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A meta-analysis approach was used to extract the frequency of avian seropositivity to West Nile Virus (WNV) (fS) and mortality from WNV (fM) from the literature published between the years 2001-2010. The indices, fS and fM , were used to represent states of resistance (seropositivity with no or limited mortality) and susceptibility in birds (detection of WNV with mortality), respectively. The indices fS and fM were calculated for each species across publications into different taxonomic strata, year, combined time intervals, and geographic location. The data were analyzed by logistic regression to assess the status of WNV infection amongst different avian taxonomic strata and infer changes of WNV infection status over time. Using level of peak viremia as a proxy for host competence among the avian species, the value of the phylogenetic signal was tested by Blomberg's K after transformation of the branch lengths. Sixty-three publications were used for the final dataset representing studies performed from 1999 to 2008 and published between 2001-2010. Analysis of both fS and fM demonstrated variability in avian responses to WNV across order, family, and species. When the data were analyzed for fS , the families and species of the orders Ciconiiformes, Galliformes, and Columbiformes had significantly higher fS than that of the Passeriformes ($P < 0.0001$). Within the Passeriformes, the Corvidae and Cardinalidae families had higher fS than any other family. When the data were analyzed for fM , dead Corvidae and Paridae were most likely to test positive for virus. Temporally, a positive trend of increasing fS was detected in several avian species while the American crow (*Corvus brachyrhynchos*) was the only species with a significant negative trend in fM ($P = 0.042$). Little to no interaction of geographic location with time was detected within species of birds. A weak but significant phylogenetic signal corresponded to host range, suggesting that the rate of spread of WNV through the avian community was partially dependent of the relatedness of hosts. The meta-data predicted that species within families, and even order, were heterogeneous in their disease susceptibility traits to WNV. The Passeriformes contributed most to this variation. Several trends in various families and orders of birds were observed from which new studies could be pursued. The pooling of data was key in detecting areas where data was lacking and

identified aspects of avian responses to WNV could be expanded.

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Introduction

Emerging pathogens can cause large outbreaks and new diseases symptoms after a successful introduction to a new area. The introduction of pathogens into new host populations is of special concern because in the absence of a shared evolutionary history between the pathogen and its new hosts, pathogenicity may not be constrained (Woolhouse et al. 2005). Multihost pathogens such as West Nile virus (WNV) have an ecologically complex life cycle driving its spread and establishment compared to single host pathogens since the mechanisms required for infection, exploitation, and transmission are highly variable among hosts (Gandon et al. 2008). Multihost pathogens can exploit the heterogeneity of susceptibility traits of various hosts to spread more rapidly (Dobson & Foufopoulos 2001; Yates et al. 2006).

Dobson and Foufopoulos proposed in 2001 that multihost pathogens rely on complex matrices where the persistence of a pathogen depends on the reproductive rates of infection (R_0) and heterogeneity of host traits such as host age, sex, and sexual activity (Dobson & Foufopoulos 2001). This mathematical model is composed of matrices representing all infected hosts and emphasizes the average duration of infection for an individual of each species capable of transmitting the pathogen. In the end, the components of the matrix that predict the force of infection of each species rely on the mortality rate, the virus induced mortality rate, and the recovery rate of each host species. This particular modelling provided three important insights into how avian heterogeneity could contribute to the overall growth of the WNV epizootic and its subsequent establishment (Dobson & Foufopoulos 2001). First, the American crow (*Corvus brachyrhynchos*) would contribute to the epizootic only if the species was present in high enough numbers to overcome its susceptibility to WNV. Second, species

such as the house sparrow (*Passer domesticus*) which exhibited limited mortality and high abundance would be important reservoirs. Third, diversity in susceptibility across species may assist in the establishment of WNV by providing many hosts that increase the resources available to the vector which, would in turn, further transmission.

Cataloging of spontaneous infections of wild birds (Gamino & Hofle 2013; Hofmeister 2011; Komar 2003), experimental infections (Perez-Ramirez et al. 2014), and detailed modelling studies (Cruz-Pacheco et al. 2005; Gomez et al. 2008; Griffing et al. 2007; Kilpatrick et al. 2010b; Kilpatrick et al. 2006b; LaDeau et al. 2007; Mughini-Gras et al. 2014; Yiannakoulis et al. 2006) have provided results either supporting these predictions or providing new hypotheses for avian host susceptibility traits. Analyses of bird abundance data based on the Breeding Bird Survey (BBS) indicates that some species have declined significantly since the arrival of WNV in the United States, while others have remained unaffected (Koenig et al. 2007; LaDeau et al. 2007; McLean et al. 2002; Wheeler et al. 2009). In terms of susceptibility, corvids, including crows and jays, have been shown to have high mortality from WNV, whereas sparrows and pigeons show lower mortality rates. Experimental infections of birds demonstrate that individual species exhibit a broad spectrum of pathology and disease, from asymptomatic infections to severe neurological syndromes or sudden death, which in some cases is correlated to the viral load (Basile et al. 2010; Komar et al. 2003; Lopes et al. 2007; McLean et al. 2002; McLean et al. 2001; Nemeth et al. 2006a; Nemeth et al. 2006b; O'Brien et al. 2010; Senne et al. 2000; Swayne et al. 2000; Wunschmann et al. 2004; Wunschmann et al. 2005). Taken together, these studies have indicated a role for the house sparrow as important in the spread of WNV because of its high viral load and survival to infection. The American robin (*Turdus migratorius*)

is also an important driver of transmission because of its abundance and high sustained virus load during nesting. The American crow is more limited in sustaining outbreaks because of its susceptibility to mortality during WNV infection (Komar 2003; Komar et al. 2003; LaDeau et al. 2007).

Birds belong to highly complex taxonomic strata and individual species may not represent the same disease susceptibility traits across families, even within closely related species of the same order. Because of the large number of field studies, combining data and reanalyzing via meta-analysis can provide information for the gaps in knowledge of WNV infection of birds.

A meta-analysis was performed on serologic, virologic, and mortality data published on field studies performed in the U.S. during the first decade of WNV encroachment into North America. Data from these studies was combined based on three assumptions: 1) that positive serological status could be used as an indicator of the survivorship of the bird to WNV infection; 2) that a dead bird infected with WNV was susceptible to the pathogen; and 3) that studies published between 2001-2010 were performed in the context of local disease transmission and had taken into account other co-morbidities or factors resulting in the death of the bird. In this study, the literature was surveyed using the two basic parameters of seropositivity and mortality rate within each study to examine differences across taxonomic strata using order, family, and species level combined as meta-data. Using experimental data for 24 species, host competence as defined by level of viremia was tested for phylogenetic relatedness. Statistically, phylogenetic signal is a measurement of the “non-independence” of particular species traits based on phylogenetic relatedness (Revell et al. 2008). The goal of this meta-

analysis was to determine if combining data from several field studies could provide new information on avian hosts not commonly examined under normal experimental challenge conditions. In addition, it was hypothesized that this type of analysis would be comparable to other more extensive WNV modelling and experimental infection studies already published for studying wild populations of birds.

Methods

Database Search and Dataset Construction

This study did not require approval by an animal ethics committee since no live animals or tissues from avian species were used in this study. The guidelines as defined by the “preferred reporting items for systemic reviews and meta-analyses [PRISMA, (Moher et al. 2009)] were followed for this meta-analysis (Additional Files 1 and 2) In this study, the literature was reviewed for the years 1999 through 2010, included mortality and seroprevalence to WNV, and consisted only of natural infections of avian species. A search of peer-reviewed publications within the ISI Web of Science and PubMed was performed by querying for any article containing words (“West Nile virus” [MeSHTerms] OR ‘WNV’ [All Fields]) AND (“birds”[MeSHTerms OR All Fields] OR “avian”[All Fields]). In total, 421 references were found after the initial search for articles generated from US publications from 2001 to 2010 to obtain data generated from collection years 1999 to 2008 (Figure 1). Papers were chosen only if there was primary data consisting of the common name or species of bird, the total number of birds tested and the number of each species tested, the status of the bird (alive or dead), the numbers of birds tested, the testing format, the number of positive birds, and the location (state level of each bird). A sample size greater than 15 in the original study was required for

inclusion of the data from each paper to avoid oversaturation of the dataset with small numbers of birds that precluded recalculation of the frequency of seropositivity (fS) and mortality (fM) of that species. The order and family of each species was also added to the database. Data were coded to subgrouping into three time periods consisting of 1999 through 2001 (period 1), 2002 through 2004 (period 2), and 2005 through 2008 (period 3). If low bird counts precluded analysis as three time periods, two time periods consisting of 1999 through 2004 (period 1) and 2005 through 2008 (period 2) were used. Data were grouped and analyzed as two geographic zones designated as East (CT, FL, GA, IL, KY, LA, MA, MN, MS, NJ, NY, OH, PA, VA, WI) and West (CA CO, KS, MT, ND, OK, TX, WY). The reference was also coded and included for each bird. Each method of testing was recorded for each entry. The method of WNV detection was recorded and included detection of live virus by culture, detection of viral antigen, and detection of viral RNA by rt-PCR. The method of WNV serology was recorded and included ELISA (IgM WNV ELISA, IgG WNV ELISA, or competitive inhibition ELISA) and PRNT. For those studies based in experimental infections, the mean viremia peak (as \log^{10} pfu/ml) was included.

