UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

FACULTAD DE CIENCIAS

VECTORES, RESERVORIOS Y VIRUS: ESTUDIO MACROECOLÓGICO DEL VIRUS DEL OESTE DEL NILO

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTORA EN CIENCIAS BIOLÓGICAS

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P r e s e n t e

Me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día **8 de abril de 2019**, se aprobó el siguiente jurado para el examen de grado de **DOCTORA EN CIENCIAS** de la alumna **TOLSÁ GARCÍA MARÍA JOSÉ** con número de cuenta **512012861** con la tesis titulada: **"Vectores, reservorios y virus: estudio macroecológico del Virus del Oeste del Nilo"**, realizada bajo la dirección del **DR. GERARDO SUZÁN AZPIRI:**

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Sin otro particular, me es grato enviarle un cordial saludo.

A T E N T A M E N T E
"POR MI RAZA HABLARA EL ESPIRITU"
Ciudad Universitaria, Cd. Mx., a 20 de junio de 2019

DR. ADOLFO GERARDO NAVARRO SIGÜENZA
COORDINADOR DEL PROGRAMA

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ÍNDICE

RESUMEN

Las enfermedades zoonóticas influyen directamente en la salud humana y animal, la conservación de la vida silvestre, la política y la economía. Por esa razón, se han desarrollado diferentes aproximaciones que ayudan a entender las dinámicas de transmisión entre patógenos zoonóticos y hospederos; así como prevenir y /o mitigar sus efectos. Uno de los patógenos con importantes impactos en la salud humana y en las poblaciones de aves silvestres es el Virus del Oeste del Nilo (VON). Éste se transmite principalmente entre mosquitos y aves, y en ambos grupos existe una red compleja de interacciones con el virus. El presente trabajo está compuesto por cuatro capítulos, los cuales tienen como objetivo general evaluar a través de una perspectiva macroecológica (mundial y regional en los Estados Unidos) y local (Sonora, México) las dinámicas de infección del VON en aves y mosquitos. En el primer capítulo se realizó estudio comparativo a nivel global donde se identificaron y propusieron especies de aves importantes para la transmisión del VON, así como factores asociados a la susceptibilidad de las aves a este virus. En el segundo capítulo se desarrolló un meta-análisis donde se determinó que algunas de las historias de vida de las aves hospederas son predictoras de su supervivencia y mortalidad por VON. En el tercer capítulo, utilizando minería de datos espaciales, se identificó una alta asociación geográfica entre el género de mosquitos *Culex* y la mortalidad de aves por VON en los Estados Unidos. Con base en estas asociaciones se propusieron zonas de riesgo en áreas importantes para la conservación de las aves en este país. Finalmente, en el cuarto capítulo se realizó un estudio de campo en Bahía de Kino, Sonora, con el fin de determinar mediante técnicas moleculares la presencia de VON en las aves. Todas las muestras de este estudio fueron negativas a VON. Como conclusión general, se resalta la necesidad de usar aproximaciones analíticas a diferentes escalas que permitan generar nuevo conocimiento sobre las dinámicas de transmisión entre el virus, sus hospederos y vectores. Así mismo, se identificaron sesgos importantes en la investigación de VON y de la historia natural de las aves a nivel mundial. Con las aproximaciones macroecológicas (regional y mundial) se identificaron y propusieron especies de aves y mosquitos importantes en las dinámicas de transmisión del virus y en la conservación de las aves silvestres. Finalmente, el estudio realizado en Bahía de Kino sugirió que en el momento en que se realizaron los muestreos no estaba circulando el virus entre las aves y que se necesitan estudios longitudinales para determinar su presencia. Las enfermedades zoonóticas representan un problema global que debe de abordarse desde diversas escalas y perspectivas. Éstas permitirán desarrollar modelos analíticos y espaciales que identifiquen zonas de riesgo y nos permitan conocer aspectos de su transmisión y el impacto que puedan tener en la conservación de la vida silvestre y la salud humana y animal.

Palabras clave

Virus del Oeste del Nilo, macroecología, aves, mosquitos, susceptibilidad, prevalencia serológica, prevalencia molecular, mortalidad, historias de vida, ecología, Bahía de Kino, Sonora

ABSTRACT

Zoonotic diseases directly influence human and animal health, wildlife conservation, politics and economics. For this reason, different approaches have developed to help and understand the transmission dynamics between zoonotic and host pathogens; as well as preventing and/or mitigating its effects. One pathogen with the greatest impact on human health and bird populations is the West Nile Virus (WNV). It is transmitted mainly between mosquitoes and birds, and in both groups exist a wide variability in their associations with the virus. This work is composed by four chapters, which have the general aim to evaluate through a macroecological perspective (global and regional in the United States) and local (Mexico, Sonora) the infection dynamics of West Nile Virus in birds and mosquitoes. In the first chapter, a global comparative study was conducted where important bird species were identified and proposed for the WNV transmission, well as factors associated with the susceptibility of birds to this virus. In the second chapter, a meta-analysis was developed where it was determined that the life histories of the host birds are predictors of their survival and mortality by WNV. In the third chapter, using spatial data mining, a high geographic association was identified between *Culex* mosquito genus and bird mortality by WNV in the United States. Based on these associations, risk areas were proposed in important areas for the conservation of birds. Finally, in the fourth chapter a field study was conducted in Bahía de Kino, Sonora; in order to determine by molecular techniques the presence of WNV in birds. The sampling had negative results. As a general conclusion, the use of different analytical approaches in this work allowed us to generate new knowledge about the transmission dynamics between the virus, its hosts and vectors. Likewise, important biases were identified in the investigation of WNV and the natural history of birds worldwide. With the three macroecological approaches, it was possible to identify and propose birds species and mosquitoes important in the dynamics of virus transmission and in the conservation of wild birds. And the study carried out in Bahía de Kino allowed a better understanding of the transmission cycle of the virus at the local level. Zoonotic diseases represent a global problem that must be addressed from different scales and analytical perspectives. This will allow us to develop analytical and spatial models that allow us to know aspects of their transmission, identify risk areas that affect the wildlife conservation and human and animal health.

