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# West Nile Virus activity in central Iowa bird populations and the utility of wildlife rehabilitation centers in monitoring wildlife disease

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**West Nile Virus activity in central Iowa bird populations and the utility of  
wildlife rehabilitation centers in monitoring wildlife disease**

by

**Natalie Jean Randall**

A thesis submitted to the graduate faculty  
in partial fulfillment of the requirements for the degree of

**MASTER OF SCIENCE**

Major: Wildlife Ecology

Program of Study Committee:  
Julie A. Blanchong, Major Professor  
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Iowa State University

Ames, Iowa

2011

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## ABSTRACT

Little is known about the frequency, occurrence, or impacts of West Nile virus (WNV) on many Iowa wildlife species, including birds. The lack of knowledge about WNV in Iowa bird populations is partially due to difficulties associated with monitoring wildlife health. We evaluated the utility of wildlife rehabilitation centers for providing information about wildlife population health and disease monitoring, as well as assessing where on the central Iowa landscape birds have the highest risk of exposure to WNV. Specifically, we: 1) examined the records of two ecologically important groups of birds (raptors [orders Acciptriformes, Falconiformes, and Strigiformes] and corvids [family Corvidae]) submitted over a ten year period to the Wildlife Care Clinic (WCC), a rehabilitation facility in central Iowa, and analyzed whether there was significant variation in submission rates and diagnoses among years, seasons, and species, 2) sampled peridomestic birds submitted to the WCC as well as free-living peridomestic birds within the geographic area from which most of the WCC's submissions originated, and compared WNV prevalence between these groups, and 3) collected blood samples from free-living birds captured at central Iowa sites comprising gradients of urban, agriculture, and natural land uses, and tested them for exposure to WNV. Our results indicated that submission rates and diagnoses to the WCC varied among years, season, and species. In most cases, records were not of sufficient detail to draw specific conclusions regarding the actual causes of morbidity. However, the signature of WNV emergence in corvid submissions was evident within the rehabilitation data. We also found that the seroprevalence of WCC and field-captured birds were similar, suggesting WNV exposure in wildlife submitted to the WCC was representative of current WNV exposure rates in the wild. Across the central Iowa land uses, our results suggest that a bird's risk of WNV exposure increased in urban areas, possibly because urban landscapes often harbor standing water favorable for ornithophilic mosquito vector reproduction. We also found that the risk of exposure was significantly different between years (potentially due to weather differences), and among taxonomic families, where the Cardinalidae had the highest WNV seroprevalence. Overall, our findings provide insights regarding the frequency and occurrence of WNV in central Iowa bird populations and suggest wildlife rehabilitation

centers have the potential to provide useful wildlife health and disease prevalence information about free-living populations.

## CHAPTER ONE: GENERAL INTRODUCTION

Historically, disease in wildlife populations only became a concern to the general public in cases where human health or the health of domestic livestock were affected (Wobeser 2006). However, in the past two decades, scientists and researchers have become increasingly interested in other important aspects of wildlife disease, such as the role it plays in wildlife population ecology and its effect on species of conservation need (Daszak et al. 2000, Wobeser 2006). The development of the field of wildlife disease ecology has in turn facilitated the discovery of many evolutionarily old and new disease agents. Understanding the factors contributing to the emergence and spread of these disease agents in wildlife populations are critical to both public health and wildlife agencies for creating and implementing disease management strategies.

In 1999, a new zoonotic disease was detected in the New York City area of New York, USA. West Nile Virus (WNV), an arthropod-borne *Flavivirus* (family Flaviviridae), has since spread across North America, causing cases of illness and death in a variety of wildlife species, as well as domestic animals (particularly horses) and humans (CDC 2004). First discovered in the West Nile region of Uganda in 1937 (CDC 2004), WNV is considered one of the most widely distributed viruses in the world, with different strains reported in Africa, Asia, Australia, Europe, and North, Central, and South America (WHO 2008, Komar and Clark 2006). West Nile Virus exhibited a rapid spread across naïve North America, taking only six years for viral activity to expand throughout the lower 48 U.S. states. West Nile Virus was first detected in eastern Iowa (the state in which this study was conducted) in 2001, with all Iowa counties reporting WNV activity in 2002 (IDPH 2010). The virus is maintained primarily within a bird-mosquito-bird transmission cycle, where mosquitoes are the primary vector and birds the primary amplifying host (Kilpatrick et al. 2007). In particular, Komar et al. (2003) reported that several members of the orders Passeriformes and Charadriiformes experimentally infected with WNV were highly competent reservoirs. Kilpatrick et al. (2006) identified American Robins (*Turdus americanus*) as potential “super spreaders” of WNV in the Maryland/Washington D.C. area, and Komar et al. (2005) found that Northern Cardinals (*Cardinalis cardinalis*) and House Sparrows (*Passer domesticus*)

were the primary amplifying hosts in St. Tammany Parish, Louisiana. Although mammals can develop an infection if bitten by an infectious mosquito, this group is often considered a dead-end or spillover host in the transmission cycle, as they usually do not produce a viremia high enough to infect a biting arthropod vector (Kilpatrick et al. 2007).

Thus far, tens of thousands of individual birds have tested positive for WNV in North America but actual estimates of avian exposure, infection, and mortality rates have been difficult to ascertain (LaDeau et al. 2007). However, researchers have documented significant declines associated with WNV for certain avian groups, particularly those in the family Corvidae, but also for some species within the Turdidae and Paridae families (LaDeau et al. 2007). These population declines can affect an entire bird community dynamic, as well as the ecological integrity within a system due to the cascading effects of species losses. For example, declines in avian species that act as scavengers may result in the persistence of diseased carcasses in the environment, potentially increasing the risk of disease exposure for animals within that environment. Also, many tree and plant species are dependent on a threshold bird population for effective seed and nut dispersal (LaDeau et al. 2008). Additionally, bird species losses may lead to lower diversity within the bird community. Allen et al. (2009) found that increased WNV activity was correlated with low bird diversity, for which they suggest bird communities comprised of highly competent species may amplify WNV, without low reservoir competent species to dilute the virus circulating in the environment.

The presence of WNV (and other diseases) in wildlife populations is often identified through some method of passive or active surveillance (CDC 2003). Passive surveillance occurs when sick or dead animals are opportunistically collected, often by the general public, and submitted to public health or wildlife agencies (Stallknecht 2007). Although this method is a relatively inexpensive source of disease information for wildlife agencies, the lack of routine sample collection may result in underreporting or delayed detection (Eidson 2001). Alternatively, active surveillance focuses on collecting samples from a target wildlife population or searching for a particular disease agent within a wildlife community. This method has been identified as one of the best methods for early detection and response to disease in wild animals (Stallknecht 2007, Stitt et al. 2007). However, implementing



targeted and systematic surveillance programs for extended periods is often cost and time prohibitive for state and federal wildlife agencies (Eidson 2001). Specific surveillance methods used for detecting WNV activity have included passive forms, such as dead bird surveillance, and active forms, such as sentinel chicken flocks and mosquito trapping (CDC 2003).

In addition to traditional disease surveillance methods, wildlife rehabilitation centers may be an untapped resource for wildlife disease surveillance, as they regularly receive and care for a variety of wildlife species (Stitt et al. 2007). These facilities may be useful for disease surveillance because sick or injured wildlife submitted for rehabilitation may a) have been debilitated by the disease agent of interest, or b) sustained an unrelated injury allowing the disease agent to infect more easily (Wobeser 2006). Thus, wildlife rehabilitation patients tested for disease may have a higher prevalence compared to randomly sampled free-living animals in the wild. Although other studies examining morbidity and mortality in wildlife rehabilitation centers have suggested these facilities may play a role in wildlife disease surveillance (Wendell et al. 2002, Kelly and Sleeman 2003, Nemeth et al. 2007), this idea has not been rigorously evaluated. If these facilities are to be useful for wildlife disease surveillance, it is important to determine if disease frequency in wildlife rehabilitation patients is similar to disease frequency in the populations from which they originate. Wildlife rehabilitation centers may also have the potential to be sources of useful general information about wildlife health by tracking trends in rehabilitation submissions, as many states require that licensed rehabilitators keep detailed records of their patients.

Along with the use of surveillance to monitor the occurrence and frequency of disease in wild animal populations, public health and wildlife management agencies' ability to predict where the risk of exposure may be greatest, and to which populations, is important for devising disease management strategies that efficiently use limited resources. In the case of WNV, a number of studies have been conducted to ascertain the link between risk of exposure to WNV and land use. For example, Bradley et al. (2008) found that landscape factors associated with urban areas, such as impervious surface coverage by streets, houses, and buildings in the metropolitan area of Atlanta, Georgia corresponded with an increase in WNV antibody prevalence in songbirds. The authors suggested this may be in part due to the

availability of WNV vector breeding habitat associated with urban landscapes, as many mosquito species require fetid standing water, such as that found in sewers and catch basins, to reproduce (Epstein 2001). Gomez et al. (2008) also cited increased levels of urbanization as one of the factors associated with elevated seroprevalence in small mammals sampled in the mid-Atlantic region of the U.S. In the Midwest, DeGroote et al. (2008) and Liu et al. (2008) found that the incidence of WNV in humans and mosquitoes (respectively) was positively correlated with the presence of agriculture. However, research pertaining to whether the prevalence of WNV in avian species varies with land use in the Midwest is relatively scant. Given that the above studies have found increased prevalence of WNV in both humans and mosquito vectors in areas with a high proportion of agriculture, it would be useful to know if the primary amplifying hosts in the WNV cycle (birds) show similar exposure patterns. If so, birds could act as an indicator of WNV risk to humans and domestic animals in agricultural areas.

The objectives of my research were to evaluate the utility of wildlife rehabilitation centers for wildlife disease surveillance, as well as provide insight regarding how wildlife disease risk varies with land use. My first objective was to determine how useful routinely collected wildlife rehabilitation data are for extracting information about wildlife health and population disturbances. Secondly, I evaluated whether WNV exposure patterns in avian submissions to a wildlife rehabilitation facility reflected exposure patterns in free-living birds, which could provide valuable information regarding the prevalence of disease in the wild. For my last objective, I evaluated whether the risk of exposure to WNV in Midwestern peridomestic birds varied with land use in order to identify locations where birds were at greatest risk of encountering the virus. Overall, my research can provide wildlife managers with insights about the utility of wildlife rehabilitation data regarding wildlife health and disease surveillance. This research can also provide guidance to wildlife management agencies regarding where on the landscape the risk of WNV exposure may be greatest, as well as which wildlife species have the greatest risk of exposure. This information can be useful for devising efficient disease surveillance and management strategies.

## Thesis Organization

This thesis is composed of five chapters. Chapter 1 is a general introduction to topics covered within Chapters 2, 3, and 4. In Chapter 2, I investigated how useful routinely collected wildlife rehabilitation data are for deducing information about wildlife health and population disturbances. This chapter will likely be submitted to *The American Midland Naturalist*. In Chapter 3, I evaluated whether WNV exposure patterns in avian submissions to a wildlife rehabilitation facility reflected exposure patterns in free-living avians. In Chapter 4, I assessed whether the risk of exposure to WNV in Midwestern peridomestic birds varied with land use in order to identify locations where birds were at greatest risk of encountering the virus. Modified versions of the latter two chapters are intended for submission to the *Journal of Wildlife Diseases*. Chapter 5 is a general conclusion of my findings, management implications, and suggestions for future research. Mary Ann Nieves, former director of the Iowa State University Wildlife Care Clinic, oversaw data collection for Chapter 2, for which I (Natalie J. Randall) completed data analysis and written text, with editorial assistance from Julie A. Blanchong. I also completed data collection, analysis, and written text for Chapters 3 and 4, with editorial assistance from Julie A. Blanchong and laboratory assistance from Bradley J. Blitvich.

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## **CHAPTER 2: A TEN-YEAR RETROSPECTIVE STUDY OF AVIAN POPULATION TRENDS FROM WILDLIFE REHABILITATION SUBMISSIONS**

A paper to be submitted to *The American Midland Naturalist*

Natalie J. Randall, Mary Ann Nieves, Julie A. Blanchong

### **Abstract**

Identifying factors affecting wildlife health is of increasing importance to wildlife managers. Wildlife rehabilitation centers have the potential to be sources of useful information about wildlife health through trends in rehabilitation submissions, as licensed rehabilitators regularly receive and care for many wildlife patients, and are required to keep detailed records. To determine how useful routinely collected rehabilitation data are for deducing information about wildlife health and population disturbances, we examined the records of two ecologically important groups of birds (raptors [orders Accipitriformes, Falconiformes, and Strigiformes] and corvids [family Corvidae]) submitted over a ten year period to the Wildlife Care Clinic, a rehabilitation facility located in central Iowa. We analyzed rehabilitation submissions using a binomial model with an identity link to evaluate whether there was significant variation in submission rates and diagnoses among years, seasons, and species. Submission rates and diagnoses for raptors and corvids varied among years, seasons, and species. In most cases, records were not of sufficient detail to draw specific conclusions regarding the actual causes of morbidity within these groups. Despite a lack of formal disease testing, however, the signature of West Nile virus (WNV) emergence in corvid populations was strongly evident in the rehabilitation data. Specifically, the sharp rise in unsolicited American Crow submissions during the initial emergence of WNV in central Iowa, followed by a significant decline in these submissions during the next five years, is consistent with other, independent data suggesting populations of crows were depressed after the initial wave of disease. For wildlife rehabilitation center data to provide useful information about wildlife health to wildlife managers, we recommend that 1) more

detailed information should be collected and recorded about the circumstances (e.g., location, habitat, surroundings, weather, animal's behavior) under which wildlife rehabilitation patients are found, and 2) samples for disease testing should be routinely collected and archived. Finally, and perhaps most importantly, wildlife managers should communicate with wildlife rehabilitators regarding management objectives and species and diseases of particular concern. These actions would enhance the ability of this wealth of wildlife data to be useful to wildlife managers in making inferences regarding trends in wildlife populations from which rehabilitation patients originate.

## **Introduction**

The detection of disease in wildlife often occurs after the disease has become widespread, making control or eradication difficult (Woodroffe 1999). Establishment of such a disease may result in negative impacts to wildlife health, and, in the case of zoonotic disease, has the potential to spread to humans and domestic animals. For instance, West Nile Virus (WNV) has been associated with a significant decrease in corvid populations (Family Corvidae) since its introduction to North America in 1999 (LaDeau et al. 2007), and has subsequently resulted in cases of illness and death in both domestic animals (particularly horses) and humans (CDC 2004). Due to the negative impacts of WNV and other emerging infectious diseases (EIDs), developing effective sentinel methods has become a priority to detect wildlife disease early in its emergence. However, surveillance and monitoring can be costly and time consuming for wildlife management agencies to implement (Eidson 2001). Recently, wildlife rehabilitation centers have been identified as potential sources of wildlife health information because 1) the infrastructure is already in place to collect and examine many wildlife species from a relatively large geographic area, and 2) licensed wildlife rehabilitators are required to keep detailed records of patient submissions (Stitt et al. 2007).