Frequency of Mortality and Seropositivity.

The parameter fS was calculated on the extracted data by dividing the number of each species' plasma or serum which tested WNV-antibody positive by the total number of that particular species tested. The fM was calculated on the extracted data by dividing the number of each species' carcass which tested positive by WNV by the total number of that particular species tested. These indices were recalculated for each publication and then the raw numbers

were pooled and calculated for taxonomic rank (order and family and species) for all data from 1999 through 2008, for each separate year, for each period, and for region.

Data Analysis

Recalculated parameters were used to infer resistance (fS) and susceptibility (fM) to WNV in the subsequent data analysis. Both fS and fM were log transformed and plotted to visualize general patterns based on taxonomic, temporal, and geographic classification (Additional File 3). The indices, fS and fM were compared at the level of order, family, and species using contingency tables and analyzed by the adjusted Mantel-Haenszel chi-square test and odds ratio (OR) (Dosemeci & Benichou 1998).

Two logistic regression models were constructed with time as a continuous regressor. The reference article was included in this analysis as a random effect. Thus, any possible correlation between observations obtained from the same article was incorporated and considered in the model (Casella 1988; de Palma et al. 2008; McCulloch 1985; Searle 1995). The first regression was performed using the following logistic equation:

$$\text{logit}(\hat{\pi}) = \log\left(\frac{\hat{\pi}}{1 - \hat{\pi}}\right) = \hat{\beta}_0 + \hat{\beta}_1 x + \hat{\gamma}_j + \hat{\mu}$$

where β_0 is the intercept from the equation, β_1 is the regression coefficient (fixed but unknown), $\hat{\pi}$ consisting of fS or fM , x as the time period, $\hat{\gamma}_j$ as the random effect to account for any correlation that may be among publications (j), and $\hat{\mu}$ is the normal error of the model. A second regression model incorporated the interaction of geographic location and time period and was performed using the equation,

$$\text{logit}(\hat{\mu}) = \log\left(\frac{\hat{\mu}}{1 - \hat{\mu}}\right) = \hat{\beta}_0 + \hat{\beta}_1 x_1 + \hat{\beta}_2 x_2 + \hat{\beta}_3 x_1 x_2 + \hat{\mu}$$

with f_S or f_M as $\hat{\mu}$, x_1 as the year period, x_2 as the geographic area (East or West), $x_1 x_2$ as the interaction term, and $\hat{\mu}$ as the normal error of the model. In this model, the focus of interest was the term $\hat{\beta}_3$. Slopes were inspected first from the linear regression and then, the interaction effect was incorporated into the model to assess the significance of the term. All statistical analyses were conducted using web-based and commercially available computer statistical packages (R v 2.15, The R Foundation for Statistical Computing, 2012 and SAS 9.3 SAS Institute, Cary, North Carolina).

From these same publications, data from inoculations of 24 avian host species were used to estimate detectable phylogenetic signal for the mean viremia peak (MVP) from each species. The MVP was used as a proxy for host competence since a bird is infectious to feeding mosquitoes when the mean viremia titer is greater than $10^{5.0}$ pfu/ml (Komar et al 2003). For analysis, the trait was categorized into two values as competent (MVP > $10^{5.0}$ pfu/ml) or noncompetent (MVP < $10^{5.0}$ pfu/ml) hosts. A set of 1,000 randomly-selected topologies that included the species in the dataset were downloaded from a web available global phylogeny of birds [<http://birdtree.org>, (Jetz et al. 2014)]. Branch lengths were transformed using an Ornstein-Uhlenbeck (OU) transformation model (Blomberg et al. 2003) with *ad-hoc* functions which applies trait data using phylogenetic regression to estimate the contribution of traits compared to the expected phylogenetic relationships. Phylogenetic signal was calculated based on Blomberg's K which transforms the tip of a phylogenetic tree according to a trait value

(Blomberg et al. 2003). Statistical significance was estimated after 10,000 randomizations. Analyses were conducted using a statistical software package [R v. 3.15, Picante: R tools for integrating phylogenies and ecology v 1.6.2; (Kembel et al. 2010)].

Results

Database Search and Study Selection

From the 421 article references initially found (244 articles with serological data and 137 articles with mortality data), 87 were selected for full review (Figure 1). A meta-analysis approach was used to extract the frequency of avian seropositivity (fS) and the frequency of avian mortality (fM) from each publication. Sixty-three articles were included for final analysis consisting of 39 articles for the fS data and 24 articles for the fM data (Additional Files 3 and 4). The highest number of birds sampled occurred in 2004 (Figure 1).

The final dataset included 142 avian species for which there was an antibody response detected against specific WNV antigens using either ELISA and/or a plaque reduction neutralization testing (PRNT) (Additional File 3). The final dataset included 88 species of dead birds that tested positive for WNV, WNV antigen, or WNV genes detected by viral culture, antigen-based commercial kits, or reverse transcription polymerase chain reaction (rt-PCR), respectively (Additional File 4). In total, 174 species of birds were included in the meta-analysis that had 1) 15 or more samples collected per species and 2) were either positive for WNV virus, specific antibodies against WNV, WNV antigen, or WNV genetic material (Figure 2).

Passeriformes represented 77% of the meta-data, followed by Columbiformes and Galliformes (Figure 2). For data with serological status (recalculated to fS), the house sparrow and the white-crowned sparrow (*Zonotrichia leucophrys*) were most commonly represented,

followed by the house finch (*Haemorhous mexicanus*), the mourning dove (*Zenaida macroura*), and Gambel's quail (*Callipepla gambelii*). For data with mortality status (recalculated to *fM*), the American crow was most commonly represented followed by other members of the Corvidae family including the Florida scrub jay (*Aphelocoma coerulescen*) and blue jay (*Cyanocitta cristata*).

Overall Analysis

For the indices, *fS* and *fM*, association with taxonomic class was performed using contingency tables and odds ratios (OR). Variable responses to WNV infection were observed for both *fS* and *fM* across order, family, and species. For the analysis at the taxonomic rank of order, Passeriformes were designated as the reference group, with Ciconiiformes, Falconiformes, Galliformes, and Columbiformes more likely to be WNV-seropositive than Passeriformes with each respective OR = 1.56, 2.81, 2.47, and 2.61 ($P < 0.0001$; Table 1). Piciformes and Anseriformes were significantly less likely to test seropositive ($P < 0.001$; Table 1). Strigiformes and Gruiformes were not significantly different than the Passeriformes for *fS* overall. Passeriformes were the most likely order to have dead birds in which WNV was detected ($P < 0.0001$; Table 1).

For the comparison of *fS* and *fM* data at the taxonomic rank of family, the Corvidae were used as the reference group. Corvidae and Cardinalidae families were more likely to have seropositive species than any other family (Table 2). The Corvidae and Paridae families were more likely to have dead birds that were positive for WNV antigen compared with other families (Table 2). Data were obtained for 32 families of birds and the Anatidae, Emberizidae, Parulidae, and Columbidae families showed no significant differences in *fS* or *fM*. Except for the

Cardinalidae, all families were less likely to be seropositive for WNV than the Corvidae. However, the Corvidae demonstrated the highest mortality. In families with evidence of variable responses to WNV among species, differences were disproportionally represented by one or two species of a family as in the Corvidae with the American crow (Figure 3). Within the Icteridae family, the common grackle (*Quiscalus quiscula*) was most likely to be seropositive of the other 11 species. The house finch, American robin, and blue jay were most likely to be seropositive within the Fringillidae, Turdidae, and Corvidae families, respectively ($P < 0.0001$). Within the Galliformes, the Phasiandea were 31 times more likely to be seropositive than the Tetraonidea.

