Wild Birds as Vectors for Salmonella on Ohio Dairies

THESIS

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Abstract

Salmonella is a gram negative bacilli that is capable of producing serious disease in both animals and humans. It is estimated that non-typhoidal Salmonella causes 1.2 million illnesses a year in the US, resulting in 450 deaths. Food producing animals are the primary reservoir for human salmonellosis. Birds are attracted to farms due to the presence of feedstuffs, and their close contact with livestock provides the potential for pathogen transmission. Enteric pathogens such as Salmonella can colonize a wide variety of species and thus could be transmitted between livestock and wild birds.

The goal of this study was to determine if wild birds play a role in the transmission of Salmonella as either a mechanical vector by transmitting Salmonella on their body surface, or as a biological vector by transmitting Salmonella through their feces on Ohio dairy farms.

Dairy farms were identified for participation in this study because they were clients of the Ohio State University Large Animal Clinic located in Marysville, Ohio. Three Ohio dairy farms were selected to participate based on appropriate bird habitat near barns, owners allowed access, and convenience of location of farm. External and cloacal swabs were taken from 346 live wild birds captured with mist nets. Wild birds were mist netted close to the barn (≈5 ft.) and far from the barn (≈600 ft.). Environmental cow fecal samples (n=100 per farm) were collected from the barns at
each dairy. All samples were cultured for the presence of *Salmonella* using standard culture techniques.

Four *Salmonella* positive samples were recovered from the wild birds surrounding the three Ohio dairy farms. A bird was considered to be positive if either the cloacal or surface swab was positive for *Salmonella*. There was a significant association observed between farm and wild bird *Salmonella* positive outcome (p-value=0.020).

The prevalence of *Salmonella* in wild birds close to the barn was 1.95% and 0.52% in wild birds far from the barn. The distance at which the birds were captured in relation to the barn was not associated with wild bird *Salmonella* status in our analysis (p-value= 0.327).

The prevalence of *Salmonella* in non-migratory birds was 1.19% and 1.06% in migratory birds. There was no observed association between wild bird *Salmonella* positive outcome and migratory bird status (p-value= 1.000).

Based on our findings, the overall prevalence of *Salmonella* in wild birds is low (1.16%). The dairy cows on the three farms sampled had a high prevalence of *Salmonella* shedding (71-94%), but this was not reflected in the prevalence of *Salmonella* in wild birds that were within 600 feet of the barn. Our results suggest that wild birds likely do not play an important role in the transmission of *Salmonella* on Ohio dairy farms.


Dedication

This document is dedicated to my parents for their
never ending love and support of my dreams.
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Organism

*Salmonella* is a gram negative bacilli that is capable of producing serious disease in both animals and humans (Yan et al., 2004). *Salmonella* genus bacteria belong to the *Enterobacteriaceae* family. Similar to other bacteria in this family, *Salmonella* are intracellular facultative anaerobes, motile, oxidase negative, and able to ferment glucose (Yan et al., 2004). The intestinal tract of animals provides an ideal environment for *Salmonella*. Over 2500 serovars of *Salmonella* have been identified and are divided between two species; *S. enterica* and *S. bongori* (Coburn et al., 2007). The two species have been divided into six subspecies, which are further divided based on characteristics of the flagella, and lipopolysaccharide and carbohydrate (LPS) structures (Coburn et al., 2007).

The *Salmonella* bacterium is the most common food-borne source of bacterial disease in the world (Forshell & Wierup, 2006). Contaminated food and water are responsible for most *Salmonella* infections (Ohl & Miller, 2001). Direct transmission person-to-person and indirect transmissions by fomites are also possible sources of infection. *Salmonella* infections are often food-borne and symptoms in humans include gastroenteritis, and if invasive, may result in systemic infection and death.
(Yan et al., 2004). The signs and symptoms of *Salmonella* are observed after the innate immune system has launched a response to the invading organism and are caused by cytokine production and inflammation (Ohl & Miller, 2001). Food producing animals are the primary reservoir for human salmonellosis (Forshell & Wierup, 2006). Eggs, meat, dairy, and poultry are the food products most associated with contamination with this bacterium (Yan et al., 2004). Infection acquired through eggs is rare today due to industry eradication efforts that have almost eliminated *Salmonella* in eggs by maintaining breeding flocks that are *Salmonella* free.

Contamination of dairy products is only a concern when products are not pasteurized, as the pasteurization process kills the *Salmonella* bacterium. The food products that have the highest level of contamination in the food industry today are fresh ground meat products (USDA, 2013). Ground poultry products have been shown to have the highest level of contamination when compared to other fresh meat (USDA, 2013).

*Salmonella* prevalence in food producing animal populations can range from 0-100%; depending on the region, species, and production system (Forshell & Wierup, 2006).

The pathogenesis of *Salmonella* infections relies heavily on the virulence factors of the bacterium including the ability to infect macrophages, which serve as a host cell that allow the pathogen to replicate (Ibarra & Steele - Mortimer, 2009).

Intestinal macrophages serve as a host which allow the bacterium to survive, replicate and may be distributed through the body if systemic, using phagocytosis of macrophages as the method to spread the infection cell-to-cell (Achouri et al., 2015; Guiney, 2005). The ability of the *Salmonella* bacterium to adjust to changing environments inside the host demonstrates the adaptability of the organism. A low
gastric pH increases the *Salmonella* infectious dose, yet salmonellae have adapted to this initial barrier to infection by exhibiting an acid-tolerance response (Ohl & Miller, 2001). *Salmonella* are able to reach their destination of the intestinal epithelium by inducing their uptake into these cells by crossing the intestinal mucus layer and using fimbriae to adhere to intestinal epithelial cells (Ohl & Miller, 2001). Virulence of the organism is credited to the complex combination of several coordinated virulence factors, which allow the bacterium to evade the host’s innate immune response (Guiney, 2005; Ohl & Miller, 2001).

**Impact on Public Health**

It is estimated that non-typhoidal *Salmonella* causes 1.2 million illnesses a year in the United States, resulting in 450 deaths (CDC, 2013). Symptoms of *Salmonella* infection in humans generally present 12 to 72 hours after infection and the illness may last 4 to 7 days (CDC, 2014b). Most cases of infection do not require treatment, but in severe cases hospitalization is necessary. As many as 23,000 people per year are hospitalized in the US for *Salmonella* infections (CDC, 2013). The highest mortality rates from *Salmonella* infections are primarily in the poorest developing world (Sánchez-Vargas et al., 2011).

Symptoms of enteritis can be severe, especially in young or immunocompromised hosts, resulting in septicemia or death. The infection is often short lived and can be resolved by innate immunity (Guiney, 2005), but in
immunocompromised patients the infection cannot be eliminated due to a defective CD4 T cells, which are responsible for an acquired immune response (Libby et al., 2002). This defect in CD4 T cells is often found in patients with HIV infection and may result in chronic and severe disseminated salmonellosis (Jones & Falkow, 1996).