The Wildlife Care Clinic (WCC), a wildlife rehabilitation center at Iowa State University in Ames, Iowa, has been providing rehabilitative care to wildlife since its establishment in 1984, with continuous records of wildlife submissions kept from 1999 to present. Most of the wildlife submitted to the WCC originate from central Iowa; however, submissions have come from across the state. Although most wildlife patients are birds and

mammals, the WCC also treats and cares for reptiles and amphibians. A recent review of WCC records indicated that, on average, they have received 483 patients annually over the last 10 years, representing a total of 26 orders, 64 families, and 153 species (WCC unpublished data).

In this retrospective review of WCC records, we examine two ecologically different yet important groups of birds regularly admitted to the WCC to determine if these data provide insights into the health of these free-living bird populations. These two groups are comprised of raptors (Orders Falconiformes and Strigiformes) and corvids (Family Corvidae).

Many raptors have been identified as important species of conservation need and additional knowledge about the condition of their populations may be useful for their conservation (Iowa Department of Natural Resources Wildlife Action Plan: <http://www.iowadnr.gov/wildlife/diversity/plan.html>). The presence of raptors in an ecosystem has been correlated with high biodiversity of various plant and animal taxa as well as ecosystem function, likely because these top predators select prey-productive habitats (Sergio et al. 2006). Thus, the presence of raptors may serve as an indicator of high biodiversity in an area. Also, decreases in top predator populations may suggest trophic level disturbance, as they often control populations of herbivorous prey species, whose numbers in turn affect vegetation composition, density, and structure (Schmitz et al. 2000, Berger et al. 2001). Lastly, raptors living in or near agricultural areas may moderate rodent populations often associated with large-scale farming, as rodents comprise a substantial part of their diet. For these reasons, monitoring raptor submission trends at rehabilitation centers may be important, since these records can indicate disturbances in local or regional raptor populations. In addition, it may be valuable to know the final disposition of raptors admitted to rehabilitation clinics and thus how often rehabilitative care results in returning these animals to the wild.

The careful tracking of submissions to rehabilitation centers may identify changes in submission patterns, potentially suggesting the presence of some disturbance event in wild populations, such as an EID, that may warrant further investigation. To explore this possibility, we examined corvid submissions to the WCC from 1999 to 2008. Because this



time frame includes the emergence of WNV in Iowa (2001), to which corvids have been found to be especially susceptible (Komar et al. 2003), post hoc examination of these records for changes in corvid submission rates or temporal patterns may indicate a signal of WNV occurrence in the environment.

Our objective for this review was to examine WCC records of raptors and corvids between 1999 and 2008 with the goal of drawing inferences about how useful these data are for deducing information about wildlife population trends and health, rehabilitation success rates, and whether changes in submission patterns can detect population disturbances (such as WNV). These results could provide useful information about local wildlife populations to wildlife managers, and may also identify gaps in existing data collection procedures that indicate additional types of data rehabilitators could collect so that stronger inferences can be made regarding the causes of morbidity.

## **Materials and Methods**

All raptors and corvids submitted for rehabilitation to the WCC were given a case ID number, and a submission form indicating the date, species, presenter, and location found (the latter was noncompulsory of the presenter and therefore ranged in degree of specificity) was completed. A physical exam was performed on each animal (aka patient) by WCC staff, and a course of action, based on the diagnosis, was implemented (e.g., rehabilitated, euthanized). Once a resolution was determined, patient information additional to the submission form, such as diagnosis, resolution, date of resolution, release/transfer site, and band number (if applicable) was entered into an annual report.

We organized the annual report records from 1999-2008 into a single database consisting of only raptor and corvid cases, and assigned each patient record to one of six diagnostic categories: Trauma, Orphaned, Nutritional Disease, Infectious Disease, Other, and Unknown. We further classified the trauma category into gunshot, vehicle collision, impact injury (where the bird impacts a stationary object, such as a window), animal attack, electrocution, and unknown but with injuries consistent with trauma. Orphaned patients were young birds that had little chance of survival without feeding intervention. Nutritional disease included patients which were diagnosed as starving, emaciated, or dehydrated.

Infectious disease was assigned to patients where a pathogen was identified through laboratory testing. The Other category consisted of relatively rare causes (e.g., toxicosis, neoplasia) in which the diagnosis in raptors and corvids was seen on average less than once per year. Lastly, the Unknown category was assigned to those patients for whom a cause of submission could not be determined, based on the information provided in the WCC records. In addition, we assigned each patient one of three resolutions: Released, Died, or Transferred. Released patients were those returned to the wild. Died described patients that were euthanized or died post submission. Transferred patients referred to those which were moved to another wildlife care center or placed in a permanent home.

From the classifications described above, we characterized 1) the percentage of each diagnosis, 2) the proportion of total annual submissions for each group (raptors and corvids) relative to the total WCC submissions, and 3) the proportion of diagnoses each year for each group. We also described the percentage of each resolution type for these species.

For both corvids and raptors, we used a binomial model with an identity link to evaluate whether there were significant differences in submission rates among years. Specifically, we compared the proportion of each group's submissions within each year (e.g., # raptors in year  $i$  / total # of WCC submissions in year  $i$ ) to the overall proportion of submissions during the 10-year period (e.g., total # of raptors submitted over 10 years / total # of WCC submissions over 10 years). This approach is analogous to comparing treatment means to a grand mean in an analysis of variance (ANOVA). We also used this binomial model to evaluate whether diagnoses varied over time in either raptors or corvids. For each diagnostic category, we compared the proportion of animals with that diagnosis each year to the proportion of patients with that diagnosis across the 10-year period.

To evaluate whether the frequency of different diagnostic categories varied seasonally, we grouped each diagnosis into four seasons over the entire 10-year period: spring (March-May), summer (June-August), fall (September-November), and winter (December-February). Using a binomial model, we compared the proportion of each diagnosis in each season to the proportion of that diagnosis across the 10-year period for raptors and corvids.

Lastly, we evaluated whether diagnoses varied among species within the raptor and corvid groups. Again, we used a binomial model to compare the proportion of a particular species having a certain diagnosis to the proportion of all species with that diagnosis. Within the raptor group, we compared the six most common species submitted to the WCC (Cooper's Hawk [*Accipiter cooperii*], Red-tailed Hawk [*Buteo jamaicensis*], American Kestrel [*Falco sparverius*], Eastern Screech-Owl [*Megascops asio*], Great Horned Owl [*Bubo virginianus*], and Barred Owl [*Strix varia*]) using one diagnostic category at a time. We made the same comparison for corvids, which only comprised two species (Blue Jay [*Cyanocitta cristata*] and American Crow [*Corvus brachyrhynchos*]).

We conducted all statistical analyses in SAS Version 9.1 (SAS Institute, Inc., Cary, NC, USA). Sequential Bonferroni corrections were applied to adjust *P*-values for multiple comparisons (Rice 1989). Results were considered significant if *P*-values were less than or equal to the sequential Bonferroni value adjusted from an alpha value of 0.05.

## Results

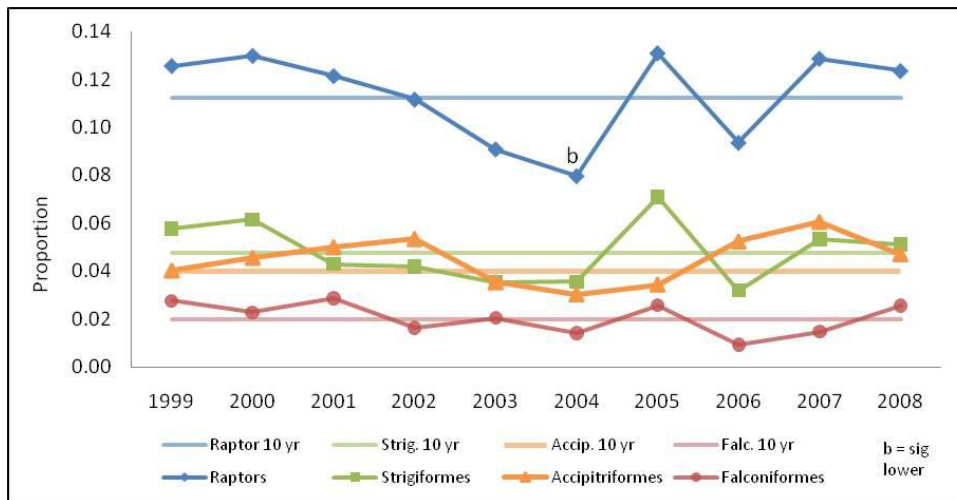
### Raptors

From 1999 to 2008, the WCC received 544 raptor submissions comprising 20 species (Table 1) from three orders (Accipitriformes, Falconiformes, and Strigiformes) and four families (Accipitridae, Falconidae, Tytonidae, and Strigidae). The most common diagnosis of raptors submitted to the WCC was Trauma (~58%). The majority of these Trauma cases had an unknown cause (59%), followed by vehicular collision (30%). The remaining trauma cases included entrapment and impact injury (3% each), followed by animal attack (2%), electrocution (1%), and gunshot (1%).

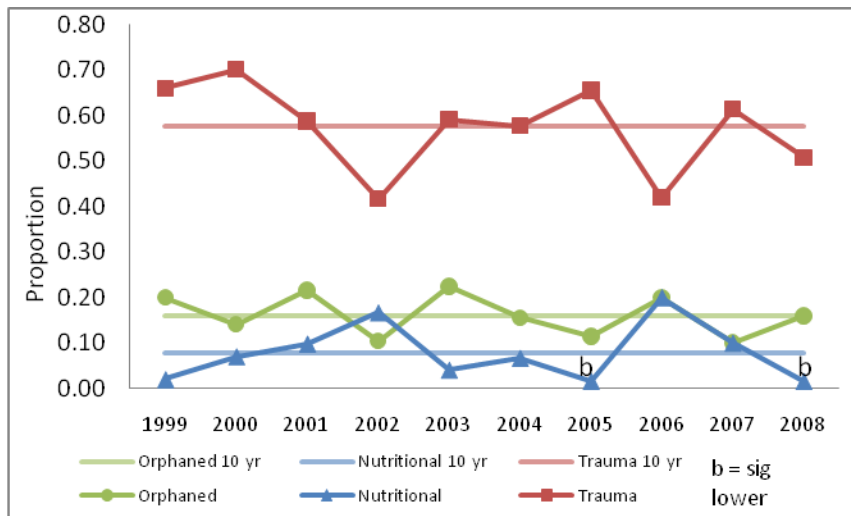
During the 10-year period, 11% of WCC submissions were raptors. We detected significant variation in raptor submission rates among years (test-statistic=17, df=9 [hereafter  $t_{df}$ ],  $P=0.048$ ). Specifically, a significantly lower proportion of raptors were submitted to the WCC in 2004 relative to the proportion of raptors submitted during the 10-year period ( $P=0.003$ , estimate=0.033, 95% CI= [0.011, 0.054], hereafter given as “*P*-value, estimate [95% CI]”; Fig.1).

**Table 1.** Number of raptor species and the diagnostic breakdown (by percent) for each species admitted to the WCC from 1999-2008, as well as for all raptors combined.

Species	Number	Diagnosis (%)					
		Trauma	Orphaned	Nutritional Disease	Infectious Disease	Other	Unknown
Osprey ( <i>Pandion haliaetus</i> )	2	100	0	0	0	0	0
Mississippi Kite ( <i>Ictinia mississippiensis</i> )	2	50	50	0	0	0	0
Bald Eagle ( <i>Haliaeetus leucocephalus</i> )	11	45.5	0	0	0	45.5	9
Northern Harrier ( <i>Circus cyaneus</i> )	2	100	0	0	0	0	0
Sharp-shinned hawk ( <i>Accipiter striatus</i> )	12	75	0	0	8.3	16.7	0
Cooper's Hawk ( <i>Accipiter cooperii</i> )	33	57.6	12.1	9.1	12.1	9.1	0
Northern Goshawk ( <i>Accipiter gentilis</i> )	1	0	0	0	100	0	0
Broad-winged Hawk ( <i>Buteo platypterus</i> )	2	50	50	0	0	0	0
Red-tailed Hawk ( <i>Buteo jamaicensis</i> )	149	69.1	2.7	12.1	3.4	10.7	2
Rough-legged Hawk ( <i>Buteo lagopus</i> )	3	100	0	0	0	0	0
American Kestrel ( <i>Falco sparverius</i> )	93	30.1	46.2	4.3	1.1	14	4.3
Merlin ( <i>Falco columbarius</i> )	1	100	0	0	0	0	0
Peregrine Falcon ( <i>Falco peregrinus</i> )	3	33.3	0	0	0	66.7	0
Barn Owl ( <i>Tyto alba</i> )	2	100	0	0	0	0	0
Eastern Screech-Owl ( <i>Megascops asio</i> )	60	55	30	1.7	1.7	11.6	0
Great Horned Owl ( <i>Bubo virginianus</i> )	91	56	7.7	11	13.2	12.1	0
Barred Owl ( <i>Strix varia</i> )	62	69.3	9.7	9.7	0	9.7	1.6
Long-eared Owl ( <i>Asio otus</i> )	4	75	0	0	0	25	0
Short-eared Owl ( <i>Asio flammeus</i> )	5	60	20	0	0	20	0
Northern Saw-whet Owl ( <i>Aegolius acadicus</i> )	6	66.6	16.7	0	0	16.7	0
<b>All Raptors</b>	<b>544</b>	<b>57.7%</b>	<b>15.8%</b>	<b>7.7%</b>	<b>4.6%</b>	<b>12.5%</b>	<b>1.7%</b>

**Figure 1.** The annual proportion of raptor, Accipitriformes, Falconiformes, and Strigiformes submissions relative to the total WCC submissions for each year from 1999-2008. The horizontal line for each group ('Raptor 10 yr', 'Accip 10 yr', 'Falc 10 yr', and 'Strig 10 yr') is the number of submissions in each group for the 10-year period divided by the total WCC submissions for the 10-year period. Years which are significantly lower are indicated by "b" ( $\alpha = 0.05$ ).

Of all diagnostic categories attributable to raptor submissions, Trauma and Nutritional Disease were significantly different among years ( $t_9=18.4$ ,  $P=0.030$  &  $t_9=26.3$ ,  $P=0.002$ , respectively). However, none of the individual years showed a significant difference in the frequency of Trauma after  $P$ -values were adjusted using sequential Bonferroni correction (years 2000, 2002, and 2006 were all  $0.01 < P < 0.05$ ). The proportion of Nutritional Disease submissions was lower in 2005 and 2008 ( $P < 0.001$ ,  $0.061$  [0.025, 0.096] &  $P < 0.001$ ,  $0.061$  [0.027, 0.096], respectively; Fig. 2). We did not find significant differences over time in the proportion of Orphan submissions ( $t_9=8.1$ ,  $P=0.522$ ). We did not analyze the Infectious Disease or Unknown categories because there were several years in which no raptors were submitted with these diagnoses. Lastly, because the Other category is a catch-all for an assortment of relatively rare diagnoses, analysis of this category would be unlikely to indicate anything substantive about raptor health.



