Pathogen Risks Related to the Movement of Birds Frequenting Livestock and Fresh Produce Growing Areas in the Southwestern U.S.

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ABSTRACT: Concentrated animal feeding operations (CAFOs) are sometimes located in close proximity to fresh produce fields, both of which serve as easily accessible food and water sources for wild birds. When birds travel between these two areas, they have the potential to transfer pathogens from cattle, a documented source of enteric zoonotic foodborne pathogens, to fresh produce crops through fecal deposition. However, the presence of pathogens in wild birds is not a risk unless the birds or their fecal material come into contact with fresh produce crops. Therefore, the objective of this study was to determine if birds visiting CAFOs use flyways that cross fresh produce fields, thereby increasing the risk for contaminating fresh produce intended for human consumption. During 2014, birds trapped at a CAFO in southern Arizona were fitted with Lotek nano-coded radiotransmitters. Two receivers were placed at the CAFO and two receivers were placed in nearby fresh produce fields. A total of 103 birds were fitted with radiotransmitters, including 66 red-winged blackbirds, 21 Eurasian collared doves, 11 brown-headed cowbirds, four common ravens, and one European starling. Over four million data points were collected indicating the date, time, and bird ID number for each time a bird was recorded within 1 km of a receiver. Radiotelemetry results showed that birds travel regularly between the CAFO and fresh produce fields. Using PCR and culture techniques, 2 (1.9%) birds tested positive for *Salmonella*, and 5 (4.9%) tested positive for non-O157 Shiga toxin-producing *Escherichia coli* (STEC). During the same time period, *Salmonella* (4%), STEC O157 (16%), and non-O157 STEC (44.5%) were detected in 400 cattle fecal samples from the CAFO. Our results will aid in determining the pathogen risks that birds pose to fresh produce when they are frequent visitors to a CAFO and fresh produce fields.

KEY WORDS: *Agelaius phoeniceus*, birds, concentrated animal feeding operation (CAFO), *E. coli*, food safety, leafy greens, red-winged blackbird, *Salmonella*, STEC

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INTRODUCTION

Over 400 species of birds live in or visit the Sonoran Desert. The area serves as a major flyway for migratory birds, and as a permanent home for a variety of others. Due to long flights of migratory birds and high temperatures, many birds take respite in agricultural areas, often visiting a number of locations within a single area (Taylor et al. 2011). With 350,000 cattle marketed in Arizona each year from feed yards and cow/calf operations all over the state, there are numerous concentrated animal feeding operations (CAFOs) where birds can safely stop by or take up residence to be close to food, water, and shelter.

Many of the CAFOs in the southwest desert are surrounded by fresh produce fields. When birds visit these CAFOs, foodborne pathogens may be transferred through birds' interactions with cattle and their surrounding environment (Pedersen and Clark 2007). Growers have reported birds often travel to roosting sites from the CAFOs, many times passing over fresh produce fields to get there, and sometimes stopping in or around the fields for food or water along the way. When large flocks of birds land in a fresh produce field, they may destroy produce and leave behind fecal material potentially contaminated with zoonotic enteric foodborne pathogens.

Food safety is a top priority for all fresh produce growers in the desert produce production region. Tremendous resources are spent each year to ensure that produce is safe to eat when it reaches consumers (Calvin et al. 2004). Despite these efforts, 48 million Americans get sick from foodborne pathogens each year, and approximately half of those incidents are attributed to fresh fruits and vegetables (Scallan and Mahon 2012). *Salmonella* and STEC (i.e. Shiga toxin-producing *Escherichia coli*) are two of the major zoonotic pathogens that cause foodborne outbreaks in the United States (Scallan and Mahon 2012). These pathogens live commensally in the gastrointestinal tracts of cattle and may be shed in feces (Smith 2014), where flocking birds such as European starlings (*Sturnus vulgaris*) can make contact and transport the pathogens elsewhere (Wetzel and LeJeune 2006, LeJeune et al. 2008).

In 2010, there was a multi-state outbreak of *E. coli* O145 that was traced-back to romaine lettuce grown in Yuma, AZ (Taylor et al. 2013). This was the first time that an outbreak related to leafy greens was linked to the Southwest produce-growing region. Dust, mud, wildlife, and irrigation water were all investigated as potential sources; however, no definitive source was identified. During the same year, a study focusing on feces of dogs (*Canis familiaris*) and coyotes (*C. latrans*), often seen by produce growers in and around their fields in the Yuma area, concluded that neither was a significant source of STEC, although *Salmonella* was prevalent in stray dog and coyote feces (Jay-Russell et al. 2014 b). In a subsequent study,

Salmonella and STEC were detected in feral swine (*Sus scrofa*) and javelina (*Pecari tajacu*) (Jay-Rusell et al. 2014 a).