Key words

West Nile virus macroecology, birds, mosquitoes, susceptibility, serological prevalence, molecular prevalence, mortality, life history, ecology, United States, Kino Bay, Sonora

INTRODUCCIÓN GENERAL

Los patógenos zoonóticos son aquellos transmitidos entre animales y humanos, los cuales representan una amenaza constante para la conservación de la vida silvestre, la salud humana y animal y la economía. Por ejemplo, estos patógenos han provocado disminuciones en las poblaciones de organismos terrestres y marinos poniendo en riesgo significativo a sus poblaciones (Pedersen *et al.*, 2007; George *et al.* 2015). Con respecto a la economía, el banco mundial estimó que las enfermedades zoonóticas tuvieron un costo directo de 20 billones de dólares y 200 billones indirectamente entre el 2000 y 2010 (Webster *et al.*, 2015).

Debido a la urgente necesidad de prevenir y/o mitigar los efectos de las enfermedades zoonóticas, se han desarrollado diferentes aproximaciones analíticas, las cuales se complementan al tener como objetivo común el proveer una mejor comprensión de las dinámicas de transmisión de las enfermedades (Stephens et al. 2016). Existen estudios que realizan monitoreos epidemiológicos en hospederos silvestres y vectores (mosquitos, garrapatas, etc), otros prueban hipótesis con experimentos realizados bajo condiciones controladas en laboratorio y algunos estudios sintetizan y analizan la información existente con el fin de encontrar patrones comunes entre las especies hospederas y de patógenos, así como asociaciones ecológicas y distribuciones geográficas (Calzolari *et al.*, 2012; Kamiya *et al.*, 2014; Strauss *et al.*, 2016).

La macroecología la cual es una disciplina que estudia las interacciones ecológicas a través del uso de bases de datos a grandes escalas espaciales y temporales y analizadas con métodos estadísticos (Partel *et al.* 2016; Gurevich *et al.* 2018). Recientemente se le ha postulado como una disciplina que complementa a la epidemiología, salud pública y animal, ecología y evolución de enfermedades infecciosas, al identificar a gran escala patrones comunes en la relación patógeno-hospedero (Díaz y Madin 2011; Gaidet *et al.*, 2012; Stephens *et al.*, 2016). Además, la macroecología permite generar y probar hipótesis al proveer un contexto más comprensivo con respecto a los estudios realizados a escala local (Civitello *et al.*, 2015; Gurevitch *et al.*, 2018).

Las dinámicas de transmisión de los patógenos zoonóticos y de la relación patógeno-hospedero pueden ser estudiadas a través de la macroecología. Lo que implica en primer lugar la identificación y caracterización de los hospederos (Cronin *et al.*, 2010; Han *et al.*, 2015). Para lo cual se requiere reconocer a las especies que sobreviven al

contacto con un patógeno, las que mueren a causa de la infección o a las que tienen el potencial para contribuir desproporcionalmente a la transmisión incrementando el riesgo de infección a otros hospederos (Wheeler *et al.*, 2010; Martin et al. 2018). Particularmente, se ha enfatizado la necesidad de identificar a estas últimas, denominadas reservorios competentes (Cronin *et al.*, 2010; Gervasi *et al.*, 2015).

Se ha observado en diversas enfermedades que existe una amplia variabilidad en la competencia como reservorio, debido a que los hospederos exhiben diferencias fisiológicas (inmunidad, estrés, morfología, diversidad genética) y ecológicas (migración, comportamiento social, alimentación) importantes en la relación parásito-hospedero (Figuerola *et al.*, 2008; Johnson *et al.*, 2009; Gervasi *et al.*, 2015).

La identificación y caracterización de las especies hospederas es el primer paso para explorar una extensa variedad de asociaciones con sus patógenos, vectores (en el caso de las enfermedades transmitidas por vector), factores ambientales, evolutivos, geográficos, microbiológicos, inmunológicos, fisiológicos entre muchos otros (Keesing et al. 2006; Stephens *et al.*, 2009; Grubaugh *et al.*, 2015). Posteriormente, la información generada en estas asociaciones puede ser incorporada a una amplia variedad de modelos matemáticos, estadísticos, evolutivos y geográficos. Dichos modelos permiten extender el conocimiento, sugerir medidas preventivas para la conservación de vida silvestre, proponer soluciones y ser incorporadas a criterios de políticas públicas.

El Virus del Oeste de Nilo (VON) es uno de los patógenos zoonóticos más importantes a nivel mundial por sus impactos en la conservación de la avifauna y en la salud humana (Busani *et al.*, 2011; Bakonyi *et al.*, 2013). Perteneciente al género Flavivirus, familia Flaviviridae, se clasifica en 7 linajes y estos a su vez se dividen en una alta diversidad de genotipos y cepas, los cuales difieren en su virulencia (Brault *et al.*, 2004; Davis *et al.*, 2004; Pérez-Ramírez et al. 2014). El ciclo de transmisión del VON es extremadamente complejo, es transmitido principalmente por la picadura de mosquitos *Culex* a las aves; aunque, se ha demostrado experimentalmente la transmisión directa por la ruta oral-fecal (Komar et al. 2003). En el ciclo de transmisión intervienen una amplia diversidad de hospederos y vectores con diferente diversidad, abundancia y habilidad para transmitir el virus (Durand *et al.*, 2017).

El presente trabajo se conforma por cuatro capítulos desarrollados con formato de artículos científicos. En el capítulo 1 se realizó un análisis comparativo a nivel mundial

sobre la susceptibilidad de las aves al VON considerando: la prevalencia serológica, prevalencia molecular y la mortalidad. Los objetivos fueron primero determinar si nuestros estimados de susceptibilidad podrían ser predictores de la competencia como reservorios del VON; y segundo, investigar si algunos factores asociados con el hospedero (filogenia), virus (cepa), tiempo-espacio y sesgos metodológicos están asociados con la susceptibilidad de las aves a este virus. Para realizarlo se utilizaron métodos comparativos filogenéticos y modelos lineales mixtos generalizados asociados a aproximaciones Bayesianas.

El segundo capítulo se desarrolló tomando como base las historias de vida y ecología de las aves hospederas para determinar si ambas son buenas predictoras de la supervivencia (prevalencia serológica) y mortalidad asociada al VON. Se consideraron para las historias de vida la masa corporal, periodo de incubación y tamaño de nidada. En cuanto a la ecología se consideraron la migración y hábitat debido a que son las variables ecológicas más importantes asociadas a la exposición de las aves al VON. Se efectuó un meta-análisis para probar estas asociaciones.