**Figure 2.** Proportion of annual raptor diagnoses of Orphaned, Nutritional Disease, and Trauma for each year from 1999-2008. The horizontal line for each diagnosis ('Orphaned 10 yr', 'Nutritional 10 yr', and 'Trauma 10 yr') is the number of raptor submissions in each diagnostic category for the 10-year period divided by the total number of raptor submissions for the 10-year period. Years which are significantly lower are indicated by "b" ( $\alpha = 0.05$ ).

We found significant seasonal variation in raptor submissions for Trauma ( $t_3=30.5$ ,  $P < 0.001$ ), Orphaned ( $t_3=76.3$ ,  $P < 0.001$ ), and Infectious Disease ( $t_3=10.2$ ,  $P=0.017$ ). Trauma submissions were higher during the fall and winter ( $P=0.006$ ,  $-0.10$  [-0.172, -0.028] &  $P < 0.001$ ,  $-0.188$  [-0.276, -0.101], respectively) and significantly lower in the summer

( $P < 0.001$ , 0.118 [0.069, 0.167]). Our review of the Trauma subcategories indicated the majority of fall and winter Trauma cases were of unknown cause (48% and 61%, respectively), followed by vehicle collision (45% and 25%, respectively). Orphaning was higher in spring and summer ( $P = 0.042$ , -0.068 [-0.134, -0.003] &  $P < 0.001$ , -0.106 [-0.144, -0.068], respectively) and lower in fall and winter ( $P < 0.001$ , 0.152 [0.121, 0.183] &  $P < 0.001$ , 0.148 [0.112, 0.183], respectively). Infectious Disease submissions were lower in the spring ( $P < 0.001$ , 0.037 [0.015, 0.059]). We did not find any significant seasonal trends in Nutritional Disease submissions ( $t_3 = 1.2$ ,  $P = 0.749$ ).

We also found differences in the frequency of diagnoses among raptor species in Trauma ( $t_5 = 38.9$ ,  $P < 0.001$ ), Orphaned ( $t_5 = 92.0$ ,  $P < 0.001$ ), Nutritional Disease ( $t_5 = 10.5$ ,  $P = 0.062$ ), and Infectious Disease ( $t_5 = 20.6$ ,  $P < 0.001$ ). Relative to raptors as a group, American Kestrels had a significantly lower proportion of traumatic injuries ( $P < 0.001$ ; 0.262 [0.176, 0.348]), while Red-tailed Hawks had a higher proportion ( $P < 0.001$ ; -0.121 [-0.184, -0.059]). In addition, American Kestrels and Eastern Screech-Owls both showed evidence of higher orphaning ( $P < 0.001$  &  $= 0.014$ ; -0.304 [-0.390, -0.217] & -0.131 [-0.236, -0.026], respectively), whereas Great Horned Owls and Red-tailed Hawks had significantly lower orphaning (both  $P < 0.001$ ; 0.092 [0.039, 0.144] & 0.142 [0.108, 0.176], respectively). Relative to raptors as a whole, Eastern Screech-Owls had a significantly lower proportion of Nutritional Disease ( $P < 0.001$ ; 0.068 [0.030, 0.105]). American Kestrels had lower proportions of submissions attributed to Infectious Diseases ( $P = 0.003$ ; 0.038 [0.013, 0.064]) while Great Horned Owls had a significantly higher proportion of Infectious Disease submissions ( $P = 0.005$ ; -0.083 [-0.141, -0.024]).

Regarding the resolution of the six raptor species most frequently submitted for rehabilitation ( $n = 488$ ), 41% were released, 55% died, and 4% were transferred (Table 2). American Kestrels had the highest release rate (69%), followed by Eastern Screech-Owls (45%). Great Horned Owls accounted for the highest percentage that died of the six raptor species.

**Table 2.** The resolution (by percent) of the six raptor species most frequently submitted to the WCC from 1999 to 2008.

Species	Died	Released	Transferred
Coopers Hawk ( <i>Accipiter cooperii</i> )	60.6	36.4	3.0
Red-tailed Hawk ( <i>Buteo jamaicensis</i> )	63.8	34.9	1.3
American Kestrel ( <i>Falco sparverius</i> )	29.0	68.8	2.2
Eastern Screech Owl ( <i>Megascops asio</i> )	46.7	45.0	8.3
Great Horned Owl ( <i>Bubo virginianus</i> )	71.4	24.2	4.4
Barred Owl ( <i>Strix varia</i> )	52.4	39.5	8.1
<b>Overall</b>	<b>54.8%</b>	<b>41.3%</b>	<b>3.9%</b>

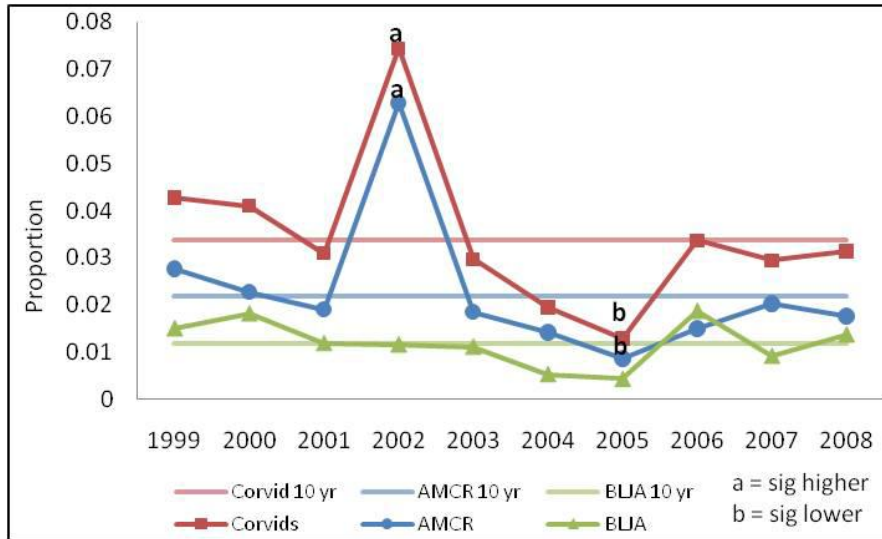
### Corvids

From 1999 to 2008, the WCC received 163 corvid submissions of two species, Blue Jay and American Crow. The most frequent diagnosis of corvids was Orphaned (46%), followed by Trauma (33%) (Table 3). Similar to Trauma in raptors, the majority of corvid traumas were of unknown cause (71%), followed by vehicular collision (17%).

**Table 3.** The number of corvid species and the diagnostic breakdown (by percent) for each species admitted to the WCC from 1999-2008, as well as for all corvids combined.

Species	Number	Diagnosis (%)					
		Trauma	Orphaned	Nutritional Disease	Infectious Disease	Other	Unknown
Blue Jay ( <i>Cyanocitta cristata</i> )	57	14	74	2	0	5	5
American Crow ( <i>Corvus brachyrhynchos</i> )	106	42	31	4	4	15	4
<b>All Corvids</b>	<b>163</b>	<b>33%</b>	<b>46%</b>	<b>3%</b>	<b>2%</b>	<b>12%</b>	<b>4%</b>

We found significant variation among years in the proportion of corvid submissions ( $t_9=18.4$ ,  $P=0.030$ ). A significantly higher proportion of corvids were submitted to the WCC in 2002 ( $P<0.001$ ,  $-0.041$   $[-0.064, -0.018]$ ), followed by a significantly lower proportion of corvid submissions in 2005 ( $P<0.001$ ,  $0.021$   $[0.010, 0.031]$ ; Fig. 3). At the species level, American Crows had a significantly higher proportion of submissions in 2002 ( $P<0.001$ ,  $-0.041$   $[-0.062, -0.020]$ ) and lower proportion of submissions in 2005 ( $P=0.002$ ,  $0.013$   $[0.005, 0.022]$ ). The proportion of Blue Jay submissions, however, did not show significant variation among years ( $t_9=9.6$ ,  $P=0.386$ ), although 2005 was a 10-year low for this species.



**Figure 3.** The proportion of annual corvid, American Crow (AMCR), and Blue Jay (BLJA) submissions relative to the total WCC submissions for each year from 1999-2008. The horizontal line for each group ('Corvid 10 yr', 'AMCR 10 yr', and 'BLJA 10 yr') is the number of submissions in each group for the 10-year period divided by the total WCC submissions for the 10-year period. Years which are significantly higher are indicated by "a" and years which are significantly lower are indicated by "b" ( $\alpha = 0.05$ ).

The frequency of both Trauma ( $t_9=34.4$ ,  $P<0.001$ ) and Orphaned ( $t_9=20.3$ ,  $P=0.016$ ) diagnoses for corvids also varied significantly among years. Trauma diagnoses were significantly higher in 1999 ( $P<0.001$ ,  $-0.498$  [ $-0.672$ ,  $-0.325$ ]) and Orphaned diagnoses were significantly lower in 1999 ( $P<0.001$ ,  $0.343$  [ $0.188$ ,  $0.497$ ]). We did not analyze the remaining categories due to several years in which no corvids were submitted with these diagnoses.

Seasonal trends in corvid diagnoses were only evaluated for Trauma ( $t_3=46.7$ ,  $P<0.001$ ) due to several seasons where no corvids were submitted with the other types of diagnoses. The proportion of corvid Trauma submissions was significantly higher in winter ( $P<0.001$ ,  $-0.580$  [ $-0.704$ ,  $-0.455$ ]) and lower in summer ( $P<0.001$ ,  $0.194$  [ $0.130$ ,  $0.258$ ]).

We also found differences in the frequency of Trauma ( $t_1=14.8$ ,  $P<0.001$ ) and Orphaned ( $t_1=27.8$ ,  $P<0.001$ ) diagnoses among corvid species. Relative to corvids as a group, American Crows had significantly higher Trauma ( $P<0.001$ ,  $-0.099$  [ $-0.145$ ,  $-0.054$ ]) while Blue Jays had significantly lower Trauma ( $P<0.001$ ,  $0.185$  [ $0.100$ ,  $0.270$ ]). Conversely, American Crows had a lower proportion of Orphaned submissions ( $P<0.001$ ,  $0.149$  [ $0.098$ ,  $0.199$ ]), whereas Blue Jays had a higher proportion ( $P<0.001$ ,  $-0.277$  [ $-0.371$ ,  $-$



0.183]). There were no differences found in Nutritional Disease submissions between the two species ( $t_1=0.6$ ,  $P=0.456$ ).

Overall, 71.8% of corvids submitted for rehabilitation died in care, followed by 27.6% which were released, and 0.6% transferred (Table 4).

**Table 4.** The resolution (by percent) of the two corvid species submitted to the WCC from 1999 to 2008.

Species	Died	Released	Transferred
Blue Jay ( <i>Cyanocitta cristata</i> )	72.8	27.2	0.0
American Crow ( <i>Corvus brachyrhynchos</i> )	71.1	28.0	0.9
<b>Overall</b>	<b>71.8%</b>	<b>27.6%</b>	<b>0.6%</b>

## Discussion

Previous retrospective studies of wildlife rehabilitation data have focused on causes of morbidity in a wide range of taxa (reptiles [Brown and Sleeman 2002], red foxes [Kelly and Sleeman 2003], and raptors [Fix and Barrows 1990, Wendell et al. 2002]) submitted to wildlife rehabilitation centers. However, few have focused on what fluctuations in submissions among years, seasons, and species may indicate about the free-living population(s) from which these animals originate. Tracking these patterns in fluctuations may allow inferences to be made about the health of wildlife in these populations. Subsequently, this information may provide insight for local and regional wildlife management agencies, potentially highlighting environmental irregularities for which further investigation may be necessary.

We identified a number of interesting patterns within the data. For example, we found a significantly lower proportion of raptor submissions in 2004, lower rates of raptor Nutritional Disease in 2005 and 2008, lower rates of Infectious Disease in American Kestrels and higher rates in Great Horned Owls. For corvids, we found significantly lower Orphaning and significantly higher Trauma in 1999, relative to other years. However, we can only speculate about the specific reasons for these patterns, as the rehabilitation data recorded were not sufficient to draw meaningful conclusions. To make such conclusions, it is important that detailed information is collected about the circumstances surrounding the discovery of an animal submitted for rehabilitation (e.g. location, habitat, weather, behavior), as well as the primary cause of submission and the existence of any underlying disease. It is

also important to account for human-related bias when making population-level inferences from wildlife submitted for rehabilitation, as sick or injured peridomestic wildlife species are more likely to be discovered due to their proximity to human activity (Fix and Barrows 1990, Wendell and Sleeman 2002).

### Raptors

Because the primary cause of raptors admitted to the WCC was Trauma of unknown cause, it is difficult to make inferences about the actual mechanisms leading to traumatic injury in raptors. Vehicular collision was the second most frequent cause of Trauma, which suggests many incidences of raptor morbidity are tied to human activity and is in keeping with the findings of similar studies (Fix and Barrows 1990, Wendell et al. 2002, Hager 2009). Orphaned raptors were the second most frequent cause of submission. Often, there was not enough information provided by the presenter to assess the context in which the “orphan” was found, making it difficult to determine the reasons by which raptor orphaning was occurring. For example, a young, recently fledged bird may appear orphaned to a well-meaning person and be relatively easy to capture, even though an adult bird may still be nearby providing post-fledging care.

The higher frequency of Trauma for raptors in the fall and winter seasons could be related to live prey becoming scarcer due to hibernation or periods of dormancy, relegating raptors to scavenge road-killed carrion more frequently and subsequently suffer traumatic injury by a moving vehicle. In addition, power poles along roadsides are often used as perches, which can lead to electrocution (Hager 2009). This is supported by our finding that Red-tailed Hawks, which commonly perch near roadsides to search for prey or scavenge (Hager 2009), had the highest frequency of traumatic injuries. However, American Kestrels, which are also known to search for prey in this manner, had a significantly lower amount of Trauma. Potentially, the large size of a Red-tailed Hawk relative to an American Kestrel (Peterson 2002) may increase its chances of both surviving and being detected after traumatic injury. In contrast to our Trauma results, Fix and Barrows (1990) reported no significant differences in the frequency of Trauma between species in a study of raptors submitted to the WCC from 1986-1987.

Not surprisingly, Orphaned submissions were highest in the spring and summer, which coincides with the nesting season. Our finding that American Kestrels and Eastern Screech-Owls each had a high frequency of Orphaning may be attributed to their tolerance for nesting in nest boxes or tree cavities associated with human activity, where orphaned young may be easily discovered (Smallwood and Bird 2002, Gehlbach 1995). Cavity nesters in residential areas may also be at risk of tree-felling by landowners during spring yard work activities (as trees with cavities are more likely to be dead and subsequently culled). In addition, the small size of immature American Kestrels and Eastern Screech-Owls relative to that of immature Red-tailed Hawks and Great Horned Owls (Peterson 2002) may mean people are more likely to attempt to capture and submit these smaller species. This may also explain, in part, the significantly lower frequency of Orphaned Red-tailed Hawks and Great Horned Owls submitted to the WCC.