The Arizona Leafy Green Marketing Agreement (AZ LGMA) is a voluntary collaborative effort by the Arizona produce industry that establishes food safety guidelines for growers of fresh produce crops intended for human consumption. Currently, they advise growers to plant their crops at least 400 ft away from a CAFO, depending on other risk factors, to prevent food safety risks (AZ Leafy Greens Marketing Agreement 2015). To date, there is only one study indicating that CAFOs may pose a potential threat to the safety of fresh produce crops when grown up to 400 ft away from the CAFO (Berry et al. 2015). However, the study did not examine the potential for foodborne pathogen transmission between CAFOs and wildlife.

The objectives of this study were to determine: 1) if birds visiting CAFOs use flyways that cross fresh produce fields, and 2) if these birds harbor the same strains of foodborne pathogens, specifically of *Salmonella*, *E. coli* O157, and non-O157 STEC, that exist at the CAFO. These preliminary data will indicate if birds occupying areas around livestock operations may be a food safety risk to nearby fresh produce.

METHODS

Study Sites

The study was conducted from 2013 to 2015 in a leafy green production area in southern AZ that is in close proximity to a cattle feed yard.

Passerines and Columbiformes

Mist nets were set approximately 30 min before sunrise in areas where birds were observed on a daily basis. Nets were checked every 15-20 min after the first birds were observed in the area. Trapping continued until most bird activity ceased or until trapping success declined, usually by 11:00 a.m.

Birds caught in the mist nests were removed and placed in pillowcases for transport. After all nets were checked, the captured birds were brought to a nearby area with appropriate protection from the weather for processing. We recorded the species of each bird, and collected fecal samples directly using a cloacal swab (BD Diagnostic Systems, Sparks, MD) or by catching fresh droppings into a microcentrifuge tube. Samples were stored in a cooler with ice and shipped to the lab for processing within 24 hours of sample collection. After being fitted with a backpack radiotransmitter, birds were released directly from the processing area.

Corvids

A decoy and net launcher were set up in an area containing animal carcasses that were heavily visited by ravens. The decoy was set up 30 days before trapping to acclimate the ravens to the presence of the equipment. On the day of trapping, a functional net launcher replaced the decoy two hours before sunrise, and animal carcasses were strategically placed as bait within the range of the net launcher. A technician laid in wait in a blind until the ravens arrived, at which time the technician launched the net over the ravens, safely trapping them underneath. Once a raven was successfully removed from the net, we immediately collected fecal samples. We fixed the birds with backpack radiotransmitters.

Cattle

Cattle feces were collected monthly from the feed yard. Five samples were collected from each of 10 randomly-selected cattle enclosures representing all age classes of the cattle. Fresh samples were collected from the pen floor, either upon deposition by the cattle or within minutes of deposition. Samples were collected into sterilized cups (National Scientific, Claremont, CA) using sterile scoops (Bel-Art, Wayne, NJ). Samples were stored on ice and shipped to the lab for processing within 24 hours of sample collection.

Radiotelemetry

In order to track bird movement patterns, birds were fitted with backpack radiotransmitters: Lotek model NTQB-3-2 for passerines and columbiformes, and NTBQ-6-2 for ravens (Lotek Wireless Inc., Newmarket, ON, Canada). Based on early studies of bird radiotelemetry (Karl and Clout 1987), birds were only fitted with a radiotransmitter if the transmitter weighed less than 3% of the bird's total weight. Transmitters were affixed to the birds as backpacks using Teflon ribbon and glue. The transmitters intended for passerines and columbiformes had a battery life of 3 months, while the raven transmitters had a battery life of six months. The backpacks were constructed so that around the time that the battery stopped working, the backpack material would unravel and the unit would fall off the bird. Half of the birds were fitted with backpack radiotransmitters in spring, while the remainder were deployed in fall.