Trend Analysis by Species

Incorporating the reference article as a random effect, a logistic regression was constructed to determine if fS or fM increased or decreased as a function of time. A series of descriptive and exploratory approaches were initially used to define and select the species with appropriate and valid data (Figure 4). Nineteen species were selected for fS analysis and 9 species for fM analysis. Using this model, most of the species showed a positive trend of increasing fS in time in which all had positive β estimates. Eight species, including the red winged blackbird (*Agelaius phoeniceus*), the California quail (*Callipepla californica*), Gambel's quail, the northern cardinal (*Cardinalis cardinalis*), the house finch, the rock dove (*Columba livia*), the American crow, the fish crow (*Corvus ossifragus*), and the song sparrow (*Melospiza melodia*) showed a significant increase in fS over time (Table 3). The other eight species showed no temporal changes in fS . The common grackle and northern mockingbird (*Mimus polyglottos*) had negative trends which did not significantly change over time.

The odds of being WNV seropositive increased with time (Figure 4). The OR for seropositivity increasing between the time periods was highest for Gambel's quail ($e^{\beta} = 80.7$) followed by the northern cardinal ($e^{\beta} = 15.4$) and the rock dove ($e^{\beta} = 10.62$). When analyzed as two time periods, the first period being 1999 to 2004 and the second period being 2005 to 2008, the change in seropositivity was lower across all species during the second period. However, the change in fS over time was different amongst species. Analyzed as two time periods, the change in fS between periods 1 and 2 [$fS_{\text{period2}} - fS_{\text{period1}}$] were highest for the cardinal, rock dove, and the fish crow with differences equal to 0.149, 0.173, and 0.122, respectively. The house finch and the American crow demonstrated the smallest changes between the two time periods equal to 0.03, and 0.04, respectively. The house sparrow, northern cardinal, and rock dove, when analyzed over three periods (1999-2001, 2002-2004, and 2005-2008) had the highest changes in fS between year-period 2 to year-period 3 with ($fS_{\text{period3}} - fS_{\text{period2}}$) equal to 0.65, 0.58, and 0.52, respectively. The American crow was the only species that showed a significant negative trend in fM across the years ($e^{\beta} = 0.65$) (Table 3, Figure 4). There was a smaller decrease in the odds of death from WNV between the first and second time periods (-0.098), with an increase in the rate during the second to third periods (-0.105).

When the interaction between year-period and geographic location was included in the model, the rock dove showed an increase in fS in the East whereas for the West, the slope was close to zero. No other interactions between year-period and geographic location was observed for species present in both geographic locations.

Relationship between Viremia and Species Phylogeny

If WNV exploited a particular avian host for transmission, then the level of viremia could theoretically be more highly associated with related species. Thus, the probability that two particular species share similar competence in host susceptibility or resistance to the virus should decline with increasing phylogenetic distance between those species. The degree of phylogenetic signal was tested by quantifying the value of Blomberg's K after transformation of the branch lengths and the value of K was significant for host competence to WNV ($K=0.42$, $P=0.024$) indicating that the contribution of phylogeny to viral load (or host competence) was non-random (Figure 5).

Discussion

West Nile virus is currently the most widely distributed arbovirus in the world (Kramer et al. 2008). This particular disease was used as a focus of a meta-analysis because of the large amount of data collected during the US outbreak which recorded seropositivity, virus detection, and dead bird counts in field-based routine surveillance papers and hypothesis driven research papers. Birds are the primary hosts for WNV throughout its worldwide geographical distribution and the exchange of virus between mosquitoes and birds represents the annual transmission cycle (Campbell et al. 2013; Lanciotti et al. 1999). Not all bird species produce sufficient viremia to sustainably contribute to the overall virus transmission cycle. However, bird abundance can overcome inefficient transmission (Komar et al. 2003; Lanciotti et al. 1999). The dissimilar mortality rates and the broad spectrum of pathology amongst species of birds suggest that host heterogeneity contributes to susceptibility to WNV (Bowen & Nemeth 2007; Busquets et al. 2012; Del et al. 2014a; Kilpatrick et al. 2006a; Komar et al. 2003; Lanciotti et al. 1999; Langevin et al. 2001; Nemeth et al. 2011; Oesterle et al. 2009; Wheeler et al. 2011;

Ziegler et al. 2013). The final meta-analysis of the data focused only on field-based studies of the first 10 years of the WNV epizootic because, although, bird sampling may have had year-to-year sampling biases, the majority of diagnostic testing for public health surveillance occurred throughout this period. Once a major decline in WNV surveillance occurred with the onset of swine influenza as documented in the literature, comprehensive data was more difficult to rely on (Couzin-Frankel 2010). Census and diagnostic laboratory data was not used because of concerns with overlap with the published data.

Many avian diseases, especially those that are not zoonotics do not have compelling modelling studies and experimental challenge data. The hypothesized that multiple, small field WNV studies analyzed by meta-analysis would contribute to the understanding of a host pathogenesis was investigation. This approach provided information regarding trends in mortality and seropositivity for several species of birds and determined if there were temporal and/or geographical impacts to these trends.

Field studies were utilized because experimental inoculations of (usually) locally captured wild birds may not be truly representative of viral load since susceptibility can vary geographically within the same hosts. Since the emergence of WNV in North America in 1999, the number of articles pertaining to avian WNV infections was several thousand in the first ten years, yet, only a small fraction of the literature focused on its epidemiology. The inclusion parameters required consistent serological and viral detection methods which resulted in 63 contributing publications. WNV infection and serological status was cataloged for 174 species of birds since 2001 in the US-based studies included in this analysis. Multiple studies have been performed examining susceptibility through experimental inoculation of representative species

of North American Passeriformes (Brault et al. 2004; Brault et al. 2011; Del et al. 2014b; Kipp et al. 2006; Komar et al. 2003; Komar et al. 2005; McLean et al. 2001; Nemeth et al. 2009b; Nemeth et al. 2008; Nemeth et al. 2011; Owen et al. 2006; Reisen et al. 2006a; Reisen & Hahn 2007; Reisen et al. 2006b; VanDalen et al. 2013; Wheeler et al. 2012), Piciformes (Komar et al. 2003), Anseriformes (Banet-Noach et al. 2003; Blitvich et al. 2003), Strigiformes (Komar et al. 2003; Nemeth et al. 2006a; Nemeth et al. 2006b; Nemeth et al. 2009c), Gruiformes (Komar et al. 2003; Olsen et al. 2009), Falconiformes (Busquets et al. 2012; Nemeth et al. 2009c; Ziegler et al. 2013), Columbiformes (Guerrero-Sanchez et al. 2011; Komar et al. 2003; Panella et al. 2013; Reisen et al. 2006a; Reisen et al. 2005a), and Galliformes. Recently, Perez-Ramirez et al. (2014) described experimental infection of 77 species of birds worldwide since 1955 (Perez-Ramirez et al. 2014). By studying WNV, the sheer volume of published studies provides a rich dataset for mining of new hypotheses. Despite intrinsic limitations of metadata, differences in f_S and f_M were consistently found throughout taxonomic classes for which data was obtained.

Differences in f_S and f_M are found throughout Orders

Across orders, the Ciconiiformes, Falconiformes, Galliformes, and Columbiformes were more likely to be seropositive than Passeriformes. These analyses supplement general trends seen in studies already described. Similar to the field data, most experimental infections have been performed in Passeriformes and the data from this group provides the most variable responses to WNV (Perez-Ramirez et al. 2014). These currently combined data were highly represented by house sparrows and the American crow in the Passeriformes. The house sparrow was also found to be resistant to experimental infection but have persistent WNV infection (Perez-Ramirez et al. 2014). From the data, these birds had one of the highest

degrees of seropositivity throughout and this did not change over time likely given its profound resistance to this virus. The odds of having a high f_M were also mainly found in the Passeriformes. Upon experimental infection, the American crow (Brault et al. 2007; Brault et al. 2004; Brault et al. 2011; Kipp et al. 2006; Komar et al. 2003; McLean et al. 2001; Nemeth et al. 2011; Weingartl et al. 2004), the house finch, common grackle (Bunning et al. 2002), and the tufted titmouse (*Baeolophus bicolor*) (Kilpatrick et al. 2013) demonstrated high viremia with high mortality while most of the other Passeriformes demonstrated moderate to low viremia and low mortality.

