 82 (4%) to be positive for *Salmonella* spp. and eight (10%) to be positive for *Campylobacter* spp., with greater prevalence of both noted in multiage flocks (Keerthirathne et al., 2022). The Etter lab also conducted research on *S. enterica* prevalence in BYP in Vermont over 2019-2021, with eight of 42 flocks (19%) testing positive during this time (Larsen et al., 2022). Four flocks were also tested for *Campylobacter* by the lab in 2021, one (25%) of which was positive.

The objectives of this study were to monitor the prevalence of both *Salmonella enterica* and *Campylobacter* in adult BYP in the state of Vermont for 2022. We hypothesized that of the two zoonotic pathogens under study, *Campylobacter* would be more prevalent among the Vermont BYP population compared to *S. enterica.* Additionally, we hypothesized that multiage flocks or flocks with younger birds would show greater positivity for *S. enterica* compared to single-aged or older flocks.

Materials and Methods

Sample Collection: In order to find people who would be willing to participate in this study, advertisements were placed on Front Porch Forum which circulated around different regions of Vermont in order to create a more geographically comprehensive study. Information about the study was also spread by word-of-mouth. 58 flocks were sampled from across the state of Vermont between September and November of 2022. Samples consisted of soiled bedding (generally shavings or straw) or feces. Details were noted about each farm, including the number of birds in each flock, the age and breeds of birds in a flock, and whether the flock was located in a rural or urban area (i.e. in a large yard/close to a forested area vs. in an area surrounded by other houses). It was also noted if a flock was always kept enclosed, or if they were free ranged for part or all of the day.

Testing for *S. enterica*: 25g of soiled bedding, or 25g of feces in the case of feces-only samples, were transferred to a WhirlPak bag and enriched in 100mL of BPW. Samples were then homogenized in a stomacher for 60 seconds (USDA-APHIS, n.d.). At this point, samples were incubated at 37°C for four hours (USDA-APHIS, n.d.). Following incubation, 1mL of sample was transferred to a tube containing 10mL of tetrathionate (TT) enrichment broth and incubated at 37°C for 24 hours, while 100uL of sample was transferred to a tube containing 10mL of Rappaport-Vassiliadis (RV) enrichment broth and incubated at 42°C for 24 hours (USDA-APHIS, n.d.). If the RV and TT tubes exhibited growth (based on turbidity) post-incubation, samples were streaked onto *Salmonella* (Nontyphoidal) Chromogenic Plating Medium agar (R&F Products, Downers Grove, IL). Plates were then incubated for 48 hours at 35°C while monitoring for *S. enterica* growth (presence of pink or red colonies on a colorless background) (R&F Products, 2023).

Testing for *Campylobacter*: 25 grams of soiled bedding samples were inoculated in 100 mL of BPW and homogenized in a stomacher. These samples were then tested for *Campylobacter* through use of a BAX, which identifies *C. jejuni*, *C. coli,* and *C. lari* via PCR (Hygiena, 2022). This system is operated by preparing a lysis reagent which contains 12.5uL protease for every milliliter of lysis buffer (Hygiena, 2022). 200uL of this reagent was added to cluster tubes

(Hygiena, 2022) along with 5uL of the homogenized sample-BPW mixture. Cluster tubes were heated in thermal blocks at 37°C for 20 minutes and then at 95°C for 10 minutes (Hygiena, 2022). The tubes were next placed in a cooling block at 2-8°C for five minutes (Hygiena, 2022). 30uL of the lysate solution was then transferred from the cluster tubes to tubes containing PCR tablets, following which the PCR tubes were placed in a Q7 cycler. The program could then analyze the samples and report results (Hygiena, 2022).

Growth of *Campylobacter*: Once a positive sample was identified, 10g of fecal material was enriched in 25mL of BPW and homogenized in a stomacher (Etter et al., unpublished). 1.2mL of this fecal suspension was then transferred into a tube containing 10.8mL of Bolton broth, and incubated under micro-aerophilic conditions by placing tubes in an AnaeroPack jar along with an AnaeroPouch MicroAero Gas Generator. Samples were incubated at 42°C for 48 hours (Etter et al., unpublished).

Post-incubation, 100uL of the Bolton broth mixture was plated onto R&F *C. jejuni/C. Coli* Chromogenic agar. Plates were incubated at 42°C for 24-48 hours, again under micro-aerophilic conditions, and monitored for the growth of salmon-colored colonies, which would indicate positive *C. jejuni/C. Coli* (Etter et al., unpublished).

