

# Role of the European Starling in the Transmission of *E. coli* O157 on Dairy Farms

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**ABSTRACT:** Routes that cattle become exposed to and contaminated with *Escherichia coli* O157 remain enigmatic. To ascertain the potential role of wild birds, particularly European starlings, in the transmission of *Escherichia coli* O157:H7 among dairy farms, the ecology of this pathogen in these birds was studied. Bird movement from roost sites to farms was monitored using radio-telemetry (n = 49). Concurrently, frequency of livestock feed contamination and *E. coli* O157:H7 carriage in birds and in cattle was ascertained. Most of the birds tagged from farms in the limited geographic region roosted communally at a location within 20 km of the farms where they were tagged. Individual birds returned frequently to the same farm on a daily basis for feeding. *E. coli* O157:H7 was cultured from approximately 3% of starlings and 4% of cattle in the study population. We have previously reported the isolation of indistinguishable molecular subtypes of *E. coli* O157 from starlings from different farms. Clearly, European starlings can serve as a vehicle to disseminate this pathogen from farm to farm. What remains to be determined is the magnitude of the contribution that birds have in the overall ecology of *E. coli* O157 in livestock populations, and how this might be mitigated.

**KEY WORDS:** disease, *E. coli* O157, European starling, food safety, *Sturnus vulgaris*, wildlife

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## INTRODUCTION

The Foodborne Diseases Active Surveillance Network, established by the Centers for Disease Control and Prevention (CDC), U.S. Department of Health and Human Services, estimates that 76 million cases of acute diarrheal illness occur annually in the U.S. (Anonymous 2004). Both cattle and wild birds harbor the microorganisms responsible for 6 of the 9 human diseases tracked by the CDC in this program including *Campylobacter*, *Listeria*, *Salmonella*, Shiga toxin-producing *Escherichia coli* (STEC), *Yersinia*, and *Cryptosporidium*. Control of these pathogens in cattle at the pre-harvest stages of food production is believed to positively impact food safety (Tollefson et al. 1996). For example, Jordan et al. (1999) predicted, based on mathematical modeling, that a 50% decrease in the concentration of fecal *E. coli* O157 carriage among slaughter cattle would drastically reduce the incidence of post-harvest carcass contamination. Elder et al. (2000) further provided empirical evidence that the rate of carcass contamination was proportional to the prevalence of hide and fecal carriage of animals presented for slaughter. To date, very few on-farm control measures have been proven to reduce the carriage of *Salmonella* spp., *Campylobacter jejuni*, or Shiga-toxin producing *E. coli* in cattle. Given the evidence above, and the fact that foods of bovine origin are the primary foodborne route of human infection with *E. coli* O157, there is a strong desire by farmers, food processors, and public health/food safety organizations to identify on-farm interventions to control foodborne pathogens at the preharvest stage of production.

Given that wild birds can harbor these bacteria, our objective in this study was to determine the potential role that European starlings (*Sturnus vulgaris*), a frequent nuisance pest on dairy farms, have in the transmission of *E. coli* O157 among dairy farms. Our ultimate goal is to determine whether control of these pests on farms would likely have positive effects on food safety. This information will provide immediate and tangible benefits to food producers, the public, and the public health infrastructure. Reducing the carriage of foodborne pathogens, even by small amounts, would have significant impacts, both on public health and on the economy. For example, the estimated annual cost of illnesses caused by *E. coli* O157 and other Shiga toxin-producing *E. coli*, is approximately \$1 billion (USDA 2004), and the average cost of *E. coli* O157 alone to the cattle industry exceeds \$267 million annually (NCBA 2004).

## METHODS

### Radio Telemetry

In September 2007, in accordance with The Ohio State University's Animal Care and Use Committee guidelines, European starlings were mist-netted on 5 dairy farms in Wayne County, OH, that had previously reported an abundance of starlings. Ten birds on each of 4 farms and 9 on a fifth farm (n = 49) were fitted with radio transmitters (model ANTC-M4-2L, Lotek Engineering, Ontario, Canada) and released. At each of these dairy farms, we established permanent receiver sites to detect radio-tagged birds. Additionally, we placed temporary receiver systems at 17 other dairies located near the 5 main dairies. Tem-

porary telemetry sites were kept active for approximately one week. Two mobile telemetry setups were also used daily and traveled throughout the study area, generally avoiding the sites where fixed systems were located. We used the mobile systems to locate radioed birds using sites outside the range of the net of fixed telemetry systems. To allow the birds to behaviorally adjust to wearing the radio transmitters, we did not analyze data that were collected within 3 days of the tagging date.

A bird was considered stationary if it was detected by radio-telemetry at a site during a 15-minute or greater period. If the bird moved and was recorded at another site, the 15-minute period was reset.