Experimental infection of Columbiformes demonstrated low viral loads in adult infection of mourning doves consisting of $> 10^3$ to $< 10^6$ plaque forming units (*pfu*)/ml with very limited mortality (Komar et al. 2003; Reisen et al. 2005a; Reisen et al. 2006b). One of these studies also demonstrated limited transmissible viremia in mourning doves except when nesting (Reisen, Fang and Martinez 2005).

Regarding the Falconiformes, viremia varied during experimental infections depending on species infected, but kestrels and small falcons had low mortality likely contributing to the higher f_S in our data (Ziegler et al. 2013). Falconiformes also had one of the highest OR's for f_M . Infection of large falcons can result in substantial mortality even at low challenge doses (Nemeth et al. 2009c; Wunschmann & Ziegler 2006; Ziegler et al. 2013). In studies using large falcons, a two week duration of viremia also occurred (Ziegler et al. 2013). The meta-data for f_M also contained Cooper's hawk (*Accipiter cooperii*), sharp-shinned hawk (*Accipiter striatus*), red-tailed hawk (*Buteo jamaicensis*) and red-shouldered hawks (*Buteo lineatus*) in which high

mortality has been reported mainly in field data (Wunschmann et al. 2004; Wunschmann et al. 2005).

In the Galliformes, viral loads upon experimental infection were moderately low consisting of $< 10^4$ pfu/ml, but partridges and quails were more likely to die from infection (Clark et al. 2006; Escribano-Romero et al. 2013; Sotelo et al. 2011; Taylor et al. 2013; Urban et al. 2013). The sage grouse (*Centrocercus sp.*) was highly susceptible to WNV mortality and these birds developed viremia of around 10^6 pfu/ml. The field data in this current analysis contained limited numbers of turkeys, California quails (*Callipepla californica*), and the ruffed grouse (*Bonasa umbellus*) which likely was not reflected in the f_M overall for Galliformes.

Anseriformes demonstrated limited seropositivity and limited mortality. This is likely due to the variability found across species wherein the experimental infection of the Mallard (*Anas platyrhynchos*) demonstrated limited mortality (Komar et al. 2003) while the Common Canada goose (*Branta canadensis*) exhibited moderate mortality with high viremia (Banet-Noach et al. 2003). As of this manuscript, no experimental infections of US Ciconiiformes (long-legged wading birds) are detailed in the literature; however a field study predicted that nesting black-crowned night herons (*Nycticorax nycticorax*) would be competent hosts with $> 10^5$ pfu/ml of virus while egrets were likely incompetent hosts with $< 10^5$ pfu/ml of virus (Reisen et al. 2005b).

Differences in f_S and f_M in Avian Families

Understanding comparative data from field and infection studies is complex; field data is affected by relative bird abundance, the methods employed for capturing birds, and the location of a field study (Ward et al. 2006) while experimental infection is affected by the innate

differences in resistance of locally acquired wild birds or the loss of genetic robustness in bred captive birds (Langevin et al. 2005). Families with evidence of heterogeneity were frequently disproportionally represented by a one or two species of a family.

Data were obtained for 32 families and the Anatidae, Emberizidae, Parulidae, and Columbidae families showed no significant differences in f_S or f_M . By comparison, all families were less likely to be seropositive for WNV than the Corvidae except for the Cardinalidae, however the Cardinalidae exhibited very low f_M . The Lanidae had a high OR for f_M , though there is limited data regarding Lanidae experimental infection (Ward et al. 2006). However, in a group of captive loggerhead shrike (*Lanius ludovicianus*) in Canada, birds that died demonstrated a high amount of virus as detected by rt-PCR (Bertelsen et al. 2004). Although the house finch was most likely to be found seropositive in the Fringillidae, this species was the only Fringillidae reported with mortality in this field data. The American robin was the only species of the Turdidae to develop seropositivity and this bird was resistant to WNV infection when experimentally challenged (Kilpatrick et al. 2010a; VanDalen et al. 2013). The blue jay was more likely to be seropositive within the Corvidae, however, these birds are likely equal in susceptibility to WNV as the American crow with experimental infection (Weingartl et al. 2004). Within the Galliformes, the Phasiandea were 31 more times likely to be seropositive than the Tetraonidea. The Tetraonidea is a highly variable family when exposed to WNV experimentally, with pheasants demonstrating low mortality (Wunschmann & Ziegler 2006).

Logistic regression to analyze temporal trends

In order to assess temporal trajectories of f_S and f_M in different avian species, a logistic regression model was used incorporating a random effect which accounted for the reference

articles. From these initial analyses, 19 species were selected for the f_s dataset and nine species for f_M . Using this model, it was found that the f_s for American crow changed over time at a lower magnitude compared with other passerines and this was the only species in which f_M decreased over time. The data also showed the f_s significantly increased over time for nine of nineteen species.

Although the trends are clear from the descriptive statistics, in order to perform standard inferences (P-values, confidence intervals, hypothesis testing) we considered a logistic regression model. The number of observations and the "unbalanced" structure of the data (not having same number of observations in the same periods and periods with no observations) were challenging, thus a partition of longer time periods allowed analysis using this model. A random effect was introduced because some of the measurements obtained over time are from the same publications; this controlled for possible correlations between observations coming from the same studies or groups of researchers.

Since susceptibility of American avian species to WNV are highly heterogeneous, phylogenetic signal analysis was used to explore the relationship of viral load and species relatedness (Blomberg et al. 2003; Revell et al. 2008). Phylogenetic signal quantitates the relationship between phylogeny and ecologically similar traits. Viremia was used as a marker because the higher the circulating virus, the higher chance of transmission from that reservoir host to a vector. A K value is derived in these analyses and zero represents a random or convergent pattern of evolution, whereas a K value of 1 or above, indicates that a trait is strongly conserved. The results showed a significant albeit weak association between phylogeny and viral load indicating that factors associated with viral load are predicted by the

phylogeny. Further studies are warranted utilizing more metadata derived from experimental infections.

Limitations of meta-analysis

While metadata on limited studies relying upon basic indices such as f_S and f_M can be used to derive information on the variability in susceptibility traits across species, this type of analysis is of limited value in actually modelling the transmission dynamics of each species in WNV epizootics. Modelling the transmission dynamics of WNV in a large field study incorporating local vector and bird abundance, infectivity rates for mosquitoes, and infectivity rates for birds, demonstrated that the American robin was responsible for the majority of WNV infections in mosquitoes (Griffing et al. 2007; Kilpatrick et al. 2006a; Kilpatrick et al. 2010a; VanDalen et al. 2013). Field studies and experimental studies predicted that based on abundance, frequent infection, and persistence of infection, house sparrows would be important amplifying hosts in WNV epizootics (Komar et al. 2003; Langevin et al. 2005; Nemeth et al. 2009a; Nemeth et al. 2008). To further complicate this modelling, bird behavior can influence transmission (Janousek et al. 2014). For instance, in roosting communities, birds placed in the center of the roost seroconverted less slowly than those outside the roost indicating that transmission is diluted by the chance of encounter (Janousek et al. 2014). Finally, this analysis does not indicate ecologically the drivers of the WNV epizootic, nor does it demonstrate a causal relationship between heterogeneity in bird susceptibility and epidemiological trends in human infection.

Nonetheless, meta-analysis of field data coupled with experimental data can indicate trends in avian species not present in over-abundance but important in the maintenance of

WNV within the avian and mosquito community. The use of meta-analysis could indicate trends that would be relevant for further field testing and modelling for less intensively studied species and other disease outbreaks. Overall, the data demonstrates that at the taxonomic level of order, the data can be biased by the over contribution of a limited number of species, however the results demonstrate that susceptibility as a trait is most variable within the Passeriformes, which did have the most abundance.

Conclusions

Metadata on limited studies relying upon basic indices such as fS and fM can be used to derive information on the variability in susceptibility traits across species which could be tested in further experimental challenge studies and focused field studies. Because not all diseases generate rich field datasets, the use of meta-analysis on experimental infection studies, large multi-host field studies, and extensive mathematical modelling could indicate trends that would be relevant for further field testing for less intensively studied outbreaks. The data indicated that only certain species of birds contributed more heterogeneity to WNV outcomes and these birds were primarily Passeriformes. Using the data retrieved, we were able to determine that differences in fS and fM are consistently found throughout taxonomic classes as has been demonstrated by experimental infections.