_Salmonella_ epidemiology is complex, so many different approaches and methods must be taken to control the disease. Patterns of _Salmonella_ infections vary depending on climate, food harvesting and processing technologies, farm animal density, and preparation of food by consumers (Kemal, 2014). Dairy, beef feedlot, swine, and poultry farms in particular can have a very high prevalence of _Salmonella_ on farms depending on their hygiene and operational practices because of high animal density. Wild birds serve as a potentially important source of _Salmonella_ transmission, since they may be in close contact with cattle and their feedstuffs.

Monitoring _Salmonella_ infections in the United States is essential to evaluate and understand trends in illness. The ability to track _Salmonella_ outbreaks based on serotypes allows for the identification of the source of the outbreak as well a timely response to contain the outbreak. Fecal samples are collected and isolates are obtained from approximately 35,000 cases of _Salmonella_ yearly and then serotyped by public health laboratories (CDC, 2014c). This data is then submitted to the Centers for Disease Control and Prevention in order to monitor trends in _Salmonella_ cases at the local, state, and national level to determine if an outbreak is ongoing or the cases are sporadic.

The health care costs associated with treatment of non-typhoidal _Salmonella_ are estimated to be about $365 million annually (CDC, 2013). A large public health
concern is the emergence of antimicrobial resistant *Salmonella* infections. The emergence of these resistant strains is found in part to be a result of the use of antimicrobials in food animals. Due to the wide usage of antimicrobials, selective pressure has allowed bacteria that are anti-microbial resistant to emerge and disseminate (Angulo et al., 2004).

The percentage of antimicrobial resistant *Salmonella* cases has been steadily increasing over time, and has risen to approximately 5% of non-typhoidal *Salmonella* isolates tested by the CDC being resistant to five or more types of antibiotics (CDC, 2013). With all types of resistance to antimicrobials included, there are approximately 100,000 drug resistant *Salmonella* infections per year (CDC, 2013). Antimicrobial resistant *Salmonella* have been found to be more likely to cause bloodstream infections and hospitalization (Varma et al., 2005). The severity of bloodstream infections and other severe complications that can result from antimicrobial resistant *Salmonella* increases the costs of hospitalization. Treatment costs for antimicrobial resistant *Salmonella* are much higher than for susceptible *Salmonella* infections due to the infections being more severe, requiring longer hospitalization, and treatment being less effective or failing (Angulo et al., 2004; Varma et al., 2005).
Surveillance

Antimicrobial resistant *Salmonella* infections in the United States are monitored by a public health surveillance system, the National Antimicrobial Resistance Monitoring System- Enteric Bacteria (NARMS). The system tracks resistance of enteric and food-borne bacteria. The system is a coordinated partnership among the US Food and Drug Administration (FDA), the US Centers for Disease Control and Prevention (CDC), and the US Department of Agriculture (USDA). Antimicrobial resistance is monitored in enteric bacteria from retail meats (FDA), humans (CDC), and food animals (USDA) (CDC, 2014a).

The emergence of antimicrobial resistant *Salmonella* infections in humans is of great public health concern, as some antibiotics used to treat these severe infections are no longer effective. Children and pregnant women are of special concern because fewer antibiotics are FDA approved for use in these populations and once resistant to the approved antibiotics, little treatment options exist. Several studies have provided evidence linking resistant animal infections to human infections.

Ceftiofur resistant *Salmonella* Heidelberg infections in retail chicken in Canada have been found to be closely related to *Salmonella* Heidelberg infections in humans (Dutil et al., 2010). This relationship is reflected in the changing levels of ceftiofur use in hatcheries and the mirroring impact on the changing levels of ceftiofur resistance of Salmonella recovered from chickens. This study provides evidence that ceftiofur use in chickens translates to extended-spectrum cephalosporin resistance in bacteria from poultry and humans (Dutil et al., 2010). The use of
extended-spectrum cephalosporins in food animals is a concern because it is leading to resistance to a variety of cephalosporins that are used to treat human infections. For example; ceftriazone is another extended-spectrum cephalosporin that is used to treat severe or invasive salmonellosis in children and pregnant women and now *Salmonella* resistant ceftriazone infections have emerged (Dunne et al., 2000).

The emergence of multidrug resistant *Salmonella* Newport has been linked to exposure to a dairy farm based on indistinguishable pulsed-field gel electrophoresis patterns and antibiotic resistance profiles (Gupta et al., 2003). This finding is not unusual because the association between the use of antimicrobial agents in food animals and the emergence of multi-drug resistant *Salmonella* infections in humans has been demonstrated through various studies (Angulo et al., 2004). An increase in the number of *Salmonella* serotype Newport strains and especially MDR *Salmonella* Newport strains was first recognized by the Centers for Disease Control and Prevention (CDC) and several state health departments in 2000 (CDC, 2000). A veterinary diagnostic laboratory sent two *Salmonella* isolates from ill dairy cattle to the Massachusetts Department of Health (MDPH) for serotyping, which in turn ended up having the same Newport-MDR AmpC resistance pattern as two isolates from ill children that were also submitted to the MDPH.

Although, the number of cases of *Salmonella* in humans in the United States has been decreasing since 1996, the number of resistant *Salmonella* strains has been increasing (CDC, 2001; Gupta et al., 2003). The emergence of *S*. Newport-MDR AmpC resistant strains in human infections has directly coincided with the emergence of *S*. Newport MDR AmpC infections found in cattle (Gupta et al., 2003). While
some of the infections found in humans may be due to direct contact on a dairy farm, it is also likely that some infections are due to improper handling of contaminated food such as beef. Gupta et al, isolated the *S. Newport*-MDR AmpC strain from healthy and ill cattle on dairy farms. The *S. Newport*-MDR AmpC strain was also found in ground beef samples from inspected chilled carcasses (Joyce et al., 2000). Since dairy cattle contribute to the beef supply (Troutt et al., 2001), it is likely consumption of contaminated food products may serve as a source of infection.

**Transmission to Humans**

*Salmonella* are generally transmitted to humans from contaminated food in developed countries. Products of animal origin such as fresh dairy products, meat, poultry, and eggs have been traced as the source of infections in the past. *Salmonella* may be transmitted in other pathways such as person-to-person spread in institutions where hygiene is lacking, or direct or indirect contact with infected animals at a petting zoo (Kemal, 2014). Those particularly at risk for infection are those who are under five years of age, the elderly, immunocompromised individuals, antibiotic users, or those who use antacids that decrease the acidity in their stomachs (CDC, 2014b; Kemal, 2014).