El tercer capítulo es una aproximación macroecológica a nivel regional (Estados Unidos) donde se evalúa la relación geográfica entre las aves con evidencia de mortalidad por VON y los géneros de mosquitos positivos para este virus. Basado en estas asociaciones se propusieron zonas de alto riesgo de mortalidad aviar en áreas de importancia para la conservación de las aves (IBAs, por sus siglas en inglés). Para realizarlo se utilizó una aproximación de minería de datos geográficos.

Finalmente, en el cuarto capítulo se realizó un estudio local en Bahía de Kino, Sonora con el objetivo de identificar la presencia del VON en aves migratorias y residentes en tres diferentes hábitats (vegetación halófita, mezquital y manglar). Se consideraron tres temporadas de muestreo. La presencia del VON se determinó mediante hisopados cloacales utilizando métodos moleculares (Reacción en Cadena de la Polimerasa, PCR) con primers específicos para Flavivirus y para VON.

Debido a la necesidad de comprender mejor a las enfermedades infecciosas, se utiliza al VON como modelo. Con el objetivo general de evaluar a través de una perspectiva macroecológica (mundial y regional en los Estados Unidos) y local (México, Sonora) las dinámicas de transmisión entre este virus, sus hospederos (aves) y vectores (mosquitos).

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Review

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Macroecology of birds potentially susceptible to West Nile virus

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Zoonotic diseases transmitted by wildlife affect biological conservation, public and animal health, and the economy. Current research efforts are aimed at finding wildlife pathogens at a given location. However, a meta-analytical approach may reveal emerging macroecological patterns in the host–pathogen relationship at different temporal and spatial scales. West Nile virus (WNV) is a pathogen with worldwide detrimental impacts on bird populations. To understand macroecological patterns driving WNV infection, we aimed to recognize unknown competent reservoirs using three disease metrics—serological prevalence (SP), molecular prevalence (MP) and mortality (M)—and test if these metrics are correlated with the evolutionary history, geographical origin of bird species, viral strain, time–space and methodology. We performed a quantitative review of field studies on birds sampled for WNV. We obtained 4945 observations of 949 species from 39 countries. Our analysis supported the idea that MP and M are good predictors of reservoir competence, and allowed us to identify potential competent reservoirs. Furthermore, results indicated that the variability of these metrics was attributable to phylogeny, time–space and sample size. A macroecological approach is needed to recognize susceptible species and competent reservoirs, and to identify other factors driving zoonotic diseases originating from wildlife.

1. Introduction

Most of the emerging infectious diseases that affect humans are caused by zoonotic pathogens transmitted from animals to humans [1]. Surveillance of these pathogens is of international concern for ensuring human health, socio-economic development and wildlife conservation [2,3]. To this end, current research efforts aim to sample wildlife and detect zoonotic pathogens that can infect them as well as humans [4,5].

Individually, these studies are essential for epidemiological surveillance at a given location. However, comparisons between studies may reveal macroecological patterns emerging from the host–pathogen transmission dynamics [6]. In these terms, a vast amount of data remain underexplored [7,8].

Although data obtained from epidemiological surveillance are heterogeneous and imperfect, they are valuable under the framework of macroecology. Using a macroecological approach, we can identify emerging patterns and underlying processes of epidemics and epizootics, while considering a wide range of species at different temporal and spatial scales, generating hypotheses and accounting for sampling bias and other confounding effects [6,9,10].

Macroecology investigates generalized patterns at large spatial and temporal scales [6,11], and it has been applied to make comparisons between hosts and pathogens [12,13]. In addition, macroecology has been employed to investigate the general relationships between host species diversity and disease risk

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Table 1. Description of host, viral, time-space and methodological factors associated with bird susceptibility to WNV.

factors	variable	justification	classification	references
host	phylogeny (Ph)	closely related species may have similar interactions with their pathogens	phylogeny [30]	[31,32]
	geographical origin (GO)	WNV is endemic to the Old World; New World birds could thus be more susceptible to the virus than Old World birds, which may be better adapted to WNV	Old World (Palaearctic, Afrotropical, Oriental and Australian species), New World (Nearctic, Neotropical), and Old/New	[17,33,34] electronic supplementary material, appendix 1
viral	strain (S)	strains have evolved independently in different parts of the world and might differ in their biological properties and virulence	Eg101, Hu04, Is90-STI, Ita98, Italy2011/23743, Italy 2009/j-225677, Magpige/10, NY99, Romania 96, WN02	[17,35] electronic supplementary material, appendix 1
environment	time–space (ST)	time–space may reflect local conditions like vector and host community composition, human socio-economic conditions, and environmental factors	country and year of sampling	[36,37]
methodological	sample size (N)	prevalence data could depend highly on the number of sampled hosts	individuals sampled and times sampled for each species	[38]

[14–16]. This information contributes to preventing diseases, and it can be incorporated into predictive models to anticipate the risk of pathogens infecting wildlife, domestic animals and humans.

Currently, one of the most enigmatic zoonotic pathogens is West Nile virus (WNV). It belongs to the family Flaviviridae, genus *Flavivirus*, which has a high diversity of lineages and strains that differ in their biological properties and virulence [17]. The virus propagates in sylvatic cycles involving mosquitoes and birds as the primary host species, and humans and other vertebrates are considered incidental hosts [5,18].

While it is endemic to Africa, WNV activity has been reported in domestic and wild birds, humans, mosquitoes and horses in Europe, representing a crucial public health problem [5]. WNV was also introduced into North America in 1999 and has caused considerable public health problems and bird mortality, with persistent impacts in some populations [19–21].

WNV has been studied thoroughly in the United States because its emergence and experimental and field studies suggest substantial variability in the relationship between birds and virus (susceptibility, reservoir competence, mortality and immunity) [22]. It is worth pointing out that, in this study, we define susceptibility of a host species as the frequency at which this host species had been exposed and reacted in a quantifiable way (by allowing pathogen proliferation indicated by molecular prevalence, by generating antibodies to the considered pathogen and/or through clinical symptoms, as quantified by host mortality). This definition means that it involves jointly genetic and ecological factors that have triggered such a reaction.