Thus, susceptibility of birds to WNV infection may not be a trait specific of either an order or a family of species. Birds with higher mortality such as the house finch, the American crow, the common grackle, and the blue jay also demonstrated more seropositivity than expected. This may be because they are relatively abundant in urban and periurban areas where disease is highly prevalent and may bias these findings because they are a dominant

482 component of the published data. The American crow is still notable it susceptibility profile by
 483 displaying high mortality and the slowest increase in seropositivity over time.

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Figure 1(on next page)

Distribution of from West Nile virus publications serological status, mortality status and species identification from naturally infected North American bird.

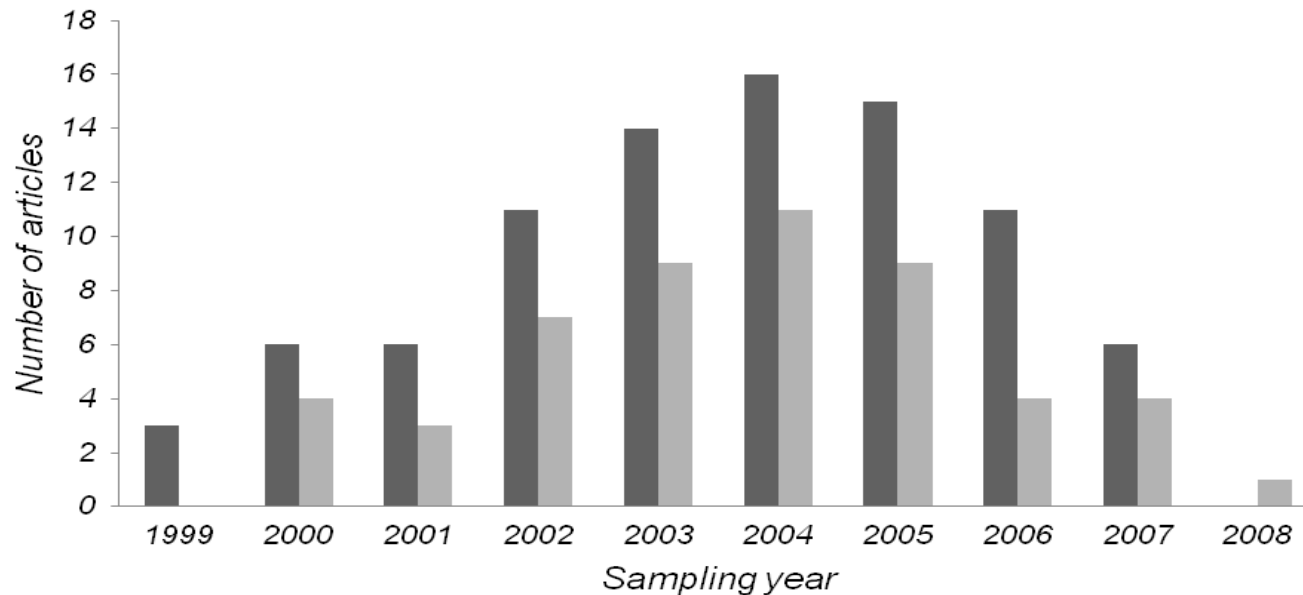
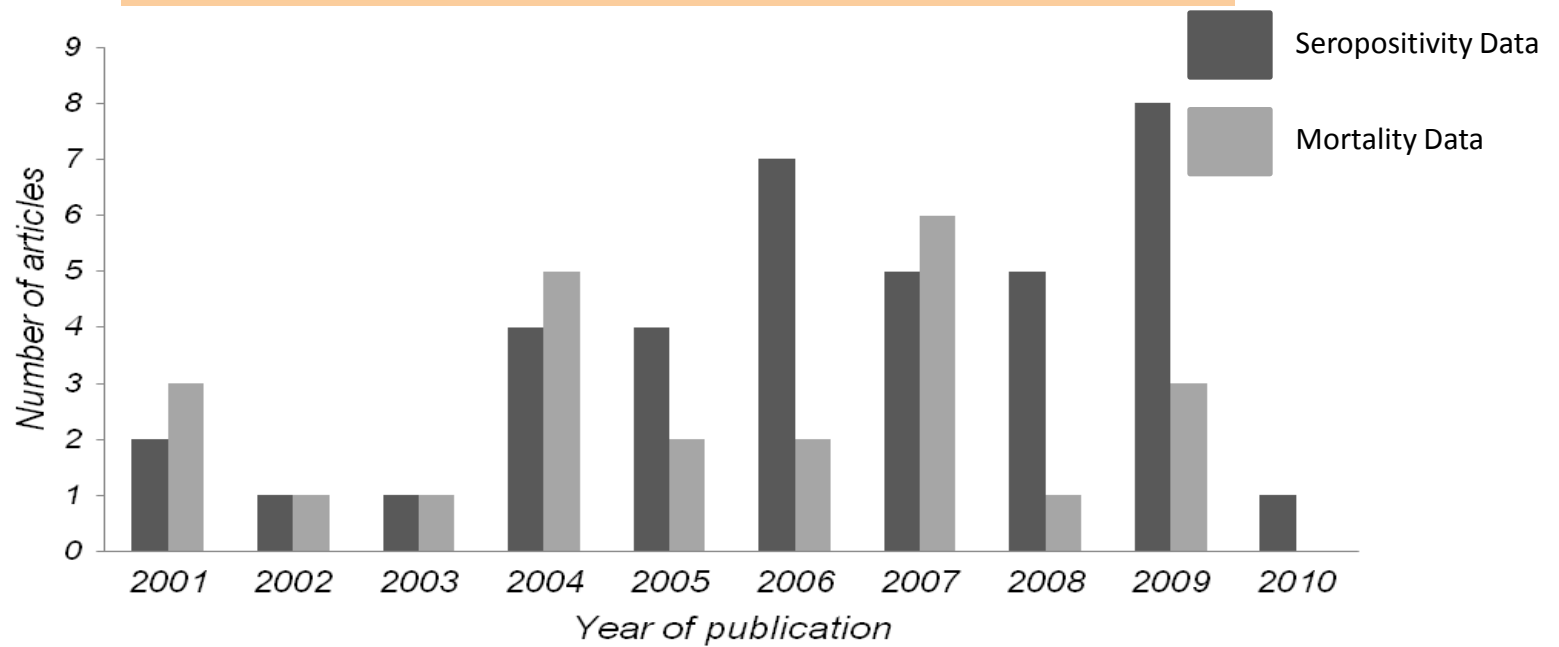


Figure 2(on next page)

The distribution of orders of North American avian hosts derived from West Nile virus publications from 2001 to 2010.

Pie chart demonstrating the distribution of orders of North American avian hosts derived from West Nile virus publications that consisted of studies performed between 1999 and 2008 (publication dates from 2001 to 2010) which contained serological status, mortality status and species identification from naturally infected birds.