Statistical Analysis: Trends in the prevalence of *S. enterica* were assessed for statistical significance by performing a Fisher's Exact analysis. Analysis was performed using Rstudio v2021.09.2. This was used to test for significance in *S. enterica* prevalence across different housing styles (free-ranged, penned, or indoor), and for significance in *S. enterica* prevalence in different regions (rural, semi-rural, or urban). Statistical significance in prevalence across different ages was also assessed. Statistical analysis was not performed for *Campylobacter* prevalence due to low incidence.

Results

A total of 58 flocks across 33 farms were sampled for *S. enterica* during the fall of 2022. Some farms contained multiple flocks which were housed separately, accounting for the higher flock compared to farm number. Of these 58 flocks, seven (11.11%) tested positive.

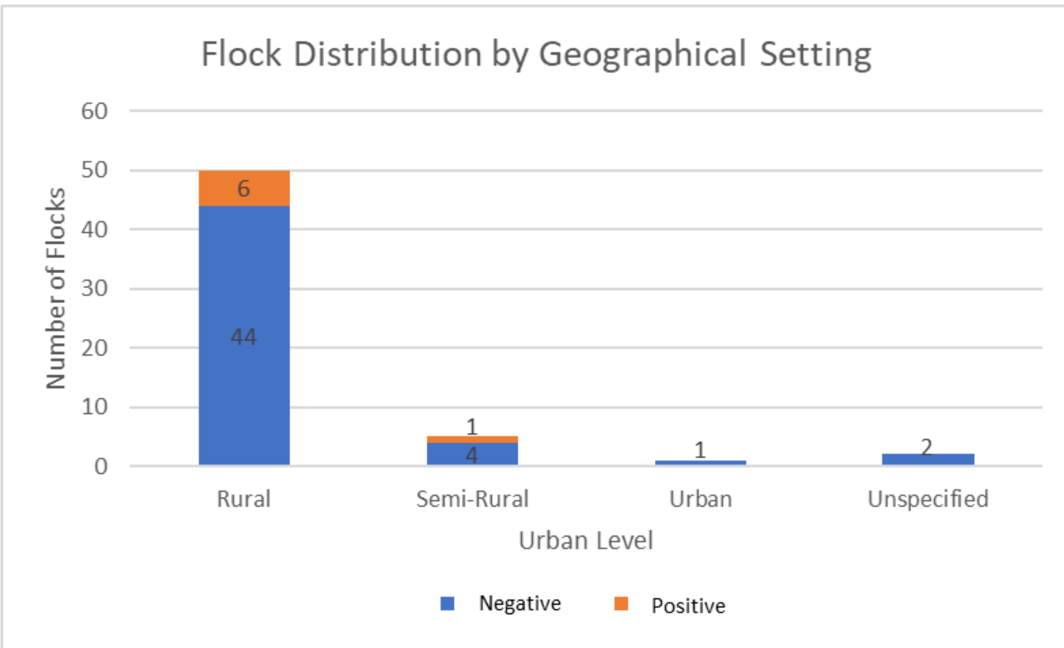

Figure One: Distribution of *S. enterica* based on geographical setting

Fifty of the sampled flocks came from rural areas while five were from semi-rural areas. One flock came from a more urban region. Information regarding the urban level of the remaining two flocks was not recorded. Across this distribution, six of the 50 rural flocks (12%) were positive for *S. enterica*, and one of the semi-rural flocks (20%) was positive. The sole urban flock was negative for *S. enterica*. The differences in positivity by geographical setting were not statistically significant (P = 0.54).