Birds were tracked using two receivers (Lotek model SRX-DL-1) placed at the feed yard and two receivers placed in nearby leafy green fields. Each receiver was connected to a solar panel and 12-volt battery to ensure constant recording throughout the season. Birds that flew within 1,000 m of a receiver were picked up by the receiver, and the date, time, and their individual ID number were recorded.

Laboratory Methods

Fecal samples from all tagged birds were pre-enriched by placing cloacal swabs collected in the field into tryptic soy broth (TSB) (BD Diagnostic Systems, Sparks, MD). Samples were then incubated for two hours at 25°C with agitation at 100 rpm, followed by eight hours at 42°C with agitation, and held overnight at 6°C, using a Multitron programmable shaking incubator (Eppendorf, Hauppauge, NY).

For detection of *E. coli* O157, immunomagnetic separation (IMS) using Dynal anti-*E. coli* O157 beads (Invitrogen/Dynal, Carlsbad, CV) was performed on TSB enrichment broths with the automated Dynal BeadRetriever (Invitrogen) per the manufacturer's instructions (Cooley et al. 2013). After incubation and washing, 50 µL of the resuspended beads were plated onto Rainbow agar (Biolog, Hayward, CA) with novobiocin (20 mg/L) and tellurite (0.8 mg/L) (MP Biomedicals, Solon, OH). Fifty µL of the resuspended beads were also plated onto

MacConkey II Agar with sorbitol supplemented with 500 µl of potassium tellurite solution and 100 µl Cefixime (CT-SMAC); plates were streaked for isolation and incubated for 24 hours at 37°C.

To detect non-O157 STEC, pre-enrichment broth was incubated in mEHEC selective media (Biocontrol, Bellevue, WA) for 12 hours at 42°C followed by plating and incubating on Chrom STEC (DRG International Inc., Springfield, NJ). Up to six presumptive STEC positive colonies were confirmed for the presence of *stx1* and/or *stx2* genes by real-time PCR (Eppendorf, Hauppauge, NY). Confirmed STEC isolates were then characterized for virulence genes (*stx1*, *stx2*, *eaeA*, *hlyA*, *fliC* and *rfbE*) using conventional PCR.

Salmonella was recovered by adding pre-enrichment broth to Rappaport-Vassiliadis (RVS) (BD Becton, Sparks, MD) and incubating for 48 hours at 42°C as described previously (Kawasaki et al. 2005). A loopful of RVS bacterial suspension was then streaked onto Xylose Lysine Tergitol 4 (XLT4) agar plates and incubated for 24 to 48 hours at 37°C for isolation.

RESULTS

We collected 400 cattle fecal samples and 103 bird fecal samples at the CAFO. We fitted all 103 birds with backpack radiotransmitters to track their movement patterns. Five species of birds were caught (Table 1). The majority (64.1%) of birds fitted with radiotransmitters were red-winged blackbirds (*Agelaius phoeniceus*) (n = 66), followed by Eurasian collared doves (*Streptopelia decaocto*) (20.4%), and brown-headed cowbirds (*Molothrus ater*) (10.7%) (Figure 1).

Sixteen (4.0%) cattle samples and 2 (1.9%) bird samples tested positive for *Salmonella*, including one raven and 1 red-winged blackbird (Table 1). Isolates from cattle were serovars Altona (n = 2), Amager (n = 1), Anatum (n = 1), California (n = 1), Dublin (n = 1), Muenchen (n = 3), Rough "O" (n = 1), and Soerenga (n = 1), plus two samples there were unable to be assigned a type. In contrast, *Salmonella* serovars from the raven and red-winged blackbird were Livingstone and Kentucky, respectively.

E. coli O157 was isolated from 64 (16.0%) of the cattle samples and none of the bird samples, whereas non-O157 STEC was cultured from 178 (44.5%) cattle and 5 (4.9%) bird samples (Table 1).

Radiotelemetry data showed that while birds were recorded at receivers in all CAFO and leafy green locations, 88.9% of bird visits were recorded on the receivers located at the CAFO, with ravens spending the most time there (98.7% of raven visits). Brown-headed cowbirds spent more time than other bird species near leafy green fields (29.2% of their visits were in leafy green fields) (Figure 2).