In this study, we were primarily interested in the role of wild birds in the transmission of *Salmonella* on farms, as either a mechanical vector by transmitting *Salmonella* on their body surface, or as a biological vector by transmitting *Salmonella* through their feces on Ohio dairy farms. *Salmonella* infected dairy cows could then
possibly contaminate meat products from culled dairy cows on these farms and lead to infections in people. Transmission of *Salmonella* through other dairy products such as milk products is not a concern due to the pathogen being killed during the pasteurization process. Dairy cattle are capable of being asymptomatic carriers of *Salmonella*, while still being able to shed the bacteria in their feces. Asymptomatic carriers are able to therefore contaminate transportation trailers, slaughter facilities, and slaughter workers clothing and equipment during slaughter without displaying clinical symptoms (Kemal, 2014). Due to high-speed slaughter facilities, which may slaughter hundreds of animals in a day, the number of carcasses that could be contaminated is large.

The spread of antimicrobial resistance through contaminated animal food products is also a large concern. The association between antimicrobial resistance in food animals and antimicrobial resistance in humans has been demonstrated by various methods including; field studies, case reports, molecular subtyping comparing isolates from human and non-human sources, epidemiological investigations of sporadic infections, outbreak investigations, and ecological and temporal associations (Angulo et al., 2004).
**Salmonella in Birds**

*Salmonella in Poultry in the U.S.*

*Salmonella* infections in domestic fowl have a long history in the commercial poultry industry. The most prevalent serovars causing infection have changed over time and with implementation of control measures. *S.* Pullorum and *S.* Gallinarum had a large impact on the commercial flocks for most of the 1990’s, causing high morality in flocks (Poppe, 2000). High mortality limited the profitability of the poultry industry and lead to the development of intervention measures to prevent and control infections. The industry developed a voluntary National Poultry Improvement Plan (NPIP) in 1935 in order to implement testing of breeding flocks to ensure that the disease would not be transmitted to egg-laying stock (Snoeyenbos, 1991). These control measures were effective in eradicating *S.* Pullorum and *S.* Gallinarum from commercial poultry flocks in the United States (Rabsch et al., 2000).

From the 1950s to the late 1970s, *S.* Typhimurium became the most common serovar identified in poultry (Williams, 1984). *S.* Enteritidis then emerged as the most common *Salmonella* serovar in the US. (Rodrigue et al., 1990). The emergence of the *S.* Enteritidis serovar has been suggested to be due to the eradication of *S.* Gallinarum, which once competitively excluded *S.* Enteritidis (Rabsch et al., 2000). *S.* Enteritidis is mostly associated with outbreaks with food containing undercooked eggs (Henzler et al., 1994). Eggs may become infected through vertical transmission through an infection in the ovary and oviduct or the eggshell may be penetrated and be contaminated with feces of hens shedding *Salmonella* (Poppe, 2000; Rabsch et al.,
2000). Therefore, the higher prevalence of *Salmonella* in a flock, the greater the chance that eggs will be contaminated with *Salmonella*.

The increase in *S. Enteritidis* infections was met by the creation of a vaccine for egg-laying hens against the infection (Cogan & Humphrey, 2003). Since the introduction of the vaccine there has been a decline in cases of *S. Enteritidis*. Other factors responsible for this decline may include; build-up of immunity in animals, and improved biosecurity for egg-laying flocks (Cogan & Humphrey, 2003).

Raw poultry products also serve as a source of *Salmonella* infections in the United States (USDA, 2013). Due to the growing concern of contamination of raw meat and poultry products, the Hazard Analysis and Critical Control Points System (HACCP) was developed to address this concern in 1996.

The objectives of this system are to:

“verify that establishments demonstrate consistent process control for preventing, eliminating, or reducing the contamination of raw meat and poultry products with disease-causing bacteria, by setting *Salmonella* performance standards that slaughter establishments and establishments that produce raw ground products should meet”

Poultry are commonly infected with *Salmonella* and while intervention strategies can be put in place to reduce prevalence of infections, there is currently no way to completely eliminate all infections.

*Salmonella Prevalence By Species of Wild Bird*

Salmonellosis is one of the most common avian diseases and is highly communicable to humans (Kabir, 2010). Although *Salmonella* infection is often found in ducks and poultry; raptors, pittacines, passerines, gulls, and pigeons also fecally excrete *Salmonella* (Hudson et al., 2000; Sanchez et al., 2002). *Salmonella* infection is possible in all species of birds but, the outcome of the infection is dependent upon the age of the bird, serovar, virulence, stress, and susceptibility to infection (Friend, 1999). Many publications suggest that wild birds may act as a reservoir for *Salmonella* and contribute to its persistent presence on farms as well as the spread within and amongst farms (Reed, 2002; Daniels, 2003 & Andres, 2013).

**Figure 1** shows the relative rates of isolation of *Salmonella* species in free-ranging wild birds as obtained by wild bird surveys in 1999 by the USGS National Wildlife Center. In more recent publications, cowbirds have been found to be commonly infected by salmonellosis (Kirk et al., 2002; Tizard, 2004). If wild birds were to be categorized by diet, the wild birds most commonly infected by *Salmonella* are birds with a carnivorous or omnivorous diet (Tizard, 2004).

It is difficult to compare the prevalence of *Salmonella* in other wild bird studies because the prevalence that is determined depends on the sampling location
and methods used in the study (Skov et al., 2008). A study of *Salmonella* prevalence in wild birds in close proximity to cattle and pig farms in Denmark found a 1.5% prevalence of *Salmonella* in wild birds surrounding *Salmonella* positive livestock populations (Skov et al., 2008). This number is fairly low when compared to some other studies. Refsum reported 441 cases of *Salmonella* in small passerines in Norway from 1969-2000 (Refsum et al., 2002). The methods of this study differed from other studies due to the isolation of the agent being conducted on the birds’ post-mortem. A study conducted in California to determine *Salmonella* prevalence in wild birds around dairy farms observed an overall prevalence of 2.5% (Kirk et al., 2002). Andres et. al determined a *Salmonella* prevalence in wild birds “near pig premises site” of 3.46% and “far from pig premises site” of 0.46%, indicating that animal production facilities may act as an amplifier among wild birds regardless of animal species (Andrés et al., 2013).
Figure 1.1 Relative rates of isolation of *Salmonella* spp. in free-ranging wild birds (Friend, 1999)
Serovars

The serovars that infect domestic and wild birds frequently differ. Whereas domestic poultry were largely impacted by pullorum disease (S. Pullorum) and fowl typhoid (S. Galinarum) in the poultry production system of the past, wild birds are more largely impacted by paratyphoid forms, which are variants of salmonellae (Friend, 1999; Poppe, 2000). S. Typhimuirum is an example of a prevalent variant. The majority of avian salmonellosis outbreaks are caused by S. Typhimurium (Thomas et al., 2008). This type of infection can cause high mortality rates in aquatic birds and songbirds (Thomas et al., 2008). In 2000, paratyphoid infections were found to be distributed worldwide and increasing in prevalence among wild birds (Friend, 1999; Hudson et al., 2000).

