THE EPIDEMIOLOGIC ASPECTS OF *SALMONELLA* SHEDDING IN WILD BIRDS OF TEXAS

A Dissertation

by

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ABSTRACT

Wildlife have become increasingly important as vectors for zoonotic disease. As reservoirs, wild birds have some of the greatest potential to disseminate pathogens across a large area. Due to its broad host range and public health importance, the transmission of *Salmonella* in birds can carry significant risks to human and animal health. The objectives of this dissertation is to determine the prevalence of *Salmonella* shedding in birds in various settings, to identify potential risks to human and animal health, and to evaluate ecological factors that may affect *Salmonella* prevalence.

Wild birds were sampled in three settings: passerines in an urban environment in eastern Texas, waterfowl in wildlife management areas along the Texas coast, and passerines at a feedlot in central Texas. Demographic and environmental data was collected for each sampled bird. Standard bacteriologic culture methods were used to isolate *Salmonella* and, in most cases, isolates were characterized by serotype and antimicrobial susceptibility.

Overall, the apparent prevalence of *Salmonella* shedding was very low compared to domestic species such as cattle and domestic poultry. In urban bird species, 1.8% (2/114) were found to be shedding *Salmonella*. This suggests that the threat these birds pose to public health is limited. However, the large numbers in which these birds congregate in urban areas, especially near grocery and retail stores, makes even the low prevalence of *Salmonella* shedding still a concern. Similarly, in waterfowl the prevalence of *Salmonella* shedding was very low (0.5%, 2/375). While the tendency of waterfowl to utilize agricultural fields and surface waters may potentially lead to contamination, the risk to public health appears minimal compared to the risk posed by *Salmonella* shedding in domestic poultry. Prevalence of *Salmonella* shedding was

dramatically different in wild birds congregating in feedlots with 29.2% (28/96). This contrast is interesting considering that many of the same species as sampled in an urban setting were also sampled at the feedlot. The prevalence of *Salmonella* shedding in birds was not significantly associated with the prevalence of *Salmonella* shedding in cattle.

This dissertation shows that prevalence of *Salmonella* shedding among wild birds in Texas can vary dramatically and suggests that environmental factors may affect the prevalence. More research will need to be done to determine how these factors influence the prevalence in birds and what the prevalence of shedding is in other bird groups in Texas.

DEDICATION

To my family, whose love and support has allowed me to accomplish so much. To my husband, Stephen, who supported me through all the long days and hard work. And to my son, William, who inspires me to be my best self and discover the world around me.

Ad astra per aspera

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TABLE OF CONTENTS

Page

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Salmonella is a major cause of gastrointestinal infections in humans in the United States and throughout the world, causing an estimated 93.6 million cases of gastroenteritis annually (Majowicz *et al.*, 2010). Though foodborne exposure is considered most important in humans, direct contact with feces or a contaminated environmental source can also be a source of transmission (Hoelzer *et al.*, 2011). In animals, *Salmonella* is most commonly associated with livestock and poultry (Pires *et al.*, 2014). However, wildlife are increasing being recognized as a reservoir (Cummings *et al.*, 2016, Grigar *et al.*, 2017, Grigar *et al.*, 2016, Hernandez *et al.*, 2012b). Wild birds, especially migratory species, are particularly concerning as reservoirs as they are able to travel great distances and utilize a variety of habitats both urban and rural. This may increase their propensity to disseminate pathogens leading to the introduction of novel pathogens, strains, or antimicrobial resistance phenotypes.

In birds, *Salmonella* typically does not cause clinical signs and thus can be isolated from healthy and diseased individuals (Benskin *et al.*, 2009). If present, signs can include lethargy, fluffed-up plumage, rapid breathing, neurologic dysfunction, drooping wings, and, rarely, arthritis (Benskin *et al.*, 2009, Friend & Franson, 1999, Daoust, 1978). Post-mortem examination reveals poor body condition, undigested food in the crop, enteritis, and gross lesions on the liver and esophagus (Benskin *et al.*, 2009, Friend & Franson, 1999).

Salmonella has been isolated from a variety of birds. Hall and Saito (2008) reviewed the U.S. Geological Survey National Wildlife Health Survey database for wild bird mortality events that occurred in the United States (U.S.) from 1985 to 2004 and attributed 5.4% (186/3472) to

Salmonella. Over 68,000 bird deaths were estimated to have resulted from these events representing 98 species from 12 orders (Hall & Saito, 2008). While many species can harbor *Salmonella*, gulls and songbirds are the groups most commonly infected (Friend & Franson, 1999, Benskin *et al.*, 2009, Hudson *et al.*, 2000). This is predominantly due to their feeding habits and ecology. Gulls typically feed near waste or sewage areas where environmental prevalence of *Salmonella* is higher (Williams *et al.*, 1976). Similarly, songbirds are typically exposed at feeding stations which are prone to a build-up of feces, and thus enteric pathogens (Hernandez *et al.*, 2012a).

There are over 2,300 serotypes of *Salmonella* and many of these have been isolated from wild birds (Tizard, 2004). There are two species of *Salmonella*, *S. bongori* and *S. enterica*. Within *S. enterica*, there are six subspecies with *S. enterica subsp enterica* being the most common (Brenner *et al.*, 2000). Herein, *Salmonella* serotypes occur within *S. enterica subsp enterica* unless otherwise stated. *Salmonella* Typhimurium is the serotype most commonly implicated in wild bird infections (Benskin *et al.*, 2009, Hall & Saito, 2008, Hudson *et al.*, 2000). Typhimurium and other serotypes isolated from wild birds are common causes of salmonellosis in humans (CDC, 2017). *Salmonella* carriage by birds has the potential to have a major public health impact. Pangloli *et al.* (2008) sampled bird droppings near milking machines, the milk bulk tank, and near the barns at a dairy in Tennessee and isolated *Salmonella* from 13–75% of samples. With the close proximity to milking machines and the bulk tank, *Salmonella* from bird droppings could easily contaminate the dairy's milk supply. Gruszynski *et al.* (2014a) identified gulls in Virginia shedding the same *Salmonella* Newport pulse-field gel electrophoresis (PFGE) pattern that was implicated in a multistate outbreak caused by contamination of tomatoes. This further illustrates the public health impact birds can have in *Salmonella* ecology.

The purpose of this review is to catalogue studies that have documented *Salmonella* shedding in bird species of North America and the risk factors associated with *Salmonella* shedding. This will allow for a broader perspective of the role that wild birds play in the ecology of *Salmonella.*

Literature Search Methods

Relevant databases were searched to identify studies conducted on the prevalence, characterization, and ecology of *Salmonella* in wild birds of North America. Databases searched include PubMed, Medline (Ovid), Agricola (EBSCO), CAB Abstracts (Ovid), Web of Science, and Wildlife and Ecology Studies Worldwide (EBSCO). Keywords used were *"Salmonella"* and "*wild bird"*. Searches were limited to studies published after 1950 in English. Abstracts and method sections of resulting studies were reviewed to identify articles that assessed *Salmonella* shedding in free-living wild birds in North America. North America was defined as Canada, the United States, Mexico, Central America, and the Caribbean. Relevant review articles were also included in article review to identify any studies that may have been missed by the database searches.

Articles were reviewed and included if they met the following criteria: 1) study was conducted in free-living wild birds and 2) identified the species or type of bird studied. Reference lists of reviewed articles were cross-referenced to ensure no pertinent studies were missed.

Results of Literature Search

Database searches resulted in 1,616 articles. After limiting to articles published after 1950 in English, removing duplicates, and discarding non-North American studies, 92 articles remained. Full article review and cross-referencing resulted in a total of 102 articles for review.

These were categorized based on species group: waterfowl, sea and shore birds, wading birds, songbirds and similar, raptors and scavengers, and other. Orders included in each category are listed in Table 1. A summary of the studies included in listed in the appendix.

Table 1 Avian orders included in bird categories

Group	*Orders Included*
Waterfowl	Anseriformes (ducks, geese, swans) Gaviiformes (loons) Podicipediformes (grebes)
Sea Birds and Shorebirds	Procellariiformes (albatrosses, petrels) Suliformes (frigatebirds, boobies, cormorants, darters) Charadriiformes (plovers, sandpipers, gulls)
Wading Birds	Ciconiiformes (storks) Pelecanformes (pelicans, herons, ibises) Gruiformes (cranes, rails, coots) Phoenicopteriformes (flamingos)
Songbirds and Similar Birds	Columbiformes (pigeons, doves) Caprimulgiformes (nightjars, swifts, hummingbirds) Trogoniformes (trogons) Passeriformes (perching birds) Coraciiformes (kingfishers) Piciformes (woodpeckers)
Raptors and Scavengers	Accipitriformes (hawks, kites, eagles) Falconiformes (caracaras, falcons) Strigiformes (owls)
Other Species	Galliformes (pheasants, turkeys, etc.) Cuculiformes (cuckoos, roadrunners, anis) Psittaciformes (parrots)

Waterfowl

Many waterfowl are migratory making them well-suited to spread pathogens across large distances. Waterfowl also typically utilize areas that are in close proximity to humans or

livestock such as parks, recreational areas, and crop fields (Edge & Hill, 2007, Fox *et al.*, 2016). This makes them of particular interest in the ecology of *Salmonella*.

Based on current literature, the role that waterfowl play in *Salmonella* ecology appears unclear. Some studies have found little to no shedding in the species sampled. Hussong *et al.* (1979) surveyed Canada Geese (*Branta Canadensis*) and Tundra Swans (*Cygnus columbianus columbianus*) wintering in the Chesapeake Bay area of Canada and were unable to isolate *Salmonella* (0/44). Milani *et al.* (2012) also sampled Tundra Swans in Alaska during molting and were unable to isolate *Salmonella* (0/100). In Wisconsin and Illinois, Bradshaw and Trainer (1966) sampled Canada Geese and Mallards (*Anas platyrhynchos*) and had no reactions to serological testing for *Salmonella* Typhimurium or *Salmonella* Pullorum (0/12 and 0/55, respectively by species). Youatt and Fay (1968) were unable to recover *Salmonella* from fecal samples of hunter-harvested ducks in Michigan (0/4). Similarly, Smith *et al.* (2002) sampled wildlife entering rehabilitation centers in California and did not isolate *Salmonella* from Western Grebes (*Aechmophorus occidentalis*) or Common Loons (*Gavia immer*) (0/14 and 0/2, respectively).

However, *Salmonella* has been isolated from some of these same species in other studies. While studying Common Loons in Florida, White *et al.* (1976) isolated *Salmonella* from 14.2% (27/190). These loons were part of a large die-off during the winters of 1970 through 1975. Characterization of isolates identified 8 different serotypes: Infantis, Agona, Saintpaul, Montevideo, Muenchen, Newport, Typhimurium, and Blockley (White *et al.*, 1976). Nine of these isolates were also resistant to at least one antimicrobial (White & Forrester, 1979). Duncan *et al.* isolated *Salmonella* from the liver and pectoral muscle of an Eared Grebe (*Podiceps nigricollis*) in California (1983). Interestingly, the bird was collected from an area that was

known to be contaminated with raw sewage. Among Mallards and Canada Geese, Fallacara *et al.* sampled birds in parks in Ohio and found only one Mallard shedding *Salmonella* Java (1/449, 0.2%) (2001). When sampling free-living birds at the Columbus Zoo in Ohio three years later, Fallacara *et al.* found 1.7% (8/450) shedding *Salmonella*, all Canada Geese (2004). In both studies, all birds were visibly healthy and isolates were resistant to 7 different antimicrobials. Resistance to antimicrobials is particularly unusual but may be related to the urban environment in which the birds lived. Siembieda *et al.* also sampled Mallards and Canada Geese entering wildlife hospitals in California and isolated *Salmonella* from 2 Mallards and 1 Canada Goose (8%, 3/37) (2011). While Milani *et al.* and Hussong *et al.* did not isolate *Salmonella* from swans, Pedersen isolated from 0.6% (3/459) of Mute Swans (*Cygnus olor*) in Michigan, New Jersey, Rhode Island, New York, and Indiana (2013).

Waldrup and Kocan (1985) and Grigar *et al.* (2017) both sampled ducks migrating through the Central flyway. Waldrup and Kocan sampled ducks in Oklahoma and were unable to isolate *Salmonella* (0/331), however, 1.5% and 3.0% serum samples were reactive to serogroups B and D, respectively (1985). Along the Texas coast, Grigar *et al.* (2017) were able to isolate *Salmonella* from a Redhead (*Aythya Americana*) and a Blue-winged Teal (*Spatula discors*) (2/375, 0.5%). In western Canada along the Pacific and Central flyways, Jokinen *et al.* (2011, 2010) sampled ducks and geese as well as surface water and sewage samples. In the Salmon River watershed in British Columbia, they were unable to isolate *Salmonella* from ducks (0/23) and only two geese were found to be shedding *Salmonella* (2/15, 15%) (Jokinen *et al.*, 2010). However, at the Oldman River watershed in Alberta, they found 7.9% (3/38) of ducks and 10% (8/81) of geese to be shedding *Salmonella* (Jokinen *et al.*, 2011). These isolates were also highly diverse, representing 26 different serotypes, and did not overlap with serotypes found

in other sample types (Jokinen *et al.*, 2011). In the Atlantic flyway, Gruszynski *et al.* (2014a) found similar results to Grigar *et al.* (2017) with *Salmonella* only being isolated from 1 duck (1/262, 0.4%).

