Salmonella Surveillance in Wild Passerine Populations Along an Urban - Rural Gradient

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Overview

Recent outbreaks of salmonellosis in North America and Scandinavia have resulted in widespread mortalities in passerine populations. Although these outbreaks are thought to arise from increased host contact through feeder stations and environmental stressors such as winter temperatures, little is known about the prevalence and distribution of endemic *Salmonella* spp. in free-ranging passerines. This pilot study represents a cross-disciplinary collaboration among UGA researchers in ecology and veterinary medicine. We will survey the wild avian community over a range a habitat types in the metropolitan Atlanta area for infection with *Salmonella* spp.. We will use bacterial culturing to isolate positive samples and pulsed-field gel electrophoresis (PFGE) and random amplified polymorphic DNA (RAPD) analysis to type any retrieved isolates. Information from this study will be used to identify the underlying prevalence of *Salmonella* spp. and the distribution of serotypes in an avian community not experiencing an epidemic outbreak. Habitat characteristics or avian community correlates with *Salmonella* presence and prevalence can be investigated with future studies.

Background

Salmonella spp. can infect and cause significant disease in free-ranging and captive birds. Most salmonellosis in free-ranging wild birds is documented via case reports and other clinical observation (Fig. 1), with outbreaks in wildlife birds occurring during the winter months. While many outbreaks are localized, several large-scale epizootics have occurred, yet little is known about the *Salmonella* spp. found in these free-ranging species.

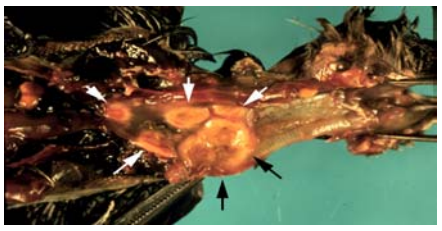
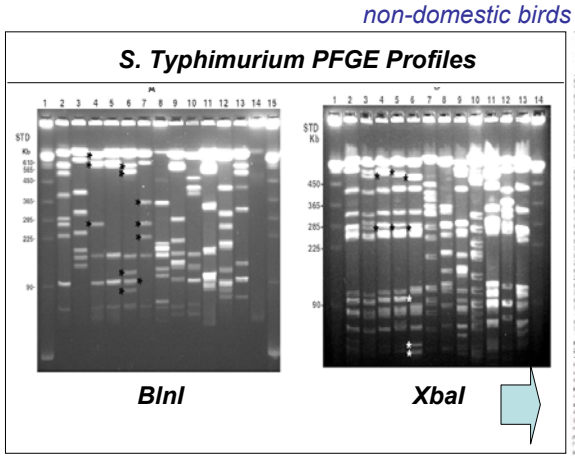


FIG. 1. *Gross pathology of an American cowbird that died of salmonellosis. The arrows delineate multiple necrotic plaques on the esophageal mucosa from which S. enterica serovar Typhimurium was isolated.*

Salmonella spp. have been isolated from numerous free-ranging avian species, including passerine, psittacine, and gallinaceous birds; free-ranging and captive waterfowl; and raptors. Cases of salmonellosis in free-ranging birds, particularly in passerines, have been reported in several countries and several eastern and midwestern states in the United States since 1957, when this disease was first documented in wild birds. The most common *Salmonella* serotype encountered is *Salmonella enterica* serovar Typhimurium, a broad-host-range serotype that is commonly associated with cases of human salmonellosis in the United States. The high incidence of *S. enterica* serovar Typhimurium among passerines and their subsequent congregation around bird feeders may offer some opportunity for the transmission of this organism to humans. A widespread epizootic of salmonellosis was reported during the winter of 1997 to 1998 in free-ranging birds across the northeastern and midwestern United States. Hundreds of birds, mostly songbirds, were reported to have died in this epizootic. We determined by pulsed-field gel electrophoresis (PFGE) that *S.* Typhimurium associated with these epizootic outbreaks were clonal (Table 1).

TABLE 1. *Genotypic and phenotypic characteristics of Salmonella isolated from non-domestic birds*



Recent comparison of PFGE patterns from these passerine isolates against a larger collection of S. Typhimurium strains, isolated from various animal species and locales, determined that these isolates represent a Typhimurium strain commonly isolated from various wild and domesticated animal species.

Specific objectives

- Document the prevalence of *Salmonella* in free-ranging songbird populations
- Examine how the presence of infection varies among sites and host species
- Quantify body condition and immune parameters in relation to host infection status

Plan for study

STUDY SITES

• 20 sites are established in the metropolitan Atlanta area and surrounding counties representing a gradient of urban development
• These sites include parks, preserves, and residences located within varied landscapes
• An urban impact score composed of human population density, distance from the urban center, and impervious surface coverage characterizes each site
• Vegetation and avian biodiversity measures further describe the habitat quality of each site

SAMPLING SCHEME

• Each site will be sampled three times throughout the summer breeding season
• Sampling occurs between 6:30a.m. and noon, using 2-6 mist nets placed around established feeder stations
• In addition to a fecal sample, morphological and physiological data is gathered in the field and the individual is released shortly after capture
• Fecal deposits around the feeder station are also sampled
• 500 samples across all sites will be collected in the sampling season

DIAGNOSTICS

• Samples will be cultured within 6-24 hours of field collection
• Serotype will be ascertained by PCR-based methods for identifying O, Phase 1 and 2 antigen types. Isolates will be strain-typed using PFGE (pulse-field gel electrophoresis).
•We will compare PFGE patterns from wild bird *Salmonella* isolates against a *Salmonella* database of PFGE profiles for matches.
• A subset of culture-negative samples will also undergo *Salmonella*-specific real-time PCR to confirm negative findings

FOCAL SONGBIRD SPECIES

All wild songbirds (Passeriformes) captured in avian mist nets will be sampled for multiple pathogens including *Salmonella* spp.

Past work indicates that half of all samples will be from Northern Cardinals (*Cardinalis cardinalis*) and Carolina Wrens (*Thryothorus ludovicianus)*

- These 2 species are year-long residents of the GA Piedmont and can be found at all points along the urban gradient
- Both demonstrate territorial behavior which facilitates tracking and trapping
- Differ in nest site preference, diet, and mating behavior which will allow for future examination of how the interaction between host traits and urbanization may affect overall condition and disease risk

Significance and future directions

From this study, we expect to determine the prevalence of *Salmonella* among wild bird populations in Georgia. Molecular typing by PFGE will allow us to determine the genetic diversity among *Salmonella* serovars commonly encountered in song birds in Atlanta and identify strains endemic to this animal population. Additional genetic screens of select plasmid and phage associated virulence genes will allow us to determine the virulence potential of these *Salmonella* strains. Ultimately, we will compare songbird *Salmonella* PFGE patterns to the human, PulseNet database at CDC to assess the potential of these wildlife isolates to cause disease in humans. This information will be important in our understanding transmission dynamics of *Salmonella* in wild bird populations and the potential for these wild bird *Salmonella* strains to cause disease in humans.