How Wild Birds Become Infected

Wild birds may become infected through two main routes; which include carnivorous behavior, or environmental contamination (Tizard, 2004). Wild birds that are carnivorous or omnivorous are therefore most commonly infected. This trend is observed due to carnivorous and omnivorous birds consuming food that is contaminated such as intestine, consuming feed that is on the ground, or feeding or living in contaminated water, which is contaminated by feces (Tizard, 2004). Since animals are capable of being asymptotically infected, they may be contaminating the environment by shedding the organism in their feces with no outward signs. Feces in the environment may serve as a reservoir for contamination for humans and
other animals (Poppe, 2000). Increasing infections in songbirds are mainly due to reforestation, habitat changes, and the encroachment of urban and suburban environments into areas that provide good bird habitat. There have been many cases of *Salmonella* outbreaks in free-ranging wild birds (Alley et al., 2002; Daoust et al., 2000; Hernandez et al., 2012; Refsum et al., 2002). Sources of environmental contamination that cause *Salmonella* infection in free-ranging wild birds has led to the distribution of the disease by allowing the infection to enter the food web and thereby infect predators of these birds (Friend, 1999). The movement of migratory birds has also allowed for the spread of disease and in the case of antimicrobial resistant strains, the spread of resistance along migration routes (Reed et al., 2003).

*How Wild Birds Spread Infection*

Birds are attracted to farms due to the presence of feedstuffs, and their close contact with livestock provides the potential for pathogen transmission (Byler, 2002). Enteric pathogens such as *Salmonella* can colonize a wide variety of species and thus could be transmitted between livestock and wild birds. Wild birds are known to be reservoirs for *Salmonella* spp. (Hamer et al., 2012). Previous studies have found that *Salmonella* is present sporadically in the intestinal flora of wild birds (Horton et al., 2013). The bird species most likely to have significant outbreaks of *Salmonella* are passerines, finches, cowbirds and house sparrows (Tizard, 2004).

Many different modes of transmission are possible and the importance of the mode depends on: behavioral and feeding patterns of the bird species, the strain of
salmonellae, and husbandry practices if human hatched and reared (Friend, 1999). Vertical transmission occurs through transovarial transmission by which the egg is infected due to an infection in the ovary or oviduct (Kabir, 2010; Poppe, 2000).

Horizontal transmission occurs by contact with infected litter, feed, water, feces, shavings, equipment, and contact with infected wild and domestic animals (Poppe, 2000). Horizontal transmission may cause the contamination of surface waters, pastures, soils, colonization of birds, cattle, and feed, causing the re-colonization of farm animals (Poppe, 2000).

Domestic and wild birds can excrete *Salmonella* in their feces for weeks to months. Therefore, infected wild birds could easily infect cattle if feeding in barns and contaminating their feed and water (Friend, 1999; Poppe, 2000). The high density of cattle in production barns allows for continuous cycles of salmonellosis within a herd. Farm practices such as using livestock feces, slurry, and sewage sludge as fertilizer serve as a source of infection for wild birds and may contribute to continuing this cycle (Friend, 1999; Wray & Davies, 2000).

Studies have identified *Salmonella* with the same MLVA (multilocus variable number tandem repeat analysis) profiles in both livestock and wild birds, strongly indicating wild birds may serve as a potential source of infection for livestock (Horton et al., 2013). Along with the transmission of *Salmonella* on farms from birds to cattle, wild birds may also be responsible for transmitting *Salmonella* between farms. Understanding the epidemiology of the transmission of *Salmonella* within a farm is key to determining the most effective methods to reduce
salmonellosis on farms and therefore reduce *Salmonella* foodborne infections in humans.

*Introduction to wildlife/livestock interaction*

Wildlife interaction serves as an important risk factor in the epidemiology of emerging zoonotic diseases due to the ability of wildlife to serve as a reservoir of zoonotic diseases (Daszak et al., 2000). Wildlife and livestock interaction is difficult to prevent for several reasons; farms many have encroached on wildlife habitat, farms provide an attractive food source for wildlife, or farms are surrounded by ideal wild bird habitat. The edge habitat created along agricultural lands is ideal for migratory and non-migratory birds. These habitats provide the best brush cover for birds, the best food sources, and allow cover to avoid predators. Human encroachment on wildlife habitat has resulted in declining wildlife habitat and the concentration of wildlife populations, which has in-turn resulted in more interactions between livestock and wildlife (Daszak et al., 2000).

The ability of *Salmonella* to be transmitted from cattle to wild birds by “spill-over” is a concern because wild birds may act as a vector for transmission of the bacteria to other locations where it may infect other animals. The ability of *Salmonella* to be transmitted from wild birds to cattle by “spill-back” is also of concern due to the serious consequences *Salmonella* infections can have on a herd’s health and productivity (Daszak et al., 2000) Free-ranging wild birds are therefore
capable of acting as a reservoir for disease and continuously infecting cattle herds with salmonellosis.

**Salmonella on Dairy/Cattle Farms**

*Background*

*Salmonella* infections are an important concern for the dairy and beef cattle industries. Infections may cause significant morbidity and mortality within a herd, can be transmitted through nonclinical infections with no signs presenting, and may contaminate animal products during the slaughter process (Wray & Davies, 2000).

A significant portion of the U.S.’s ground beef supply is supplied by culled dairy cows (Troutt et al., 2001). Culled dairy cows are more likely to be shedding *Salmonella* than dairy cows in normal production, and therefore present more concern for the possibility of contamination of the carcass with fecal material at slaughter (Troutt et al., 2001).

The most current study of *Salmonella* on U.S. cattle beef feedlots conducted by NAHMS (The National Animal Health Monitoring System’s) in 2011, found that of 202 pens in 68 feedlots, 60.3% had at least one positive test result for *Salmonella* (USDA, February 2014). It was also found that 35.6% of sampled pens were positive for *Salmonella*. These numbers highlight the importance of *Salmonella* prevention and intervention strategies on farms to prevent *Salmonella* infections.
Salmonella Infection

Salmonella clinical signs in cattle include, fever, abortion, diarrhea, anorexia, and decreased milk production and occasionally death (Fossler et al., 2005; USDA, October 2005). Cattle may have an asymptomatic infection but are still capable of shedding the pathogen in their feces for extended periods of time (Fossler et al., 2005; USDA, October 2005). Salmonella infections can be expensive to producers due to treatment costs and mortality (Fossler et al., 2005).