DISCUSSION

In this study we documented foodborne pathogens in cattle feces and wild bird fecal samples from birds that frequented a cattle feed yard. Preliminary analysis of bird movement from the CAFO demonstrates that multiple common species – including but not limited to red-winged blackbirds, brown-headed cowbirds, European starlings, Eurasian collared doves, and common ravens – frequently travel between the CAFO and nearby leafy green fields. The current industry standard for the recommended minimum distance between a CAFO and fields growing fresh produce is 400 ft (AZ Leafy Greens Marketing Agreement 2015). In this study, we documented birds traveling the maximum distance of our receivers from the CAFO (1,000 m), which is still only a fraction of the distance that many birds travel in a single day. While 400 ft may be an adequate buffer for terrestrial animals, avian species have much larger home ranges than most mammals and the ability to fly, making establishment of a buffer zone for them nearly impossible.

All of the birds caught in this study are full-time residents in the southwest desert. During the study, growers indicated that there are times when migratory birds descend on a field and destroy it in a matter of hours, which suggests to us that bird density could be an important risk factor. For example, in 2008 an outbreak of *Campylobacter* was traced back to raw peas from a field located near a sandhill crane breeding site (estimated 10,000 birds) in the Pacific flyway; samples from both the birds and the peas tested positive for *Campylobacter* with similar unique patterns from environmental samples confirmed by DNA fingerprinting (Gardner et al. 2011). While we did not witness damage to produce by migratory or breeding birds firsthand during the course of the study, a follow-up study on pathogen risks specifically associated with migratory or breeding birds should be conducted.

Only a few individual birds in our study were actively shedding any of the three pathogens for which we tested. Specifically, red-winged blackbirds, a year-round resident of the southwest desert, made up the majority (64.1%) of the birds that we trapped, but only three of them (4.5%) carried at least one of the foodborne pathogens for which we tested. While we did not find a relationship between the prevalence of foodborne pathogens in wild birds and cattle, Swirski et al. (2014) found that cattle fecal samples

Table 1. Prevalence of pathogens by bird species.

Common Name	Scientific name	*Salmonella*	Non-O157 STEC	*E. coli* O157
Brown-headed cowbird	*Molothrus ater*	0	3/11 (27.3%)	0
Common raven	*Corvus corax*	1/4 (25.0%)	0	0
Eurasian collared dove	*Streptopelia decaocto*	0	0	0
European starling	*Sturnus vulgaris*	0	0	0
Red-winged blackbird	*Agelaius phoeniceus*	1/66 (1.5%)	2/66 (3.0%)	0
Cattle		16/400 (4.0%)	178/400 (44.5%)	64/400 (16.0%)
TOTAL		**2/103 (1.9%)**	**5/103 (4.9%)**	**0**

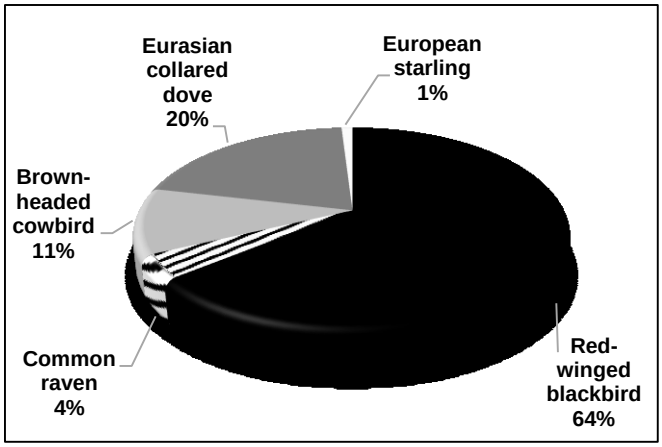

Figure 1. Birds fitted with radiotransmitters by species.

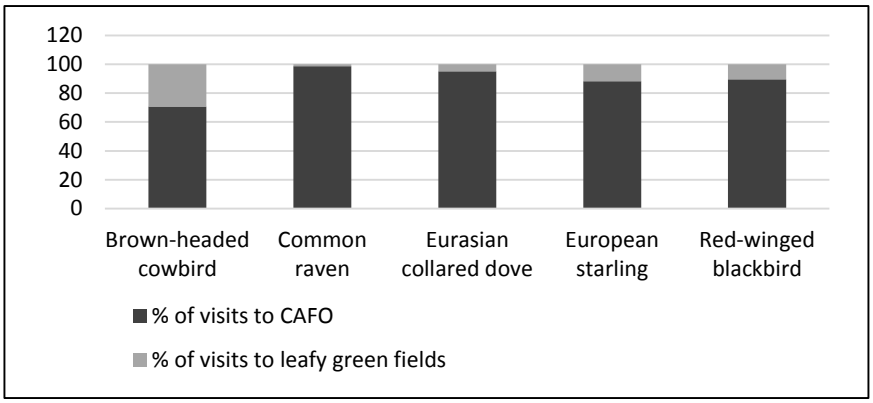

Figure 2. Percent of visits to a CAFO and to leafy green fields by bird species using radiotelemetry.