Salmonella has also been found in American Coots (*Fulica americana*). In California, Quortrup *et al.* (1957) did a serological study of waterfowl and found nine American coots (9/181, 5.0%) and one Northern Pintail (*Anas acuta*) (1/651, 0.2%) reactive to Typhimurium. Also in California, Rosen *et al.* (1957) found 8.6% (74/862) of American Coots sero-reactive to Typhimurium and 0.7% (6/862) reactive to *Salmonella* Pullorum-Gallinarum. Similarly to Waldrup and Kocan (1985), *Salmonella* was not isolated from fecal samples, however, Typhimurium and *Salmonella* Bessarek were isolated from internal organs (Rosen *et al.*, 1957). Overall, *Salmonella* is not typically shed by waterfowl in North America except when birds are exposed to areas with high prevalence of *Salmonella* in the environment such as was the case in Duncan *et al.* (1983) and Fallacara *et al.* (2004, 2001).

Sea Birds and Shorebirds

Conversely, *Salmonella* is frequently isolated from sea birds and shorebirds, especially gulls. Like waterfowl, *Salmonella* prevalence in these species are closely linked to environmental exposure. Hall and Saito (2008) reviewed avian wildlife mortality events reported in the U.S. from 1985–2004 and found Ring-Billed Gulls (*Larus delawarensis*) (10.8%, 20/186), Double-Crested Cormorants (*Phalacrocorax auritus*) (8.1%, 15/186), and American White Pelicans (*Pelecanus erythrorhynchos*) (7.5%, 14/186) to be some of the most commonly affected species.

Many studies have been done in gull species as they commonly feed at waste sites and sewage areas and are pervasive in urban and recreational areas. Overall, these species are

subclinically infected. Kinzelman *et al.* (2008) sampled Ring-Billed and Herring Gulls (*Larus argentatus*) at beaches along Lake Michigan in Wisconsin and found 0.7% (5/724) of sampled gulls shedding *Salmonella* Choleraesuis. A similarly low prevalence was found in Massachusetts by Snoeyenbos *et al.* (1967). Among Herring Gulls sampled 2.5% (10/405) were found to shed *Salmonella* and 1% (1/80) of egg contents were contaminated with *Salmonella*. In Quebec, Lévesque *et al.* (2000) isolated almost 200 strains of *Salmonella* from Ring-Billed Gull droppings and over the three study periods found concentration of *Salmonella* in droppings to range from 1.2×10^4 to 2.3×10^2 CFU/g. Seven strains were found to be serotypes known to be pathogenic in humans: Brandenburg, Agona, Hadar, Stanley, Anatum, and Typhimurium (Lévesque *et al.*, 2000). Nearby in Montreal, Quessy and Messier (1992) also sampled apparently healthy Ring-Billed Gulls and found 8.7% (23/264) to be shedding *Salmonella*. Of the four study sites, gulls at a refuse dump had the highest prevalence (13.0%) (Quessy & Messier, 1992). Serotypes isolated included: Hadar, Heidelberg, Berta, Thompson, Haardt, Typhimurium, Manilla, Kentucky, Infantis, and Montevideo. A similar prevalence (9%, 8/87) was found by Stoddard *et al.* (2008) who sampled droppings from Western Gulls (*Larus occidentalis*) in California. Serotypes include Enteritidis, Montevideo, Newport, Reading, and Saintpaul, with one gull shedding both Newport and Saintpaul (Stoddard *et al.*, 2008). Interestingly, one of the Saintpaul isolates was resistant to ceftizoxime, a third-generation cephalosporin similar to those used to treat salmonellosis in people (Stoddard *et al.*, 2008). Also on the western coast of the U.S., Berg and Anderson (1972) sampled gull feces near fish processing plants on the Oregon coast and were able to isolated *Salmonella* from 2.1% (11/521) of samples. The authors suggested that while the prevalence is low, the large numbers of gulls in the area and their proximity to plants processing fish for human consumption may have an

impact on public health. From November 2010 to July 2011, Gruszynski *et al.* (2014a) sampled droppings from gulls in Virginia and found 31% (9/29) positive for *Salmonella* by enzyme-linked immunoassay (ELISA) and confirmed with culture. The study also found 21% (5/24) of "avian" droppings positive for *Salmonella* (Gruszynski *et al.*, 2014a). These samples were from a field frequently used mainly by gulls, but the exact species the droppings came from could not be determined (Gruszynski *et al.*, 2014a). Most of these isolates were typed as Javiana which has been linked to several tomato outbreaks in the region (Gruszynski *et al.*, 2014a). To study the impact these gulls may have on public health in Virginia, Gruszynski *et al.* (2014b) sampled gull feces at four sites and found 17.2% (62/360) shedding *Salmonella*. The majority of these gulls heavily utilized landfills as feeding sites. Twenty-two serotypes were identified, including Infantis, Typhimurium, and Newport (Gruszynski *et al.*, 2014b). Of the Newport isolates, PFGE pattern 61 was most commonly found (Gruszynski *et al.*, 2014b). This pattern has been linked to multiple outbreaks in tomatoes in the region, strongly suggesting that the pattern is endemic to this region of Virginia and can be transmitted by local gull populations (Gruszynski *et al.*, 2014b).

While most gulls are subclinical, some studies have found *Salmonella* in sick or deceased birds. Faddoul *et al.* (1966) sampled birds associated with die-offs in Massachusetts and Rhode Island and isolated *Salmonella* from 10% (1/10) of gull cases. In Quebec, Mikaelian *et al.* (1997) identified *Salmonella* Typhimurium as the cause of a die-off involving 38 Ring-Billed Gulls and the cause of death in one Black-Legged Kittiwake (*Rissa tridactyla*). Nearby in New York, Brand *et al.* (1988) sampled birds found dead or moribund and isolated *Salmonella* from 5.0% (25/505) of sampled livers and intestines. Species from which *Salmonella* was isolated included Herring Gulls, Great Black-Backed Gulls (*Larus marinus*), and Black Skimmer

(*Rynchops niger*) (Brand *et al.*, 1988). In six birds, *Salmonella* was determined to be the cause of morbidity or mortality (Brand *et al.*, 1988). A wide variety of serotypes were isolated including Typhimurium, Heidelberg, Blockley, Manhattan, Thompson, Agona, Infantis, and Stanley, as well as *S. enterica* subsp. *arizonae* (Brand *et al.*, 1988). In Michigan, Radwan and Lampky (1972) isolated Typhimurium from a sick Herring Gull. Hall *et al.* (1977) necropsied six birds from a die-off in a colony of Ring-Billed and California Gulls (*Larus californicus*) at an irrigation pond in Idaho and were able to isolate *Salmonella* from two. As mentioned previously, Smith *et al.* (2002) sampled birds entering wildlife rehabilitation centers in California. In addition to waterfowl, they were unable to isolate *Salmonella* from Common Murres (*Uria aalge*) (0/14) but did isolate *Salmonella* from 6% (2/32) Western Gulls (Smith *et al.*, 2002). One isolate was 4,5,12:1 monophasic and resistant to gentamicin, while Ohio and Johannesburg were both isolated from the second gull (Smith *et al.*, 2002). A similar prevalence (6%, 1/17) in Western Gulls was found by Steele *et al.* (2005) in wildlife rehabilitation centers in California and Washington.

Like gulls, high prevalence of *Salmonella* has been documented in cormorants. In Florida, White and Forrester (1979) sampled Double-Crested Cormorants (*Phalacrocorax auritus*) and isolated *Salmonella* from 11% (12/104) of birds sampled. Serotypes included Infantis, Agona, and Typhimurium (White & Forrester, 1979). Single and multiple resistance to antimicrobials was found in Agona isolates (White & Forrester, 1979). Meteyer *et al.* (1997) sampled livers from Double-Crested Cormorants associated with a Newcastle disease outbreak in Minnesota, Nebraska, North Dakota, and South Dakota and found 28% (20/72) positive for *Salmonella*. Clavijo *et al.* (2001) also sampled Double-Crested Cormorants associated with a Newcastle disease outbreak and were able to isolate *Salmonella* from brain tissue of one bird.

The authors noted that *Salmonella* is frequently associated with Newcastle disease outbreaks, may change the presentation of Newcastle disease, and enhance the mortality rate (Clavijo *et al.*, 2001).

There is very limited research on other species of sea and shorebirds. In North America, Youatt and Fay (1968) are the only researchers to have studied other species and were unable to recover *Salmonella* from three American Woodcocks (*Scolopax minor*) harvested by hunters in Michigan.

Overall, sea and shorebirds, especially gulls, have much higher prevalence of *Salmonella* shedding than other species, presumably because of their utilization of waste and sewage sites.

Wading Birds

Similar to waterfowl, some wading birds can be common in both urban (parks, recreational areas) and agricultural environments (livestock pastures, crop fields) (Hernandez *et al.*, 2016, Phalen *et al.*, 2010). *Salmonella* prevalence in these species is also highly influenced by their feeding ecology with the exposure risk being higher in birds whose food becomes contaminated by droppings or who consume filter-feeding mollusks (Benskin *et al.*, 2009).

In urban environments, Hernandez *et al.* (2016) studied White Ibises (*Eudocimus albus*) in Florida, a species common in parks and recreational areas in the southeastern U.S. They found that 13% (33/261) of adults and 35% (22/72) of nestlings were shedding *Salmonella* (Hernandez *et al.*, 2016). Isolates represented 24 different serotypes, 33% of which are in the CDC's top 20 serotypes among laboratory-confirmed human cases, and 43 PFGE types, 44% of which matched PulseNet patterns (Hernandez *et al.*, 2016). They also compared ibises that lived in natural habitats to those that lived in urban environments and were often hand-fed by humans.

Those that lived in urban environments were more likely to be shedding *Salmonella* with a PFGE pattern matching PulseNet, illustrating the impact environmental ecology can have on *Salmonella* prevalence in birds (Hernandez *et al.*, 2016).

In agricultural areas, Cattle Egrets (*Bubulcus ibis*) are of particular interest due to their close relation to grazing cattle or other livestock. Callaway at al. (2014) sampled Cattle Egrets, as well as other species commonly associated with cattle, in central Texas and found 13% (2/16) shedding *Salmonella* Montevideo. Also in central Texas, Phalen *et al.* (2010) sampled Cattle Egret nestlings at 5 colonies and found *Salmonella* prevalence to range from 29–95%. Seventeen different serotypes were isolated with the majority of isolates being 4,5,12:i- monophasic (Phalen *et al.*, 2010). All were pan-susceptible and highly invasive in the day-old chick infection model (Phalen *et al.*, 2010). During necropsy of sampled birds, microscopic lesions were found on the livers of birds with systemic infections suggesting that the infection can be fatal in Cattle Egret nestlings as well (Phalen *et al.*, 2010). All colonies sampled were in close or direct contact with human habitations and when compared to serotypes isolated form horses at a nearby veterinary teaching hospital 12 were also found in Cattle Egrets (Phalen *et al.*, 2010). This suggests that Cattle Egrets can pose a health risk to both livestock and humans. Along with geese as mentioned earlier, Jokinen *et al.* (2011) also sampled pelicans in the Oldman river watershed of Alberta and found 26% (5/19) shedding *Salmonella*. Similar to the area study in Phalen *et al.* (2010), the Oldman river area is largely agricultural with river water used for both drinking water and crop irrigation suggesting that *Salmonella* shedding by birds and other wildlife can pose a risk to both human health and agriculture (Jokinen *et al.*, 2011).

Other species of wading and water birds that inhabit protected wildlife areas have also been found to shed *Salmonella*. Windingstad *et al.* (1977) sampled apparently healthy Sandhill

Cranes (Antigone Canadensis) in Indiana and Wisconsin and found 4% (2/48) to be shedding *Salmonella*. In New Jersey, Kirkpatrick *et al.* (1986) sampled nestlings in a colony of wading birds and isolated *Salmonella* from 5% (2/37) nests. Isolates were serotyped as Newport and Typhimurium, both resistant to penicillin, from the nest of a Black-Crowned Night-Heron (*Nycticorax nycticorax*) and Glossy Ibis (*Plegadis falcinellus*), respectively (Kirkpatrick, 1986). Also in 1986, Stroud *et al.* found a dead Whooping Crane (*Grus americana*) near a wildlife refuge in Colorado. Necropsy revealed an enlarged liver and spleen and nodules and microscopic lesions on the liver, small intestine, kidneys, trachea, and spleen (Stroud *et al.*, 1986). *Salmonella* Agona was isolated from the liver and determined to be the cause of death (Stroud *et al.*, 1986). In Yucatan, Aguirre *et al.* (1991) took cloacal swabs from moribund and dead Caribbean Flamingos (*Phoenicopterus ruber*) during a lead poisoning die off and found 11% (3/27) to be shedding *Salmonella*. As mentioned previously, Siembieda *et al.* (2011) sampled birds entering wildlife hospitals in California and found 5% (2/44) of wading birds to be shedding *Salmonella*; both were Black-Crowned Night-Herons.

Overall, there is limited research on the ecology of *Salmonella* in wading birds in North America. What studies are available indicate that these birds are a potential source of *Salmonella* and the close habitation of some species to urban or agricultural areas may pose a risk to human or livestock health.

Songbirds and Similar Birds

The vast majority of research has been done in songbirds (passerines) and similar birds as *Salmonella* has been commonly documented in outbreaks among these species. According to Hall and Saito (2008), 21.5% of avian mortality events in passerines were caused by salmonellosis with most linked to *Salmonella* Typhimurium. The most common species of

passerines involved in these events were as follows: Pine Siskin (*Spinus pinus*), American Goldfinch (*Spinus tristis*), Northern Cardinal (*Cardinalis cardinalis*), House Sparrow (*Passer domesticus*), Evening Grosbeak (*Coccothraustes vespertinus*), Brown-Headed Cowbird (*Molothrus ater*), and Common Redpoll (*Acanthis flammea*) (Hall & Saito, 2008). Many times these outbreaks are associated with backyard feeders during the winter months (Burton & Dufour, 2000) where high utilization by birds leads to accumulation of pathogens (Macdonald *et al.*, 1968).