The microbiological contact between the virus and a susceptible host (exposure) triggers two primary processes. First, birds develop an immune response that limits cell infection; for example, antibodies neutralize the virus and render it non-infectious [22]. Second, the virus enters the host cell and replicates [23,24].

In WNV, host species that develop viremia greater than 10^6 PFU ml^{-1} are considered competent reservoirs. Under experimental conditions, the amount and duration of the viremia that species develop has been measured and standardized in the reservoir competence index (RCI) [20,25,26]. The highest RCI has been observed in species of Passeriformes, Charadriiformes (gulls, auks) and Strigiformes (owls). The Columbiform (doves), Pelecaniform (herons), Psittaciform (parrots) and Galliform (pheasants, turkeys) orders are considered incompetent to transmit the virus, with viremias below 10^4 PFU ml^{-1} [27].

While the RCI is a useful measure for understanding the dynamics of the virus, experimental data include less than 1% of the approximately 10 699 bird species around the world [28]. This data scarcity is because most species are difficult to keep in captivity and because experimental approaches are incredibly challenging, hampering the possibility of obtaining robust estimates of reservoir competence. Therefore, even the most up-to-date list of competent reservoirs among bird species for WNV is far from completion [21,25]. The scientific challenge is to develop analytical tools that allow *a priori* identification of competent reservoirs and thus reduce disease risk in wild species and humans [29].

Variation in the relationships between host species and the virus has been associated with several factors. Among the most important are the phylogeny and geographical origin (GO) of the host, virus strain, time–space and methodology (table 1) [17,35,39,40]. While each of these variables has been studied independently, to our knowledge, no review integrates them in the transmission dynamics of WNV.

To understand WNV dynamics on a global scale, we performed a quantitative review of field studies in birds

considering three disease metrics: serological prevalence (SP), molecular prevalence (MP), and mortality (M). We had two main objectives: (1) to determine if our metrics could be useful to predict the species reservoir competence to WNV, and (2) to investigate if factors of the host (evolutionary history and GO), virus (strain), time–space and methodology (sample size) are associated with these disease metrics (SP, MP or M).

2. Material and methods

(a) Disease metrics

Bird species were considered susceptible to WNV if at least one positive record was found for serological or molecular diagnostic tests [20,35,41]. Three metrics (SP, MP and M) were examined independently as surrogates of different microbiological processes (table 2).

(b) Data collection

We searched for reports of birds exposed to WNV in the global databases of ISI Web of Science and PubMed by using the keywords 'West Nile virus', 'Flavivirus' and 'birds'. Additionally, we searched for published records from health institutions of the United States (Centers for Disease Control and Prevention, CDC 2015) [43], Canada (Canadian Wildlife Health Cooperative, CCWHC 2009–2014) [44] and Mexico (Centro Nacional de Programas Preventivos y Control de Enfermedades, CENAPRECE 2003–2004) [45].

Studies with records of the number of birds analysed by serological and molecular diagnostics for WNV were considered. We excluded reviews and experimental studies because one of our objectives was to understand the potential role of the time–space in bird susceptibility. We also did not take into account observations at the order, family or genus level, as we aimed at finding differences between species.

An independent observation was considered to be a record for a bird species at a particular sampling site and during a particular year (electronic supplementary material, appendix 1). This procedure allowed us to incorporate routine surveillance studies where some bird species were sampled during multiple years, sampling seasons, counties, habitats or sites. The taxonomic adscription of bird species was homogenized based on the BirdLife taxonomic checklist.

Negative observations (i.e. studies without any positive diagnoses) were excluded from the analysis. Having all negative tests suggests that the virus was not present in this time and space, but it could also mean that susceptible bird species were not caught, due to the different sampling techniques that each bird group requires.

To ensure the analysis of true negatives, we considered that prevalence was zero if there were no positive tests in a sample size $N_i > 16$. It has been suggested that 16 individuals is the minimum sample size necessary to detect a change of 1% in prevalence with reasonable confidence [46,47]. Although this threshold cannot prove that the virus is absent within a bird population, it nevertheless provides a standard for excluding from the database records whose sample size is too small to provide useful information on which populations are WNV-negative (2638 observations are remaining).

(c) Data analysis

(i) Identifying competent reservoirs of WNV

SP, MP and M were each analysed independently using the same methods. The analysis was performed in three steps. First, each prevalence observation (P) was calculated as the proportion of positive individuals (N_{ip}) among the total number of individuals tested for WNV (N_i) [43]. P was weighted by the number of individuals sampled P_w to reduce variation in sampling size, i.e. the number of individuals tested per observation (N_i) $\log_{10}$ transformed [12,38,48].

$$P_w = \log_{10}(N_i) \times P.$$

This transformation helped to avoid over-representation of species with large sample size. Note that our observations ranged from 1 to 9040 individuals tested for a given species. Finally, using the P_w values for each observation, we calculated the mean prevalence for each bird species (P_{wm}) for each metric.

Second, phylogenetic generalized least squares (PGLS) was applied to test if our three metrics (P_{wm} of SP, MP and M) independently are good predictors of the RCI. PGLS is a modification of generalized least squares that uses the phylogenetic relationships among species to generate an expected covariance in species data. Because of their more recent common ancestry, closely related species are expected to have more similar traits and produce more similar residuals from the last squares regression line generating high autocorrelation within interspecific data [49,50]. Thus, the estimates of the general linear model must be weighted based on the relatedness between taxa [51–53]. For this analysis, we used the bird phylogeny proposed by Jetz *et al*. [30].

WNV RCI has been calculated as the product of three factors: the proportion of birds infected as a result of exposure, the proportion of exposed vectors that become infectious per day and the number of days that a bird maintains an infectious viremia [25]. RCI has been estimated previously in different studies, and it was standardized for approximately 43 bird species by Kilpatrick *et al*. [26]. Therefore, we used these values in the PGLS.

PGLS was performed considering P_{wm} as the dependent variable and the RCI, phylogeny (PH) and sampling effort (SE) as predictors. For this, we paired our metrics and the available values of the RCI for each species. In the model, we considered a subset of 32 bird species for SP, 11 for MP and 31 for M. We included the SE as a predictor in our model to control for discrepancies in the number of times that species were sampled (1–99 times) [48]:

$$P_{wn} \sim \text{RCI} + \text{PH} + \text{SE}.$$

Subsequently, we carried out a second set of PGLS considering the bird species that did not have RCI values. We did this for two reasons. First, it allowed us to include the species with the highest values of the disease metrics. Second, we identify the potential competent reservoirs based on the correlation between the metrics and the RCI. A subset of 438 species for SP, 112 for MP and 333 for M were used.