The low rate of Infectious Disease submissions in the spring corresponds with the seasonal cycle of vector borne infections, which often emerge in the summer and peak in the fall (CDC 2010) due to the seasonal emergence of the vector itself (and, accordingly, Infectious Disease submissions were relatively high in the fall [ $0.01 < p < 0.05$ ]). However specific Infectious Diseases were not tested at high enough rates to be evaluated here. Implementation of regular disease testing or sample banking in conjunction with current public health and wildlife agency concerns would be necessary for any relationships between disease incidence in raptor submissions and the emergence of disease in wild raptor populations to be reliably investigated.

The large proportion of Orphaned American Kestrels submitted to the WCC coupled with the high release rate of this species may suggest they can be raised and rehabilitated successfully (although there are no data available on post-release success rates). By comparison, Great Horned Owls, most frequently diagnosed with Trauma, had the lowest release rate of the six most common raptor species, suggesting they may be more difficult to successfully rehabilitate. Overall, nearly 50% of raptors submitted to the WCC were released back into the wild, which is an improvement from the 34% release rate for raptors submitted to the WCC during 1986-1987 reported by Fix and Barrows (1990).

### Corvids

The primary cause of corvid submissions to the WCC was Orphaned, the majority of which were Blue Jays. Anecdotal accounts from WCC staff suggest that an influx of Orphan submissions often occurs after severe wind/rain storms, during which nestlings may be knocked out of trees and onto sidewalks and yards where they are subsequently found. Of the two corvid species, Blue Jay nestlings are smaller and may be more easily knocked out of the nest during inclement weather.

Corvid submissions to the WCC peaked in 2002, of which the majority were American Crows (Fig. 3). This pulse coincided with the emergence of WNV in the eastern part of Iowa in 2001 and subsequent spread across the entire state in 2002 (IDPH 2010). Corvids have been found to be more susceptible to mortality caused by WNV relative to other passerine families (Komar et al. 2003). The WCC did not implement any public awareness campaign at this time encouraging people to submit sick corvids, and therefore the 2002 rise in WCC corvid submissions may be an indicator of an increase in WNV in wild crows. Due to the lack of testing for WNV, however, we cannot definitively attribute this increase in corvid submissions to WNV emergence in Iowa. Following the spike in submissions in 2002, the frequency of corvid submissions declined through 2005, which is consistent with other data suggesting the central Iowa corvid population experienced a decline as a result of their greater susceptibility to WNV (Project FeederWatch data for American Crows in the Mid-Central Region of the U.S. [which includes Iowa; <http://www.birds.cornell.edu/pfw/DataRetrieval/trendgraphs/amecro.html>]). Specifically, the percentage of feeders visited by American Crows decreased sharply (approximately 15%) from 2002 to 2003, followed by a plateau at this decreased level thru 2008. Project FeederWatch data for Blue Jays also show a decline in this region from 2001 to 2002 (<http://www.birds.cornell.edu/pfw/DataRetrieval/trendgraphs/blujay.html>), although not to the degree seen in American Crows. Using 26 years of Breeding Bird Survey data, LaDeau et al. (2007) found that American Crow populations decreased significantly during this time period, and that regional declines in this species were positively correlated with regional WNV epidemics in humans.

The variation in corvid diagnoses among years did not appear to follow any pattern related to the emergence of WNV. Fluctuation in Trauma diagnoses throughout the 10-year period could be related to the availability of carrion and trash along roadsides and residential areas, which American Crows commonly scavenge at the risk of a vehicle collision (Verbeek and Caffrey 2002). Blue Jays are not usually scavengers and, accordingly, made up a much smaller proportion of Trauma submissions relative to American Crows. Similar to raptors, the primary cause of Trauma in corvids was unknown, and while they may have been a result of vehicle collision, more information about the circumstances in which the individual was found would be necessary to draw reliable conclusions.

Variation in seasonal diagnoses of corvid submissions was only analyzed for the Trauma category, and the results, similar to raptors, found corvid Trauma to be more frequent in the winter and less so in the summer. These results may be related to the seasonal feeding ecology of corvids, particularly American Crows, which often congregate to scavenge along roadsides or parking lots in residential areas during winter when natural food sources are scarce, increasing the risk of vehicle collision or impact injury (Verbeek and Caffrey 2002). Relative to raptors, corvid rehabilitation was less successful, with nearly 75% dying post-submission.

### Conclusion

A few noteworthy patterns in raptor and corvid submissions to the WCC were discovered in this review, particularly the signature of WNV emergence in wild corvids. Some of the significant patterns could be explained, in part, by the ecology of the taxa; however, we could only speculate about the occurrence of other patterns for which we did not have enough information on the circumstances of the animal's morbidity. In addition to very specific 'location found' data, the collection of detailed environmental data, such as the surroundings (e.g., backyard, park, highway), recent weather events (e.g., severe windstorm), and climate data for the season in which the animals were found, would be useful for evaluating the context of a submission's morbidity by helping to determine potential contributing factors. Other pertinent information that should be collected includes the behavior displayed by the animal at the time of discovery. These data, in concert with the real-time tracking of wildlife submission records, could allow wildlife managers, via

rehabilitators, to detect patterns that suggest the possibility of a population disturbance in the environment that may require further investigation. In addition, the implementation of active testing or consistent sample banking of submissions for EIDs of current public health or conservation concern may be useful in the early detection of disease outbreaks within wildlife populations. Despite the lack of formal disease testing, the trend in WCC corvid submissions paralleling the effects of WNV was clearly evident in the data. Had active testing or sample archival been employed on all WCC patient submissions, we could have learned valuable information about WNV activity in local wildlife populations with minimal extra effort. Lastly, as rehabilitation centers detect trends in submissions that may be indicative of a disease outbreak or other disturbance, this information should be communicated to wildlife managers and public health officials. Managers may then be able to use the detailed information collected by rehabilitators to focus their efforts on affected taxa and/or geographic areas to implement targeted surveillance or intervention in the case of a disease threat.

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### CHAPTER 3. WILDLIFE REHABILITATION CENTERS IN DISEASE SURVEILLANCE AND MONITORING

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#### **Abstract**

The presence of disease in wild animal populations is often identified through some method of surveillance, although implementing on-going surveillance systematically for extended periods is often cost and time prohibitive for wildlife management agencies. However, wildlife rehabilitation centers could be an untapped resource in wildlife disease surveillance, as these facilities often receive wildlife whose reason for admittance is a result of their becoming incapacitated by a disease directly, or sustaining an unrelated injury that may allow a disease to infect more easily. Thus, wildlife rehabilitation patients may have an increased prevalence of disease relative to randomly sampled free-living wildlife. Our study evaluated whether wildlife rehabilitation facilities are an efficient means for monitoring disease frequency and occurrence in wildlife. We sampled peridomestic birds submitted to the Wildlife Care Clinic (WCC), a rehabilitation facility in central Iowa, as well as free-living peridomestic birds within the geographic area from which most of the WCC's submissions originated, and tested them for exposure to West Nile Virus (WNV). Next, we compared WNV prevalence between the WCC and field-captured birds. Our results indicated that the seroprevalence of WCC and field-captured birds were similar (2.8% and 2.3%, respectively), suggesting WNV exposure in wildlife submitted to the WCC was representative of current WNV exposure rates in the wild. Thus, wildlife rehabilitation centers could provide valuable information regarding the presence, prevalence, and patterns of wildlife disease to managers at a fraction of the cost and effort of active disease surveillance. Additionally, our results indicated that raptors (specifically, Accipitriformes and Strigiformes) submitted to the WCC had the highest WNV exposure rates (nearly 40%) relative to other bird taxa and therefore may be particularly useful in WNV monitoring. We

also observed that the order Piciformes had no WNV seropositive individuals within the field or wildlife rehabilitation center, despite a sample size comparable to several other orders in which WNV exposure was detected, for which we suggest future research should explore this anomaly. We also suggest that future studies on the utility of wildlife rehabilitation centers in disease surveillance should expand to include additional rehabilitation centers, EIDs/epizootic diseases of concern, and classes of wildlife.

## **Introduction**

The continued emergence of new infectious diseases, as well as the re-emergence of existing infectious diseases, has been fueled in part by the increased contact between humans, livestock, and wildlife via habitat modification, global travel, and translocation of both wild and domestic animals (Daszak et al. 2000). Taylor et al. (2001) estimated that 75% of emerging infectious diseases (EIDs) are zoonotic, affecting both humans and animals. In addition to the health risks of wildlife-associated diseases to humans and domestic animals, diseases in wildlife have been identified as potential threats to the existence of species of conservation need (Daszak et al. 2000, Wobeser 2006). Wildlife diseases also have ecological costs in terms of biodiversity, where disease introduced to naïve wildlife populations can cause direct declines in some species and indirectly result in declines to others (Tompkins et al. 2011). Such changes in biodiversity can precipitate changes in overall ecosystem structure and/or function (Collinge and Ray 2006).

Because of their potential importance to human, livestock, and wildlife health, wildlife health officials are often faced with the difficult and costly task of monitoring for and then tracking these diseases in wildlife populations. Wildlife diseases are monitored through some method of surveillance, although individual surveillance methods may vary in their efficiency at detecting disease (Wobeser 2006). Most wildlife disease surveillance is conducted passively, where sick or dead animals are opportunistically collected, often by the general public, and submitted to public health or wildlife agencies (Stallknecht 2007). Although passive surveillance is a relatively cost effective source of wildlife disease information, the lack of routine sample collection may result in an underestimation of disease occurrence or delayed detection (Eidson 2001). On the other hand, active surveillance,

which focuses on collecting samples from a target wildlife population or searching for a particular disease agent within a wildlife community, has been identified as one of the best methods for early detection and response to disease in wild animals (Stallknecht 2007, Stitt et al. 2007). However, implementing targeted and systematic surveillance programs for extended periods is often cost and time prohibitive for state and federal wildlife agencies (Eidson 2001).

Wildlife rehabilitation centers may be an untapped resource in wildlife disease surveillance. Stitt et al. (2007) determined that of the individuals (e.g., hunters, trappers) and organizations (e.g., road maintenance crews, animal control groups) that commonly encountered wildlife on Vancouver Island (British Columbia, Canada), wildlife rehabilitation centers received the widest variety of taxa from the largest geographic area. In particular, wildlife rehabilitation centers may be useful in monitoring wildlife disease frequency and occurrence in the environment because of their potential to more easily detect disease. This is because sick or injured wildlife submitted for rehabilitation may a) have been debilitated by the disease agent of interest (primary infection), or b) sustained an unrelated injury allowing the disease agent to infect more easily (secondary infection) (Wobeser 2006), where either event may increase the probability of the animal being captured and admitted for rehabilitation. Additionally, seasonal or annual fluctuations in diseased wildlife submitted for rehabilitation may provide information about temporal trends of a particular pathogen in wild populations. Lastly, a higher number of wildlife rehabilitation submissions within a certain taxonomic group may indicate species in the wild that are more susceptible to infection by a particular disease agent. Although other studies examining morbidity and mortality in wildlife rehabilitation centers have suggested these facilities may play a role in wildlife disease surveillance (Wendell et al. 2002, Kelly and Sleeman 2003, Nemeth et al. 2007), this idea has not been rigorously evaluated. If these facilities are to be useful for wildlife disease surveillance, it is important to determine how similar disease frequency in wildlife rehabilitation patients is to disease frequency in the populations from which they originate.

West Nile Virus (WNV) may be an ideal disease for evaluating how useful wildlife rehabilitation submissions are for monitoring disease activity in wild populations. Since its

arrival in North America in 1999, WNV has spread into the 48 contiguous United States, Canada, and parts of Central and South America (Komar and Clark 2006, CDC 2011). West Nile Virus has caused illness and death in numerous wildlife species, domestic animals (particularly horses), and humans. In particular, correlations between significant population declines in certain avian groups, particularly passerines, and the emergence of WNV in North America have been reported (LaDeau et al. 2007). West Nile Virus is maintained in the environment by mosquito vectors and vertebrate host species, primarily within the class Aves (Kilpatrick et al. 2007). Komar et al. (2003) found that many urban dwelling bird species, such as House Sparrows (*Passer domesticus*) and House finches (*Carpodacus mexicanus*), are highly competent at perpetuating WNV in the environment because they can produce high viremias for long periods. Many of the species submitted to wildlife rehabilitation centers are “backyard” birds (Iowa State University Wildlife Care Clinic, unpublished data). Presumably, this is because of their proximity to human populations (peridomestic), where sick or injured birds are more likely to be detected and subsequently brought in for rehabilitation. Therefore, testing “backyard” birds for WNV at rehabilitation centers may be an efficient means of detecting and monitoring WNV activity.

Our objective was to compare WNV patterns in patients at a wildlife rehabilitation center with WNV patterns in a wild population that serves as the source of these patients to gain insight into whether wildlife rehabilitation facilities may be an efficient means for monitoring disease frequency and occurrence in wildlife. To do so, we sampled peridomestic birds submitted to the Wildlife Care Clinic (WCC), a rehabilitation facility located in central Iowa, USA, as well as free-living peridomestic birds within the geographic area from which most of the WCC’s submissions originated, and tested them for exposure to WNV. We compared WNV prevalence between the WCC and field-captured birds to ascertain if the prevalence was higher, lower, or the same between these two groups. Similar or higher prevalence of disease in wildlife rehabilitation patients relative to that found in the wild populations from which they originate may indicate that disease testing at rehabilitation centers may be an effective and efficient means of detecting and monitoring wildlife diseases.

## **Materials and Methods**

### Wildlife Care Clinic

The WCC is a wildlife rehabilitation center housed at Iowa State University in Ames, Iowa, and has been providing rehabilitative care to wildlife since its establishment in 1984. Most of the wildlife submitted to the WCC originate from central Iowa; however, submissions have come from across the state. Although most wildlife submissions are birds and mammals, the WCC also treats and cares for reptiles and amphibians. Wildlife submitted for rehabilitation are given a case ID number, and a submission form indicating the date, species, presenter, and location found is completed. A physical exam is performed on each animal (aka patient) by WCC staff, and a course of action, based on the diagnosis, is implemented (e.g., rehabilitated, euthanized). Patient information additional to the submission form, such as diagnosis, resolution, date of resolution, release/transfer site, and band number (if applicable) are then entered into an annual report.

Between May 2008 and November 2010, we drew blood samples from WCC avian patients older than fledgling stage (to decrease the likelihood of detecting maternal antibodies [Nemeth et al. 2008, Gibbs et al. 2005]) and assigned each patient an ID number corresponding to the detailed WCC patient record. We obtained the samples by brachial venipuncture using 26.5 and 27.5 gauge needles, for which no more than 0.5% of a bird's total mass was drawn. We collected the blood samples with 75-mm heparinized capillary tubes and centrifuged them for two minutes at 8,000g, after which the serum was separated and stored at -20°C. The Institutional Animal Care and Use Committee (IACUC) approved our collection methods in permit # 4-09-6724-Z.