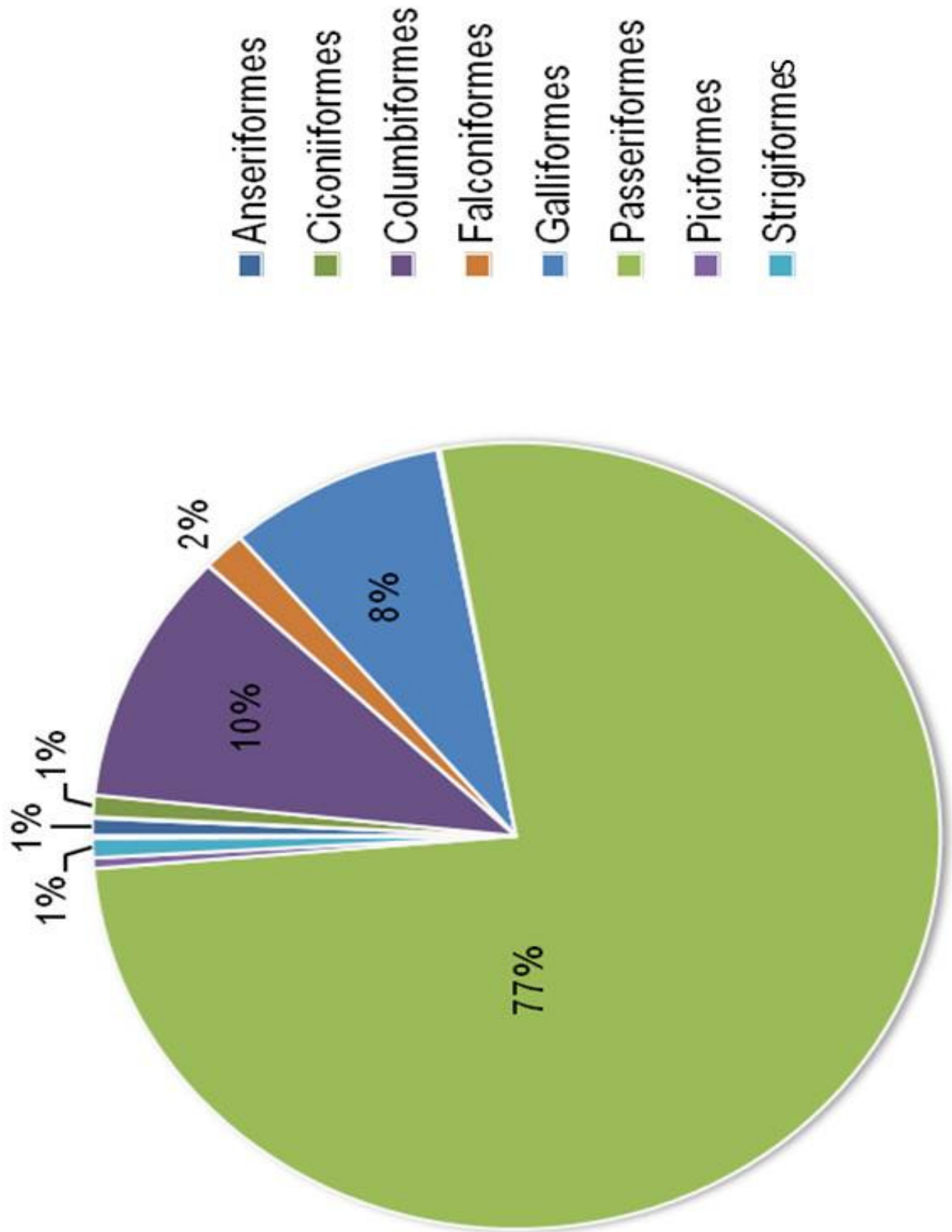


Figure 3(on next page)

Comparative West Nile virus seropositivity and mortality birds

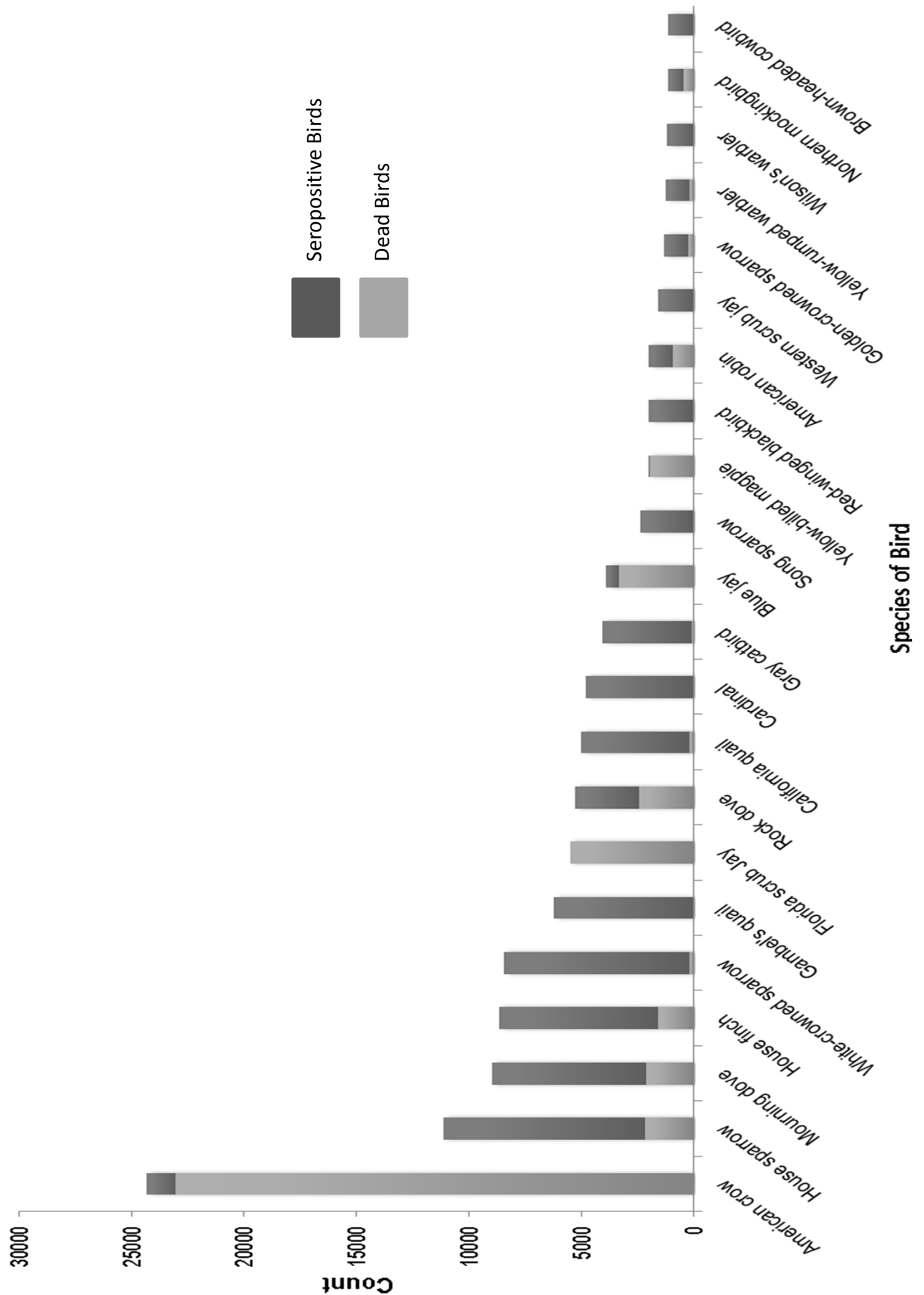
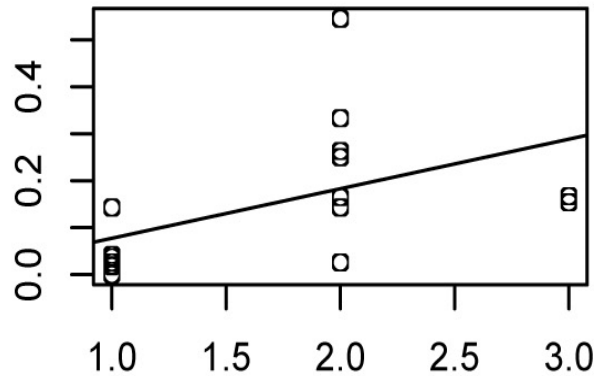
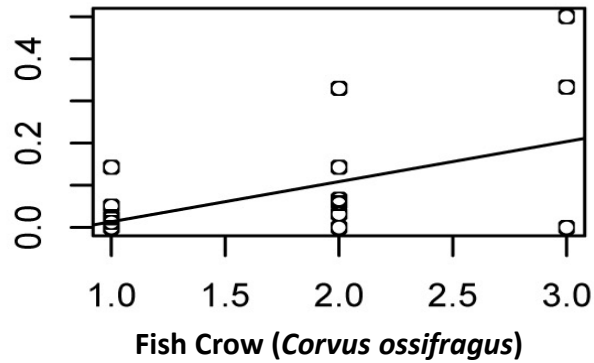


Figure 4(on next page)

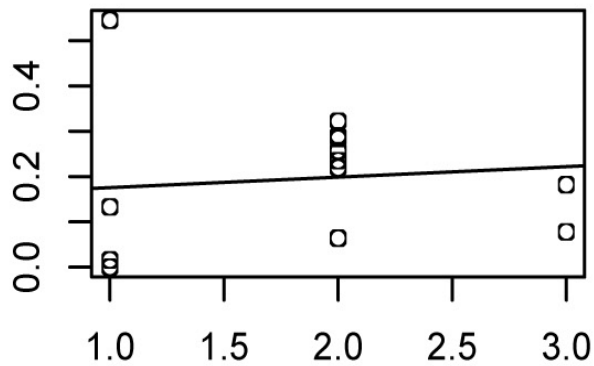
The frequency of seropositivity and mortality of the American crow (*Corvus brachyrhynchos*), the fish crow (*Corvus ossifragus*) and the rock dove (*Columbia livia*) over three time periods