An outbreak in songbirds at backyard bird feeders actually led to the first isolation of *Salmonella* Typhimurium from free-flying birds (Hudson & Tudor, 1957). Dead birds were submitted by residents in New Jersey who were concerned by recent die-offs among birds at backyard feeders (Hudson & Tudor, 1957). Three European Starlings (*Sturnus vulgaris*), seven sparrows (species not noted), one Brown-Headed Cowbird, and six Rusty Blackbirds (*Euphagus carolinus*) were necropsied, and *Salmonella* Typhimurium was isolated from tissue cultures of all necropsied birds (Hudson & Tudor, 1957). Over ten years later, Wobeser and Finlayson (1969) reported another group of die-offs associated with House Sparrows feeders in Ontario. *Salmonella* Typhimurium was isolated from tissue cultures of 92% (45/49) necropsied birds. Also in Canada, Bowes (1990) reported feeder-associated die-offs in House Sparrows in British Columbia. Two sparrows were necropsied and *Salmonella* Typhimurium was isolated from all tissues cultured. In Pennsylvania, Fichtel (1978) found sixteen birds dead at a feeding station at a nature reserve all in poor condition and severe weight loss including: six Northern Cardinals, eight Tree Sparrows (*Spizelloides arborea*), one White-Throated Sparrow (*Zonotrichia albicollis*), and one Dark-Eyed Junco (*Junco hyemalis*). At necropsy, all birds were determined to be either definitively diagnosed with salmonellosis or suspected cases (Fichtel, 1978). Similar

die-offs were reported by Locke *et al.* (1973) in Evening Grosbeaks in West Virginia and House Sparrows, American Goldfinches, and Pine Siskins in Maryland. Unlike the die-off studied in Fichtel (1978), all birds found were in fair to good condition (Locke *et al.*, 1973). Serotypes isolated from tissue cultures included Typhimurium and Enteritidis (Locke *et al.*, 1973). Hernandez *et al.* (2012b) identified an outbreak in songbirds in the eastern U.S. (Georgia, South Carolina, North Carolina, Tennessee, Virginia, and West Virginia) that mainly included Pine Siskins, American Goldfinches, and Northern Cardinals. Typhimurium PFGE type A3 was identified as the main outbreak strain and was responsible for 94% of Pine Siskin and American Goldfinch cases (Hernandez *et al.*, 2012b). In Florida, Nesbitt (1974) necropsied birds from a feeder-associated die-off and was able to isolate *Salmonella* Typhimurium from all necropsied birds. Species affected included: Blue Jay (*Cyanocitta cristata*), Tufted Titmouse (*Baeolophus bicolor*), Brown Thrasher (*Toxostoma rufum*), House Sparrow, Red-Winged Blackbird (*Agelaius phoeniceus*), Common Grackle (*Quiscalus quiscula*), Northern Cardinal, Chipping Sparrow (*Spizella passerina*), White-Throated Sparrow, and Ground Dove (*Columbina passerina*).

Occasionally outbreaks are not limited to feeders and backyard die-offs. Daoust *et al.* (2000) sampled songbirds submitted for necropsy from 1997–1998 in Newfoundland, Labrador, Nova Scotia, New Brunswick, and Prince Edward Island and identified 73 confirmed cases of salmonellosis and 263 suspected cases. Species most commonly affected included: Common Redpoll, Pine siskin, Purple Finch (*Haemorhous purpureus*), Evening Grosbeak, and American Goldfinch (Daoust *et al.*, 2000). Of the 35 isolates recovered, 34 (97%) were identified as Typhimurium phage type 40 which was linked to a concurrent outbreak in Quebec (Daoust *et al.*, 2000). During this same time, Prescott *et al.* (1998) reported another outbreak in Ontario that was also primarily affecting Common Redpolls and Pine Siskins. While the majority of sampled

birds were found near feeders, they also identified Typhimurium phage type 40 as well as phage type 160 (Prescott *et al.*, 1998). Also overlapping this time frame, Mikaelian *et al.* (1997) reported that from 1992 to 1997, 6 cases of *Salmonella* Typhimurium were identified in Ouebec. Two cases were associated with die-offs in House Sparrows and one in an individual House Sparrow (Mikaelian *et al.*, 1997). In the U.S., Faddoul *et al.* (1966) surveyed die-offs in Massachusetts and Rhode Island and identified *Salmonella* in 12% (12/100) of consignments surveyed. Eight out of twelve cases (66.7%) of Brown-Headed Cowbirds and three out of fourteen (21.4%) cases of sparrows (2 House sparrows, 1 White-Throated Sparrow [*Zonotrichia albicollis*]) were positive for *Salmonella* Typhimurium (Faddoul *et al.*, 1966). In Michigan, Radwan and Lampky (1972) sampled fifteen sick or dead birds as well as 45 healthy Brown-Headed Cowbirds and found 25% (15/60) positive for *Salmonella*. Serotypes identified included Typhimurium, Paratyphi A, Paratyphi B, and Albany. Deceased or moribund birds that yielded *Salmonella* isolates included a Red-Winged Blackbird, a Ruby-Throated Hummingbird (*Archilochus colubris*), and a Lesser Yellowlegs (*Tringa flavipes*).

Songbirds have also been found to shed *Salmonella* subclinically, and many of these species are those that live in proximity to urban or agricultural areas. In Ontario, Tizard *et al.* (1979) reported that 0% (0/22) of apparently healthy European Starlings and 15% (9/60) of House Sparrows were shedding *Salmonella* Typhimurium. Phage types included 20, 40, 160, and one untypable (rough) isolate. Janecko *et al.* (2015) sampled corvid species, a group known to heavily utilize refuse sites and anthropogenic food sources, in both the U.S. (California, Kansas, New York, Massachusetts) and Canada (Ontario, Prince Edward Island, Nova Scotia, British Columbia). In the U.S., 1.7% (10/590) of American Crows (*Corvus brachyrhynchos*) were shedding *Salmonella* with a wide variety of serotypes identified including Typhimurium,

Agona, Cerro, Braenderup, Montevideo, and Muenchen (Janecko *et al.*, 2015). In Canada, 1.8% (7/400) of American Crows and 2% (1/49) of Common Ravens (*Corvus corax*) were found to shed *Salmonella* with serotypes including Typhimurium, Typhimurium var. Copenhagen, and Brandenburg (Janecko *et al.*, 2015). Overall, Snoeyenbos *et al.* (1967) reported a similarly low prevalence among various blackbirds sampled in Massachusetts. While no *Salmonella* was isolated from Red-Winged Blackbirds, 1.9% of Common Grackles, 3.7% (11/299) of Brown-Headed Cowbirds, and 8.8% (13/148) of European Starlings were positive for *Salmonella* (Snoeyenbos *et al.*, 1967). Typhimurium was the most common serotype isolated. The authors noted that the prevalence in Brown-Headed Cowbirds was indicative of shedding in other species, as cowbirds are brood parasites that lay their eggs in the nests of other birds, thus leaving their chicks to be fostered by other species (Snoeyenbos *et al.*, 1967). Similar findings were reported by Grigar *et al.* (2016) in urban bird species in Texas (1.8%, 2/114). Species sampled were predominantly Great-Tailed Grackles (*Quiscalus mexicanus*) and European Starlings (Grigar *et al.*, 2016). Both isolates were from Great-Tailed Grackles (species prevalence 3%, 2/76) and were identified as Typhimurium (Grigar *et al.*, 2016). Also in Texas, Brobey *et al.* (2017) identified a much high prevalence in birds sampled (17.0%, 20/116) along a suburban to rural gradient. Species-specific prevalence ranged from 20.0–100.0%: Mourning Dove (*Zenaida macroura*) (20%, 1/5), Inca Dove (*Columbina inca*) (33%, 1/3), Blue Jay (*Cyanocitta cristata*) (100%, 1/1), Northern Cardinal (23%, 6/26), American Goldfinch (29%, 2/7), House Sparrow (21%, 6/29), Red-Bellied Woodpecker (*Melanerpes carolinus*) (50%, 2/4), and Yellow-Bellied Sapsucker (*Sphyrapicus varius*) (50%, 1/2) (Brobey *et al.*, 2017). In Mexico, Espinosa-Argüelles *et al.* (2010) reported a similar prevalence in doves (26.3%, 53/201) with a higher prevalence in juveniles and females. 26.4% (47/178) and 26% (6/23) of White-

Winged (*Zenaida asiatica*) and Mourning Doves were sero-positive for Gallinarum-Pullorum (Espinosa-Argüelles *et al.*, 2010). White *et al.* (1981) reported a large prevalence range among birds sampled in Florida. While *Salmonella* was not recovered from Blue Jays, Mourning Doves, Red-Winged Blackbirds, or Ground Doves, 10% (2/20) of Eastern Towhee(*Pipilo erythrophthalmus*), 8% (2/25) of Common Grackles, 14% (6/42) of American Crows, and 6% (1/18) of Northern Cardinals were found to shed *Salmonella* (White *et al.*, 1981). Conversely, Hamer *et al.* (2012) found a much lower prevalence in the Chicago area of Illinois with only one Red-Winged Blackbird (0.8%, 1/180) shedding *Salmonella* out of all birds sampled.

Many passerine species are associated with agricultural operations. Particularly when associated with livestock operations, these birds often shed *Salmonella* at a higher prevalence than the same species in other environments. This could potentially lead to spreading *Salmonella* strains around the agricultural operation or transfer strains to other environments. In Ohio, Morishita *et al.* (1999) swabbed the cloaca and choana of House Sparrows and European Starlings either on agricultural operations or within one mile of an agricultural operation and isolated *Salmonella* from 1.1% (4/373) and 7.1% (62/868) of cloacal swabs, respectively. *Salmonella* was not isolated from any choanal swabs (Morishita *et al.*, 1999). Barber *et al.* (2002) sampled bird feces at swine farms in Illinois as well as sparrows and starlings (species not defined) residing on the farm. While *Salmonella* could not be isolated from the sparrows and starlings sampled, it could be isolated from 8% (3/38) of bird feces samples (Barber *et al.*, 2002). Similarly, Craven *et al.* (2000) sampled European Starlings and House Sparrows near broiler houses in Georgia and found an overall prevalence of 10.0% (12/124). Pao *et al.* (2014) sampled birds residing in pastures with sheep and goats in Maryland and Virginia and were only able to isolate *Salmonella* from one European Starling (0.2%, 1/446). Birds residing near cattle

operations, namely concentrated animal feeding operations (CAFO) such as dairies and feedlots, are commonly studied as they are usually considered pests. These birds consume cattle feed and can cause considerable economic loss to operators. In Texas, Callaway *et al.* (2014) sampled birds commonly associated with cattle during fall migration along the Central Flyway over a four year period. Throughout the study period 12.9% (40/309) of Brown-Headed Cowbirds and 28% (14/51) of Common Grackles were found to be shedding *Salmonella* (Callaway *et al.*, 2014). Muenster, Montevideo, and Typhimurium were the most common serotypes isolated. Also in Texas, Carlson *et al.* (2011) surveyed European Starlings at CAFOs and isolated *Salmonella* from 3% (2/81) of starlings sampled. Starling numbers were found to be significantly related to *Salmonella* contamination and feed and water contamination suggesting that starlings may be a source of contamination in CAFOs (Carlson *et al.*, 2011). A much lower prevalence (0.7%, 3/434) was reported by Gaukler *et al.* (2009) in European Starlings at CAFOs in Kansas. All three starling were found to also be shedding *Escherichia coli* (Gaukler *et al.*, 2009). Two were identified as *Salmonella* subsp. *arizonae* and showed resistance to multiple antimicrobials while one remained untyped (Gaukler *et al.*, 2009). *Salmonella* prevalence among birds occupying dairies is similar. Kirk *et al.* (2002) sampled birds at dairies in California and found an overall prevalence of 2.5% (22/892). Species specific prevalence ranged from 1.2–3.2%: Brown-Headed Cowbirds (3%, 3/95), House Sparrows (3.1%, 14/451), Brewer's Blackbird (*Euphagus cyanocephalus*) (2%, 1/44), House Finch (*Haemorhous mexicanus*) (2%, 1/61), European Starling (1%, 1/80), Red-Winged Blackbird (1%, 1/78), and Rock Pigeon (*Columba livia*) (1%, 1/83) (Kirk *et al.*, 2002). Serotypes isolated were similar to birds associated with beef cattle, including Montevideo, Muenster, Typhimurium, and Meleagridis (Kirk *et al.*, 2002). Pedersen *et al.* (2006) reported a slightly higher prevalence in Rock Doves at dairies in Colorado (3.2%,

9/277). Serotypes identified included Newport, Montevideo, Typhimuirum var. Copenhagen, and Senftenberg (Pedersen *et al.*, 2006). The authors noted that Rock Doves have a small home range and therefore may amplify transmission rates throughout the dairy (Pedersen *et al.*, 2006). Very few studies have been done in birds near produce fields. Gorski *et al.* (2011) sampled birds in a major produce region in California and found 6.7% (7/105) to be shedding *Salmonella*. Species found to be shedding *Salmonella* include American Crows, Spotted Towhee (*Pipilo maculatus*), and White-Crowned Sparrow (*Zonotrichia leucophrys*) (Gorski *et al.*, 2011). Seroptypes isolated included Tyhpimurium, Give, 6,8:–:e,n,z_{15}, and IV_43:z_4,z_{23} (Gorski *et al.*, 2011).

In a few studies, *Salmonella* has not been identified. Brittingham *et al.* (1988) surveyed passerines and woodpeckers in Wisconsin were unable to isolate *Salmonella* from any birds sampled. In Montreal, Gabriele-Rivet *et al.* (2016) were also unable to isolate *Salmonella* from any Rock pigeons sampled (0/187).