### Field Study

The area from which nearly half of the WCC avian submissions originated was determined using WCC submission records from 2002 to 2007. This area encompassed a six and a half mile radius centered over Ames, Iowa, across which we selected 10 study sites (Appendix A). We sampled each site three times (three “rounds”) from May through August in 2009 and 2010 to target minimally migrant breeding birds to increase the chance that any seropositive bird we sampled had been exposed to WNV locally. This season also corresponds to the timing of WNV activity, when mosquito vectors are active. Recaptured

birds within a round were not re-sampled, but birds that were recaptured between rounds were re-sampled, as they may have been exposed to WNV within that time. The order in which sites were sampled in the first round was determined randomly and then repeated in this order for subsequent rounds. To sample all sites during each round within the constraints of the field season length, sampling at any given site did not exceed five days.

We placed feeding stations at each of the 10 sites and maintained them throughout the sampling season to concentrate birds for capture. At each site, two 12-meter long, three-meter high, 38-mm mesh mist nets were opened and monitored, primarily during the morning hours, except when weather conditions would have caused additional physiological stress to captured birds (e.g., extreme temperatures and/or precipitation). We carefully removed captured birds from the mist nets and placed them into handling sacks to minimize their stress during processing. We recorded species, age (after-hatch-year [AHY], hatch-year [HY], or unknown [U]; Pyle 1997), sex (male, female, or unknown), mass, tarsus length, and any anomalies (e.g., deformities, old injuries, ectoparasites, etc.) for each bird, after which a blood sample was collected using the methods explained above. We used a non-toxic permanent marker in red, blue, or a combination of red and blue to mark a small area of light colored plumage on the inner left wing to identify recaptures and avoid re-sampling within a round. Walk-in traps were used in conjunction with the mist nets at sites conducive to the presence of ground-feeding birds. Birds captured using walk-in traps were subjected to the same handling/sampling protocol as those that were mist netted. We stored the blood samples in a cooler until the day's sampling was complete, and then centrifuged them for two minutes at 8,000g, after which the serum was separated and stored at -20°C. IACUC approved our capture and collection methods in permit # 4-09-6724-Q. Additionally, this work was conducted under Federal Bird Banding Permit # 23285 and State of Iowa Collectors Permit # SC 871.

### Laboratory

Initially, we tested serum samples for the presence of *Flavivirus* antibodies (a genus that includes both WNV and SLEV [St. Louis Encephalitis virus, which is also known to be present in the region]) with an epitope blocking enzyme-linked immunosorbent assay (ELISA) using the 6B6C-1 monoclonal antibody (MAb). In this ELISA, we added each

serum sample to a well of incubated WNV antigen (from mosquito cultures), where *Flavivirus* antibodies, if present, were bound to the WNV antigen. MAb 6B6C-1 was subsequently added to the well, and was blocked from binding to the antigen epitope if *Flavivirus* antibodies were present within the serum sample (as the sample's antibodies had already bound to the antigen receptor). The ability of the sample to block the MAb from binding to the WNV antigen was quantified as the "percent inhibition". Samples with greater than 30% inhibition were considered to contain *Flavivirus* antibodies, as recommended in Blitvich et al. (2003). An inhibition value of 30% was the minimum inhibition value (using known positive [WNV infected horse] and negative [chicken] samples) for which we could be confident samples were positive for *Flavivirus* antibodies, as opposed to negative with "non-specific binding" (binding due to non-specific factors rather than binding due to *Flavivirus* antibodies in the serum).

We tested samples that were positive for *Flavivirus* antibodies with a plaque reduction neutralization test (PRNT), which detects the presence of WNV-specific neutralizing antibodies within *Flavivirus* positive samples. We performed the PRNTs using the NY99-35261-11 strain of WNV, as well as the TBH-28 strain of SLEV (which were included to note both the presence and prevalence of SLEV in our sample population), with African green monkey kidney (Vero) cells. The viruses were acquired from the World Health Organization Center for Arbovirus Reference and Research located at the Centers for Disease Control and Prevention, Division of Vector-Borne Infectious Diseases, Fort Collins, CO. Initially, we tested the sera with a 1:20 screening dilution. Those samples for which less than 10 plaque-forming units developed (meaning that WNV antibodies were present and inhibiting the formation of viral plaques) were then tested with all dilution levels (1:40, 1:80, 1:160, 1:320, and 1:640). Serum titers were described as the reciprocal of the lowest dilution ratio that resulted in >90% reduction in the number of plaques (PRNT<sub>90</sub>). To be diagnosed positive for WNV antibodies, the PRNT<sub>90</sub> antibody titer was required to be at least fourfold greater than that of the other *Flavivirus* (SLEV) tested.

### Analysis

We chose to analyze the WCC and field sampled birds by taxonomic order so that we could include as many of the species sampled as possible while still accounting for potential

taxonomic differences in WNV seroprevalence (Ringia et al. 2004, Komar et al. 2005, Beveroth et al. 2006). Because of the potential differences in seroprevalence between orders, only individuals belonging to orders sampled at both the WCC and field (i.e., “shared” orders) were used for calculating the prevalence of WNV antibodies for each location. Additionally, we calculated WNV and *Flavivirus* seroprevalence rates for all the orders sampled within the WCC. We used a Fisher’s Exact Test to evaluate whether a significant difference in the presence of WNV antibodies existed between the shared orders of field-sampled and WCC-sampled birds using SAS Version 9.2 (SAS Institute, Inc., Cary, NC, USA). We also used a Fisher’s Exact Test to determine if WNV antibody prevalence was significantly different among the WCC orders for which 10 or more individuals were sampled. All tests were considered significant at  $\alpha=0.05$ .

## Results

During the course of the study, we sampled 1,936 birds in the field from three taxonomic orders (Table 1, Appendix B). Within the WCC, we sampled 180 individual birds from 12 taxonomic orders (Table 2). Ninety-five WCC birds were sampled between May and August, which corresponds to the period of field sampling, and another 85 WCC birds were sampled between September and April.

Birds from the orders Columbiformes, Passeriformes, and Piciformes were sampled in both the field and WCC, of which only birds in Columbiformes and Passeriformes were seropositive (Table 1). The prevalence of WNV antibodies was not significantly different between the field (2.3%) and WCC (2.8%) sampled birds ( $P=0.68$ ).

**Table 1.** WNV summary data for taxonomic orders represented in both the field and WCC.

Order	No. Sampled		No. Sero(+)		Seroprev. (%)	
	Field	WCC	Field	WCC	Field	WCC
Columbiformes	70	22	7	1	10.0	4.5
Piciformes	102	2	0	0	0.0	0.0
Passeriformes	1764	48	37	1	2.1	2.1
<b>Overall</b>	<b>1936</b>	<b>72</b>	<b>44</b>	<b>2</b>	<b>2.3</b>	<b>2.8</b>



Several of the orders we sampled within the WCC were not sampled in the field, but had relatively high seroprevalence rates (Table 2). Of the individuals we sampled belonging to Strigiformes and Accipitriformes, approximately 40% of each order had WNV antibodies. Among the Anseriformes, Accipitriformes, Falconiformes, Columbiformes, Strigiformes, and Passeriformes (orders with  $n \geq 10$ ) we found a significant difference in WNV antibody prevalence ( $P < 0.001$ ).

**Table 2.** The taxonomic orders sampled at the WCC and the WNV and *Flavivirus* antibody prevalence for each.

Order	No. Sampled	No. <i>Flavivirus</i> (+)	No. WNV (+)	% <i>Flavivirus</i> (+)	%WNV(+)
Anseriformes	17	1	0	5.9	0.0
Galliformes	3	0	0	0.0	0.0
Podicipediformes	2	0	0	0.0	0.0
Pelecaniformes	3	0	0	0.0	0.0
Accipitriformes	32	13	12	40.6	37.5
Falconiformes	14	1	1	7.1	7.1
Charadriiformes	1	0	0	0.0	0.0
Columbiformes	22	1	1	4.5	4.5
Strigiformes	33	13	13	39.4	39.4
Caprimulgiformes	3	0	0	0.0	0.0
Piciformes	2	0	0	0.0	0.0
Passeriformes	48	1	1	2.1	2.1

## Discussion

Our findings indicate WNV seroprevalence did not differ between the taxonomic orders (Columbiformes, Passeriformes, Piciformes) that we sampled in both the field and WCC. Our data suggest that WNV seroprevalence in the WCC appeared to be representative of the current WNV seroprevalence of these orders in the wild. It does not appear, at least in the case of WNV, that rehabilitation patients are more likely to be exposed to disease than the rest of their population. Therefore, from a simple disease detection perspective, sampling rehabilitation patients for WNV does not increase the likelihood of detecting disease over sampling animals in the wild. However, given that rehabilitation patients are already “in hand”, these animals could be an efficient means for wildlife managers and public health personnel to monitor the activity and prevalence of disease in wild populations. Additionally,

monitoring the frequency of disease in wildlife rehabilitation submissions over time may be useful for tracking changes in disease activity in the wild.

Several of the taxonomic orders we sampled at the WCC were not represented in our field study due to the difficulties associated with capturing them in the wild. However, our comparison of the WCC orders for which we sampled 10 or more individuals indicated a few groups, particularly some of the raptors (Accipitriformes and Strigiformes), exhibited very high WNV seroprevalence compared to the other orders. Similarly high seroprevalence has been previously reported in some raptor species (Stout et al. 2005, Medica et al. 2007, Dusek et al. 2010). Raptors may be exposed to WNV more than other orders because they can contract the virus by ingesting infected prey, in addition to being bitten by infected mosquito vectors. Indeed, Komar et al. (2003) demonstrated that the ingestion of a WNV infected mouse caused a Great Horned Owl (*Bubo virginianus*) to become viremic. Because of the relatively high WNV exposure rate within raptors, raptors submitted to wildlife rehabilitation centers may be better at enhancing the detectability of WNV in surveillance programs compared to the other orders we tested. Nemeth et al. (2007) even suggested raptors may be good sentinels, or early indicators, of WNV activity in the wild, as they found that several raptors admitted to two wildlife rehabilitation clinics in Colorado tested positive for WNV infection earlier in the transmission season compared to other WNV surveillance systems in the area (e.g., equine cases, infected mosquito pools, human cases). Research establishing the relationship between WNV exposure in raptors submitted for rehabilitation and raptors in the wild populations from which they originate would be necessary to evaluate if those submitted for rehabilitation have higher, similar, or lower seroprevalence than raptors in the wild.

Wildlife rehabilitation data have some short-comings that limit their utility for disease surveillance that should be addressed in the context of this study. Wildlife are often submitted to rehabilitation centers by the general public, and thus wildlife submissions are often biased towards peridomestic species or species that are perceived to be charismatic by the general populous (Trocini et al. 2008). Also, due to the opportunistic nature by which the general public finds and submits wildlife to rehabilitation centers, it is difficult to predict or control the number or species composition of submissions, and this was particularly apparent

in our study. For example, the WCC historical records from 2001 to 2007 indicated that, on average, the WCC received ~54 peridomestic songbirds each year. However, we were only able to sample an average of 24 peridomestic songbirds during each year of our study (2008-2010). This smaller sample size prompted us to analyze the data at a relatively coarse taxonomic scale (order). Thus, year-to-year fluctuations in submission numbers/species to rehabilitation centers may make consistent monitoring of wildlife disease at a finer resolution (such as family or species) difficult. Additionally, our study of WNV seroprevalence took place after the initial WNV epizootic swept through Iowa in 2002 (IDPH 2010). WNV seroprevalence in our study was low and likely reflects a low-level enzootic presence within peridomestic birds. However, it is possible WCC patients would have had higher WNV prevalence relative to wild populations in the epizootic stage of this disease, when WNV had a greater impact on the bird community (as reflected in dead-bird surveillance data, IDPH 2010). During disease epizootics, it is likely that many more wild animals become incapacitated by disease, and may be subsequently submitted to rehabilitation centers, relative to when disease is enzootic.

Of the three bird orders sampled in both the field and WCC, only Piciformes did not include any members positive for WNV antibodies, despite accounting for the second largest sample size (n=104 [field and WCC combined]). The lack of seropositive cases within the Piciformes is consistent with the findings of Beveroth et al. (2006), who found that none of 39 Downy Woodpeckers (*Picoides pubescens*) sampled during the first years of WNV emergence in the state of Illinois had WNV antibodies. Although both virus particles and antibodies have been detected in wild-sampled members of the Piciformes (Komar et al. 2003 and Gibbs et al. 2006, respectively), existing literature suggests low or no WNV seroprevalence in many Piciformes species. For example, Dusek et al. (2006) tested nearly one-third of the Red-cockaded Woodpecker (*Picoides borealis*) population in Noxubee National Wildlife Refuge for WNV exposure after population declines were observed in concurrence with WNV emergence in the region. However, none of the individuals in their sample population tested positive for WNV antibodies, perhaps due to a high mortality rate associated with exposure or due to the loss of detectable antibody levels by the time the birds

were sampled. Future studies should attempt to address the anomaly of low WNV seroprevalence among the Piciformes.

In conclusion, our study was one of the first, to these authors's knowledge, to quantify the utility of wildlife rehabilitation centers in wildlife disease surveillance, by using WNV and avian hosts as an example and comparing disease prevalence in rehabilitation centers to that in the wild population. Although the WCC peridomestic birds we sampled did not show increased prevalence (and thus detectability) of WNV exposure relative to that of field-sampled birds, our findings of similar seroprevalence between the field and the WCC still have useful implications for wildlife disease monitoring. For example, sampling wildlife rehabilitation submissions for a particular disease agent does not require the same level of time and effort of capturing free-living wildlife, so that information regarding the presence, prevalence, and patterns over time of the disease in the wild can be obtained at a fraction of the effort. It should be noted that the disease surveillance potential of wildlife rehabilitation centers hinges upon the organized collection of reliable and detailed data, without which this information cannot be accurately incorporated into a disease surveillance program. Additionally, regular testing for diseases of concern in wildlife rehabilitation submissions of interest would be necessary for these centers to be of value in wildlife disease surveillance. We recommend that future research on the utility of wildlife rehabilitation centers in disease surveillance should expand to include additional rehabilitation centers, EIDs/epizootic diseases of concern, and classes of wildlife. Ultimately, coordinated testing of specific wildlife diseases could be organized across a network of rehabilitation centers to provide wildlife disease data throughout a geographic region.

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## CHAPTER 4. VARIATION OF WEST NILE VIRUS EXPOSURE IN AVIAN SPECIES WITH LAND USE.

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### Abstract

Along with the use of surveillance to monitor the occurrence of disease in wild animal populations, public health and wildlife management agencies' ability to predict where risk of exposure may be greatest, and to which populations, is important. In the Midwest, existing research suggests the prevalence of West Nile Virus (WNV) in humans and mosquitoes is linked to land use, but no data are currently available for birds, the primary amplifying host of WNV. Our study evaluated whether the risk of exposure to WNV in Midwestern peridomestic birds varied with land use in order to identify locations where birds were at greatest risk of encountering the virus. In addition, we explored whether a bird's likelihood of having WNV antibodies was associated with its taxonomic family, age, and sampling season/year. Over two summers, we collected blood samples from birds captured in sites comprising gradients of urban, agriculture, and natural land uses, and tested them for exposure to WNV. Our results suggest that a bird's risk of WNV exposure increased in urban areas, possibly because urban landscapes often harbor standing water favorable for ornithophilic mosquito vector reproduction. We also found that risk of exposure was significantly greater in 2009 than in 2010. This disparity appears to be related to differences in weather, suggesting that annual variation in environmental conditions may strongly influence WNV exposure risk. Lastly, our results indicated that seroprevalence was higher within the Cardinalidae, Columbidae, and Turdidae families relative to other taxonomic families sampled. Heterogeneity among taxa may be due to variation in exposure, physiological response to infection, or survival. Overall, this study identified several factors associated with WNV exposure in Midwestern peridomestic birds that might be useful for tailoring disease surveillance or management activities.