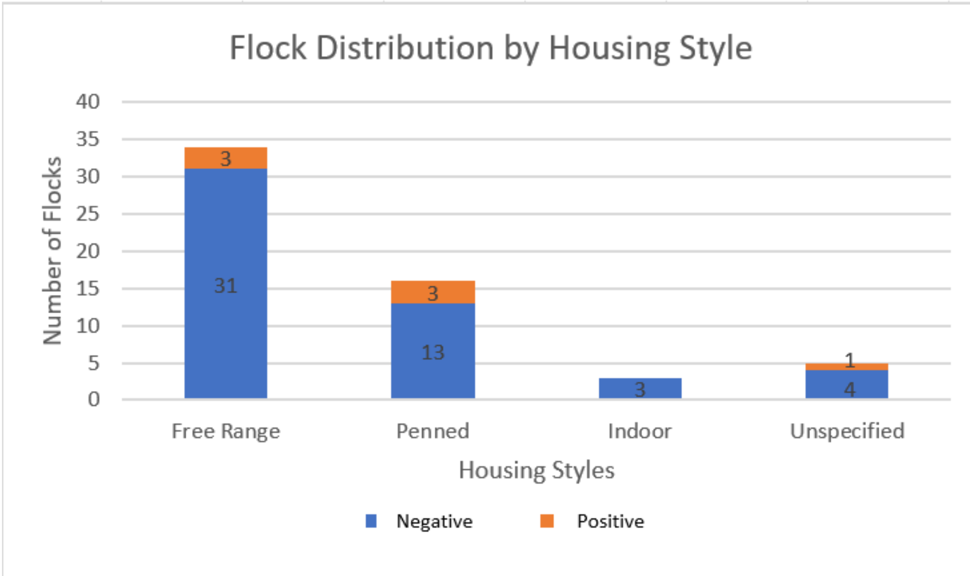

Figure Two: Distribution of *S. enterica* based on housing style

Comparisons were also made based on the housing style of the flocks. 34 flocks were free-ranged, and of these, three (8.82%) were positive for *S. enterica.* 16 flocks were kept penned, and three (18.8%) of these flocks were positive. Three flocks were kept indoors, none of which were positive. Housing style was not recorded for five flocks, and of these, one (20%) was positive for *S. enterica.* These trends were not significant (P = 0.52).

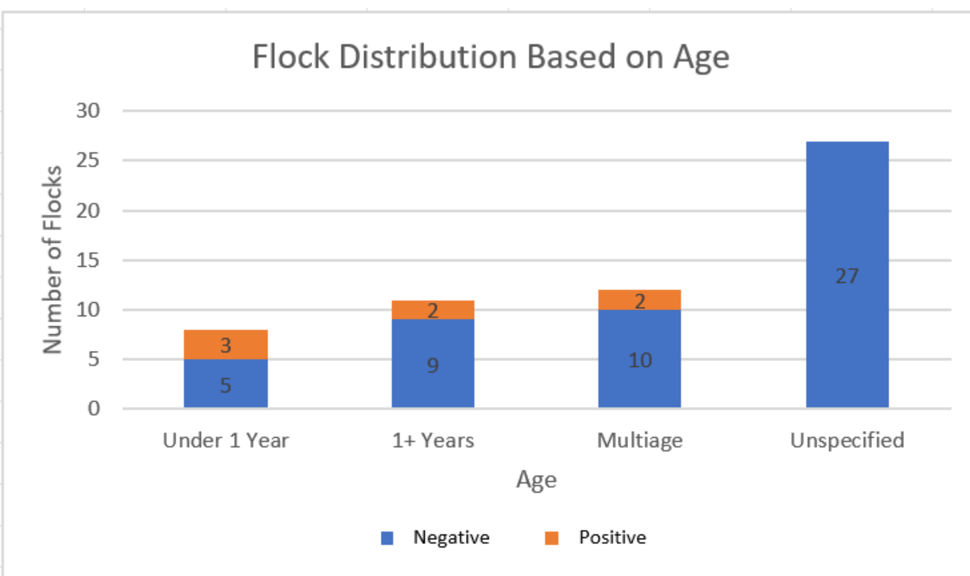

Figure Three: Distribution of *S. enterica* based on age

The distribution of *S. enterica* based on age of birds was also assessed. Of the seven positive flocks, three consisted of pullets under the age of one, two consisted of birds over the age of one, and two consisted of birds of different ages (pullets and adults). Several flocks were recorded as being adult, but ages were not specified. None of the flocks with an unknown adult age were positive for *S. enterica*. Trends in age did not prove to be statistically significant (P = 0.29).

62 flocks were tested for *Campylobacter*. This included six adult samples from 2021 which had not previously been tested in addition to the 58 flock samples which were collected during 2022. Of these flocks, only one tested positive for *Campylobacter* (1.6%). This flock consisted of adult turkeys.

Growth of the positive sample on *Campylobacter* chromogenic media was attempted, but was unsuccessful. However, the Etter lab received three *Campylobacter* isolates from R&F (Restaino & Frampton) laboratories, one each of *C. jejuni*, *C. lari*, and *C. coli*, which were frozen down and grown successfully.

Discussion

The overall prevalence of *S. enterica* in Vermont BYP for 2022 was lower (11.1%) than the prevalence recorded by the Etter lab between 2019-2021 (19%). However, the prevalence of *S. enterica* over the 2019-2021 period varied each year, with there being zero positive flocks in 2019 (n=28), one positive flock identified in 2020 (n=1), and 53.8% of sampled flocks testing positive in 2021 (n=13). One factor which could have potentially resulted in the higher *S. enterica* rate in 2021 compared to 2022 is an outbreak of the *S. enterica* serovar Typhimurium among songbirds in the spring of 2021 (Larsen et al., 2022). Four of the seven positive flocks tested positive during this outbreak, suggesting a possible correlation (Larsen et al., 2022). The lower rate of *S. enterica* among poultry in 2022 could reflect this, suggesting that there was a lower environmental risk of transmission during that year.