Overall, songbirds and other similar species often shed *Salmonella* at a fairly low prevalence. Environmental factors such as utilizing feeders and associating with agricultural operations may increase shedding risk.

Raptors and Scavengers

Though *Salmonella* is not typically found in raptors and scavengers, infection or shedding in these species may indicate the presence of *Salmonella* in other animal populations (Kirkpatrick & Trexler Myren, 1986). In New Jersey, Kirkpatrick and Trexler-Myren (1986) sampled free-living falconiformes and found 1.9% (2/105) to be shedding *Salmonella*. Isolates were obtained from healthy, juvenile Red-Tailed Hawks (*Buteo jamaicensis*), were pan-susceptible, and were serotyped as Enteritidis and Newport (Kirkpatrick & Trexler Myren,

1986). Also in New Jersey, Kirkpatrick and Colvin (1986) sampled Barn Owl (*Tyto alba*) nestlings and nests. 9% (8/94) of nestlings and 20% (5/25) of nests were positive for *Salmonella* (Kirkpatrick & Colvin, 1986). All nestlings appeared healthy (Kirkpatrick & Colvin, 1986). All isolates were resistant to penicillin (Kirkpatrick & Colvin, 1986). Serotypes isolated from nestlings were Typhimurium, Thompson, and Tuindorp. Interestingly, all isolates were from nests near agricultural sites, but preliminary research of rodents at the study sites revealed no *Salmonella* in the potential prey sampled (Kirkpatrick & Colvin, 1986). Lamberski *et al.* (2003) found that 30% (3/10) of Red-Tailed Hawks and 20% (2/10) of Cooper's Hawks (*Accipiter cooperii*) in California were shedding *Salmonella* subsp. *arizonae* 38:(k):z_{35} and Typhimurium, respectively. Mikaelian *et al.* (1997) isolated *Salmonella* from a sick Great Horned Owl (*Bubo virginianus*) that was submitted to the Canadian Cooperative Wildlife Health Centre in Quebec. In California, Smith *et al.* (2002) isolated *Salmonella* from 18% (2/11) of Northern Harriers (*Circus hudsonius*) entering wildlife rehabilitation centers. Both isolates were serotyped as Montevideo and resistant to ampicillin and amoxicillin-clavulanic acid (Smith *et al.*, 2002). Unlike Kirkpatrick and Colvin (1986), Smith *et al.* (2002) did not isolate *Salmonella* from Barn Owls sampled.

Among scavengers, Turkey Vultures (*Cathartes aura*) have been most studied and prevalence varies widely. Siembieda *et al.* (2011), as mentioned previously, also sampled Turkey Vultures entering California wildlife hospitals and isolated *Salmonella* from 5% (2/39). Also in California, Sulzner *et al.* (2014) isolated *Salmonella* from 20% (11/55) of Turkey Vultures. The study site was located near livestock operations and vultures had access to livestock remains (Sulzner *et al.*, 2014). Serotypes included Typhimurium, Newport, Give, Montevideo, Enteritidis, Anatum, and 3 stains of *S. enterica* subsp. *arizonae* (Sulzner *et al.*,

2014). In Texas, Winsor *et al.* (1981) described a similar prevalence (30%, 6/20) and three isolates were characterized as *S. enterica* subsp. *arizonae*. Everard *et al.* (1979) and Adesiyun *et al.* (1998) each isolated *Salmonella* from one Black Vulture (*Coragyps atratus*) in Trinidad.

While the public health consequences of *Salmonella* shedding in these species is very limited, they may potentially play a role as sentinels for shedding amongst other animal populations.

Other Species

In North America, the majority of studies in other species have been done on Galliformes especially turkeys and pheasants due to their importance as gamebirds and the potential threat to domestic poultry.

Wild Turkeys (*Meleagris gallopavo*) have been extensively studied due to intensive management as part of conservation efforts. The majority of studies are serological surveys for Typhimurium, Pullorum, and/or Gallinarum. Overall, prevalence is low. In Texas, both Glazener *et al.* (1967) and Trainer *et al.* (1968) tested sera from turkeys captured on in a wildlife refuge and had no reactions to Typhimurium or Pullorum (0/87 and 0/148, respectively). Also in Texas, Peterson *et al.* (2002a) found no sampled turkeys (0/70) to be serologically positive for Typhimurium or Pullorum. Similar results were also found in Arkansas by Hopkins *et al.* (1990) with no turkeys (0/44) serologically positive for Pullorum or Gallinarum. Other studies have found a limited degree of seroreactivity. In California, Charlton (2000) sampled turkeys trapped in the state or being transported to the state and found <5% seropositive for Typhimurium and Pullorum. Crupper and Applegate (2002) sampled turkeys captured in Kansas that were being transported to Utah and found 4% (2/47) seropositive for Enteritidis and 2% (1/47) seropositive for Pullorum. In turkeys captured in Texas and released in Iowa, Roslien and Haugen (1970)

found none seropositive for Typhimurium (0/20), 5% (2/39) seropositive for Pullorum, and 10% (4/39) suspected seropositive for Pullorum. Hensley and Cain (1979) also sampled turkeys in Texas and found 2.4% (6/249) seropositive for Pullorum and 2.3% (4/174) seropositive for Typhimurium. In all studies, sampled turkeys were visibly healthy. Veatch *et al.* (1998) sampled turkeys in Kansas and found 0.6% (7/1164) seropositive for Pullorum. Serological results could not be confirmed by culture of sera (Veatch *et al.*, 1998). Ingram *et al.* (2015) also used serological and culture data. They sampled hunter-harvested turkeys in Georgia and Florida and found 13% (3/24) serum samples positive for Gallinarum (2/24) and Pullorum (1/24) (Ingram *et al.*, 2015). Interestingly, 2 of these turkeys were harvested within a 12 km radius of commercial poultry operations. Tracheal, lung, and esophageal tissue were also cultured and 3% (2/64) were culture positive for *Salmonella*. Neither turkey from which *Salmonella* was isolated was tested for serological reactivity. Similar to the serologically positive turkeys, one of the culture positive turkeys was found dead in a field fertilized with chicken litter, indicating that commercial poultry may have an impact on the prevalence of *Salmonella* in wild turkeys (Ingram *et al.*, 2015). Studies that used only culture methods found similar results to serological studies. White *et al.* (1981) cultured liver and cloacal samples from turkeys in Florida and found 4.4% (18/411) positive for *Salmonella* representing 10 different serotypes. Serotypes included Typhimurium, Hartford, Miami, Rubislaw, Newport, Java, Muenchen, Manhattan, Braenderup, and Inverness (White *et al.*, 1981). Jay-Russell *et al.* (2014) isolated *Salmonella* from 23% (16/71) of wild turkeys from a California farm experiencing an outbreak of *Salmonella* in horses. The turkeys were suspected to have introduced *Salmonella* to the horses on the farm (Jay-Russell *et al.*, 2014). Conversely, Rocke and Yuill (1987) and Youatt and Fay (1968) were unable to isolate *Salmonella* from turkeys in Texas (0/511) and Michigan (0/9), respectively. However,

Rocke and Yuill (1987) were able to isolate *S. enterica* subsp. *arizonae* from the spleen and kidney of a previously sampled turkey that was found dead. The authors suggested that turkeys may shed *Salmonella* intermittently or only while stressed (Rocke & Yuill, 1987). Similarly, Howerth (1985) isolated *Salmonella* Typhimurium from the liver of an emaciated female turkey in Alabama.

Salmonella has occasionally been documented in other game and ground birds. Youatt and Fay (1968) also sampled hunter-harvested Ring-Necked Pheasants (*Phasianus colchicus*) in Michigan and were unable to isolate *Salmonella*. Also in Michigan, Belding (1955) sampled adult Pheasants (*Phasianus colchicus torquatus*) and eggs and isolated *Salmonella* in 8% (5/65) of adults and none of the eggs sampled (0/36). Among Prairie Chickens (*Tympanuchus pallidicinctus*) in Texas, Peterson *et al.* (2002b) found none (0/24) of the sampled birds to be seroreactive to Typhimurium or Pullorum. Similarly, in Kansas Williams *et al.* (2000) did a serological survey of Northern Bobwhites (*Colinus virginianus*) and found none (0/25) to be seroreactive to Pullorum or Gallinarum.

Overall, *Salmonella* is found intermittently in turkeys and rarely in other gamebirds. However, as demonstrated by Jay-Russell *et al.* (2014), turkeys can transmit *Salmonella* to domestic species and thus may have an important public health impact.

Conclusion

Salmonella has been isolated from a wide range of bird species. While most birds shed *Salmonella* subclinically, *Salmonella* can cause clinical illness in birds and may cause outbreaks especially in passerine species that utilize feeders. Environment and feeding ecology are major determinants in the epidemiology of *Salmonella* with species such as gulls that feed at refuse or

other contaminated sites having the highest prevalence. Bird feeders are also another major environmental factor. Many outbreaks in passerines are linked to bird feeder use.

Overall, relatively few studies have been done in birds in North America especially in the past decade. More research will need to be done to fully assess the current epidemiology of *Salmonella* in North American birds.

CHAPTER II

SALMONELLA SURVEILLANCE AMONG GREAT-TAILED GRACKLES (*QUISCALUS MEXICANUS*) AND OTHER URBAN BIRD SPECIES IN EASTERN TEXAS[*]

Overview

Wild birds may play an important role in maintaining and transmitting *Salmonella*. Their ability to travel large distances and their proximity to human habitations could make them a vehicle for bridging *Salmonella* from wild and domestic animals to humans. To determine the potential public health risk presented by urban birds, we investigated the prevalence of *Salmonella* among Great-Tailed Grackles (*Quiscalus mexicanus*) and other cohabiting urban bird species. Fecal samples were collected from 114 birds communally roosting in parking lots of retail locations in Brazos County, Texas from February through July of 2015. Great-Tailed Grackles and European Starlings (*Sturnus vulgaris*) were the predominant species sampled. Standard bacteriologic culture methods were used to isolate *Salmonella* from samples and isolates were characterized via serotyping and antimicrobial susceptibility testing. Overall, 1.8% (2/114) of samples were confirmed as positive for *Salmonella*. Both positive birds were Great-Tailed Grackles sampled in June, yielding a 2.6% (2/76) apparent prevalence among this species. Isolates were serotyped as *Salmonella* Typhimurium and found to be pan-susceptible based on the National Antimicrobial Resistance Monitoring System (NARMS) panel of antimicrobial agents. The occurrence of *Salmonella* in Great-Tailed Grackles represents a potential threat to

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public health, particularly considering their population size and their tendency to congregate near human establishments such as grocery stores.

Introduction

Salmonella enterica is a zoonotic pathogen that poses a considerable threat to public health. Transmission occurs through foodborne exposure as well as direct contact with the feces of infected animals. *Salmonella* is estimated to cause ~1.2 million illnesses, 20,000 hospitalizations, and 400 deaths annually in the United States (Scallan *et al.*, 2011), at an economic cost estimated to be $4.4 billion per year (Scharff, 2012). Typical disease manifestations in humans include diarrhea, fever, anorexia, abdominal pain, vomiting, and malaise (Heymann, 2015). Livestock and poultry species serve as key reservoirs for *Salmonella*, although the organism has been isolated from the gastrointestinal tract of a wide range of wild and domestic host species (Skov *et al.*, 2008). However, the epidemiology of *Salmonella* among wildlife has been poorly defined, relative to domestic species.

Wild birds may play a critical role in disseminating *Salmonella* and other enteric pathogens across the landscape, given their ability to travel great distances during migrations, dispersals, or between feeding and roosting activities. Thus, wild birds have the potential to pose a substantial risk to public health (Callaway *et al.*, 2014). To this end, there have been several studies involving birds near agricultural operations such as dairies and feedlots. For example, Callaway *et al.* (2014) conducted a survey of birds often associated with cattle in Brazos County, Texas, namely Brown-Headed Cowbirds (*Molothrus ater*), Common Grackles (*Quiscalus quiscula*), and Cattle Egrets (*Bubulcus ibis*), and found 14.9% of these birds to be shedding *Salmonella*. Phalen *et al.* (2010) estimated *Salmonella* prevalence ranging from 29% to 91% among Cattle Egret nestlings from various colonies in central Texas. The serotypes identified in

these birds were also isolated from horses in the 2 years following their study, indicating that egrets and horses may be exposed to similar sources of *Salmonella* or that transmission may be occurring between them. In addition, Kirk *et al.* (2002) captured 892 birds at nine dairies in California and isolated *Salmonella* spp. from 1.2% to 3.2% of each avian species, with Brown-Headed Cowbirds and House Sparrows (*Passer domesticus*) having the highest prevalence. Fewer studies have been conducted in nonagricultural settings. In particular, there is a dearth of research on *Salmonella* shedding among bird species that congregate in urban locations with high human activity, such as Great-Tailed Grackles (*Quiscalus mexicanus*). Hamer *et al.* (2012) collected fecal samples from 180 birds in the greater Chicago, IL area and found only one individual (0.6%) to be infected with *Salmonella*. Janecko *et al.* (2015) identified *Salmonella* in 1.7% (10/590) of samples from American crows collected in four states.