## Introduction

Along with the use of surveillance to monitor the occurrence and frequency of disease in wild animal populations, public health and wildlife management agencies' ability to predict where the risk of exposure may be greatest, and to which populations, is important for devising disease management strategies. Consequently, a number of studies have been conducted to ascertain the link between risk of exposure to West Nile virus (WNV) and land use. Since its arrival in North America in 1999, WNV has spread to the 48 contiguous United States, Canada, and parts of Central and South America (Komar and Clark 2006, CDC 2011). This virus is maintained in the environment by mosquito vectors and vertebrate host species, primarily within the class Aves (Kilpatrick et al. 2007). WNV has caused illness and death in numerous wildlife species, domestic animals (particularly horses), and humans. In particular, correlations between significant population declines in certain avian groups, particularly passerines, with the emergence of WNV in North America have been reported (LaDeau et al. 2007).

LaDeau et al. (2008) surmised that land use plays a prominent role in WNV dynamics because it affects the relationship between the host, vector, and virus. To date, many studies of WNV prevalence and land use have focused on exposure risk in areas with large human population densities (Ruiz et al. 2007, Bradley et al. 2008, LaBeaud et al. 2008). For example, Bradley et al. (2008) found that landscape factors associated with urban areas, such as impervious surface coverage by streets, houses, and buildings in the metropolitan area of Atlanta, GA corresponded with an increase in WNV antibody prevalence in songbirds. The authors suggested this may be in part due to the availability of WNV vector breeding habitat associated with urban development. Gomez et al. (2008) cited increased levels of urbanization as one of the factors associated with elevated seroprevalence in small mammals sampled in the mid-Atlantic region of the U.S. Allen et al. (2009) found that avian diversity, which is often higher in non-urban landscapes (i.e. areas with low levels of human development and minimal disturbance [Blair 1996]), was negatively correlated with WNV seroprevalence in both humans and mosquito vectors. A possible explanation for this correlation is the "dilution effect". Specifically, bird species vary in their reservoir competence; where some species may amplify the virus by producing high viremias, other

species may effectively act as dead-end hosts by producing very low viremias that cannot infect a biting mosquito vector (Komar et al. 2003). A more diverse avian population results in a wider variety of available hosts for mosquito vectors to feed on, reducing the likelihood that a vector will feed solely on a highly competent species, and ultimately resulting in reduced WNV transmission (Allen et al. 2009).

Another variable that may affect WNV prevalence in an area is the ecology of the mosquito vector. *Culex pipiens* is a common WNV mosquito vector that favors stagnant water for reproduction, such as that found in sewers, birdbaths, and wastewater treatment plants. Thus, *Cx. pipiens* is most often an urban-associated mosquito (Epstein 2001), and usually emerges mid-summer with population peaks in July and August (Lee and Rowley 2000). Although primarily ornithophilic (favoring birds), this mosquito vector sometimes feeds on, and can subsequently infect, mammal species as well. The regular availability of food sources for birds within urban and suburban areas, primarily in the form of bird feeders, can attract and support an increased number of avian hosts. This potentially increases the spatial overlap between avian hosts and these urban-dwelling mosquito vectors, which may also contribute to an increase in the prevalence of WNV within host species. The virus itself, which develops in the vector's gut, has been shown to replicate with increased efficiency at warmer temperatures (Reisen et al. 2006), where even relatively small increases in temperature, such as those found in urban microclimates, can significantly increase transmission rates (Kilpatrick et al. 2008).

Other land use types have also been associated with WNV risk. Recently, Liu et al. (2008) found that one of the major land use factors contributing to WNV outbreaks within mosquito vector pools around Indianapolis, Indiana was an increased percentage of corn and soybean dominated agriculture. The authors suggested this may be related to the dense cover provided by these crops in late summer that produces suitable temperature and moisture levels for breeding mosquito vectors. In Iowa, DeGroote et al. (2008) found that human cases of WNV were positively correlated with the presence of agriculture. However, research pertaining to whether the prevalence of WNV in avian species varies with land use in agriculturally dominated landscapes is relatively scant. Given that the above studies have found increased prevalence of WNV in both humans and mosquito vectors near agricultural

land use, it is useful to know if the primary amplifying hosts in the WNV cycle (birds) show similar exposure patterns. If so, birds could act as an indicator of WNV risk to humans and domestic animals in agricultural areas.

Besides identifying where on the landscape risk of WNV is greatest, it is also useful to identify when during the season an individual is at greatest risk of exposure, so that preventative measures may be taken. Previous research suggests risk of WNV exposure may vary seasonally (Ringia et al. 2004, Kilpatrick et al. 2006, LaBeaud et al. 2008, Liu et al. 2008). This is because the emergence of the mosquito vectors is dependent upon minimum temperatures to facilitate vector survival and adequate breeding habitat, such as warm standing water in the case of the common *Culex* vectors. As warm temperatures and standing water occur during the mid-summer months in the Midwestern U. S., the emergence of WNV mosquito vectors and subsequent WNV disease outbreaks often peak at this time of year (Liu et al. 2008). However, research on whether seasonal variation exists in disease exposure in birds in the Midwest is lacking.

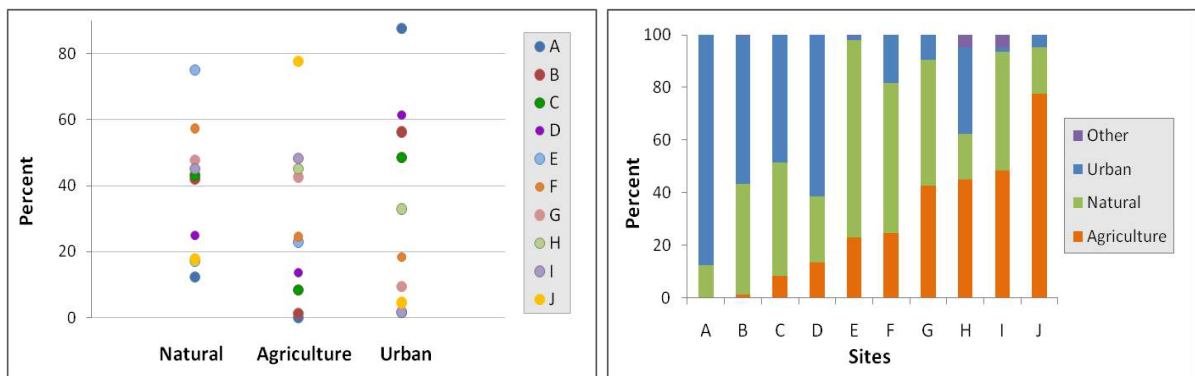
Our objective was to identify factors associated with WNV exposure in Midwestern peridomestic birds. Specifically, we evaluated whether land use is related to WNV seroprevalence in birds. First, we identified landscape features of central Iowa that are likely relevant to the ecology and transmission of WNV. We then targeted non-migrant/minimally migrant peridomestic avian species within different land use types and tested them for WNV antibodies to identify where risk of disease exposure may be greatest. Additionally, we explored whether a temporal trend existed in the number of WNV seropositive avian cases within and between years to understand when risk of exposure may be greatest. Finally, we examined whether a bird's species and age may be a factor in its risk of WNV exposure.

## **Materials and Methods**

### **Study Area**

Located in central Iowa, Story County contains a large amount of corn and soybean rowcrop, with 98% of the landcover classified as agriculture (Otto et al. 2006). Ames, a city located in southwest Story County, has a relatively large proportion of agriculture interface and provided an ideal location to sample avian species for WNV antibodies to determine exposure risk. Between mid-May and mid-August of 2009 and 2010, we captured and

sampled common peridomestic avian species within Ames and the greater Ames area (a roughly six and a half mile radius centered over the city’s geometric center)(Appendix A). Within this study area, we chose sites to reflect a gradation of three major land use categories: “Natural” (vegetated areas with no regular cultivation), “Agricultural” (crop fields with regular cultivation) and “Urban” (impervious surfaces created by streets and buildings). Each site was 0.79 km<sup>2</sup>, which was based on the average breeding season home ranges of our target avian species (Birds of North America Online 2009). The area covered by each site did not overlap that of any other sites, and we captured and sampled birds at the center of each study site. We used ArcGIS 9.2 to import the 2002 landcover layer for Story County (courtesy of the Iowa Department of Natural Resources), and calculated the percentage of each land use category within each site. Ten study sites were selected to produce a gradient spanning low, medium, and high levels of each land use category (Fig. 1).



**Figure 1.** The percentage of Natural, Agriculture, and Urban landcover at each of the 10 sites (A-J) spanning a gradient of low, medium, and high levels. The Other category in the figure on the right is landcover features that do not fit within Natural, Agriculture, or Urban (e.g., ‘barren’ and/or ‘water’). Each site was 0.79 km<sup>2</sup>, which encompassed the breeding season home ranges of the target avian species.

### Field Sampling

We sampled each site three times (three “rounds”) from May through August (about a month apart) of each year, allowing us to test for temporal trends in WNV seroprevalence. Recaptured birds within a round were not re-sampled, but birds that were recaptured between rounds were re-sampled, as they may have been exposed to WNV within that time. The order in which sites were sampled in the first round was determined randomly and then repeated in this order for subsequent rounds. The sampling period at each site varied depending on the capture rate of the birds, where our target sample size was n=30 birds per

site per round, for a total minimum sample size goal of 90 birds per site throughout the season. A logistic regression power analysis indicated that a sample size of 90 birds per site (sampling 10 sites) resulted in our having ~80% power to detect a 2.7 fold difference (or greater) in prevalence across a land use gradient. We based this power analysis estimate on findings from a similar study in Georgia, which documented a nearly fivefold increase in WNV prevalence in birds sampled across sites along a comparable land use gradient (Bradley et al. 2008). In order to sample all sites during each round within the constraints of the field season length, sampling at any given site did not exceed five days.

We primarily sampled avian species that were widespread and abundant across our study area, and for which existing literature suggested high seroprevalence (to enhance WNV detectability). These species included House Sparrows (*Passer domesticus*), House Finches (*Carpodacus mexicanus*), Northern Cardinals (*Cardinalis cardinalis*), American Robins (*Turdus migratorius*), and Mourning Doves (*Zenaida macroura*) (Gibbs et al. 2006, Beveroth 2006, Bradley et al. 2008). We placed feeding stations at each of the 10 sites and maintained them throughout the sampling season to concentrate birds for capture. At each site, two 12-meter long, 3-meter high, 38-mm mesh mist nets were opened and monitored continuously. We sampled primarily during the morning hours, except when weather conditions would have caused additional physiological stress to captured birds (e.g., extreme temperatures and/or precipitation). We carefully removed captured birds from the mist nets and placed them into handling sacks to minimize their stress during processing. We recorded species, age (after-hatch-year [AHY], hatch-year [HY], or unknown [U]; Pyle 1997), sex (male, female, or unknown), mass, tarsus length, and any anomalies (e.g., deformities, old injuries, ectoparasites, etc.) for each bird, after which a blood sample was collected by brachial venipuncture using 26.5 and 27.5 gauge needles. We collected blood samples with 75-mm heparinized capillary tubes, and no more than 0.5% of a bird's total mass was drawn. We used a non-toxic permanent marker in red, blue, or a combination of red and blue to mark a small area of light colored plumage on the inner left wing to identify recaptures and avoid re-sampling within a round. Walk-in traps were used in conjunction with the mist nets at sites conducive to the presence of ground-feeding birds. Birds captured using walk-in traps were subjected to the same handling/sampling protocol as those that were mist netted. We stored

the blood samples in a cooler until the day's sampling was complete, and then centrifuged them for two minutes at 8,000g, after which the serum was separated and stored at -20°C. The Institutional Animal Care and Use Committee (IACUC) approved our capture and collection methods in permit # 4-09-6724-Q. Additionally, this work was conducted under Federal Bird Banding Permit # 23285 and State of Iowa Collectors Permit # SC 871.

### Laboratory

Initially, we tested serum samples for the presence of *Flavivirus* antibodies (a genus that includes both WNV and SLEV [St. Louis Encephalitis virus, also known to be present in the region]) with an epitope blocking enzyme-linked immunosorbent assay (ELISA) using the 6B6C-1 monoclonal antibody (MAb). In this ELISA, we added each serum sample to a well of incubated WNV antigen (from mosquito cultures), where *Flavivirus* antibodies, if present, would bind to the WNV antigen. MAb 6B6C-1 was subsequently added to the well, and was blocked from binding to the antigen epitope if *Flavivirus* antibodies were present within the serum sample (as the sample's antibodies had already bound to the antigen receptor). The ability of the sample to block the MAb from binding to the WNV antigen was quantified as the "percent inhibition". Samples with greater than 30% inhibition were considered to contain *Flavivirus* antibodies, as recommended in Blitvich et al. (2003). An inhibition value of 30% was the minimum inhibition value (using known positive [WNV infected horse] and negative [chicken] samples) for which we could be confident samples were positive for *Flavivirus* antibodies, as opposed to negative with "non-specific binding" (binding due to non-specific factors rather than binding due to *Flavivirus* antibodies in the serum).

We tested samples that were positive for *Flavivirus* antibodies with a plaque reduction neutralization test (PRNT), which detects the presence of WNV-specific neutralizing antibodies within *Flavivirus* positive samples. We performed the PRNTs using the NY99-35261-11 strain of WNV, as well as the TBH-28 strain of SLEV (which was included to note both the presence and prevalence of SLEV in our sample population), with African green monkey kidney (Vero) cells. The viruses were acquired from the World Health Organization Center for Arbovirus Reference and Research located at the Centers for Disease Control and Prevention, Division of Vector-Borne Infectious Diseases, Fort Collins,

CO. Initially, we tested the sera with a 1:20 screening dilution. Those samples for which less than 10 plaque-forming units developed (meaning that WNV antibodies were present and inhibiting the formation of viral plaques) were then tested with all dilution levels (1:40, 1:80, 1:160, 1:320, and 1:640). Serum titers were described as the reciprocal of the lowest dilution ratio that resulted in >90% reduction in the number of plaques (PRNT<sub>90</sub>). To be diagnosed positive for WNV antibodies, the PRNT<sub>90</sub> antibody titer was required to be at least fourfold greater than that of the other *Flavivirus* (SLEV) tested.