Phylogenetic residuals were reported by each metric and bird species. Positive residuals indicate that a particular bird can replicate the virus, die from the infection and/or develop a greater immune response compared to its sister species, after accounting for other confounding factors. The PGLS models were performed using the Caper package for R software:

$$P_{wn} \sim \text{PH} + \text{SE}.$$

(ii) Factors influencing disease metrics

Finally, we tested whether disease metrics were associated with the host phylogeny, viral genotype, time–space and methodology (sample size) (table 1). Bayesian methods were used to test a generalized linear mixed model. This method has been used in previous studies to estimate disease prevalence and associated parameters in humans and wildlife. Additionally, it

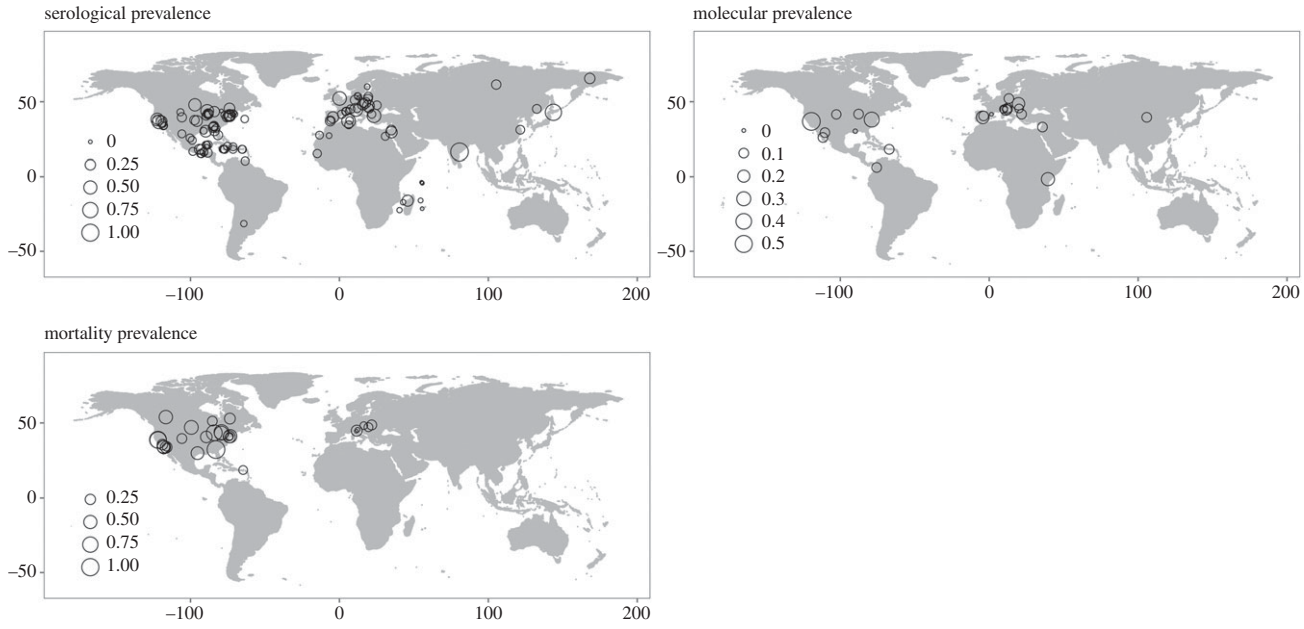

Figure 1. Bird sampling sites for each disease metric. The size of circles represents the prevalence value.

Table 2. General description and importance of disease metrics. These metrics explain, in a general way, different microbiological processes between the birds and the WNV.

disease metrics	description	relevance	references
serological prevalence (SP)	SP suggests that a host was exposed to the virus. It may or may not become infected, develop an immune response, produce antibodies and survive the exposure.	SP identifies species that survive pathogen exposure.	[22]
molecular prevalence (MP)	MP implies that the virus was able to replicate within a host and the host was alive at the time of sampling. The host may or may not subsequently survive the infection.	MP can be used to recognize species in which the virus can replicate and probably be transmitted.	[13,25]
mortality (M)	M suggests that the virus was able to replicate within the host to the point that the host developed multi-organ failure and died. Nevertheless, a dead bird that tests positive for WNV is not a definitive diagnosis of WNV as the cause of death.	M is crucial for identifying highly susceptible species and is important for wildlife conservation.	[33,42]

provides confidence intervals from the posterior distributions of the parameters of interest, which are a useful tool to predict the effects based on a quantitative model [54–57].

We regarded as fixed effects the geographical origin (GO), strain (S) and sample size (SS). The time–space (ST; country and year) and phylogeny (PH) were included as random effects. All models were tested assuming a multi-response prior with the form: $\begin{matrix} p & p{:}n \\ n{:}p & n \end{matrix}$ with a Poisson distribution for the multi-response variable Y_i= the number of positive (p) and negative (n) individuals [54]. The MCMCglmm package in R was used to estimate the posterior estimates from Markov chains built for 100 000 generations, with a burn-in of 10 000, and a thinning interval of 1000 [53].

3. Results

We found 147 published studies including 4995 records which ranged from the years 1959 to 2017 and represented 39 countries (electronic supplementary material, appendix 1). The most represented countries were the USA (59%), followed by Mexico (6%) and Canada (4%) (figure 1).

The observations included 218 814 sampled individuals and 949 different species, representing approximately 8.8% of all known bird species in the world (10 699 species). The species tested belonged to 31 orders, 114 families and 460 genera. Passeriformes, Anseriformes (ducks, geeses) and Charadriiformes were the dominant orders (figure 2).