Great-Tailed Grackles spread from Mexico to reach southern Texas by the 1880s, and their range now extends as far north and west as South Dakota, Idaho, and California (Wehtje, 2003). In the United States, the species is highly adapted to the urban environment and forms large aggregations of communally roosting individuals at night. The locations of these nocturnal roosts are frequently the trees of large, well-illuminated parking lots (Hall & Harvey, 2007). The presence of heavy loads of avian feces beneath roost trees, coupled with raucous vocalizations, result in these birds and other urban bird species gaining a reputation as pests, with many establishments attempting to haze these birds away (Fitzwater *et al.*, 1988). The objectives of this study were to estimate the prevalence of *Salmonella* shedding among roosting Great-Tailed Grackles and other urban birds in the parking lots of retail locations in Brazos County, Texas, and characterize the isolates through serotyping and antimicrobial susceptibility testing.

Materials and Methods

Bird collection and processing

On nine evenings from February through July 2015, samples were collected from roosting birds in urban parking lots of retail buildings in College Station, Brazos County, located in eastern Texas. Great-Tailed Grackles have been established since the 1940s in many metropolitan areas of Texas, including College Station (Wehtje, 2003). Sites were selected based on prior observations of Great-Tailed Grackle and other urban bird aggregations for staging and roosting. The study area was ~9 hectares in size and encompassed two gas stations, a bank, a grocery store, and several restaurants and other businesses. Nylon mist nets (12-meter length; Association of Field Ornithologists, Portland, ME) were erected next to communal roost trees as previously described (Hamer *et al.*, 2012). As roost tree canopies were above the standard height of a mist net, we adapted this protocol by connecting two rigid conduit pipes with a threaded couplet to create 6.1-meter poles that supported the nets (Figure 1A). Mist nets with 30mm mesh size were used initially, but larger birds (e.g., male great-tailed grackles) were observed escaping from these nets; thus, we switched to 61mm mesh size nets to improve capture success. The nets were erected 1–4 h before sunset as the communal birds were staging. Birds were captured in nets as they arrived at the roost trees and when 3-meter conduit poles were used to flush roosting birds into the nets. Birds were extracted immediately after being caught in many cases, and nets were monitored for additional captured birds every 15–20min. A double layer of new paper bags was used to hold birds individually until they were ready to be processed.

For each captured bird, the species, weight, wing chord, tail length, sex, and age class (hatch year or after hatch year) were recorded. Sterile cotton-tipped swabs were used to collect

fecal samples from the inner paper bag holding the bird, directly from the cloaca, or in a few cases from processing tables or technicians; samples were then deposited into Amies media (Becton-Dickinson, Sparks, MD). When voided fecal samples were available, we attempted to collect the entire amount. Standard procedures were utilized to prevent cross-contamination of samples, such as changing gloves between handling individual birds. Up to two samples were collected per bird and kept in a cooler at ~4°C. Following sample collection, birds were released at the site of capture after attaching a United States Fish and Wildlife Service (USFWS) metal leg band with a unique number, or in some cases, they were euthanized for unrelated research efforts. Fieldwork was conducted with approvals from the Institutional Animal Care and Use Committee at Texas A&M University, U.S. Fish and Wildlife Service, U.S. Geological Survey, Texas Parks

Estimate of study population

Communal Great-Tailed Grackle roosts are dynamic in the College Station, Texas, environment. The size and location of these roosts vary throughout the year, likely due to breeding activities and hazing practices to disrupt the large aggregations of birds. One technique to census communal roosting birds is to station multiple observers around the roost to count birds as they arrive (Rumbold *et al.*, 2009). As Great-Tailed Grackles arrive at their communal roost, however, they stage in large numbers and are prone to flight when disturbed. Mass departure from staging locations or roost trees occurs throughout dusk and into the evening, triggered by the many forms of disturbance that are common in parking lots of retail buildings in urban areas. To avoid duplicate counting of birds, we pursued an alternative method to estimate local population size. During the nine evenings of sampling (February through July 2015) and on frequent scouting visits before and during the sampling period, we became familiar with the trees

utilized to roost by Great-Tailed Grackles and the boundaries of the roost where trees were not used. Roost trees were primarily Southern live oaks (*Quercus virginiana*), with a typical height of ~8 meters, located in parking lots near artificial lighting. We estimated that ~55 roost trees

Figure 1 Sampling area set up and areas of avian fecal contamination.
Nylon mist nets on 6.1-meter poles next to a roost tree in the foreground with staging Great-Tailed Grackles (*Quiscalus mexicanus*) on power lines in the background (A) and white feces from Great-Tailed Grackles under two roost trees in a parking lot in College Station, Texas (B). A female Great-Tailed Grackle on grocery store shopping carts (C) and avian feces on the handle of a shopping cart (D). Color images available online at www.liebertpub.com/vbz
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were being utilized by Great-Tailed Grackles in the study area on any given night, based on our experience in the field combined with satellite imagery. Dispersal of Grackles from roost trees, in response to our sampling efforts or other human activity, allowed a conservative estimation of ~40 birds per tree. Thus, we estimated a total of 2,200 Great-Tailed Grackles within the study area, utilizing the communal roosts (Figure 1 A–D).

Microbiologic Procedure for Salmonella *Detection*

Within 1–5 h of collection, samples were brought to the research laboratory and enriched in 5mL of tetrathionate (TT) broth (Becton, Dickinson and Company, Franklin Lakes, NJ) containing 0.1mL of iodine solution. Enrichments were incubated at 42°C for 24 h and then plated to xylose lysine tergitol-4 (XLT-4) agar (Northeast Laboratories, Waterville, ME). Plates were incubated for 48 h at 37°C; these were examined for suspected colonies at 24 and 48 h. Following incubation, a single suspected colony was transferred to Kligler iron agar (KIA) slants (Becton, Dickinson and Company) and incubated for 18–24 h at 37°C. Colonies from positive slants were streaked onto trypticase soy agar (TSA) 5% sheep blood (Becton, Dickinson and Company) and incubated at 37°C for 18–24 h. After incubation, one positive colony was transferred to brain–heart infusion (BHI) broth (Becton, Dickinson and Company), incubated at 37°C for 16–24 h, and then frozen in a 15% glycerol solution at -80°C for further characterization.

Following the first month of sampling, samples were processed using the preceding method in parallel with an additional method based on Andrews and Hammack (2007). This second method was incorporated to allow future studies to characterize non-*Salmonella* bacteria that may be present in the samples and further improve *Salmonella* recovery. When two samples per bird were available, they were randomly allocated to each method. If only one sample was available, it was processed using the modified (Andrews & Hammack, 2007) method. This modified method consisted of the same steps as previously described, but with the addition of a nonspecific enrichment step in tryptic soy broth (TSB; Becton, Dickinson and Company) before the TT enrichment and an enrichment in Rappaport-Vassiliadis (RV) broth (Becton, Dickinson and Company) following TT enrichment. TSB enrichment consisted of incubating the swabs for

2 h at room temperature followed by at least 10-h incubation at 37°C. One milliliter of TSB was transferred to 9mL of TT broth and incubated for 24 h at 37°C. The remaining TSB was incubated for an additional 12 h and then frozen in the same manner as BHI in the previously described method. Then, 200 μL of the TT broth was transferred to 5mL of RV broth and incubated for 24 h at 42°C. After incubation, RV was plated to XLT-4. Steps following the 48 h incubation are the same as in the previously described method. In addition, presumptive *Salmonella* isolates were confirmed by amplification and detection of the invA gene using PCR as previously described (Kim *et al.*, 2007).

Serotyping

Confirmed *Salmonella* isolates were sent to the Clinical Microbiology Laboratories at the University of Pennsylvania Veterinary Hospital for serotyping. The xMAP® *Salmonella* Serotyping Assay (Luminex, Austin, TX) was used to identify *Salmonella* isolates. The assay simultaneously determines O and H antigen genes and also identifies serotype-specific markers in the AT (Additional Targets) test. *Salmonella* template DNA was extracted using the InstaGene Matrix as described by the manufacturer (Bio-Rad, Hercules, CA). Multiplex PCR was performed, and the amplicons were hybridized with the oligonucleotide probe-coupled bead mixture and then labeled with streptavidin-R-phycoerythrin (SAPE) reporter. The assay plate was analyzed on a Luminex® LX200™ platform, and the data were exported to Excel (Microsoft, Redmond, WA) for analysis.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility of confirmed *Salmonella* isolates was determined by the broth microdilution method. Minimal inhibitory concentrations (MIC) were established for each isolate against the National Antimicrobial Resistance Monitoring System (NARMS) Gram-

Table 2 Distribution of sex, age, season, and status of *Salmonella* shedding among 117 urban birds captured in College Station, Texas, 2015

	Great-tailed grackle	*European starling*	*House sparrow*	*Brown-headed cowbird*	*Cedar waxwing*	*Eurasian collared-dove*	Salmonella *culture positive*
Sex							
Male	11	7	3	0	0	0	0
Female	59	3	0	1	0	0	2
Unknown	6	24	1	0	1	1	0
Age							
Hatch year	5	0	1	0	0	0	0
After hatch year	69	34	3	1	1	1	2
Unknown	2	0	0	0	0	0	0
Season[a]							
Spring	49	29	2	1	1	1	0
Summer	27	5	2	0	0	0	2
Total	76	34	4	1	1	1	2

[a]Spring includes February through April; summer includes May through July.

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negative panel of 14 antimicrobial agents (plate code CMV3AGNF, Sensititre; TREK Diagnostic Systems, Cleveland, OH): amoxicillin/clavulanic acid, ampicillin, azithromycin, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and trimethoprim/sulfamethoxazole. Clinical and Laboratory Standards Institute (CLSI) guidelines were used to interpret MIC values when available(CLSI, 2012, CLSI, 2008). Otherwise, MIC values were interpreted using NARMS breakpoints (FDA, 2013). Quality control was performed weekly using *Escherichia coli* ATCC 25922.

Results

In total, 117 birds were captured, including 76 Great-Tailed Grackles, 34 European Starlings (*Sturnus vulgaris*), 4 House Sparrows, 1 Brown-Headed Cowbird, 1 Cedar Waxwing (*Bombycilla cedrorum*), and 1 Eurasian Collared-Dove (*Streptopelia decaocto*). The number of birds caught per night ranged from 1 to 22, with a median of 16 birds. Of the birds captured, 63 were female, 21 were male, and 33 were of unknown sex. The majority of birds captured (n =

109) were determined to have hatched before the calendar year of capture (Table 2). All birds were judged to be in adequate health condition based on gross appearance, with no overt signs of disease.

Fecal samples were collected from 114 birds, with 34 sampled solely from collection bags, 34 solely from cloacal swabs, 37 using both methods, 6 from other surfaces, and 3 with no record of collection method. Overall, 1.8% (2/114; 95% CI 0.2%–6.2%) of samples were confirmed positive for *Salmonella*. Both positive birds were Great-Tailed Grackles sampled in June, yielding a 2.6% (2/76; 95% CI 0.3%–9.2%) apparent prevalence among this species. The Great-Tailed Grackles were sampled at different roosting sites and by different methods (collection bag and cloacal swab). Both isolates were serotyped as *Salmonella* Typhimurium, and both were pan-susceptible to the antimicrobial agents included in the NARMS panel.

Discussion

The occurrence of *Salmonella* in Great-Tailed Grackles represents a potential threat to public health, particularly in view of their population size and preferred habitat. Great-Tailed Grackles tend to congregate near human establishments, including grocery stores, restaurants, and gas stations, creating opportunities for zoonotic transmission through direct contact or foodborne exposure. Assuming that our sample is representative of the population of Great-Tailed Grackles in the study area, we estimate that over 50 *Salmonella*-positive birds were present in this area on a given night during the study period, based on the apparent prevalence among this species (2.6%) and our conservative estimate of local population size (2,200). This is likely to be an underestimate, as fecal culture does not have perfect sensitivity for detecting the presence of *Salmonella*.

In addition to detection methods, sampling location and time of year influenced our results. Studies on *Salmonella* shedding among wild birds in agricultural settings have generated higher prevalence estimates (Callaway *et al.*, 2014), suggesting that roosting near dairies and feedlots may be a risk factor for positive *Salmonella* status among birds. Birds in urban locations may have a lower risk of *Salmonella* exposure, relative to birds that are in contact with cattle and agricultural environments. While *Salmonella* is known to persist in the environment for long periods of time, this has primarily been documented in areas used for farming or areas where another source of contamination is present (Winfield & Groisman, 2003, Toth *et al.*, 2011). Another potential factor is the time of year during which samples were collected. Although we sampled birds from February through July, the two Great-Tailed Grackles found to be positive for *Salmonella* Typhimurium were collected during the month of June. In cattle and horses, *Salmonella* shedding has been shown to follow distinct seasonal trends with summer and fall yielding the highest prevalence (Carter *et al.*, 1986, Fossler *et al.*, 2005, Cummings *et al.*, 2009). It may be that *Salmonella* prevalence would increase if samples were collected during late summer and fall, as seen in livestock. As *Salmonella* thrives in warm, moist environments, this trend in seasonality is most likely related to temperature and moisture conditions that predominate in the summer and fall months (Strawn *et al.*, 2013a, Marine *et al.*, 2015).

Resistance to antimicrobial agents that are included on the NARMS panel was not evident in the two study isolates. Presumably there is a lack of selection pressure for antimicrobial resistance among wild birds and other wildlife in most environments, relative to animals in agricultural settings. However, further research to generate a larger number of isolates would be necessary to evaluate the prevalence of antimicrobial resistance among *Salmonella* isolated from Great-Tailed Grackles.

Both isolates were serotyped as *Salmonella* Typhimurium. According to the most recent CDC data, Typhimurium is the second most commonly reported serotype in human patients with laboratory-confirmed salmonellosis in the United States (Crim *et al.*, 2015). This highlights the potential threat to public health posed by Great-Tailed Grackles. Typhimurium is also the major cause of salmonellosis in wild birds and has been implicated in widespread outbreaks among songbirds in the United States (Hernandez *et al.*, 2012b) and other areas of the world (Alley *et al.*, 2002, Fukui *et al.*, 2014). Mortality among infected birds can be high (Hernandez *et al.*, 2012b), and evidence suggests that the proportion of avian mortality attributed to salmonellosis may be increasing (Hall & Saito, 2008). On a broader scale, Typhimurium is reported to be one of the most common serotypes among clinical and nonclinical laboratory-confirmed nonhuman sources (CDC, 2014), underscoring the wide host range and complex ecology of this serotype.