### Analysis

We calculated seroprevalence for species that had one or more seropositive individuals, as well as overall seroprevalence for each year and the entire study period. We used binary response logistic regression in SAS Version 9.2 (SAS Institute, Inc., Cary, NC, USA) to test for associations between the presence of WNV antibodies in sampled birds and the three land use types. Each candidate model included either one or two land use variables (the proportion of a site in: Natural, Agriculture, Urban), as well as the categorical variables of Year, Round, Species, and Age. In total, we evaluated six candidate models. Using Akaike information criterion (AIC) scores, we ranked the candidate models and used model averaging to calculate final estimates, standard errors, odds ratios (OR) and OR 95% confidence intervals for each variable based on the AIC weights. We also used AIC weights to calculate relative importance values for each of the three land use variables.

## **Results**

Over the course of the study, we sampled 1,936 birds (Table 1) comprising 39 species from 19 families in three orders (Appendix B). We found 56 birds positive for *Flavivirus* antibodies, of which WNV accounted for 44, SLEV accounted for two, and the remaining 10 were an unknown *Flavivirus* (Table 1). WNV antibody prevalence in 2009 was 3.7% compared to 0.7% in 2010, resulting in 2.3% prevalence for both years combined.

Of the 11 species positive for WNV antibodies, Mourning Doves, American Robins, Northern Cardinals, and Baltimore Orioles (*Icterus galbula*), had the highest exposure rates at 10%, 9.1%, 19.4%, and 25%, respectively (Table 2). However, given the low sample size

of Baltimore Orioles (n=4), this percentage may not accurately reflect actual exposure risk in this species.

**Table 1.** Summary of birds sampled in the field for 2009, 2010, and overall.

Sampling Summary			2009	2010	Overall
Total no. birds sampled			993	943	1936
Birds <i>Flav.</i> <sup>1</sup> (+)			41	15	56
WNV <sup>2</sup> (+)			37	7	44
SLEV <sup>3</sup> (+)			1	1	2
Unknown <i>Flav.</i> (+)			3	7	10
No. species sampled			33	32	39
Species WNV (+)			9	6	11
Sex	Males		19	4	23
			14	2	16
			4	1	5
Age	AHY <sup>4</sup>		34	7	41
			1	0	1
			2	0	2

<sup>1</sup> *Flavivirus*, <sup>2</sup> West Nile virus, <sup>3</sup> St. Louis Encephalitis virus, <sup>4</sup> After-hatch-year, <sup>5</sup> Hatch-year

**Table 2.** Species we sampled in which *Flavivirus* or WNV-specific antibodies were detected, and the respective prevalence of each.

Species	No. sampled	No. WNV <sup>1</sup> sero(+)	No. <i>Flav.</i> <sup>2</sup> sero(+)	Prevalence (%)	
				WNV	<i>Flav.</i>
Mourning Dove ( <i>Zenaid macroura</i> )	70	7	7	10.0	10.0
American Robin ( <i>Turdus migratorius</i> )	66	6	8	9.1	12.1
Gray Catbird ( <i>Dumetella carolinensis</i> )	54	1	1	1.9	1.9
Chipping Sparrow ( <i>Spizella passerina</i> )	59	2	2	3.4	3.4
Northern Cardinal ( <i>Cardinalis cardinalis</i> )	36	7	7	19.4	19.4
Rose-breasted Grosbeak ( <i>Pheucticus ludovicianus</i> )	21	1	1	4.8	4.8
Red-winged Blackbird ( <i>Agelaius phoeniceus</i> )	64	0	2	0.0	3.1
Common Grackle ( <i>Quiscalus quiscula</i> )	105	0	1	0.0	1.0
Brown-headed Cowbird ( <i>Molothrus ater</i> )	59	1	1	1.7	1.7
Baltimore Oriole ( <i>Icterus galbula</i> )	4	1	1	25.0	25.0
House Finch ( <i>Carpodacus mexicanus</i> )	425	10	10	2.4	2.4
American Goldfinch ( <i>Carduelis tristis</i> )	338	1	4	0.3	1.2
House Sparrow ( <i>Passer domesticus</i> )	291	7	11	2.4	3.8

<sup>1</sup> West Nile virus, <sup>2</sup> *Flavivirus*

We replaced the species variable in our models with a “Family” variable, since there were many species we sampled that had no seropositive individuals, which created quasi-



complete separation of the data within our analysis. Family consisted of several taxonomic families (Columbidae, Turdidae, and Cardinalidae,) in which we a priori expected higher seroprevalence based on existing literature (Beveroth et al. 2006). Species sampled that were not members of these families were grouped into an “Other” category.

Our six candidate models each produced fairly similar AIC values and the  $\Delta$ AICs were all within five integers of the lowest AIC score (Table 3). The subsequent model averaged estimates indicated that taxonomic Family and sampling Year were significantly related to the probability a bird tested positive for WNV antibodies, while Round was not (Table 4). Although weakly significant in the individual candidate models ( $P < 0.05$ ), both Age and land use variables were not statistically significant in the final averaged model (Table 4).

**Table 3.** Akiake Information Criterion (AIC) scores for each candidate land use model (“Urb”= Urban, “Ag”=Agriculture, “Nat”=Natural, “all var.” = Age, Round, Year, Family) and its respective AIC weight ( $w$ ).

Model	AIC	$\Delta$ AIC	$w$
Urb+all var.	358.73	0.00	0.43
Urb+Ag+all var.	360.68	1.95	0.16
Urb+Nat+all var.	360.70	1.97	0.16
Ag+Nat+all var.	360.87	2.14	0.15
Ag+all var.	362.76	4.03	0.06
Nat+all var.	363.66	4.93	0.04

Within taxonomic Family, members of the Cardinalidae that we sampled were significantly more likely to have antibodies to WNV (Table 4). Birds in the families Columbidae and Turdidae were also both more likely than birds from Other families to have WNV antibodies, though not significantly so. Although Age was not statistically different, AHY birds were more than twice as likely (OR=2.136) as HY birds to have WNV antibodies (Table 4). The Round within a season in which a bird was sampled did not have a significant effect on seroprevalence. Birds sampled in 2009 were significantly more likely (OR= 2.335) to have WNV antibodies compared with those sampled in 2010 (Table 4).

**Table 4.** Model averaged results for each variable's effect on a bird's probability of having WNV antibodies.

Variable	Estimate	SE	95% CI	OR	OR SE	OR 95% CI
Intercept	-3.888	0.911	(-5.674, -2.101)	0.020	0.019	(0.003, 0.122)
Landuse						
Urban	0.013	0.009	(-0.006, 0.031)	1.013	0.010	(0.994, 1.032)
Agriculture	-0.003	0.009	(-0.022, 0.016)	0.997	0.009	(0.979, 1.016)
Natural	-0.004	0.009	(-0.022, 0.015)	0.996	0.009	(0.978, 1.015)
Age						
AHY <sup>1</sup>	0.759	0.441	(-0.105, 1.624)	2.136	0.942	(0.900, 5.072)
U <sup>2</sup>	0.860	0.603	(-0.322, 2.042)	2.363	1.425	(0.725, 7.705)
Year						
2009	0.848	0.211	(0.434, 1.262)	2.335	0.493	(1.544, 3.533)
Family						
Cardinalidae	0.844	0.357	(0.144, 1.544)	2.326	0.831	(1.155, 4.685)
Columbidae	0.436	0.368	(-0.286, 1.158)	1.547	0.570	(0.752, 3.185)
Turdidae	0.288	0.370	(-0.437, 1.012)	1.333	0.493	(0.646, 2.751)
Round						
1	-0.139	0.272	(-0.672, 0.394)	0.870	0.237	(0.510, 1.483)
2	-0.019	0.237	(-0.484, 0.446)	0.981	0.233	(0.616, 1.561)

<sup>1</sup>After-hatch-year, <sup>2</sup>Unknown

The model averaging process added enough uncertainty to the final model standard errors that none of the land use variables were considered statistically significant. However, the models with the lowest AIC values all included the Urban land use variable (Table 3). Moreover, the Urban land use variable was always significant ( $P < 0.05$ ) in the models where it was included, whereas Agriculture and Natural variables were not always so. The importance value calculated from the AIC weights also indicated that the effect of Urban land use was nearly twice as important in explaining our results (0.757) compared to either Agriculture (0.369) and Natural (0.347) land uses. In addition, the positive estimate for the effect of Urban land use indicated an increased risk of WNV exposure to birds as the proportion of a study site in Urban land use increased, whereas the negative estimates for Agriculture and Natural land use suggested a decreased risk of exposure (Table 4).

## Discussion

Our findings revealed that land use in central Iowa was weakly related to peridomestic birds' risk of WNV exposure. Specifically, birds captured in more urban landscapes were somewhat more likely to have WNV antibodies than birds in more agricultural or natural landscapes. A similar study by Bradley et al. (2008), found WNV

antibody prevalence in songbirds was 2.5 times higher in urban areas compared to non-urban areas in the Atlanta, Georgia metropolitan area. Our results may not have shown as strong a relationship between WNV seroprevalence and urban land use due to: 1) differences in the level of urbanization between the two study areas (Ames, IA is much less metropolitan than Atlanta, GA), and 2) the virus having reached endemic levels at the time of our study (eight years post emergence) compared with the more epizootic levels of WNV during the GA study (three years post emergence). Several characteristics of urbanized areas may increase a bird's likelihood of being exposed to the virus. First, the impervious surfaces created by urban associated structures, such as sewers and catch basins, may allow water runoff to pool and stagnate, providing ideal vector breeding habitat (Epstein 2001, Su et al. 2003).

Secondly, increased WNV reproduction and transmission have been linked to warmer temperatures (Kilpatrick et al. 2008, Turell et al. 2005). Urban environments may provide a warmer microclimate relative to undeveloped landscapes due to the heat retaining capabilities of urban structures comprised of brick, cement, and asphalt. Lastly, the reservoir competency of different avian assemblages present across various land uses may be a factor, as the viremia produced by different bird species can vary in duration and infectiousness. In the context of WNV and bird diversity, the dilution effect would predict that more avian diversity within the environment reduces the chances of a mosquito vector feeding on a highly reservoir competent bird, essentially diluting the circulation of the virus.

Accordingly, Allen et al. (2009) found an increase in WNV prevalence as bird diversity decreased, for which they suggested a dilution effect mechanism may be responsible. If urban associated bird communities are comprised primarily of highly competent species and lack non- or low-competent species to dilute the virus circulating in the environment, the chances of an individual bird being exposed to WNV in this habitat is increased. Although our study did not address a dilution effect variable, future research should attempt to ascertain the role the dilution effect plays in wildlife disease in urban landscapes.

In contrast to the positive relationship between WNV seroprevalence and urban land use, both agriculture and natural land use were associated with a slightly decreased probability that a bird would have WNV antibodies. Our finding that agricultural land use had little association with WNV risk in peridomestic birds differs from other Midwestern

studies that found increased occurrence of WNV linked to agricultural landcover (DeGroot, et al. 2008, Liu et al. 2008). This may be attributed to habitat differences between ornithophilic and mammalophilic mosquito vector species. DeGroot et al. (2008) reported higher proportions of the relatively more mammalophilic mosquito species, *Cx. tarsalis*, in rural agricultural areas of Iowa, in which they found the highest incidence of WNV in humans. Accordingly, *Cx. tarsalis* prefers irrigated crop fields and newly created pools of water for breeding habitat (Riesen 1993, 2002). The primarily ornithophilic *Cx. pipiens*, however, is more abundant in urban areas, which may explain why WNV exposure for birds in agricultural areas was not as high as what has been observed for humans. Future studies of how WNV circulates among birds and mammals in agricultural areas may identify whether mosquito vector feeding preferences are driving these observations.

Our analyses indicated that the likelihood of a bird having antibodies to WNV in 2009 (3.7%) was much greater than in 2010 (0.7%). Since the initial WNV epidemic in Iowa in 2002, the virus has likely reached endemic levels within the state, and subsequent year-to-year differences may be best explained by environmental variability. According to mosquito capture data for Iowa over the course of our study, mosquito pools showed a similar pattern in seroprevalence to the trends we detected, with a decrease from 2.5% WNV positive *Culex* pools in 2009 to 1.3% in 2010 (Bartholomay, L.C., unpublished data). However, this pattern does not appear to be related to mosquito abundance, as there were more than three times the number of *Cx. pipiens* captured per trap event in Story County, IA in 2010 compared to 2009. Epstein (2001) identified mild winters followed by spring droughts to be ideal conditions for amplifying WNV transmission within the environment. Potentially, overwinter survival of hibernating mosquito vectors infected with WNV in 2010 was reduced by colder winter weather, ultimately reducing the overwinter survival of the virus as well. The National Oceanic and Atmospheric Administration (NOAA) records for Ames indicated there were fewer days below freezing in the winter preceding summer 2009 than in the winter preceding summer 2010 (65 and 75 days, respectively). In addition, the precipitation data indicate that there was 6.7 inches less rainfall over April, May, and June in 2009 than in 2010 (NOAA 2011), which is in keeping with the drier spring conditions Epstein identified as useful for WNV amplification. Likely, drier spring conditions create stagnant pools of

water early in the mosquito breeding season, allowing vector (and virus) reproduction to begin sooner. Although anecdotal, these weather differences may have been a factor in the differences in WNV seroprevalence between years. Further studies on the relationship between local vector, host, and weather interactions in WNV endemic areas are necessary.

We observed no significant temporal trends in WNV seroprevalence over either sampling season. These results are consistent with those of Bradley et al. (2008), who reported no significant difference in avian seroprevalence by month, but deviate from other studies that reported seasonal differences in WNV infections in crows, mosquitoes, and humans (Ludwig et al. 2010, Lui et al. 2008, DeGroote et al. 2008, respectively). Although both exposure and infection data may indicate WNV activity in an area, these data differ in the temporal information they can provide. The viremic period in birds may last up to a week after exposure to the virus (Komar et al. 2003), which only allows a short period of time for detecting actual infection. However, the discovery of an infected individual provides a relatively definitive timeline for recent WNV activity in an area. In contrast, WNV neutralizing antibodies are usually produced within one to two weeks post-exposure (Komar et al. 2003, Styer et al. 2006) and can be long lasting (Komar et al. 2003, Gibbs et al. 2005), making it difficult to determine when a bird was actually exposed to WNV. Although antibody persistence likely varies by species, Gibbs et al. (2005) found that Rock Pigeons (*Columba livia*) were capable of maintaining detectable antibody levels beyond a one-year period. If antibodies persist for long periods, testing for exposure instead of actual infection may not provide useful information regarding seasonal trends in WNV exposure.

Our findings of high seroprevalence within the Cardinalidae are similar to those of other studies that identified Northern Cardinals as having high WNV antibody prevalence (Gibbs et al. 2006, Beveroth et al. 2006). The natural history traits of members of the Cardinalidae we sampled (Northern Cardinals and Rose-breasted Grosbeaks [*Pheucticus ludovicianus*]) were the only seropositive members of this group), such as preferred breeding habitat or mosquito defense behavior, may contribute to an increased likelihood of being fed upon by WNV mosquito vectors. For example, Northern Cardinals often place their nests within dense foliage or thickets (Halkin and Linnville 1999). This habitat may create ideal soil moisture and temperature conditions for WNV vectors, such as *Cx. pipiens*, increasing

the overlap, and thus exposure potential, between the host and vector. Additionally, Cardinalid physiology may subsequently produce a long-lasting detectable antibody response, where birds exposed to WNV in the previous year(s) retain a minimum antibody titer, increasing the chance that a sampled bird will test positive. We also found that Mourning Doves (the only member of Columbidae sampled) and American Robins (the only seropositive member of Turdidae sampled) had relatively high seroprevalence (though not significantly so), which is consistent with the findings of Beveroth et al. (2006). Higher seroprevalence among the species we sampled within these three families suggests they are likely to survive after being bitten by an infected mosquito vector rather than succumbing to infection. High seroprevalence combined with the abundance and widespread distribution of these species in central Iowa makes them ideal sampling targets for detecting the presence of WNV in the environment.