Frequency of Seropositivity

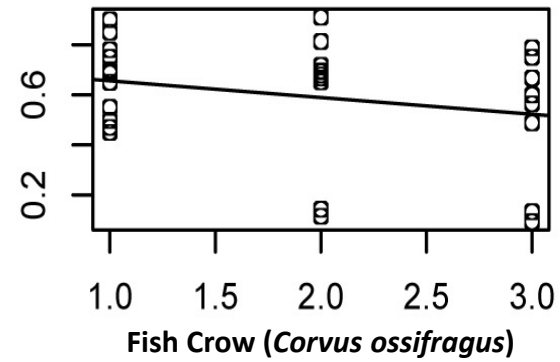
American Crow (*Corvus brachyrhynchos*)



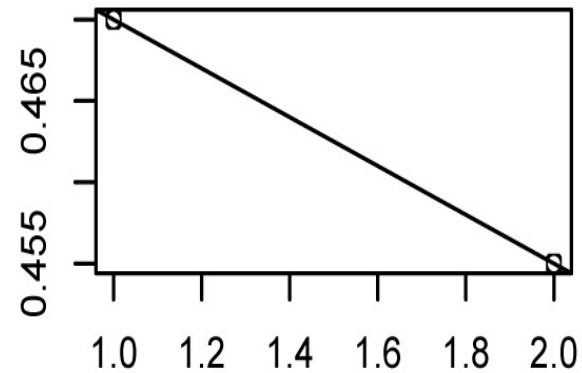
Rock Dove (*Columbia livia*)



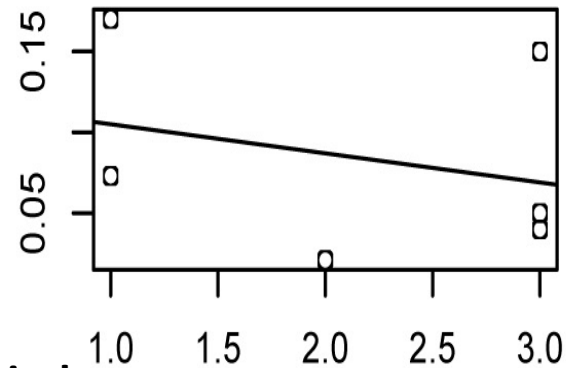
American Crow (*Corvus brachyrhynchos*)



Frequency of Mortality



Rock Dove (*Columbia livia*)



Time Period

Figure 5 (on next page)

Polar tree root dendrogram of the WNV viremia as a trait across bird species

Dendrogram formed from analysis of mean West Nile virus load as a trait conserved across species. Analysis performed through using the global phylogeny of birds (birdtree.org) and statistical significance was estimated after 10,000 permutations of the tip roots. After transformation of the branch lengths, the contribution to viral load was non-random ($P=0.024$). WNV competent host species are denoted in red. Species which are not competent WNV hosts are depicted in green.

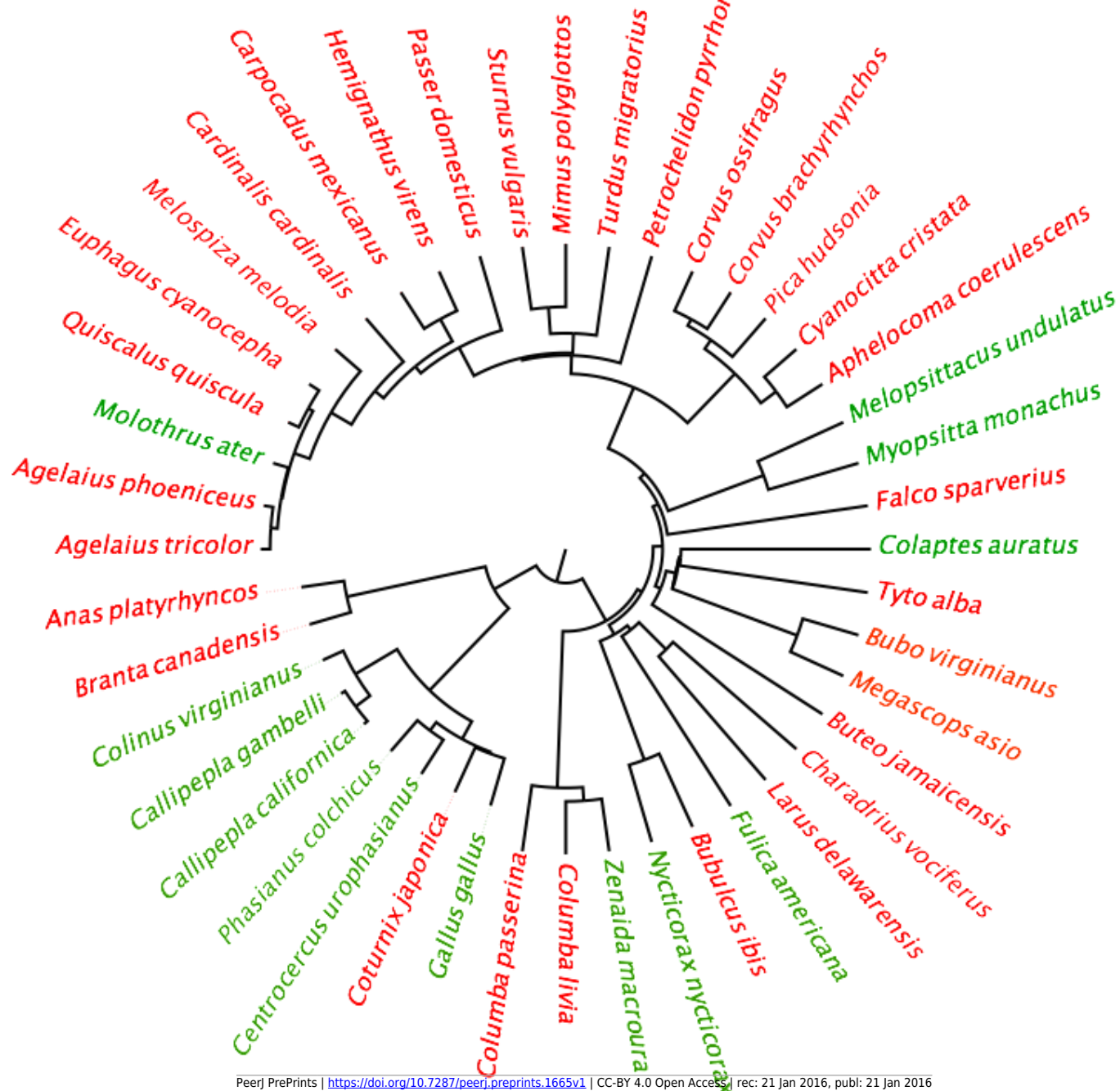


Table 1(on next page)

Odds ratios, confidence intervals, and P-values comparing the recalculated frequency of WNV seropositivity (f_S) and mortality (f_M) amongst orders of North American birds

Table 1 Calculated odds ratios, confidence intervals, and P-values comparing the recalculated frequency of WNV seropositivity (*fS*) and mortality (*fM*) amongst orders of birds, using the Passeriformes as controls. Data was obtained and pooled from studies performed between 1999 through 2008 (publication dates from 2001 through 2010) which contained serological status, mortality status, and species identification from naturally infected birds.

	Frequency of Seropositivity (<i>fS</i>)			Frequency of Mortality (<i>fM</i>)		
	Odds ratio	Confidence Interval	P-value	Odds ratio	Confidence Interval	P-value
Passeriformes	1	--	--	1	--	--
Piciformes	0.19	0.06-0.46	0.001	0.0114	0.07-0.17	0.0001
Anseriformes	0.54	0.36-0.77	0.001	0.167	0.11-0.25	0.0001
Strigiformes	0.6	0.33-1.01	0.08	0.233	0.19-0.28	0.0001
Gruiformes	0.82	0.20-2.23	0.74	0.055	0.02-0.15	0.0001
Ciconiiformes	1.56	1.26-1.92	0.0001	0.22	0.15-0.32	0.0001
Falconiformes	2.81	2.31-3.39	0.0001	0.331	0.29-0.37	0.0001
Columbiformes	2.61	2.45-2.78	0.0001	0.085	0.07-0.09	0.0001
Galliformes	2.47	2.32-2.63	0.0001	0.229	0.17-0.29	0.0001

Table 2 Calculated odds ratios, confidence intervals, and P-values comparing the recalculated frequency of WNV seropositivity (f_S) and mortality (f_M) between families of birds. Data was obtained and pooled from studies performed between 1999 through 2008 (publication dates from 2001 through 2010) which contained serological status, mortality status, and species identification from naturally infected birds. Within the Passeriformes, the Corvidae family was used as the reference group. Within the Galliformes, the Tetraonidae family was used as the reference group.