In terms of housing style, *S. enterica* prevalence was equivalent for free-ranged and penned flocks. Meanwhile, no indoor-only flocks tested positive. This suggests that there is perhaps an environmental risk of exposure in an outdoor setting which is not present in an indoor setting. Although *S. enterica* is often transmitted vertically from hen to chick, it can also be transmitted horizontally through vectors such as wildlife (Liljebjelke et al., 2005). Those flocks which were allowed to free-range or kept in an outdoor pen would be more likely to come in contact with wildlife compared to indoor flocks, especially considering that all positive flocks came from rural or semi-rural areas where wildlife might be more common.

Six of the seven *S. enterica* positive flocks came from rural locations while the final positive came from a flock in a semi-rural location. One flock from an urban location was also tested, and came back negative. While these results suggest that there may be a relationship between *S. enterica* prevalence and rural settings, this trend did not prove to be statistically significant ($P = 0.4427$). The majority of flocks sampled in this study came from rural areas, and as such, it is understandable that a larger number of them came back positive compared to other geographical settings. It may be worth assessing this trend further in the future if more samples from urban areas can be collected.

The majority of *S. enterica* positives came from flocks which were under the age of one or which consisted of birds of different ages. This is consistent with what was hypothesized, and supports what has been found in past studies on *S. enterica* prevalence in BYP (Berchieri et al., 2001 & Keerthirathne et al., 2022). However, this trend was not statistically significant (P = 0.29).

The rate of *Campylobacter* prevalence identified in this study was much lower than expected, at only 1.6%. One study performed in Ontario (Brochu et al., 2019) identified a 35% positivity rate, while one performed in Australia identified a 10% positivity rate (Keerthirathne et al., 2022). Based on these international studies and our earlier studies in Vermont, a higher rate was anticipated. It was also expected that the prevalence of *Campylobacter* would be greater than that of *S. enterica* based on the results of these studies, yet this did not occur.

One factor which could have affected the level of *Campylobacter* prevalence witnessed is the level of stress the sampled flocks were experiencing. It has been identified that a heightened level of stress can increase the shedding of *Campylobacter* in the feces of poultry (Whyte et al., 2001). It is therefore possible that more backyard flocks of Vermont are carriers of *Campylobacter* than this study reflects, yet are not currently shedding it due to low stress in their environment. The method of sample collection also imposed little stress on the birds as bedding samples were collected rather than cloacal swabs. It is also possible that there is currently low environmental risk for *Campylobacter* exposure in Vermont. *Campylobacter* is commonly transmitted horizontally, and poultry may be exposed to it through sources such as wildlife, or contaminated water (Keerthirathne et al., 2022). Analyzing *Campylobacter* levels in forms of wildlife could provide insight into this. The Etter lab has some songbird samples which could be analyzed for *Campylobacter* and provide information in this area.

The one sample which was positive for *Campylobacter* was streaked onto R&F *C. jejuni/C. Coli* Chromogenic agar, but no bacterial growth occurred. As the sample tested positive by PCR for *Campylobacter* DNA, it is more likely that the condition of the sample was not ideal for growth rather than that it was a false positive. By the time the sample had been identified as positive and materials for further enrichment were available for it to be plated, about a month had passed since its collection. During that time, it was stored at -20°C without preservatives. Because of these conditions and the sensitivity of *Campylobacter* to environmental factors such as oxygen exposure, it is possible that the *Campylobacter* present in the sample was no longer viable. With this in mind, an effort will be made to decrease the time period between *Campylobacter* testing and subsequent plating procedures in the future. This could be done by coordinating schedules of lab members in order to ensure there is time to test the gathered samples on the same day they are collected, or within a few days of collection. Getting results back in a more timely manner would allow samples to be plated sooner after sampling, leaving less time for loss of bacterial viability. Samples could alternatively be stored with preservatives so that they can be kept for a longer period if increasing the efficiency of the laboratory process does not prove realistic.

Although the identified positive sample could not be cultured, the isolates the Etter lab received from R&F laboratories were grown with success, indicating that the lab's established protocol for growing *Campylobacter* is appropriate.

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Conflict of Interest

The author has no conflicts of interest to declare.

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