Trends

The trend observed is of increasing prevalence of Salmonella on dairy farms in the U.S. based on the results of dairy industry studies conducted by NAHMS in 1996 and 2007. The number of dairy operations with positive Salmonella cultures in 1996 of 21.1% almost doubled in 2007, with 39.7% of operations sampled testing positive (USDA, July 2009). A similar trend was observed in the percentage of cows testing positive for Salmonella, with the percentage of 5.4% in 1996 and 13.7% in 2007. Although, a higher percentage of cases of salmonellae are observed on the state level, with 75% of California dairies found to have Salmonella infections present, there could be several reasons for low percentage finding on the national level (Smith et al., 1994). This leads to the conclusion that Salmonella infections on dairy farms may be steadily increasing over time.
How Cattle Become Infected

Introduction of *Salmonella* infection into herds is often due to adding infected cattle into the herd as replacements or calves for rearing (Wray & Davies, 2000). Infected adult cattle that remain asymptomatic once added to the herd may continue shedding the pathogen for extended periods of time, thereby infecting other cattle in the herd. Asymptomatic carriers of infection may begin showing signs if stressed, during such events as pregnancy, calving, and transportation (Troutt et al., 2001; USDA, December 2003).

Sources of possible *Salmonella* infection on farm include feed, water, the environment, contact with other animals, introduction of new animals to a herd, and interaction with wildlife including rodents and wild birds (Wells et al., 2001). On-farm control strategies may be effective in reducing contamination from these sources. Cows that are passive asymptomatic carriers of disease are a large problem on dairy farms. The animals are capable of spreading disease, yet show no clinical signs, therefore are left untreated for infection and continue shedding the disease. The environment is a highly important source of contamination that may act as a reservoir for infection.

If the environment is contaminated with *Salmonella*, such as the pen floor or pasture, animals are continuously exposed to *Salmonella* from this source and are continuously re-infected with the bacteria, which may then produce clinical or subclinical disease. If the bacterium is removed from the environment, the cattle cease to acquire the infection (Sanchez et al., 2002). *Salmonella* is capable of surviving in the environment for long periods of time on dairy farms (Giles et al.,
Overall, it is very difficult to remove the pathogen from farm environments due to porous surfaces such as wood and dirt, but it is possible to clean and disinfect surfaces such as; trailers which may serve as a source of infection. In the production industry of today, cows are often raised in high population-density environments, which provides an ideal environment for the spread of *Salmonella* among cattle. While decreasing population densities may reduce the transmission of the pathogen, due to how the production industry is designed, this may currently be an impractical solution.

Contamination of feed is a key route of transmission in animals and therefore preventing introduction of *Salmonella* to feed is important in preventing infections (Fedorka-Cray et al., 1995). Contamination of feed may occur by wild birds when the feed is placed out for the cows to consume or during storage, if the feed is not properly stored. Livestock that are housed indoors are at a higher risk for consuming contaminated feed due to there being no other available food source (Daniels et al., 2003). It has been demonstrated that there is a significant difference in rate of contamination of feed depending on season, with an increase of contamination from November to February (Daniels et al., 2003). This difference was possibly due to the low availability of natural sources of food for wild birds at this time. Many cattle are housed and fed in open sided barns, which allows for contamination of feed by wild birds and rodents. A study conducted in cattle-feeding operations to control European starlings in Texas, USA found an 8% decrease in contamination in feed bunks once the starling population was controlled (Carlson et al., 2011). The dose of bacterium ingested impacts the risk of clinical infection and amount of shedding (Wray &
Davies, 2000). Controlling the wild bird population around the farm may be able to reduce the amount of contamination of feed and water and result in a reduction of *Salmonella* infections. Risk factors for *Salmonella* infections and shedding in cattle include age of cattle, herd size, season, manure management and disposal methods, and feed storage (Carlson et al., 2011; Fossler et al., 2005; Wells et al., 2001).

Preventing contamination of feed and thereby infection through feed is important to prevent animals from acquiring an infection, reducing the probability of contaminated animal food products and production losses.

Herd size is an important risk factor for shedding *Salmonella* in cattle herds. Studies have implicated the relationship between herd size and fecal shedding of *Salmonella* on dairy farms (Huston et al., 2002). These findings have been supported by several other studies. The NAHMS Dairy 2007 study found that herd size impacted the percent of *Salmonella* infections, with 30.9% of large farms (500 or more) having *Salmonella* infections, whereas only 5.1% of small farms having confirmed *Salmonella* infections in the past 12 months (USDA, September 2008). A Netherlands case-control study also indicated herd size as a significant risk factor for *S. Dublin* infections (Vaessen et al., 1998). When the NAHMS 1996 Dairy study was analyzed by Kabagambe et al., it was found that larger herds had a higher odds of having cows shedding *Salmonella* than smaller herds. The reasons for herd size having a large impact on *Salmonella* shedding may be accounted for by large cattle operations having a higher tendency to add animals to the herd (USDA, 1996) or a higher population density. As mentioned earlier, introduction of new animals to a
herd may allow for the introduction of asymptomatic carriers of *Salmonella* to the herd and lead to exposure and infection of other animals in the herd.

*Prevention*

Prevention of *Salmonella* infections on dairy and cattle operations must focus on preventing contamination of feed, water, and the environment. Biosecurity measures must be developed to prevent contact with other animals, allow safe introduction of new animals to a herd, and prevent interaction with wildlife including rodents and wild birds. An effective way to decontaminate farm environments is yet to be developed and would be vital to stopping the on-farm transmission cycle that exists on production farms and allows for continuous reinfection of animals.

The high prevalence of *Salmonella* that exists on dairy farms today is due to the lack of effective interventions in current production systems.

*Limitations*

Although many studies have been conducted on the possible risk factors for *Salmonella* infections on dairy farms, the risk factors determined in each study vary greatly. For instance, Veling et al. found greater risk among farms that used purchased manure due to large acreage and had a non-seasonal calving pattern. Warnick et al. found greater risk among farms on which: poultry manure was spread
on bordering property, signs of rodents were present in cattle housing or feed-storage areas, and wild geese had contact with cattle or feed. The results of these studies and others demonstrate the varied sources of *Salmonella*, the many risk factors that may exist for each individual farm, and that infection is highly dependent on the farm itself. Reducing shedding of *Salmonella* positive animals by on-farm management techniques may not be possible due to the limited knowledge and research that exists as to what induces shedding of the bacteria.

Further research will be needed to determine the role of wild birds as mechanical or biological vectors for *Salmonella*. Cattle products have the possibility of being contaminated by *Salmonella* bacteria throughout the slaughter process by fecal contamination, but managing and reducing *Salmonella* infections on farms could reduce the pathogen load throughout the food chain (Wells et al., 2001).