(a) Disease metrics

We considered 608 (64%) species susceptible to WNV—462 species that had positive SP, 127 with MP and 335 with M—while the remaining bird species did not show sufficient evidence of exposure to the virus according to our criteria. The most frequently tested species were those highly associated with urban areas, such as the house sparrow (*Passer*

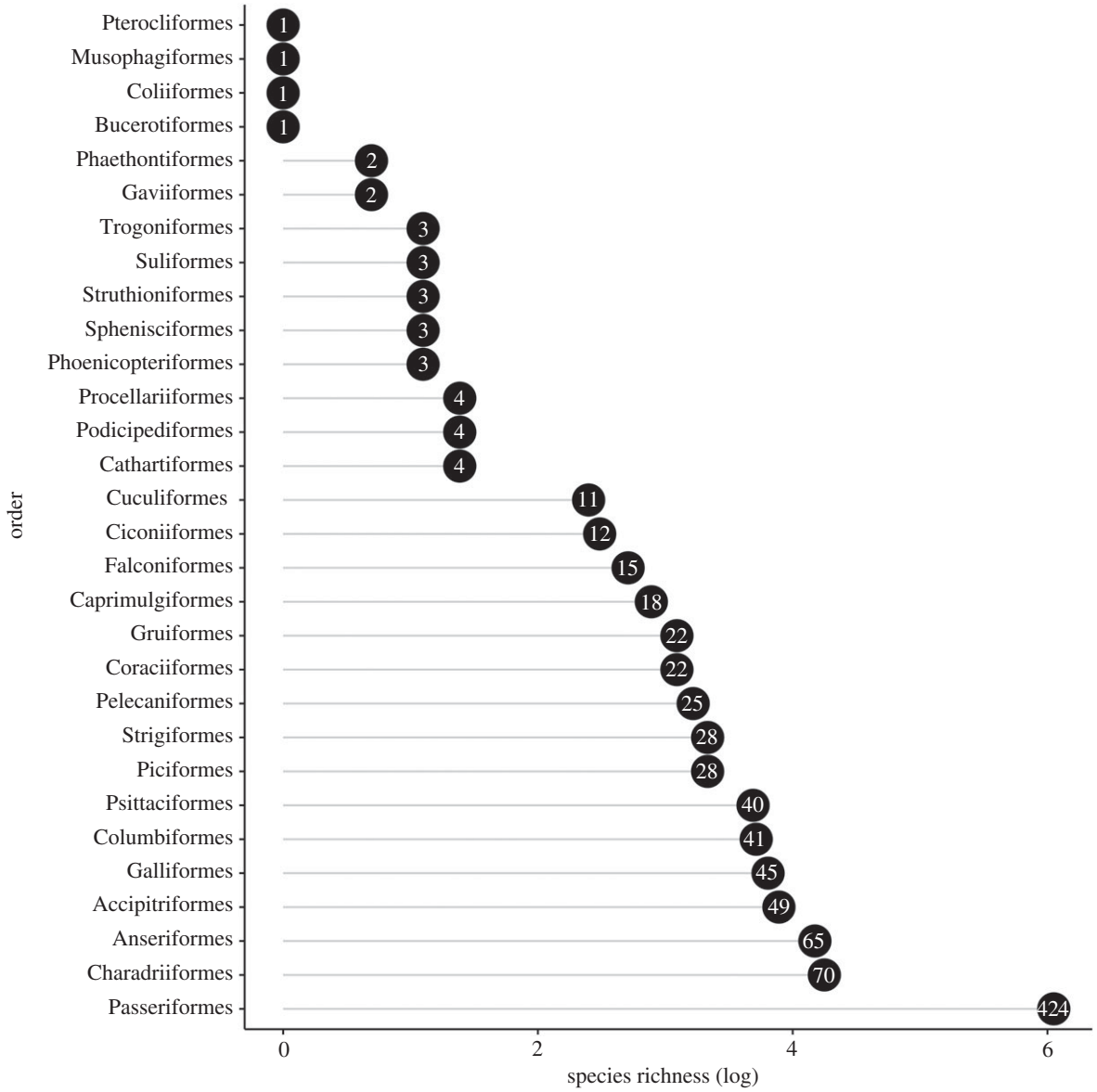

Figure 2. Number of species tested in each bird order.

domesticus, 99 records), rock dove (*Columba livia*, 76) and common starling (*Sturnus vulgaris*, 66). At the same time, there were 352 species that were sampled only once.

Bird species with the highest number of positive observations for WNV were the house sparrow (proportion = 0.70, $n = 99$), American crow (*Corvus brachyrhynchos*; proportion = 0.94, $n = 55$) and rock dove (*Columba livia*; proportion = 0.61, $n = 76$) (electronic supplementary material, appendix 2).

PGLS revealed that the orders with the highest values of SP were the Pelecaniformes: great white egret (*Casmerodius albus*, phylogenetic residuals = 0.096), Columbiformes: common ground-dove (*Columbina passerina*, 0.082) and Passeriformes: northern parula (*Setophaga americana*, 0.058). MP was the highest in Passeriformes, including the European greenfinch (*Carduelis chloris*, 0.103), Eurasian blackbird (*Turdus merula*, 0.102) and hawfinch (*Coccothraustes coccothraustes*, 0.098). Finally, M had the highest values in two Passeriformes—the northern mockingbird (*Mimus polyglottos*, 0.055) and western tanager (*Piranga ludoviciana*, 0.029), and a Gruiform, the sandhill crane (*Antigone canadensis*, 0.029) (figure 3; electronic supplementary material, appendix 3).

(b) Association between disease metrics and the RCI

We found that MP ($R^2 = 0.54$, $p = 0.01$) and M ($R^2 = 0.6$, $p \leq 0.01$) were highly positively correlated with the RCI, while SP showed no association ($R^2 = 0.04$, $p = 0.193$). Also, the number of studies that a given species was sampled had significant effects on MP and M (table 2).

(c) Factors associated with disease metrics

The posterior distributions of the three disease metrics showed significant associations with the sample size. Likewise, these metrics showed a negative effect of the GO, and the strain did not present a significant association (tables 3 and 4). Moreover, posterior distributions of the phylogeny and time–space showed a substantial effect on the three disease metrics.