collected at dairy farms in Ohio where European starlings roost overnight positively correlated with a higher prevalence of *E. coli* O157:H7 than farms that did not have roosting starling populations. Other studies on dairy farms in Ohio indicated that European starlings play a role in the transmission of *E. coli* O157 among dairy farms, carrying highly related molecular subtypes among the starling populations (LeJeune et al. 2006). Like starlings, red-winged blackbirds are not a protected species, so growers make reasonable efforts to prevent them from landing in their fields by using visual, auditory, and olfactory deterrents, as well as lethal measures as a last resort to prevent crop damage and to protect public health.

Cattle are reservoirs of foodborne pathogens, particularly of *E. coli* O157 and non-O157 STEC. Prevalence ranges from 4% to 83% in feedlot cattle (Fegan et al. 2004, Arthur et al. 2009, Venegas-Vargas et al. 2016). This marked difference in prevalence is likely due to a number of dynamic risk factors, including environment (Stanford et al. 2016), animal age (Mir et al. 2015), diet (Venegas-Vargas et al. 2016), season (Stanford et al. 2016), and year (Venegas-Vargas et al. 2016). Contrary to STEC prevalence in cattle, the prevalence of STEC in wild birds is consistently lower. A study in Japan found 5-25% of wild birds carried isolates with specific Shiga toxin genes (Kobayashi et al. 2009), while 10.8% of feral pigeons carried STEC in Italy. In Colorado, only 7.9% of pigeons carried genes for Shiga toxin (Pedersen et al. 2006). In a review by Langholz and Jay-Russell (2013), the authors identified 23 *E. coli* studies conducted on wild birds, documenting positive results in 0% to 5% of samples. A larger study was conducted in London showing that while 50.8% of fecal samples were presumptive positive for *E. coli*, only 1.5% to 7.9% of wild birds carried a Shiga toxin gene (Hughes et al. 2009). Our data were similar with 44.5% of cattle and 4.9% of bird samples positive for STEC, indicating that birds are not a significant carrier of STEC.

In this study, cattle shed different *Salmonella* serovars than birds, suggesting no relationship between these populations. The cattle fecal samples contained 10 serovars, similar to the findings of another study in nearby Texas that found 13 (Carlson et al. 2011). Other studies have shown similar *Salmonella* prevalence to the 4% we found in our cattle fecal samples, including 5.4% in Nebraska feedlots (Schmidt et al. 2015) and less than 1% in cattle in the central California coast produce production region (Gorski et al. 2011). Unlike cattle, wild birds are considered a reservoir for *Salmonella*. Numerous studies investigating *Salmonella* prevalence in wild birds have been conducted in agricultural areas. For example, Callaway et al. (2014) found that 14.9% of wild birds in Texas that are often associated with cattle, including brown-headed cowbirds that were also in our study, were shedding *Salmonella*. In a study of egret nestlings in central Texas, *Salmonella* prevalence ranged from 29% to 91% (Phalen et al. 2010). These prevalence rates are much higher than those that we measured (1.9%) in southern Arizona and California. This could be due to the differences in temperature, humidity, geography, and other factors that could affect transmission and shedding that differ between our field sites and those in the other studies.

Bird intrusions into vegetable fields are perceived as a food safety risk by fresh produce growers, and this study indicates that transmission of foodborne pathogens from a CAFO to leafy green fields via wild birds is possible. However, based on radiotelemetry data, the proportion of visits birds made to the CAFO in this study was higher compared with their visits to nearby fresh produce fields (Figure 2). In order to address the bigger picture of bird intrusion in agricultural fields and the potential risk for pathogen transmission from animal operations and livestock to fresh produce fields, further studies should be conducted to determine which species of birds are present and if they carry foodborne pathogens. To maintain the highest level of food safety and minimize risks, growers should make every effort to keep birds from landing in their fields. Future studies will examine spatial and temporal relationships of birds moving between CAFOs and fresh produce fields.

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