**Purpose**

The purpose of this study was to determine if wild birds play a role in the transmission of *Salmonella* as either a mechanical vector by transmitting *Salmonella* on their body surface, or as a biological vector by transmitting *Salmonella* through their feces on Ohio dairy farms.
Objectives

1. Measure prevalence of *Salmonella* in wild birds surrounding Ohio dairy farms
2. Determine whether wild birds serve as a mechanical or biological vector of infection by collecting external and cloacal swab samples
3. Compare prevalence of *Salmonella* in wild birds close to the barn (≈ 5 ft.) and far from the barn (≈ 600 ft.)
4. Compare prevalence of *Salmonella* in migratory and non-migratory birds

Summary

*Salmonella* is an important zoonotic food-borne pathogen that causes approximately 1.2 million illnesses a year in the United States (CDC, 2014b). *Salmonella* infections range from mild to life threatening and therefore limiting the number of infections is vital. Effective vaccines are not available for non-typhoidal *Salmonella* for animals and humans and thus, prevention of this infection is important. Raw animal products are a large source of contaminated products, yet consumer education about preparation of foods is also necessary. Food production has become a mechanized technical process that involves many steps that require adherence to certain guidelines set by the USDA, such as the Hazard Analysis Critical Control Points (HACCP). In order to prevent disease, disease transmission as well as mechanisms of contamination must be understood to halt these mechanisms from occurring (Tauxe, 1997). In order to prevent future outbreaks of infectious disease
that can be transmitted by free-ranging wild birds, infectious diseases in birds and migration patterns of birds must be better understood (Reed et al., 2003). Prevention of *Salmonella* infections on farms will most likely be obtained by adopting a comprehensive disease management plan that takes into account all sources of *Salmonella* exposures. *Salmonella Typhimurium* is a serovar of particular importance to animal and human health due to its broad host range and multiple antimicrobial resistance profiles and therefore prevention of this serovar is especially important (Wells et al., 2001).
Chapter 2: Methods and Materials

Farm Selection

The dairy farms that participated in this study were clients of the Ohio State University Large Animal Clinic located in Marysville, Ohio. Contact information was available for these farms as well as an existing working relationship with University personnel. Six dairy farms were initially selected for convenience sampling and contacted about participation in the study. These farms were selected because they had appropriate bird habitat near barns, owners allowed access, and convenience of location of farm. The farm owners were contacted via email and phone. Three of the six selected farms in central Ohio agreed to participate and were included in the study. The locations of the farms can be observed in Figure 2.1. One of the farms housed approximately 110 cows, one housed approximately 1,000 cows, and one approximately 2,000 cows.
Figure 2.1 Map of Farm Locations in Central Ohio

**Bird Mist Netting**

Wild bird samples were collected from participating farms at 18 time points during May and June of 2014. This time period was chosen to include the time period of migration across Ohio for migratory birds. Wild birds were captured in the morning hours from sunrise to approximately noon at each location using six to eight
mist nets. The nets were checked every fifteen minutes. Upon capture, the birds’ feet were swabbed with gauze, which was moistened with buffered peptone water (BPW) and the sterile gauze was placed in a sterile whirl pack. Birds were carefully removed from the net under the supervision of a trained individual with a U.S. Federal Bird Banding and Marking Permit and a Wild Animal Banding Permit from the state of Ohio. Birds were placed in cloth bags to transport them to the sampling area, and then were removed and cloacal samples were obtained using sterile swabs moistened with BPW and swabbing the cloacal area. The swabs were then placed in a 2 mL sterile microtube and transported back to the laboratory.

After collection of the cloacal swab sample, birds received an identifying metal band obtained from the U.S. Federal Bird Banding and Marking Permit from the U.S. Department of the Interior. Metal bands were placed on birds to ensure that a single bird was not sampled more than once. The birds were then released to the area from which they were captured.

Samples from Cattle

Fifty individual fecal samples were collected from the floor of freestall barns housing lactating cows at the participating farms on two occasions; once before bird sampling and once after bird sampling. Approximately 10 g of fecal material was collected in each sample and was placed in 50 mL sterile tubes on the farm. Upon returning to the laboratory, 4 grams of fecal material was then weighed and placed in a sterile tube with 36 mL tetrathionate broth (TTB) with iodine and tergitol
supplementation. This mixture was incubated at 37°C for 24 hours. Next, 100 mL of this mixture was transferred into 10mL of Rappaport Vassiliadis (RV) broth and incubated at 42°C for 24 hours. After incubation, a xylose lysine desoxycholate 4 (XLT-4) plate was inoculated with each swab and incubated for 24 hours at 37°C. Positive cultures were identified as those that grew black colonies on the XLT-4 agar that were characteristic of *Salmonella* spp. Positive cultures were placed on triple sugar iron slants and incubated for 37°C for 24 hours to determine if the results demonstrated those of *Salmonella* spp. which are: red slant, black butt of the slant, and gas bubbles, indicating hydrogen sulfide production. Positive cultures were also agglutination tested with polygroup antisera for somatic groups O.

**Samples from Wild Birds**

Samples were transported directly to the laboratory after collection, where both cloacal and external swabs were incubated in buffered peptone water (BPW) for 24 hours at 37°C. Swabs were then placed in Rappaport Vassiliadis (RV) broth and incubated 18-24 hours at 42°C. After incubation, a xylose lysine desoxycholate 4 (XLT-4) plate was inoculated with each swab and incubated for 24 hours at 37°C. Positive cultures were identified as those that grew black colonies on the XLT-4 agar that were characteristic of *Salmonella* spp. Positive cultures were placed on triple sugar iron slants and incubated for 37°C for 24 hours to determine if the results demonstrated those of *Salmonella* spp. which are: red slant, black butt of the slant,
and gas bubbles, indicating hydrogen sulfide production. Positive cultures were also agglutination tested with polygroup antisera for somatic groups O.

**Data Analysis**

The primary outcome of interest was recovery of *Salmonella* from wild birds. A bird was considered to be positive if either the cloacal or surface swab was positive for *Salmonella*. A positive outcome was defined as a sample that grew black colonies on XLT-4 agar and demonstrated results of those of *Salmonella* spp. when placed on triple sugar iron slants and had a positive polygroup antisera somatic group O agglutination test. Fisher’s exact test was used to analyze risk factor variables for *Salmonella* positive outcome in wild birds using JMP statistical software. The significance of the association was based on a p-value < 0.05.
Chapter 3: Results

Cow fecal samples and wild bird external and cloacal samples were collected from three central Ohio dairy farms between May 2014 and June 2014. The Salmonella prevalence in cows on these farms ranged from 71-94% (Table 3.1). Study herds were from three different counties in central Ohio. The herd size ranged from 110-2000 head. All herds were predominantly Holstein cows. The overall prevalence of Salmonella in wild birds was 1.16%, whereas the prevalence in non-migratory birds was 1.19%, and the prevalence in migratory birds was 1.06% (Table 3.2). There was a significant association between farm and wild bird Salmonella positive outcome (p-value=0.020) (Table 3.3).