4. Discussion

Based on a quantitative review of WNV as a model, this study allowed us to (i) identify highly susceptible bird

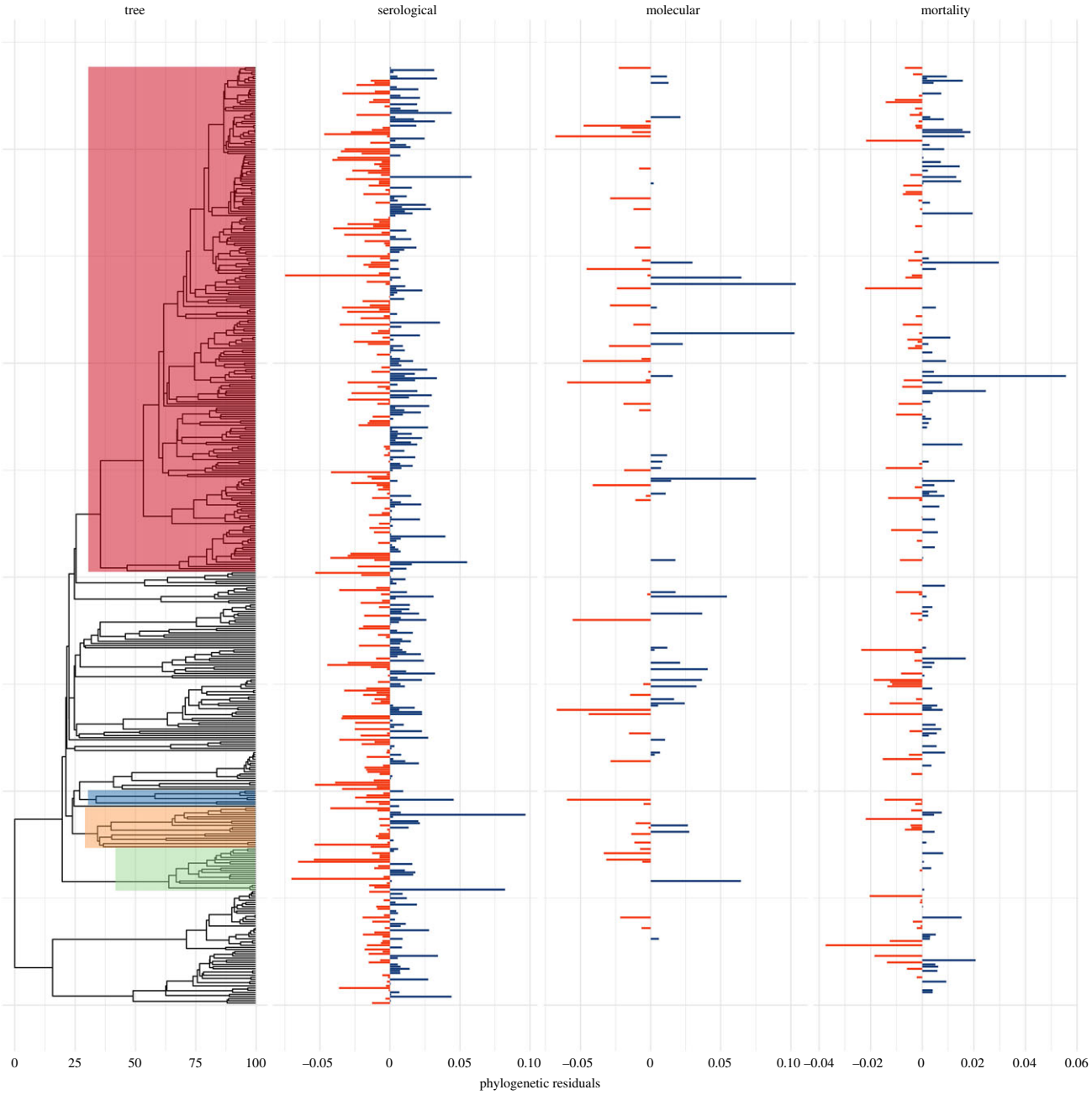

Figure 3. Bird phylogeny and phylogenetic residuals for each metric. The blue bars represent positive residuals (values higher than those predicted by the phylogenetically controlled model), and the red bars the negative residuals in each bird species. The orders with the highest values are indicated with the following colours: Passeriformes (red), Gruiformes (blue), Columbiformes (green) and Pelecaniformes (orange).

species, (ii) propose potentially competent reservoirs and (iii) identify relevant factors associated with bird species risk of WNV. To our knowledge, this is the first study to consider field data on a global scale to examine SP, MP and M, and to test their associations with experimental data.

(a) Characterizing susceptible bird species: survival, transmission and death from WNV exposure

We identified bird species recognized as highly susceptible to WNV. For example, 93% of the records for the American white pelican (*Pelecanus erythrorhynchos*) were positive ($n = 14$ positive records), and the virus has been considered a significant concern for this species in the US [58]. Also, we found that the Spanish imperial eagle (*Aquila adalberti*) and snowy owl (*Bubo scandiacus*) are highly sensitive due to their high number of positive records (electronic supplementary material, appendix 2). These results are relevant because these species are classified as Vulnerable on the IUCN red list [59].

SP results coincided with previous studies in suggesting that Columbiformes and Pelecaniformes are tolerant to infection [19,20]. Passeriformes had the highest values of MP. These data are consistent with prior knowledge suggesting that these are the primary reservoirs to WNV. Nevertheless, we detected other groups that are less recognized as reservoirs, such as the Accipitriformes (hawks) and Picifomes (figure 3). Passeriformes had the highest M values; this group is known to have some species that die by infection [21]. However, our results revealed other species of concern, such as the Gruiformes (coots, rails) and Psittaciformes. These species could die due to WNV infection (figure 3).

Table 3. Phylogenetic generalized least squares.

disease metrics	reservoir competence index	number of studies	complete model
serological prevalence	estimate = 0.008, s.e. = 0.049, $t = 3.3013$, $p = 0.83$	estimate = 003, s.e. = 0.0498, $t = 3.3013$, $p = 0.07$	R^2 adj = 0.04; $F_{2,29} = 1.74$, $p = 0.193$
molecular prevalence	estimate = 0.570, s.e. = 0.194, $t = 0.018$, p = 0.01	estimate = 0.−0.172, s.e. = 0.151, $t = -1.135$, $p = 0.01$	R^2 adj = 0.54; $F_{2,8} = 6.87$, $p = 0.01$
mortality	estimate = 0.107, s.e. = 0.030, $t = 0.001$, $p \leq 0.01$	estimate = 0.010, s.e. = 0.002, $t = 4.490$, $p < 0.01$	R^2 adj = 0.6; $F_{2,27} = 34.26$, $p \leq 0.01$

(b) Molecular prevalence and mortality metrics as predictors of the RCI

The strong correlations between MP and RCI ($R^2 = 0.54$) and between M and RCI ($R^2 = 0.6$) may be because viral replication is measured in each of these metrics [25]. Therefore, high values of MP and M could suggest a high potential to be competent reservoirs. It has been proposed that mortality could facilitate WNV amplification because hosts that die have higher viral loads than those that survive [19]. As could be expected, SP is not associated with birds' capacity to transmit the virus. As such, it is important to point out that despite being the most frequently used metric, SP should not be used as a proxy for competence.