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Order	Family	Frequency of Seropositivity f_S			Frequency of Mortality f_M		
		Odds ratio	Confidence Interval	P-value	Odds ratio	Confidence Interval	P-value
Passeriformes	Corvidae	1	--	--	1	--	--
	Cardinalidae	0.99	0.89-1.11	0.955	0.129	0.08-0.2	0.52
	Emberizidae	0.06	0.05-0.07	0.0001	0.07	0.05-0.08	0.0001
	Fringillidae	0.63	0.56-0.71	0.0001	0.176	0.15-0.19	0.026
	Hirundinidae	0.07	0.03-0.17	0.0001	0.125	0.064-0.24	0.59
	Icteridae	0.27	0.23-0.32	0.0001	0.063	0.05-0.08	0.0001
	Lanidae	0.43	0.21-0.89	0.024	0.45	0.28-0.71	0.0001
	Mimidae	0.29	0.25-0.33	0.0001	0.1	0.08-0.13	0.008
	Paridae	0.05	0.02-0.09	0.0001	0.104	0.07-0.148	0.04
	Parulidae	0.04	0.03-0.05	0.0001	0.104	0.07-0.148	0.04
	Passeridae	0.53	0.47-0.58	0.0001	0.11	0.09-0.127	0.0004
	Sturnidae	0.273	0.15-0.49	0.0001	0.068	0.05-0.089	0.0001
	Turdidae	0.467	0.39-0.557	0.0001	0.115	0.09-0.135	0.006
	Tyrannidae	0.066	0.03-0.14	0.0001	0.07	0.03-0.138	0.02
Galliformes	Tetraonidae	1	--	--	1	--	--
	Odontophoridae	6.28	3.11-12.7	0.0001	0.06	0.02-0.14	0.0001
	Phasianidae	31.22	14.72-66.22	0.0001	Limited Data	Limited Data	Limited Data

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26 **Table 3** Results of logistic regression estimates of random effects and P-values using the
 27 recalculated frequency of WNV seropositivity (f_S) and mortality (f_M) amongst species of birds.

Data was obtained and pooled from studies performed between 1999 through 2008 (publication dates from 2001 through 2010) which contained serological status, mortality status and species identification from naturally infected birds.

Species	Common Name	Frequency of Seropositivity		Frequency of Mortality	
		Estimate of Random Effects	P-value	Estimate of Random Effects	P-value
<i>Agelaius phoeniceus</i>	Red-winged blackbird	1.0144	0.091	ND	ND
<i>Bubo virginianus</i>	Great horned owl	ND	ND	-0.8367	--
<i>Callipepla californica</i>	California quail	0.9472	0.003	ND	ND
<i>Callipepla gambelii</i>	Gambel's quail	4.3908	0.018	ND	ND
<i>Cardinalis cardinalis</i>	Cardinal	2.7375	<0.0000	ND	ND
<i>Carduelis tristis</i>	American goldfinch	1.7764	0.181	ND	ND
<i>Haemorhous mexicanus</i>	House finch	1.4309	<0.0000	ND	ND
<i>Columba livia</i>	Rock dove	2.3624	<0.0000	-0.2807	--
<i>Corvus brachyrhynchos</i>	American crow	1.1418	0.002	-0.4262	0.042
<i>Corvus ossifragus</i>	Fish crow	1.3817	<0.0000	-0.04879	--
<i>Cyanocitta cristata</i>	Blue jay	2.3681	0.116	1.0632	0.16
<i>Dumetella carolinensis</i>	Gray catbird	0.8083	0.132	ND	ND
<i>Geothlypis trichas</i>	Common yellowthroat	0.1406	--	ND	ND
<i>Melospiza melodia</i>	Song sparrow	1.3801	0.047	ND	ND
<i>Mimus polyglottos</i>	Northern mockingbird	-0.04756	0.961	ND	ND
<i>Molothrus ater</i>	Brown-headed cowbird	1.0071	0.104	ND	ND
<i>Passer domesticus</i>	House Sparrow	0.5017	0.236	-0.4381	0.161
<i>Quiscalus quiscula</i>	Common grackle	-0.2327	0.697	-0.6584	0.386
<i>Sturnus vulgaris</i>	Common starling	--	--	0.0592	0.927
<i>Turdus migratorius</i>	American robin	0.7509	0.184	-0.1708	--

Figure Legends

Figure 1 Distribution of from West Nile virus publications that consisted of studies performed between 1999 and 2008 (publication dates from 2001 to 2010) which contained serological status, mortality status and species identification from naturally infected birds. The top graph contains publications plotted by the year of publication and the bottom graph contains the publications plotted by the year of data collection.

Figure 2 Pie chart demonstrating the distribution of orders of avian hosts derived from West Nile virus publications that consisted of studies performed between 1999 and 2008 (publication dates from 2001 to 2010) which contained serological status, mortality status and species identification from naturally infected birds.

Figure 3 Stacked bar graph demonstrating the comparative West Nile virus seropositivity and mortality of 22 species of birds (represented by common name) for which there were greater than 1,000 birds tested. Data was derived from publications that consisted of studies performed between 1999 and 2008 (publication dates from 2001 to 2010) which contained serological status, mortality status and species identification.

Figure 3 Trend lines comparing the frequency of seropositivity and mortality of the American crow (*Corvus brachyrhynchos*), the fish crow (*Corvus ossifragus*) and the rock dove (*Columbia livia*) over three time periods. Data was derived from publications that consisted of studies performed between 1999 and 2008 (publication dates from 2001 to 2010) which contained serological status, mortality status and species identification. Time periods 1, 2 and 3 consisted of years 1999-2001, 2002-2004, and 2005-2008, respectively. If two time periods are graphed, time periods 1 and 2 consist of 1999-2004 and 2005-2008, respectively.

Figure 4 The degree of phylogenetic signal was tested by quantifying the value of Blomberg's K after transformation of the branch lengths. WNV competent host species are denoted in red. Species which are not competent WNV hosts are depicted in green.

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Table 2 (on next page)

Odds ratios, confidence intervals, and P-values comparing frequency of WNV seropositivity (f_S) and mortality (f_M) between families of North American birds.

Table 2 Calculated odds ratios, confidence intervals, and P-values comparing the recalculated frequency of WNV seropositivity (f_S) and mortality (f_M) between families of birds. Data was obtained and pooled from studies performed between 1999 through 2008 (publication dates from 2001 through 2010) which contained serological status, mortality status, and species identification from naturally infected birds. Within the Passeriformes, the Corvidae family was used as the reference group. Within the Galliformes, the Tetraonidae family was used as the reference group.

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Order	Family	Frequency of Seropositivity f_S			Frequency of Mortality f_M		
		Odds ratio	Confidence Interval	P-value	Odds ratio	Confidence Interval	P-value
Passeriformes	Corvidae	1	--	--	1	--	--
	Cardinalidae	0.99	0.89-1.11	0.955	0.129	0.08-0.2	0.52
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Galliformes	Tetraonidae	1	--	--	1	--	--
	Odontophoridae	6.28	3.11-12.7	0.0001	0.06	0.02-0.14	0.0001
	Phasianidae	31.22	14.72-66.22	0.0001	Limited Data	Limited Data	Limited Data

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Table 3 (on next page)

Random effects and P-values using the recalculated frequency of WNV seropositivity (f_S) and mortality (f_M) amongst species of birds.

Table 3 Results of logistic regression estimates of random effects and P-values using the recalculated frequency of WNV seropositivity (f_S) and mortality (f_M) amongst species of birds. Data was obtained and pooled from studies performed between 1999 through 2008 (publication dates from 2001 through 2010) which contained serological status, mortality status and species identification from naturally infected birds.

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<i>Molothrus ater</i>	Brown-headed cowbird	1.0071	0.104	ND	ND
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