CHAPTER III

PREVALENCE OF *SALMONELLA* AMONG WATERFOWL ALONG THE TEXAS GULF COAST[*]

Overview

Migratory waterfowl may play a role in the ecology and transmission of zoonotic pathogens, given their ability to travel long distances and their use of varied habitats. Our objectives were to estimate the prevalence of *Salmonella* among waterfowl along the Texas Gulf coast and to characterize the isolates. Fecal samples were collected from hunter-harvested waterfowl at four wildlife management areas from September through November 2016. Standard bacteriologic culture methods were used to isolate *Salmonella* from samples, and isolates were characterized by serotyping and anti-microbial susceptibility testing. The apparent prevalence of fecal *Salmonella* shedding was 0.5% (2/375). Serotypes identified were Thompson and Braenderup, and both isolates were susceptible to all anti-microbial agents tested. Although fecal contamination of agricultural fields or surface waters could serve as a potential source of zoonotic *Salmonella* transmission, waterfowl along the Gulf coast during the fall hunting season appear to pose minimal risk.

Introduction

Waterfowl and other migratory birds are unique among wildlife in their potential to carry zoonotic pathogens across a widespread geographic area. Many species of waterfowl travel long distances during spring and fall migrations, crossing state and national borders and utilizing a

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variety of habitats to rest and feed. This could allow pathogens to be disseminated over a broad range in a relatively short time period. In the process, waterfowl may introduce emerging pathogens to a region, as well as novel strains of established pathogens or novel anti-microbial resistance phenotypes (Fries *et al.*, 2015, Guenther *et al.*, 2010, Luechtefeld *et al.*, 1980, Middleton & Ambrose, 2005).

Salmonella enterica is a leading cause of acute bacterial enteritis among people in the United States and throughout the world (Majowicz *et al.*, 2010). Exposure is typically foodborne (Scallan *et al.*, 2011), but transmission can also occur through direct contact with feces or contact with an environmental source contaminated by feces (Hoelzer *et al.*, 2011). *Salmonella* is commonly associated with livestock and poultry (Pires *et al.*, 2014), although various wildlife species can also serve as reservoirs (Cummings *et al.*, 2016, Grigar *et al.*, 2016). Research on *Salmonella* in wildlife has been relatively limited, but previous work in Europe and the United States indicates the potential for carriage among certain waterfowl species. Fecal deposits were collected from ducks in the United Kingdom over two winters (n = 477), and 4% were culture positive for *Salmonella* (Mitchell & Ridgwell, 1971). Conversely, no birds were found to be positive for *Salmonella* (n = 318) during the 2008–2010 waterfowl hunting seasons in Spain (Antilles *et al.*, 2015). In the United States, investigators were unable to culture *Salmonella* from 331 samples collected from ducks in Oklahoma (Waldrup & Kocan, 1985). However, 1.5% and 3.0% of serum samples collected were reactive to *Salmonella* serogroup B and D antigens, respectively. A similarly low prevalence (0.2%) was found among waterfowl in metropolitan parks in central Ohio (Fallacara *et al.*, 2001).

Although these prevalence estimates are low, the overall environmental impact of *Salmonella* shedding is magnified by the large size of some migratory and residential flocks. In

urban and suburban areas, large flocks of waterfowl can generate substantial accumulations of feces in recreational parks and private lawns (Conover & Kania, 1991, Feare *et al.*, 1999). Waterfowl and other birds have also been found to be a significant source of fecal material in recreational waters (Edge & Hill, 2007, Gorham & Lee, 2015, Meyer, 2005). In rural areas, waterfowl frequently occupy agricultural fields and surface waters for resting and feeding (Fox *et al.*, 2016), with possible implications for food safety. Furthermore, *Salmonella* may survive in the environment for extended durations (Feare *et al.*, 1999, You *et al.*, 2006). Thus, the objectives of this study were to estimate the prevalence of *Salmonella* among waterfowl along the Texas Gulf coast and to characterize the isolates.

Materials and Methods

Fecal samples were collected via cloacal swab from hunter-harvested waterfowl during the beginning of the 2016 hunting season (September–November). Samples were collected at four wildlife management areas (WMAs) along the Texas Gulf coast: Justin Hurst WMA in Brazoria County (September 17), Mad Island WMA in Matagorda County (September 24 and November 5), J.D. Murphree WMA in Jefferson County (October 29) and Guadalupe Delta WMA in Calhoun County (November 26). Species, sex and age group were recorded for each bird sampled. Cloacal swabs were collected within 6 hr of harvest and were placed into 10 mL of buffered peptone water (World Bioproducts, Woodinville, WA). Swabs were transported on ice to the research laboratory within 10 hr of collection and were then incubated for 18–24 hr at 37°C. Culture methods from Grigar *et al.* (2016) were used to isolate *Salmonella* from samples. Presumptive *Salmonella* isolates were confirmed by amplification and detection of the *invA* gene using PCR as previously described (Kim *et al.*, 2007).

Confirmed *Salmonella* isolates were sent to the Clinical Microbiology Laboratories at the University of Pennsylvania Veterinary Hospital for serotyping using the xMAP® *Salmonella* Serotyping Assay (Luminex, Austin, TX), as described in Grigar *et al.* (2016). Anti-microbial susceptibility of confirmed *Salmonella* isolates was determined by use of the broth microdilution method. Minimal inhibitory concentrations (MICs) were established for each isolate against the National Antimicrobial Resistance Monitoring System (NARMS) Gram-negative panel of 14 anti-microbial agents (Sensititre; TREK Diagnostic Systems, Cleveland, OH): amoxicillin/clavulanic acid, ampicillin, azithromycin, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline and trimethoprim/sulfamethoxazole. Clinical and Laboratory Standards Institute (CLSI) guidelines were used to interpret MIC values when available (CLSI, 2012, CLSI, 2008). Otherwise, MIC values were interpreted using NARMS breakpoints (Centers for Disease Control and Prevention) (CDC, 2016a).

Results

From September through November of 2016, 375 fecal samples were collected from hunter-harvested waterfowl along the Texas Gulf coast, with a median of 82 birds sampled per collection date. The following species were represented: Blue-winged Teal (*Anas discors*, 260), Gadwall (*Anas strepera*, 26), Green-winged Teal (*Anas crecca*, 25), Northern Shoveler (*Anas clypeata*, 18), Black-bellied Whistling-Duck (*Dendrocygna autumnalis*, 17), Northern Pintail (*Anas acuta*, 8), American Coot (*Fulica americana*, 7), American Wigeon (*Anas americana*, 6), Redhead (*Aythya americana*, 3), Ring-necked Duck (*Aythya collaris*, 2), Cinnamon Teal (*Anas cyanoptera*, 1), Mottled Duck (*Anas fulvigula*, 1) and Ruddy Duck (*Oxyura jamaicensis*, 1).

Among all sampled waterfowl, 243 (64.8%) were male and 132 (35.2%) were female. A total of 332 (88.5%) were recorded as adults and 43 (11.5%) as juveniles.

Two samples (0.5%) were confirmed positive for *Salmonella* (95% CI, 0.1%–1.9%). Both samples were collected from adult female birds at Mad Island WMA in Matagorda County: a Blue-winged Teal sampled in September and a Redhead sampled in November. Serotypes identified were Thompson (Blue-winged Teal) and Braenderup (Redhead). Both isolates were susceptible to all anti-microbial agents tested.

Discussion

To our knowledge, this is the first study to investigate *Salmonella* shedding among waterfowl along the Gulf coast of the United States. The apparent prevalence of *Salmonella* shedding in this sample of birds was <1%, although this is presumably an underestimate of the true prevalence given the sensitivity of fecal *Salmonella* culture (House *et al.*, 1993, Smith *et al.*, 1994). Waterfowl in this region during the fall hunting season thus appear to pose minimal risk of *Salmonella* transmission to humans or domestic food animal species. Nevertheless, fecal contamination of agricultural fields or surface waters could serve as a potential source of zoonotic transmission through foodborne or waterborne routes. In addition, the dynamics of fecal *Salmonella* shedding may be different among northbound waterfowl during spring migration.

Salmonella Braenderup and *Salmonella* Thompson, identified in this study, are among the top 15 serotypes isolated from human patients with laboratory-confirmed salmonellosis in the United States (CDC, 2016b). Both serotypes have been implicated in human salmonellosis outbreaks traced to the consumption of produce, particularly tomatoes (Bennett *et al.*, 2015). These serotypes have also been frequently isolated from environmental sources, including

produce fields and irrigation ponds, in the United States (Bell *et al.*, 2015, Li *et al.*, 2014, Maurer *et al.*, 2015, McEgan *et al.*, 2014, Strawn *et al.*, 2013b). *Salmonella* Typhimurium, the major cause of salmonellosis among passerines in the United States and other areas of the world (Hall & Saito, 2008, Hernandez *et al.*, 2012b), was not identified among waterfowl in this study, underscoring host ecologic differences with corresponding discrepancies in pathogen exposure.

Resistance to anti-microbial agents that are included on the NARMS panel was not detected in the two *Salmonella* isolates from this study. Anti-microbial selection pressure faced by enteric bacteria of waterfowl and other wildlife is presumably negligible in most environments, relative to animals raised in agricultural settings. However, additional research to generate a larger number of isolates would be necessary to adequately evaluate the prevalence of anti-microbial resistance among *Salmonella* isolated from waterfowl.

CHAPTER IV

THE INFLUENCE OF WILD BIRDS AND ENVIRONMENTAL FACTORS ON THE PREVALENCE *SALMONELLA* IN CATTLE AT A TEXAS FEEDLOT

Overview

Wild birds commonly reside at feedlots in large numbers during winter months when food resources are scarce. These birds have the potential to introduce and spread novel pathogens or phenotypes throughout the feedyard. The objective of this study was to determine the prevalence of *Salmonella* in cattle and birds, the factors associated with *Salmonella* shedding, and the effect that wild birds have on the burden of *Salmonella* among cattle. Birds were harvested by hunters from January–February of 2016. For cattle, fecal pats were collected three times over the course of the feeding period from four pens during the winter and summer. For all samples, demographic, meteorological, and environmental data were collected. Standard bacteriologic methods were used to isolate *Salmonella*. Antimicrobial susceptibility testing was performed on a subset of cattle isolates and all avian isolates. The apparent prevalence in birds was 29.2% (95% confidence interval [CI], 20.1–38.3), with sex significantly associated with positive status. Overall apparent prevalence in bovine samples was 72.5% (95% CI 69.2–75.8). Pen number, number of days on feed, temperature at sampling, and low temperature on sampling were all significantly associated with *Salmonella* shedding in cattle. Presence of birds was not significantly associated with *Salmonella* shedding among cattle. Resistance to antimicrobials was not detected in either avian or bovine isolates. Given this, birds may not play an important role in the epidemiology of *Salmonella* among feedlot cattle.

Introduction

Salmonella is a common pathogen in cattle with most individuals shedding subclinically (Dodd *et al.*, 2011). In feedlots, the prevalence of *Salmonella* shedding can vary dramatically (Dodd *et al.*, 2011, Dargatz *et al.*, 2003, Smith *et al.*, 2016). Many of the serotypes associated with beef cattle production are also some of the most important serotypes involved in human salmonellosis cases including Typhimurium, Newport, Montevideo, and Muenchen (Dargatz *et al.*, 2003, Dodd *et al.*, 2011). Given the recovery of *Salmonella* from hides (Khaitsa *et al.*, 2007) and lymph nodes in beef carcasses (Gragg *et al.*, 2013) there is particular concern that *Salmonella* prevalence in beef cattle may have an impact on public health. Guo *et al.* (2011) estimate that 29% of *Salmonella* infections in the U.S. are the result of consumption of contaminated beef.

Wildlife can act as reservoirs and passive carriers of Salmonella (Skov *et al.*, 2008). European starlings (*Sturnus vulgaris*), Brown-Headed Cowbirds (*Molothrus ater*), Common Grackles (*Quiscalus quiscula*), and Rock Doves (*Columba livia*) commonly congregate in concentrated animal feeding operations such as feedlots and dairies and are known to shed *Salmonella* (Callaway *et al.*, 2014, Pedersen *et al.*, 2006). These birds concentrate at feedlots during the winter months when food resources are scarce to exploit the open-trough feeding system used in many operations (Homan *et al.*, 2010) leading to economic loss for producers (Besser *et al.*, 1968).

The objective of this study was to determine the prevalence of *Salmonella* shedding in cattle and birds in the study area, the factors associated with *Salmonella* shedding, and the effect that wild birds have on the burden of *Salmonella* among cattle.

Methods

Bird Collection and processing

Birds were harvested by hunters at three time points during January and February 2016. Harvested birds were kept in a cooler at approximately 4°C for transport to the lab. Species, age category (adult or juvenile), and sex were recorded for each bird. Once at the lab, birds were dissected under a biosafety cabinet. The cloacal contents were removed and placed into 10 mL of buffered peptone water (World Bioproducts, Woodinville, WA). Samples were then processed using methods described in Grigar *et al.* (2016).