## Microbiology

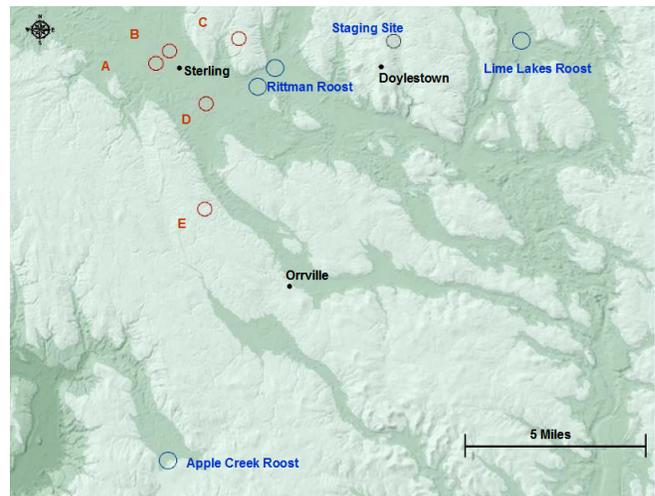
Birds captured in excess of the 10 birds required for radio telemetry, and birds captured on surrounding dairy farms, were euthanized by cervical dislocation, and the entire distal gastrointestinal tract (from proventriculus to the cloaca) was removed and placed in a sterile bag. Buffered Peptone Water (BPW) at the ratio of 1:10 was added to whole intestinal contents and homogenized in a laboratory stomacher for 30 secs at 5 strokes/sec and incubated at 37°C for 24 hours for enrichment. *Escherichia coli* O157:H7 present in any 1-ml aliquot of the overnight culture, along with a positive control, was concentrated with anti-O157 immunomagnetic beads (Dynal, Oslo, Norway), and 75 µl of bead mixture was plated on sorbitol-MacConkey agar plates containing cefixime (50 ng/ml) and potassium tellurite (2.5 mg/ml) (Sigma Chemical Co., St. Louis, MO) and incubated at 37°C for 24 hrs. Up to 5 sorbitol-negative (clear/whitish) colonies were picked from each plate and further identified as *E. coli* O157:H7, based on lactose fermentation and the inability to cleave 4-methylumbelliferyl-β-glucuronide to a fluorescent product. Confirmation of *E. coli* O157:H7 was determined by a latex agglutination test for the presence of the O157 antigen (Oxoid, Basingstoke, Hampshire, UK).

## RESULTS

### Telemetry

#### Daily Bird Movement

We recorded about 600,000 raw telemetry fixes at the 22 dairies (17 temporary sites, 5 permanent) between 21 September and 31 October 2007. After applying our criteria to adjust for birds remaining stationary, the number of radiolocations was reduced to 19,094. Forty birds provided locations beyond the 3-day acclimation period. The average number of locations per bird was 413 (SD = 62.1). The pared-down dataset indicated that most radio-tagged birds showed a strong day-to-day fidelity to the dairy sites of their capture, and use of other dairy sites was infrequent, except during the transit period to and from the roosts. The average time of first arrival at a dairy site was 1015 h, with an average departure of 1530 h. Because most human activity at the dairy associated with milking, cleaning, etc. occurred during the early morning and again late afternoon, we suspect that the birds were avoiding these periods of high human activity. The overall low frequency of locations recorded by the mobile receivers was probably the result of rather small-sized daily activities, which were often centered on the tagging-site



**Figure 1. Digital elevation map of the study area. The 3 roosts are named and labeled, while the 5 dairies where radio-tagging occurred are indicated by letters A through E. For spatial reference, the Lime Lakes Roost was about 10 miles from the town of Sterling, OH, which was surrounded by 4 of the 5 dairies used for radio-tagging. We captured and radio-tagged 49 birds by mist-netting the entrances to the dairy barns.**

dairies. The use of small-sized ranges for daily activities can be rather typical behavior for European starlings during the late-summer and fall periods (Caccamise 1991). Transit times from the roost were rapid, and the birds would often cover the 20-km distance from the main roost in about 20-25 mins. In contrast, the return transit was leisurely and could take >1.5 hrs, with staging areas spaced intermittently along the return route. The primary staging area for birds using the main roost was about 6 km from the roosting site.

### Roost Sites

Three roost sites were identified (Figure 1). The Lime Lakes Roost was the largest roost; 34 of the total 49 birds used this roost at least once. Seventeen birds used the Rittman Roost Complex, and of these, 12 also used the Lime Lakes Roost. Only one bird was found using the Apple Creek Roost; this was a bird radio tagged at Dairy E. This bird also used the Lime Lakes Roosts, as did most of the E-site radio-tagged cohort. The Lime Lakes Roost was the largest roost. This roost was used by approximately several million birds, including red-winged blackbirds and common grackles in addition to starlings. The Rittman Roost Complex was the smallest roost, with about 25,000 birds. Both of these roosts were wetland-type roosts. The Apple Creek Roost, an upland roost, had between 500,000 and 1,000,000 birds (all starlings) at its maximum. The roosting sites of 12 radio-tagged birds were never found.

### Microbiology

We trapped 316 starlings on 31 farms for microbiological culture on 31 different farms. Farms were visited repeatedly to trap birds; however, on some farms, no birds were trapped, either because of the absence of bird populations or unsuccessful trapping attempts. Birds were trapped on 10 farms. Seven (7/316, 2.2%) of intestinal

contents yielded colonies phenotypically consistent with *E. coli* O157. The highest prevalence of *E. coli* O157 in birds on an individual farm on one sample date was 1/11 (9%).