Although not statistically significant, our results indicated that AHY birds were more likely to have been exposed to WNV compared to HY birds, a trend also reported by Bradley et al. (2008) and Beveroth et al. (2006). Possibly, AHY birds have a greater likelihood of being antibody positive because they experience a longer period of potential exposure to mosquito vectors during the summer season relative to HY birds. The higher seroprevalence in AHY birds may also be a result of antibody persistence from the previous season. Conversely, seropositive HY birds indicate exposure to an infected mosquito vector within the current season, although it is possible that very young HY birds will still harbor maternal antibodies (Nemeth et al. 2008, Gibbs et al. 2005). A study by Nemeth et al. (2008) found that maternal antibodies to WNV were no longer detectable after nine days post hatch in House Sparrows. Since this species does not fledge the nest until ~14 days post hatch (Lowther and Cink 2006), antibodies within a seropositive individual captured by mist net are not likely derived from the mother. However, Gibbs et al. (2005) reported that Rock Pigeon squabs can retain detectable maternal antibodies from 19 to 33 days post hatch, which is also the window in which this species fledges (Johnston 1992), and thus the potential exists for a fledged bird captured in a mist net to be seropositive as a result of maternal antibodies. Clearly, the retention of maternally derived antibodies to WNV varies by species, and the

difficulties associated with differentiating maternal antibodies from non-maternal antibodies may complicate the interpretation of WNV exposure rates in HY birds.

In conclusion, we identified several factors associated with a bird's risk of WNV exposure in central Iowa. The weak positive relationship between seropositive birds and urban land use may be linked to the preferred habitat of ornithophilic mosquito species being present within urban landscapes. The increased seroprevalence for birds in 2009 relative to 2010 appears to be related to differences in weather, which suggests that annual variation in environmental conditions may strongly influence WNV activity. Lastly, the higher seroprevalence within the Cardinalidae, Columbidae, and Turdidae families relative to the other taxonomic families sampled may be due to variation in exposure, physiological response to infection, or survival. Overall, our findings can provide useful information to both wildlife management and public health agencies for tailoring disease surveillance or management activities in central Iowa. For example, surveillance efforts that focus on sampling adult Cardinalids in urban landscapes may enhance the detectability of WNV activity in the environment. On the other hand, peridomestic birds may not be useful indicators of WNV activity in agricultural areas. Additionally, our study established baseline WNV seroprevalence information for peridomestic birds in this region. Future studies of WNV epidemiology in Iowa can expand on our findings by further studying the relationship between the environment, vectors, and host species. In particular, these studies should investigate the role weather and land use play in the survival and distribution of WNV mosquito vectors, whether mosquito species feeding preferences between birds and mammals are influencing WNV prevalence in different taxa on the landscape, and whether physiology, mosquito defense/avoidance behavior, and/or habitat overlap explain the differences in WNV seroprevalence among avian taxa.

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## CHAPTER 5: GENERAL CONCLUSIONS

My research evaluated i) if routinely collected rehabilitation data are useful for deducing information about wildlife health and population disturbances (Ch. 2), ii) whether West Nile Virus (WNV) exposure patterns in avian submissions to a wildlife rehabilitation facility reflected exposure patterns in free-living avians, which could provide valuable information regarding the prevalence of disease in the wild (Ch. 3), and iii) whether the risk of exposure to WNV in Midwestern peridomestic avian species varied with land use in order to identify locations where birds were at greatest risk of encountering the virus (Ch. 4). For specifics regarding the in-depth objectives and study details, please refer to the individual chapters within this thesis. What follows are the general findings for each study, brief management implications, and suggestions for future research.

My general findings for Chapter 2, “A Ten-year Retrospective Study of Avian Population Trends from Wildlife Rehabilitation Submissions”, were that wildlife rehabilitation submission rates and diagnoses for the avian groups of raptors and corvids varied among years, seasons, and species. In most cases, records were not of sufficient detail to draw specific conclusions regarding the actual causes of morbidity within these groups. Despite a lack of formal disease testing, however, the signature of WNV emergence in corvid populations was strongly evident in the rehabilitation data. Specifically, the sharp rise in unsolicited American Crow submissions during the initial emergence of WNV in central Iowa, followed by a significant decline in these submissions during the next five years, is consistent with other, independent data suggesting populations of crows were depressed after the initial wave of disease. For wildlife rehabilitation center records to be useful to wildlife managers, I recommend that 1) more detailed information (e.g., location, habitat, surroundings, weather, animal’s behavior) should be collected and recorded about the circumstances under which wildlife rehabilitation patients are found, and 2) samples for disease testing should be routinely collected and archived. Finally, and perhaps most importantly, wildlife managers should communicate with wildlife rehabilitators regarding management objectives and species and diseases of particular concern. These actions would enhance the ability of this wealth of wildlife data to be useful to wildlife managers in making

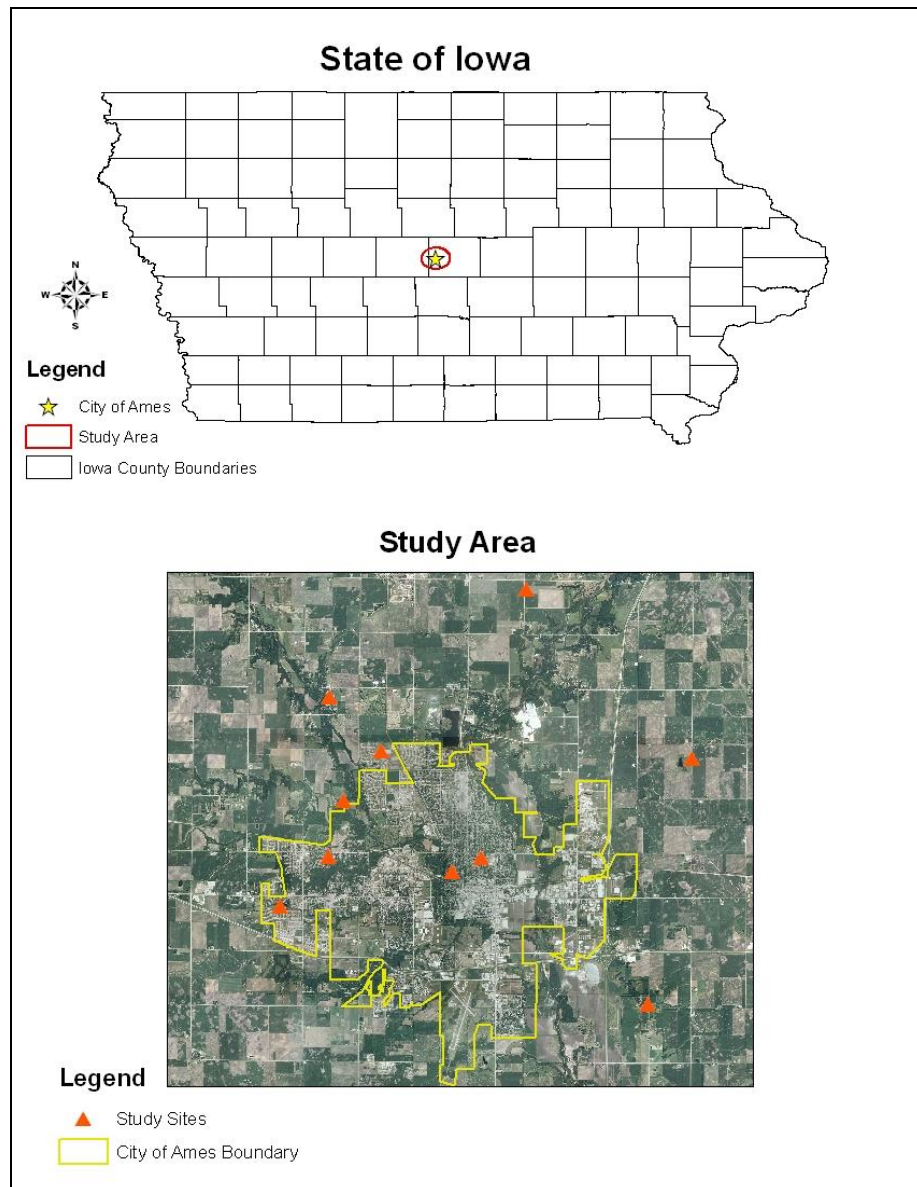
inferences regarding trends in wildlife populations from which rehabilitation patients originate.

The results for Chapter 3, “Wildlife Rehabilitation Centers in Disease Surveillance and Monitoring”, indicated that WNV antibody prevalence in both wildlife rehabilitation center and field-sampled birds was similar, suggesting WNV exposure in wildlife submitted to this facility is representative of current exposure rates in the wild. Thus, wildlife rehabilitation centers could provide valuable information regarding the presence, prevalence, and patterns of wildlife disease to managers at a fraction of the cost and effort of active disease surveillance. Some shortcomings of using wildlife rehabilitation centers in disease surveillance include annual variation in the number and species composition of submissions, so that continuously monitoring wildlife disease at the taxonomic level of family or species may be difficult. Additionally, whether a disease is occurring at epizootic or enzootic levels may make a difference in how the prevalence is reflected in wildlife rehabilitation submissions, as it is likely that many more wild animals become incapacitated by disease during epizootics, and are subsequently submitted to rehabilitation centers. However, WNV appeared to reflect a low-level enzootic presence within our study. I also found that raptors submitted to the wildlife rehabilitation center had the highest WNV exposure rates relative to other bird taxa and therefore may be particularly useful in WNV surveillance and monitoring. Lastly, I observed that the order Piciformes had no WNV seropositive individuals within the field or wildlife rehabilitation center, despite a sample size comparable to several other orders in which WNV exposure was detected, for which I suggest future research should explore this anomaly. I also suggest that future studies on the utility of wildlife rehabilitation centers in disease surveillance should expand to include additional rehabilitation centers, EIDs/epizootic diseases of concern, and classes of wildlife.

The findings of my Chapter 4 study, “Variation of West Nile Virus Exposure in Avian Species with Land Use”, suggest that a bird’s risk of WNV exposure increased in urban areas compared to agricultural and natural areas, possibly because urban landscapes often harbor standing water favorable for ornithophilic mosquito vector reproduction. Potentially, agricultural and natural areas provided breeding habitat that was preferred by non-ornithophilic mosquito species, so that birds in these habitats were less likely to be

exposed to WNV relative to birds in urban habitats. I also found that risk of exposure was significantly greater in 2009 than in 2010. This disparity appears to be related to temperature and precipitation differences in weather between years, suggesting that annual variation in environmental conditions may strongly influence WNV activity. My Chapter 4 results also indicated that seroprevalence was higher within the Cardinalidae, Columbidae, and Turdidae families relative to other taxonomic families sampled. Heterogeneity among taxa may be due to variation in exposure, physiological response to infection, or survival. Overall, this study identified several factors associated with WNV exposure in Midwestern peridomestic birds that might be useful for tailoring disease surveillance or management activities. Future studies of WNV epidemiology in Iowa can expand on my findings by further studying the relationship between the environment, vectors, and host species. In particular, these studies should investigate the role weather and land use play in the survival and distribution of WNV mosquito vectors, whether mosquito species feeding preferences between birds and mammals are influencing WNV prevalence on the landscape, and whether a bird's physiology, mosquito defense/avoidance behavior, and/or vector habitat overlap explain the differences in WNV seroprevalence among avian taxa.

## APPENDIX A. STUDY AREA



## APPENDIX B. ORDERS, FAMILIES, AND SPECIES CAPTURED

Order	Family	Common Name	Scientific Name	No. Sampled
Columbiformes	Columbidae	Mourning Dove	<i>Zenaida macroura</i>	70
Piciformes	Picidae	Red-bellied Woodpecker	<i>Melanerpes carolinus</i>	3
		Downy Woodpecker	<i>Picoides pubescens</i>	85
		Hairy Woodpecker	<i>Picoides villosus</i>	14
Passeriformes	Tyranidae	Eastern Phoebe	<i>Contopus virens</i>	2
		Eastern Wood-Pewee	<i>Sayornis phoebe</i>	6
	Vireonidae	Warbling Vireo	<i>Vireo gilvus</i>	2
	Corvidae	Blue Jay	<i>Cyanocitta cristata</i>	6
	Hirundinidae	Barn Swallow	<i>Hirundo rustica</i>	1
	Paridae	Black-capped Chickadee	<i>Poecile atricapillus</i>	107
		Tufted Titmouse	<i>Baeolophus bicolor</i>	2
	Sittidae	White-breasted Nuthatch	<i>Sitta carolinensis</i>	40
	Trogloditidae	House Wren	<i>Troglodytes aedon</i>	3
	Turdidae	Eastern Bluebird	<i>Sialia sialis</i>	5
		Veery	<i>Catharus fuscescens</i>	1
		Swainson's Thrush	<i>Catharus ustulatus</i>	1
		American Robin	<i>Turdus migratorius</i>	66
		Gray Catbird	<i>Cumetella carolinensis</i>	54
		Brown Thrasher	<i>Toxostoma rufum</i>	5
	Sturnidae	European Starling	<i>Sturnus vulgaris</i>	25
	Bombycillidae	Cedar Waxwing	<i>Bombycillia cedrorum</i>	3
	Parulidae	Tennessee Warbler	<i>Oreothlypis peregrina</i>	1
		Yellow Warbler	<i>Dendroica petechia</i>	1
	Emberizidae	Chipping Sparrow	<i>Spizella passerina</i>	59
		Vesper Sparrow	<i>Poocetes gramineus</i>	1
		Song Sparrow	<i>Melospiza melodia</i>	13
	Cardinalidae	Northern Cardinal	<i>Cardinalis cardinalis</i>	36
		Rose-breasted Grosbeak	<i>Pheucticus ludobicianus</i>	21
		Indigo Bunting	<i>Passerina cyanea</i>	9
		Dickcissel	<i>Spiza americana</i>	2
	Icteridae	Red-winged Blackbird	<i>Agelaius phoeniceus</i>	64
		Yellow-headed Blackbird	<i>Xanthocephalus xanthocephalus</i>	4
		Common Grackle	<i>Quiscalus quiscula</i>	105
		Brown-headed Cowbird	<i>Molothrus ater</i>	59
	Fringillidae	Baltimore Oriole	<i>Icterus galbula</i>	4
		Purple Finch	<i>Carpodacus purpureus</i>	2
		House Finch	<i>Carpodacus mexicanus</i>	425
		American Goldfinch	<i>Spinus tristis</i>	338
	Passeridae	House Sparrow	<i>Passer domesticus</i>	291



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