A total of 346 birds were captured, with 252 being classified as non-migratory and 94 being classified as migratory. The suspected flycatcher was included in the migratory bird counts. The number of birds captured per farm was; farm A 85, farm B 83, and farm C 178. The species captured included: American goldfinches, American robins, European starlings, house sparrows, song sparrows, barn swallows, chipping sparrows, grey catbirds and indigo buntings (Table 3.4). Figure 3.1, displays the distribution of migratory wild birds by date, showing that as the period of migration of birds through Ohio progressed, the migratory birds captured declined over time.
Four wild bird swabs collected were *Salmonella* positive, with three swabs being collected from birds captured within five feet of the barn and one swab being collected from a bird approximately 600 feet from the barn. The species of birds that had positive swabs were: red-winged black bird, house sparrow, and barn swallow (Table 3.5). The number of swabs collected per farm ranged from 83-178 (Table 3.6). The prevalence of *Salmonella* per farm ranged from 0-3.6% (Table 3.7). Three of the 346 cloacal swabs collected were positive for *Salmonella* for a prevalence of 0.87%. One of the 346 external swabs collected was positive for *Salmonella* for a prevalence of 0.29%. The ratio of *Salmonella* determined from each sampling technique (cloacal and external) was therefore, relatively similar.

<table>
<thead>
<tr>
<th>Farm</th>
<th>Approximate Number of Cows</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>2000</td>
<td>94%</td>
</tr>
<tr>
<td>B</td>
<td>1000</td>
<td>82%</td>
</tr>
<tr>
<td>A</td>
<td>110</td>
<td>71%</td>
</tr>
</tbody>
</table>

Table 3.1 *Salmonella* Prevalence in Cow Fecal Samples on 3 Ohio Dairy Farms (n=100 per farm)

<table>
<thead>
<tr>
<th></th>
<th>Positive Swabs</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Overall</strong></td>
<td>4/346</td>
<td>1.16%</td>
</tr>
<tr>
<td><strong>Non-Migratory Birds</strong></td>
<td>3/252</td>
<td>1.19%</td>
</tr>
<tr>
<td><strong>Migratory Birds</strong></td>
<td>1/94</td>
<td>1.06%</td>
</tr>
</tbody>
</table>

Table 3.2 *Salmonella* Prevalence in Wild Birds on 3 Ohio Dairy Farms
Table 3.3 Number of *Salmonella* Positive and Negative Birds by Migratory Status, Location, and Distance Captured

<table>
<thead>
<tr>
<th>Variable</th>
<th>Salmonella Positive</th>
<th>Salmonella Negative</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Migratory</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1 (1.06%)</td>
<td>93 (98.94%)</td>
<td>1.000</td>
</tr>
<tr>
<td>No</td>
<td>3 (1.19%)</td>
<td>249 (98.81%)</td>
<td>-</td>
</tr>
<tr>
<td>Farm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>1 (1.18%)</td>
<td>84 (98.82%)</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>3 (3.61%)</td>
<td>80 (96.39%)</td>
<td>-</td>
</tr>
<tr>
<td>C</td>
<td>0 (0%)</td>
<td>178 (100%)</td>
<td>0.020</td>
</tr>
<tr>
<td>Distance to Barn</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Far</td>
<td>1 (0.52%)</td>
<td>191 (99.48%)</td>
<td>0.327</td>
</tr>
<tr>
<td>Close</td>
<td>3 (1.95%)</td>
<td>154 (98.05%)</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3.4 Number of Birds Captured by Mist Net Techniques by Species on 3 Ohio Dairy Farms (n=346)

<table>
<thead>
<tr>
<th>Bird Species</th>
<th>Number Captured</th>
<th>Positive Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Migratory Birds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>American Goldfinch</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>American Robin</td>
<td>29</td>
<td>-</td>
</tr>
<tr>
<td>Brown-headed Cowbird</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Downy Woodpecker</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>European Starling</td>
<td>14</td>
<td>-</td>
</tr>
<tr>
<td>House Sparrow</td>
<td>169</td>
<td>2</td>
</tr>
<tr>
<td>Northern Cardinal</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>Red-Winged Blackbird</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Song Sparrow</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>252</strong></td>
<td><strong>3</strong></td>
</tr>
<tr>
<td>Migratory Birds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acadian Flycatcher</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>American Redstart</td>
<td>2</td>
<td>-</td>
</tr>
</tbody>
</table>

Continued
Table 3.4 Continued

<table>
<thead>
<tr>
<th>Bird Species</th>
<th>Number Captured</th>
<th>Positive Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Migratory Birds</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barn Swallow</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Blue Grosbeak</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Brown Thrasher</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Chipping Sparrow</td>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td>Common Yellowthroat</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Eastern Wood Pewee</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Grey Catbird</td>
<td>18</td>
<td>-</td>
</tr>
<tr>
<td>Grey-Checked Thrush</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>House Wren</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Indigo Bunting</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>Lincoln’s Sparrow</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Mourning Warbler</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>Northern Waterthrush</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Palm Warbler</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Rough-Winged Swallow</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Spotted Sandpiper</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Swainson’s Thrush</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>Trail’s Flycatcher</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Warbling Vireo</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>White-crowned Sparrow</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>White-throated Warbler</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Wilson’s Warbler</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Wood Pewee</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Wood Thrush</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Yellow-rumped Warbler</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Unknown (Suspected Flycatcher)</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>91</td>
<td>1</td>
</tr>
<tr>
<td><strong>Cumulative Total Birds</strong></td>
<td>346</td>
<td>4</td>
</tr>
</tbody>
</table>
**Figure 3.1** Distribution of Migratory Birds Captured By Date

<table>
<thead>
<tr>
<th>Farm ID</th>
<th>Bird Species</th>
<th>Migratory Bird Type</th>
<th>Samples Per Farm</th>
<th>Distance From Barn Sample Collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>198 Red-Winged Black Bird</td>
<td>No</td>
<td>85</td>
<td>Far</td>
</tr>
<tr>
<td>B</td>
<td>291 House Sparrow</td>
<td>No</td>
<td>83</td>
<td>Close</td>
</tr>
<tr>
<td>B</td>
<td>294 House Sparrow</td>
<td>No</td>
<td>83</td>
<td>Close</td>
</tr>
<tr>
<td>B</td>
<td>295 Barn Swallow</td>
<td>Yes</td>
<td>83</td>
<td>Close</td>
</tr>
</tbody>
</table>