During this same period, 1,860 bovine fecal samples were cultured for this organism. Forty-eight (48/1869, 2.6%) of bovine fecal samples tested positive for *E. coli* O157. Bovine *E. coli* O157 prevalence ranged from 0-33%. Additional genotypic confirmation and characterization of the isolates is under way.

## DISCUSSION

In ancient Greek mythology, the sixth labor of Hercules was to rid the Stymphalian countryside of the flocks of birds that were breeding at an astonishing rate, destroying the crops and fruit trees, and locally terrorizing the inhabitants. Contact with the bird's sharp metallic feathers would result in death. Although fictional, this story is also somewhat prophetic of problems facing modern intensive agriculture in the U.S. European starlings were first introduced to the United States in 1890 with the romantic notion of populating New York's Central Park with all the species of birds mentioned in Shakespeare's works (Chapman 1925, Bent 1950). Today, it is estimated the population of starlings has grown to 140-200 million birds (Johnson and Glahn 1992). During the winter in Ohio, it is common to observe flocks of 500 to over 2,000 birds with some large winter roosts containing 400,000 - 600,000 birds (Peterjohn 2001). Herein we report a winter roosting site with multiple bird species exceeding an estimated population size of 1,000,000 birds.

Because of their foreign origin and aggressive behavior, European starlings are considered an invasive species in the U.S. They often out-compete and displace native species of birds. Ignoring the potential costs associated with the transmission of foodborne bacteria that this proposal is aimed at addressing, it is estimated that these birds cause \$800 million in damage to agriculture annually in the U.S. (Pimentel et al. 1999). At livestock operations, starlings may preferentially select high-protein components of cattle rations, leaving the ration protein deficient and resulting in sub-optimal growth and milk production (Johnson and Glahn 1992). The negative impact these birds may have on food safety, and the resulting public health and economic costs, have not been determined.

Numerous species of wild birds can be found either inhabiting, or at least visiting, dairy farms. However, starlings by far are among the most frequent and numerous avian farm visitors at Ohio dairies. It is well known that birds, in general, are commonly colonized with *Salmonella* spp. and thermophilic *Campylobacters*. An example of birds contributing to the epidemiology of foodborne disease is the recent multi-state tomato-borne outbreak of *Salmonella* Newport infections, in which a tomato processing plant was contaminated with bird droppings (Kretsinger et al. 2003). In yet another study, *Campylobacter jejuni* strains isolated from starlings were indistinguishable from isolates obtained from humans (Broman et al. 2004).

Starlings are very versatile and adaptive to multiple habitats. They may fly between 24 and 48 km to feed (Johnson and Glahn 1992). However, starlings will increase their flying distance from roosting sites to feeding

areas farther away, if a desirable source of food is plentiful at a more distant location. Although this particular study is not complete, in a previous study, in which one of the authors (JL) was involved, indistinguishable *E. coli* O157 subtypes were isolated from two feedlots approximately 100 km apart, and wild birds were determined to be among the only potential common vehicles shared between the two feedlots (Van Donkersgoed et al. 2001). We have also previously reported indistinguishable subtypes of *E. coli* O157 being isolated from bird excrement collected on two geographically separate dairy farms in Ohio (Wetzel and LeJeune 2006). The evidence cited above has led us and other workers in the field to speculate that European starlings may play an important role in the maintenance and dissemination of foodborne pathogens on livestock operations (Nielsen et al. 2004).

On farms frequented by birds, feed contamination with droppings is not uncommon. The potential for feeds to become contaminated with pathogens of significance is dependant upon the rate of contamination and the prevalence of pathogen carriage by the vector species. Daniels et al. (2003) developed a method to estimate the frequency of livestock feed contamination with wildlife excreta, and they determined that this route of disease transmission could account for the dissemination of a large percentage of several livestock diseases. Cross-sectional studies have identified that farms on which birds have access to livestock feeds were more likely to have cattle positive for *Campylobacter* spp. and *Mycobacterium avium* subsp. *paratuberculosis* than farms that had stricter feed hygiene protocols (Wesley et al. 2000, Fredriksen et al. 2004). Although not reported for dairy farms or feedlots, molecular comparisons of *Salmonella* strains isolated from wild birds on commercial poultry operations demonstrated a sharing of common strain types between the wild birds and the laying hens (Liebana et al. 2003). Experimentally, *E. coli* O157 has been reported to replicate and persist in the digestive tract of pigeons for approximately 21 days (Cizek et al. 2000).

We conclude that the patterns of bird movement, preliminarily described here, along with the isolation of the pathogen *E. coli* O157 from both birds and cattle, further support the hypothesis that birds play a critical role in the dissemination of important foodborne bacteria among farms. The available data, however, are not sufficient to determine the extent or magnitude that this mode of pathogen dissemination contributes to the overall epidemiology of foodborne pathogens in dairy cattle. It remains unknown if intensive wild-bird exclusion programs on farms would have an impact on food safety.

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