Cattle fecal sample collection and processing

Cattle pens were selected to be geographically dispersed throughout the feedlot and to hold cattle that had just entered the lot. Two groups of four cattle pens were sampled. The first group consisted of pens sampled during the winter months when birds were ubiquitous at the feedlot and the second group was sampled during the summer months when birds were scarce in the feedlot. Cattle pens were sampled three times over the course of the feeding period: entrance into the feedlot, 90 days on feed, and shortly prior to harvest. Thirty samples were taken from voided fresh fecal pats evenly collected throughout the pen during each collection. Special attention was taken to collect samples from fecal pats near feed bunks and water troughs where both cattle and birds spend more time. Fecal samples were placed into sterile 50 mL Falcon tubes (BD Biosciences, Bedford, MA) and kept in a cooler at ~4°C. Once at the lab, samples were processed using methods described in Cummings *et al.* (2016).

Collection of meteorological and environmental data

To determine the effect that environmental factors have on *Salmonella* prevalence, meteorological and feedlot environmental data were collected at each sampling time.

Meteorological data included: high and low temperature for the sampling day, temperature at sampling, relative humidity at sampling, dew point at sampling, total precipitation during the week prior to sampling, total precipitation during the day prior to sampling, total precipitation on sampling day, wind speed at sampling, and wind direction at sampling. All meteorological data were obtained from the National Weather Service for the feedlot's location. If local data were not available, data from the closest weather forecast office was used. Environmental conditions at the feedlot were measured using a mud score at each sampling period as described in Grandin (2016).

Salmonella *characterization*

Sixty isolates were selected for antimicrobial susceptibility testing including all avian isolates and a subset of 32 bovine isolates. Bovine isolates were randomly selected equally from each pen during every sampling period. The broth microdilution method was used to determine antimicrobial susceptibility. Minimal inhibitory concentrations (MIC) were established for each isolate against the National Antimicrobial Resistance Monitoring System (NARMS) Gram-negative panel of 14 antimicrobial agents (Sensititre; TREK Diagnostic Systems, Cleveland, OH): amoxicillin/clavulanic acid, ampicillin, azithromycin, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and trimethoprim/sulfamethoxazole.

Statistical analysis

Statistical analysis was performed using SAS (version 9.4; SAS Institute Inc., Cary, NC). Avian and bovine samples were analyzed separately. To relate the two groups of samples, a variable was created describing the prevalence of the opposite group during the same point in the sampling period. For example, when analyzing avian samples a variable was created that

described the prevalence in bovine samples during the corresponding time period. Variables were individually assessed, and those with p-values ≤ 0.20 were eligible for inclusion in a multivariable analysis. Multivariate logistic regression was used to develop a model to describe avian samples while a generalized estimating equation approach was used for bovine samples. To account for longitudinal sampling in bovine samples, an auto-regressive correlation structure was used. Clustering was also present in the bovine samples and was controlled at the highest level which was determined to be sampling group. A model was developed using backwards elimination with p-values < 0.05 considered significant.

Results

Over the course of the study period 96 birds were harvested and sampled from January to February of 2016. Species sampled included Brown-Headed Cowbirds (n = 44), Common Grackles (n = 4), Eurasian Collard-Doves (*Streptopelia decaocto, n* = 19), European Starlings (n = 1), Great-Tailed Grackles (*Quiscalus mexicanus, n* = 6), House Sparrows (*Passer domesticus*, n = 1), and Rock Doves (n = 21). All sampled birds were adults. The sex of 41.7% of birds could not be determined, 38.5% were determined to be male, and 19.8% were female. Overall prevalence of *Salmonella* shedding in birds was 29.2% (95% confidence interval [CI], 20.1–38.3). Bivariable analysis concluded that prevalence differed significantly by sex (p = 0.0009) and species (p < 0.0001).

A total of 720 samples were collected from January to July of 2016. Winter pens were sampled in January, February, and May. Summer pens were sampled in March, June, and July. Overall prevalence of *Salmonella* shedding in cattle was 72.5% (95% CI 69.2–75.8). The prevalence within winter pens was 74.7% (269/360, 95% CI 70.2–79.2). Prevalence within summer pens was 70.3% (253/360, 95% CI 65.6–75.0). Bivariable analysis showed that all

variables except presence of birds was significantly associated. Multivariable analysis concluded that number of days on feed, head of cattle per pen, temperature at sampling, high temperature on sampling day, and mud score were significantly associated (Table 3). No interaction terms were found to be significant. For every additional day on feed the odds of *Salmonella* isolation increased by 0.94 (95% CI 0.91–0.97, $p < 0.0001$) when all other variables are held constant. The stocking density of the pens had a slight effect on the odds of *Salmonella* isolation (OR 0.99, 95% CI 0.98–1.00, $p = 0.0234$). A similar effect was illustrated for temperature at sampling (OR 1.64, 95% CI 1.22–2.20, $p = 0.0009$) and high temperature (OR 0.70, 95% CI 0.56–0.86, $p = 0.0009$). When compared to a mud score of 1 the odds of *Salmonella* isolation was higher as mud score increased. The odds for *Salmonella* isolation by 0.47 times (95% CI 0.21–1.01, $p = 0.0534$) higher when compared to a score of 2 and 37.13 times (95% CI 1.71–249.88, $p = 0.0002$).

Antimicrobial susceptibility test found all tested isolates to be pan-susceptible.

Table 3 Generalized estimating equation parameter estimates for final model of bovine samples

			95% CI			
Parameter	*β*	*SE*	*Lower*	*Upper*	*Z*	*p*
Intercept	-3.18	1.94	-6.99	0.63	-1.64	0.1017
Number of Days on Feed	-0.07	0.01	-0.09	-0.04	-4.84	< 0.0001
Cows per Pen	-0.01	0.01	-0.02	0.00	-2.15	0.0318
Sampling Temperature	0.50	0.14	0.23	0.77	3.61	0.0003
High Temperature	-0.33	0.09	-0.51	-0.14	-3.48	0.0005
Mud Score[1]						
1 vs. 2	-0.82	0.50	-1.79	0.16	-1.65	0.0995
1 vs. 4	3.44	0.69	2.09	4.79	4.99	< 0.0001

[1]No study periods had a mud score of 3

Discussion

While there have been some studies on the effect of wild birds at CFAOs (Callaway *et al.*, 2014, Dodd *et al.*, 2011), few have looked at both the interaction of wild birds and the environment on the prevalence of *Salmonella* shedding in a feedlot. The apparent prevalence in wild birds in this study was 29.2%, which is much higher than what has been reported in the same or similar species in non-agricultural environments (Grigar *et al.*, 2016). This may indicate that proximity to CAFOs is a risk factor for *Salmonella* shedding in birds. Multivariable analysis concluded that sex had a significant effect on the prevalence of *Salmonella* shedding. This may be affected by the species of the bird as the sex of Rock Doves and Eurasian Collared Doves could not be determined and therefore made up the majority of the reference category (unknown). Descriptive analysis found that most of the birds sampled from these two species were not shedding *Salmonella* and bivariable analysis concluded that species had a significant effect. However, species was not significant when included in a multivariable model.

Interestingly, the presence of birds did not have a significant effect on the prevalence of *Salmonella* among cattle samples. As stated previously, this may be an indication that cattle have an effect on *Salmonella* shedding in bird populations but that the reverse is not true. Number of days on feed, cattle per pen, temperature at sampling, the high temperature on sampling day, and mud score were all found to have a significant effect on the odds of isolating *Salmonella* from bovine samples. The increase of *Salmonella* shedding over the course of the feeding period has been demonstrated in numerous studies (Dodd *et al.*, 2011, Khaitsa *et al.*, 2007). As the presence of birds was not found to have a significant effect, this increase may be due to other factors such as the stocking density of CFAOs. This is illustrated by the effect that the number of cattle per pen had on the multivariable model. Higher stocking density may lead

to higher prevalence of *Salmonella* shedding as there are more available hosts. *Salmonella* is known to have seasonality and shedding in cattle and horses typically increases during the summer and fall (Cummings *et al.*, 2009, Dargatz *et al.*, 2003, Pangloli *et al.*, 2008). Given this, it is not unexpected that temperature at sampling and the high temperature for the sampling day had a significant effect on *Salmonella* prevalence in cattle samples. *Salmonella* thrives in warm, moist environments which likely increase exposure and infection of cattle especially in CFAOs (Cummings *et al.*, 2016). Mud score was also found to have a significant effect on *Salmonella* shedding. This further illustrates the preference of *Salmonella* for warm, moist environments.

Resistance was not detected in any of the isolates tested. For birds this is similar to other studies (Grigar *et al.*, 2016). Prevalence of antimicrobial resistance is typically higher in cattle, (Dargatz *et al.*, 2003); however, the small sample size of cattle isolates tested ($n = 31$) may not reflect the true prevalence of resistance.

CHAPTER V

CONCLUSIONS

The prevalence of *Salmonella* in North American birds varies widely between species and geographical areas. Prevalence among birds appears to be closely linked to habitat and feeding activities. Gulls and passerine species typically exhibit the highest prevalence and frequently suffer from associated outbreaks. These species' utilization of human-associated environments and food sources, such as feeders and landfills, greatly impact the prevalence of *Salmonella* in these species. Given the proximity that these and other species have to human environments, the investigation of the potential risks birds pose to human and animal health is critical to public health. The studies discussed in this dissertation demonstrate the variability of *Salmonella* shedding in wild birds in Texas.

Urban birds were found to have a low prevalence of *Salmonella* shedding. However, these birds also congregate in very large numbers near human habitations suggesting that while shedding is low, these birds may still pose a risk to public health. Furthermore, the serotype isolated from urban birds, Typhimurium, is very important in the epidemiology of *Salmonella* in humans with large numbers of human cases linked to Typhimurium every year. Like urban birds, waterfowl along the Texas coast were rarely found to shed *Salmonella*. Nevertheless, the migratory nature of waterfowl may increase the propensity for these species to disseminate *Salmonella* across a large area despite only shedding intermittently. The serotypes isolated from waterfowl, Thompson and Braenderup, are also commonly linked to outbreaks in produce. Waterfowl commonly utilize agricultural fields and surface waters, which may be used for

irrigation. Therefore, *Salmonella* shedding among waterfowl may still pose a slight risk to human health.

Conversely, birds sampled in a Texas feedlot had a much higher prevalence of *Salmonella* shedding than waterfowl or urban birds. This comparison is particularly interesting considering that many of the same species were sampled in an urban environment. This suggests that there may be environmental factors, such as proximity to cattle, which affect the prevalence of *Salmonella* shedding in these birds. Multivariable analysis concluded that *Salmonella* shedding in cattle was not significantly affected by the prevalence in birds. However, these species also utilize urban areas and may spread *Salmonella* to other areas where it could be more easily transmitted to humans.

Overall, the studies presented here help to build a more accurate picture of the epidemiology of *Salmonella* in wild birds. A strength of these studies is that culture isolation methods are used. This allows any results to more accurately indicate that the individuals from which *Salmonella* was isolated are actively shedding *Salmonella*. The reliance on culture methods also present limitations. Viable but not culturable (VBNC) cells may be missed as well as *Salmonella* that may be shed in small numbers. These cells may be more accurately accounted for through the use of molecular methods. Another limitation of these studies are the small scales on which they were performed. The results found in the limited locations studied may not accurately represent the epidemiology of *Salmonella* in wild birds elsewhere. Specifically regarding the study described in Chapter IV, the collection of fresh fecal pats may not accurately describe the prevalence of shedding in the cattle as some cattle could be shedding *Salmonella* at a higher rate than others in the pen.

Future studies should focus on expanding these studies to other areas as well as year-round sampling. Further studies on waterfowl will benefit from evaluating the effect of spring migration and flyway. During the winter months waterfowl may be exposed to more sources of *Salmonella* and could shed *Salmonella* during spring migration back to summer breeding grounds. Prevalence of *Salmonella* shedding may also differ by flyway. Studies evaluating birds in feedlots may benefit from direct fecal collection from cattle as well as carcass or hide sampling at harvest. This will allow a more accurate evaluation of the shedding among cattle and what effect this shedding may have on contamination at harvest.

In conclusion, *Salmonella* shedding by wild birds has the potential to pose a threat to human and animal health. The studies described here illustrate that a multitude of factors, such as habitat and population numbers, impact the prevalence of *Salmonella* in birds. Future research should be focused on more fully describing the epidemiology of *Salmonella* in wild birds including seasonal trends, geographical factors, and human interactions.