**Table 3.5** *Salmonella* Recovery in Wild Birds Captured Around 2 Ohio Dairy Farms (n=346)

<table>
<thead>
<tr>
<th>Farm</th>
<th>Total Swabs Collected</th>
<th>Close Distance to Barn</th>
<th>Far Distance to Barn</th>
<th>Migratory Birds</th>
<th>Non-Migratory Birds</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>85</td>
<td>17/85</td>
<td>68/85</td>
<td>47/85</td>
<td>38/85</td>
</tr>
<tr>
<td>B</td>
<td>83</td>
<td>26/83</td>
<td>57/83</td>
<td>34/83</td>
<td>49/83</td>
</tr>
<tr>
<td>C</td>
<td>178</td>
<td>111/178</td>
<td>67/178</td>
<td>13/178</td>
<td>165/178</td>
</tr>
</tbody>
</table>

**Table 3.6** Wild Bird Samples on 3 Ohio Dairy Farms, Cloacal and External Swabs Combined

---

37
<table>
<thead>
<tr>
<th>Farm</th>
<th>Positive Swabs</th>
<th>Prevalence</th>
<th>Positive Swabs Close Distance to Barn</th>
<th>Positive Swabs Far Distance to Barn</th>
<th>Positive Swabs Migratory Birds</th>
<th>Positive Non-Migratory Birds</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1/85</td>
<td>1.2%</td>
<td>0/17</td>
<td>1/68</td>
<td>0/47</td>
<td>1/38</td>
</tr>
<tr>
<td>B</td>
<td>3/83</td>
<td>3.6%</td>
<td>3/26</td>
<td>0/57</td>
<td>1/34</td>
<td>2/49</td>
</tr>
<tr>
<td>C</td>
<td>0/178</td>
<td>0%</td>
<td>0/111</td>
<td>0/67</td>
<td>0/13</td>
<td>0/165</td>
</tr>
</tbody>
</table>

*Table 3.7* *Salmonella* Prevalence in Wild Bird Samples on 3 Ohio Dairy Farms, Cloacal and External Swabs Combined
Chapter 4: Discussion

We observed only four *Salmonella* positive samples, for an overall prevalence of *Salmonella* of 1.16% in wild birds surrounding three central Ohio dairy farms with high rates of *Salmonella* shedding in cows. These findings are similar to findings in a previous study, which determined an overall *Salmonella* prevalence of 2.5% in wild birds surrounding nine dairies in California (Kirk et al., 2002). *Salmonella* were recovered from wild birds captured at two of the three farms. The prevalence of *Salmonella* in wild bird samples was 1.2% at farm A and 3.6% at farm B.

There was a significant difference observed between farm location and presence of *Salmonella* (p-value = 0.020). This result is not surprising because the risk factors for *Salmonella* vary by farm and are dependent on many factors such as: amount of wild bird habitat surrounding the farm, farm practices, and herd size.

In our study, the majority of *Salmonella* positive samples were collected from house sparrows (2 of 4), which is consistent with previous findings; the bird species most likely to have significant outbreaks of *Salmonella* are passerines, finches, cowbirds and house sparrows (Tizard, 2004).

The prevalence of *Salmonella* in cloacal and external swabs was similar. No bird sampled tested positive for *Salmonella* in both external and cloacal swab. This is a suspicious finding since it would be expected that if a wild bird was fecally
excreting the pathogen, the bird would also have the pathogen on their feet, thus serving as a biological and mechanical vector. In contrast, it would be possible for the wild bird to serve as a mechanical vector, with the pathogen just on the external body surface and not have a *Salmonella* infection and be shedding the pathogen in their feces.

The number of wild bird samples varied by farm with the lowest number of samples being collected being 83 and the highest being 178. The possible reason for the variation in the number of samples could be due to; the amount of available feedstuffs the birds had access to (with larger farms having more feedstuffs), the amount of suitable habitat surrounding the farm, and the weather. Poor weather conditions could cause the birds to remain inactive and sheltered versus flying around the barn.

There was no observed association between the distance at which the birds were captured in relation to the barn and presence of *Salmonella* (p-value= 0.327). Three of the four positive samples were collected at approximately five feet from the barn. One positive sample was collected approximately 600 feet from the barn. At the two smaller farms (A and B), more birds were captured at a far distance from the barn (approximately 600 feet), whereas at the larger farm, more birds were captured at a close distance from the barn (approximately 5 feet). These findings are consistent with what would be expected, since farms provide a food source for wild birds and birds fly in and out of barns to eat the animals grain, thereby landing on potentially contaminated surfaces while consuming grain and/or consuming grain that has been contaminated with infected cow feces. Domestic and wild birds can excrete
*Salmonella* in their feces for weeks to months, therefore, infected wild birds could easily infect cattle if feeding in barns and contaminating their feed and water (Friend, 1999; Poppe, 2000). Farm practices such as using livestock feces, slurry, and sewage sludge as fertilizer serve as a source of infection for wild birds and may contribute to continuing this cycle (Friend, 1999; Wray & Davies, 2000).

Our results also indicate there is no association between presence of *Salmonella* and migratory bird status (p-value= 1.000). The prevalence of *Salmonella* in non-migratory birds was 1.19% and 1.06% in migratory birds. While the movement of migratory birds has allowed the spread of disease and, in the case of antimicrobial resistant strains, the spread of resistance along migration routes (Reed et al., 2003), migratory birds were not found to contribute significantly to the spread of *Salmonella* in this study. This result suggests that migratory and non-migratory birds are infected with Salmonella and could therefore serve as vectors for spreading this bacterium both within and between farms. However, the low prevalence of infection suggests that they likely do not play an important role in the transmission of this pathogen.
Limitations

The limitations of this study include the variation of risk factors for *Salmonella* infections by farm. It may be difficult to reduce shedding of *Salmonella* on the farm due to the limited knowledge of what induces shedding of the pathogen in cows. It is difficult to compare the prevalence of *Salmonella* in other wild bird studies because the prevalence determined depends on sampling location and methods. The lack of geographical diversity in our sample locations could have possibly affected the prevalence of *Salmonella* in wild birds we detected. Our study had a relatively small sample size of n=346 wild birds samples. A larger sample size would provide more strength and precision to our estimates and a better comparison with the prevalence of *Salmonella* in cows on the farms. The few number of *Salmonella* positives detected in wild birds n=4 made it hard to statistically detect differences between groups during comparison.

Conclusion

In our study, the overall prevalence of *Salmonella* in wild birds was low (1.16%). Many publications suggest that wild birds may act as a reservoir for *Salmonella* and contribute to its persistent presence on farms as well as the spread within and amongst farms (Andrés et al., 2013; Daniels et al., 2003; Reed et al., 2003); although this is possible, it was not demonstrated in our study. The dairy cows on the three farms sampled had a high prevalence of *Salmonella* shedding, but this
was not reflected in the prevalence of *Salmonella* in wild birds that were within 600 feet of the barn. Based on our findings of low prevalence of *Salmonella* in wild birds, we conclude that wild birds likely do not play a major role in the transmission of *Salmonella* on Ohio dairy farms sampled in these locations.
Bibliography