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APPENDIX

Group	Study	Location	Species	Apparent Prevalence
Waterfowl	Hussong *et al.* 1979	Chesapeake Bay	Canada Goose	0.0% (0/44)
			Tundra Swan	
	Milani *et al.* 2012	Alaska	Tundra Swan	0.0% (0/100)
	Bradshaw & Trainer 1966	Wisconsin	Canada Goose	0.0% (0/12)
		Illinois	Mallard	0.0% (0/55)
	Youatt & Fay 1968	Michigan	Ducks	0.0% (0/4)
	Smith *et al.* 2002	California	Western Grebe	0.0% (0/14)
			Common Loon	0.0% (0/2)
	White *et al.* 1979	Florida	Common Loon	14.2% (27/190)
	Duncan *et al.* 1983*	California	Eared Grebe	100.0% (1/1)
	Fallacara *et al.* 2001	Ohio	Mallard	0.2% (1/449)
			Canada Goose	

Group	Study	Location	Species	Apparent Prevalence
	Fallacara *et al.* 2004	Ohio	Canada Goose	1.7% (8/450)
	Siembieda *et al.* 2011	California	Mallard	8.1% (3/37)
			Canada Goose	
	Pedersen *et al.* 2013	Michigan	Mute Swan	0.6% (3/459)
		New Jersey		
		Rhode Island		
		New York		
		Indiana		
	Waldrup & Kocan 1985	Oklahoma	Ducks	0.0% (0/331)*
	Grigar at al. 2017	Texas	Redhead	0.5% (2/375)
			Blue-Winged Teal	
	Jokinen *et al.* 2010	British Columbia	Ducks	0.0% (0/23)
			Geese	13.3% (2/15)

Group	Study	Location	Species	Apparent Prevalence
	Jokinen *et al.* 2011	Alberta	Ducks	7.9% (3/38)
			Geese	9.9% (8/81)
	Gruszynski *et al.* 2014a	Atlantic Flyway	Ducks	0.4% (1/262)
	Quortrup *et al.* 1957	California	American Coots	5.0% (9/181)
			Northern Pintail	0.2% (1/651)
	Rosen *et al.* 1957	California	American Coots	9.3% (80/862)
Sea Birds	Hall & Saito 2008	United States	Ring-Billed Gulls	10.8% (20/186)
and			Double-Crested Cormorants	8.1% (15/186)
Shorebirds			American While Pelicans	7.5% (14/186)
	Kinzelman *et al.* 2008	Wisconsin	Ring-Billed Herring Gulls	0.7% (5/724)

Group	Study	Location	Species	Apparent Prevalence
	Snoeyenbos *et al.* 1967	Massachusetts	Herring Gulls	2.5% (10/405)
			Egg Contents	1% (1/80)
	Lévesque *et al.* 2000[†]	Quebec	Ring-Billed Gulls	1.2×10^4–2.3×10^2 CFU/g
	Quessy & Messier 1992	Montreal	Ring-Billed Gulls	8.7% (23/264)
	Stoddard *et al.* 2008	California	Western Gulls	9.2% (8/87)
	Berg & Anderson 1972	Oregon	Gulls	2.1% (11/521)
	Gruszynski *et al.* 2014a	Virginia	Gulls	31.0% (9/29)
	Gruszynski *et al.* 2014b	Virginia	Gull	17.2% (62/360)
	Faddoul *et al.* 1966	Massachusetts	Gulls	10.0% (1/10)
		Rhode Island		
	Mikaelian *et al.* 1997*	Quebec	Ring-Billed Bulls	100.0% (39/39)
			Black-Legged	
			Kittiwake	

Group	Study	Location	Species	Apparent Prevalence
	Brand *et al.* 1988	New York	Herring Gulls	5.0% (25/505)
			Great Black-Backed Gulls	
			Black Skimmer	
	Radwan & Lampky 1972*	Michigan	Herring Gull	100.0% (1/1)
	Hall *et al.* 1977	Idaho	Ring-Billed Gulls	33.3% (2/6)
			California Gulls	
	Smith *et al.* 2002	California	Common Murres	0% (0/14)
			Western Gulls	6.3% (2/32)
	Steele *et al.* 2005	California	Western Gulls	5.9% (1/17)
		Washington		
	White & Forrester 1979	Florida	Double-Crested Cormorants	11.5% (12/104)

Group	Study	Location	Species	Apparent Prevalence
	Meteyer *et al.* 1997	Minnesota Nebraska North Dakota South Dakota	Double-Crested Cormorants	27.8% (20/72)
	Clavijo *et al.* 2001*	Alberta	Double-Crested Cormorants	100.0% (1/1)
	Youatt & Fay 1968	Michigan	American Woodcocks	0.0% (0/3)
Wading Birds	Hernandez *et al.* 2016	Florida	White Ibises	
			Adults	12.6% (33/261)
			Nestlings	30.6% (22/72)
	Callaway *et al.* 2014	Texas	Cattle Egrets	12.5% (2/16)
	Phalen *et al.* 2010	Texas	Cattle Egrets	29.0–95.0%(4/29–21/22)
	Jokinen *et al.* 2011	Alberta	Pelicans	26.0% (5/19)

Group	Study	Location	Species	Apparent Prevalence
	Windingstad *et al.* 1977	Indiana	Sandhill Cranes	4.2% (2/48)
		Wisconsin		
	Kirkpatrick *et al.* 1986	New Jersey	Black-Crowned Night-Heron	5.4% (2/37)
			Glossy Ibis	
	Stroud *et al.* 1986*	Colorado	Whooping Crane	100.0% (1/1)
	Aguirre *et al.* 2009	Yucatan	Caribbean Flamingos	11.1% (3/27)
	Siembieda *et al.* 2011	California	Black-Crowned Night-Herons	4.5% (2/44)
	Hudson & Tudor 1957*	New Jersey	European Starlings	100.0% (3/3)
			Sparrows	100.0% (7/7)

Group	Study	Location	Species	Apparent Prevalence
Songbirds and Similar Birds			Brown-Headed Cowbird	100.0% (1/1)
			Rusty Blackbirds	100.0% (6/6)
	Wobeser & Finlayson 1969	Ontario	House Sparrows	91.8% (45/49)
	Bowes 1990*	British Columbia	House Sparrows	N/A
	Fichtel 1978*	Pennsylvania	Northern Cardinals	100.0% (6/6)
			Tree Sparrows	100.0% (8/8)
			White-Throated Sparrow	100.0% (1/1)
			Dark-Eyed Junco	100.0% (1/1)

Group	Study	Location	Species	Apparent Prevalence
	Locke *et al.* 1973*	West Virginia	Evening Grosbeaks	100.0% (7/7)
		Maryland	House Sparrows	N/A
			American Goldfinches	N/A
			Pine Siskins	100.0% (1/1)
	Hernandez *et al.* 2012*	Georgia	Pine Siskins	93.5 (87/93)%
		South Carolina	American Goldfinches	
		North Carolina		
		Tennessee	Northern Cardinals	
		Virginia	Other Species	
		West Virginia		

Group	Study	Location	Species	Apparent Prevalence
	Nesbitt 1974*	Florida	Blue Jay	100.0%
			Tufted Titmouse	
			Brown Thrasher	
			House Sparrow	
			Red-Winged Blackbird	
			Common Grackle	
			Northern Cardinal	
			Chipping Sparrow	
			White-Throated Sparrow	
			Ground Dove	

Group	Study	Location	Species	Apparent Prevalence
	Daoust *et al.* 2010*	Newfoundland	Common Redpoll	100.0% (336/336)
		Labrador	Pine Siskin	
		Nova Scotia	Purple Finch	
		New Brunswick	Evening Grosbeak	
		Prince Edward Island	American Goldfinch	
	Prescott *et al.* 1998*	Ontario	Common Redpolls	N/A
			Pine Siskins	
	Mikaelian *et al.* 1997*	Quebec	House Sparrow	100.0% (6/6)
	Faddoul *et al.* 1966*	Massachusetts	Bird Consignments	12.0% (12/100)
		Rhode Island	Brown-Headed Cowbirds	66.7% (8/12)
				21.4% (3/14)
			Sparrows	

Group	Study	Location	Species	Apparent Prevalence
	Radwan & Lampky 1972	Michigan	Red-Winged Blackbirds	25.0% (15/60)
			Rudy-Throated Hummingbird	
			Lesser Yellowlegs	
			Brown-Headed Cowbirds	
	Tizard *et al.* 1979	Ontario	European Starlings	0.0% (0/22)
			House Sparrows	15.0% (9/60)
	Janecko *et al.* 2015	United States	American Crows	1.7% (17/990)
		Canada	Common Ravens	2.0% (1/49)

Group	Study	Location	Species	Apparent Prevalence
	Snoeyenbos *et al.* 1967	Massachusetts	Red-Winged Blackbirds	0% (0/42)
			Common Grackles	1.9% (2/108)
			Brown-Headed Cowbirds	3.7% (11/299)
			European Starlings	8.8% (13/148)
	Grigar *et al.* 2016	Texas	Great-Tailed Grackles	1.8% (2/114)
			European Starlings	

Group	Study	Location	Species	Apparent Prevalence
	Brobey *et al.* 2017	Texas	Mourning Dove	20.0% (1/5)
			Inca Dove	33.3% (1/3)
			Blue Jay	100.0% (1/1)
			Northern Cardinal	23.1% (6/26)
			American Goldfinch	28.6% (2/7)
			House Sparrow	20.7% (6/29)
			Red-Bellied Woodpecker	50.0% (2/4)
			Yellow-Bellied Sapsucker	50.0% (1/2)

Group	Study	Location	Species	Apparent Prevalence
	Espinosa- Argüelles *et al.* 2010	Mexico	White-Winged Doves	26.3% (53/201)
			Mourning Doves	
	White *et al.* 1981	Florida	Blue Jays	0.0% (0/21)
			Mourning Doves	0.0% (0/22)
			Red-Winged Blackbirds	0.0% (0/21)
			Ground Doves	0.0% (0/21)
			Eastern Towhee	10.0% (2/20)
			Common Grackles	8.0% (2/25)
			American Crows	14.3% (6/42)
			Northern Cardinals	5.6% (1/18)
	Hamer *et al.* 2012	Illinois	Red-Winged Blackbirds	0.8% (1/180)

Group	Study	Location	Species	Apparent Prevalence
	Morishita *et al.* 1999	Ohio	House Sparrows	1.1% (4/373)
			European Starlings	7.1% (62/868)
	Barber *et al.* 2002	Illinois	Sparrows	0.0% (0/8)
			Starlings	0.0% (0/1)
			Birds Feces	7.9% (3/38)
	Craven *et al.* 2000	Georgia	European Starlings	9.7% (12/124)
			House Sparrows	
	Pao *et al.* 2014	Maryland	European Starlings	0.2% (1/446)
		Virginia		
	Callaway *et al.* 2014	Texas	Brown-Headed	12.9% (40/309)
			Cowbirds	27.5% (14/51)
			Common Grackles	
	Carson *et al.* 2011	Texas	European Starlings	2.5% (2/81)
	Gaulker *et al.* 2009	Kansas	European Starlings	0.7% (3/434)

Group	Study	Location	Species	Apparent Prevalence
	Kirk *et al.* 2002	California	Brown-Headed Cowbirds	3.2% (3/95)
			House Sparrows	3.1% (14/451)
			Brewer's Blackbirds	2.3% (1/44)
			House Finch	1.6% (1/61)
			European Starlings	1.3% (1/80)
			Red-Winged Blackbirds	1.3% (1/78)
			Rock Pigeons	1.3% (1/83)
	Pedersen *et al.* 2006	Colorado	Rock Doves	3.2% (9/277)

Group	Study	Location	Species	Apparent Prevalence
	Gorski *et al.* 2011	California	American Crows	6.7% (7/105)
			Spotted Towhee	
			White-Crowned Sparrow	
	Brittingham *et al.* 1988	Wisconsin	Passerines	0.0% (0/387)
			Woodpeckers	
	Babriele-Rivet *et al.* 2016	Montreal	Rock Pigeons	0.0% (0/187)
Raptors	Kirkpatrick & Trexler-Myren 1986	New Jersey	Red-Tailed Hawks	1.9% (2/105)
and	Kirkpatrick & Colvin 1986	New Jersey	Barn Owl	
Scavengers			Nestlings	8.5% (8/94)
			Nests	20.0% (5/25)
	Lamberski *et al.* 2003	California	Red-Tailed Hawks	30.0% (3/10)
			Cooper's Hawks	20.0% (2/10)
	Mikaelian *et al.* 1997*	Quebec	Great Horned Owls	100.0% (1/1)

Group	Study	Location	Species	Apparent Prevalence
	Smith *et al.* 2002	California	Northern Harriers	18.2% (2/11)
			Barn Owl	0.0% (0/17)
	Siembieda *et al.* 2011	California	Turkey Vultures	5.1% (2/39)
	Sulzner et al 2014	California	Turkey Vultures	20.0% (11/55)
	Winsor *et al.* 1981	Texas	Turkey Vultures	30.0% (6/20)
	Everard *et al.* 1979	Trinidad	Black Vulture	16.7% (1/6)
	Adesiyun *et al.* 1998	Trinidad	Black Vulture	33.3% (1/3)
Other	Glazner *et al.* 1967	Texas	Wild Turkey	0.0% (0/87)
Species	Trainer *et al.* 1968	Texas	Wild Turkey	0.0% (0/148)
	Peterson *et al.* 2002a	Texas	Wild Turkey	0.0% (0/70)
	Hopkins *et al.* 1990	Arkansas	Wild Turkey	0.0% (0/44)
	Charlton 2000	California	Wild Turkey	4.3% (14/324)
	Crupper & Applegate 2002	Kansas	Wild Turkey	4% (2/47)
	Roslien & Haugen 1970	Texas	Wild Turkey	15.4% (6/39)

Group	Study	Location	Species	Apparent Prevalence
	Hensley & Cain 1979	Texas	Wild Turkey	4.0% (10/249)
	Veatch *et al.* 1988	Kansas	Wild Turkey	0.6% (7/1164)
	Ingram *et al.* 2015	Georgia	Wild Turkey	12.5% (3/24)
		Florida		
	White *et al.* 1981	Florida	Wild Turkey	4.4% (18/411)
	Jay-Russel *et al.* 2014	California	Wild Turkey	22.5% (16/71)
	Rocke & Yuill 1987	Texas	Wild Turkey	0.0% (0/511)
	Youatt & Fay 1968	Michigan	Wild Turkey	0.0% (0/9)
			Ring-Necked Pheasants	0.0% (0/105)
	Howerth 1985*	Alabama	Wild Turkey	100.0% (1/1)

Group	Study	Location	Species	Apparent Prevalence
	Belding 1955	Michigan	Ring-Necked Pheasants	
			Adult	7.7% (5/65)
			Eggs	0.0% (0/36)
	Peterson *et al.* 2002b	Texas	Prairie Chickens	0.0% (0/24)
	Williams *et al.* 2000	Kansas	Northern Bobwhites	0.0% (0/25)

*Associated with a die-off, outbreak, or necropsy of a ill bird
†Concentration of *Salmonella* in droppings