Passeriformes, Charadriiformes, Falconiformes (falcons and caracaras) and Strigiformes had the highest MP and M values. These results are consistent with previously published studies suggesting that these groups are competent WNV reservoirs [25,26]. Moreover, in this study, we found potential reservoirs that have not been analysed in experimental studies, including the Accipitriformes and Piciformes (electronic supplementary material, appendix 3). Experimental and field studies are needed to confirm the capacity of these groups to transmit the virus, and these species may need particular attention during a WNV epidemic.

(c) WNV disease metrics are associated with sample size, phylogeny and time–space

The MCMCglm models showed that our three disease metrics were affected mainly by sample size, phylogeny and time–space. These results are consistent with several studies that have suggested that a larger sample size is correlated with high levels of prevalence [48,60]. Also, phylogeny is determinant for host–pathogen interactions because sister species could provide similar environments for the parasite, or because they share a coevolutionary history with the virus [31,40].

Our variable time–space could reflect, in a general way, ecological and climatic conditions of the sampling sites where each bird species was sampled. For example, some studies have suggested that the composition of vector and hosts communities, temperature and rainfall patterns are crucial for WNV transmission [36,61,62].

Viral strain did not apparently influence MP and M metrics. This result contradicts experimental studies that demonstrate that bird susceptibility to WNV is strain-dependent [35,63]. This apparent contradiction may be due to the scales of analysis. Experimental studies focus on a small taxonomic spectrum, whereas our review encompasses many species. At this macroecological scale, strains which are highly virulent in one area may be less virulent in others. Therefore, this situation needs further experimental and field studies. At this scale, GO did not influence MP and M.

(d) Strengths and limitations of the macroecological approach

The methodology presented here could be used in different host species, pathogens and epidemiological variables using field data such as incidence, intensity, density, pathogen species richness and abundance, among others. This methodology could be applied as an initial screening to propose new competent reservoirs for pathogens of interest, though the results should be confirmed by experimental studies.

Our results can be useful to direct sampling efforts towards less-studied species and geographical regions. Of the 10 699 recognized bird species, 949 (8.8%) have been sampled for this virus, and of these, 352 species have been tested only once for WNV. This situation highlights the urgent need for increased information on the effects of this virus on bird species.

Our disease metrics were limited by some factors, and considerable uncertainty exists in our findings and interpretations. First, concerning the host, we did not take into account its immune system status, age, co-infections and infection route [17], nor did we consider mosquitoes' feeding preferences [33,64]. Finally, variations in SE, the lack of reports of zero prevalence and the lack of follow-up on positive cases introduce uncertainty into our results [46,65]. Nevertheless, considering the large number of individuals and species sampled, we are confident that these sources of variability do not affect our general conclusions.

The macroecological approach is a powerful tool to identify general patterns in disease systems such as WNV. In the future, this approach could be more effective if the field studies report more precise information. For example, it is imperative that field studies have a suitable sample design and sample size, a wide range of host species and diagnostic tests; considering these factors when designing and reporting field studies could improve the precision of our knowledge of disease dynamics.

Data accessibility. The datasets supporting this article have been uploaded as part of the electronic supplementary material, appendix and are available from the Dryad Digital Repository at: http://dx.doi.org/10.5061/dryad.qr971vj [66].

Authors' contributions. M.J.T. designed the study, collected the data, carried out the data analysis and wrote the manuscript. G.E.G.-P. participated in the study design, the statistical analyses and writing

Table 4. MCMCglm model results showing the effects of host, viral, environmental and methodological factors on each disease metric. Asterisk indicates significance at $p < 0.001$.

model	disease metric	DIC		factor associated	post mean	lower confidence interval	upper confidence interval	effective sample
1	serological prevalence	13 998.6	sample size*		2.3341	2.3131	2.3561	722.5
			G. origin	New World*	−0.7082	−0.8925	−0.5263	656.2
				Old/New World*	−0.6857	−0.8643	−0.4876	686.1
				Old World*	−0.7099	−0.8922	−0.5224	648.3
2	molecular prevalence	1330.04	sample size*		2.3259	2.1819	2.4766	9.7.3
			G. origin	New World*	−1.4752	−1.9999	−0.9459	652.2
				Old/New World*	−1.4338	−1.9725	−0.8513	641.2
				Old World*	−1.5244	−2.0728	−0.9861	657.6
3	molecular prevalence (strain)	405.92	sample size*		2.2462	1.7679	2.8017	990
			G. origin	New World	−1.3345	−3.0092	0.3343	940.7
				Old/New World	−1.09	−2.5513	0.2338	990
				Old World	−1.1393	−2.4285	0.3413	990
			strain	Eg101	−0.4431	−2.332	1.6022	990
				Hu04	−0.841	−4.3278	2.4425	693.5
				IS90-ST1	−0.0477	−1.973	2.083	990
				Ita98	−1.0245	−3.0645	0.7967	865.8
				Italy2011/23743	−0.7497	−4.0994	2.8292	685.6
				Italy/2009/0-225677	−0.7006	−4.193	2.3076	492.7
				Magpige/10	−0.8078	−4.5642	2.7598	504.4
				NY99	−0.5054	−2.1207	1.3019	990
				Romania 96	−0.7724	−4.0438	2.011	731.6
				WN02	−0.1263	−2.4114	2.0698	835.5
4	mortality		sample size*		2.3107	2.2589	2.3567	647.2
			G. origin	New World*	−1.1397	−1.4595	−0.8022	894.4
				Old/New World*	−1.1825	−1.55	−0.8636	990
				Old World*	−1.1717	--1.5157	−0.8206	747.4
5	mortality (strain)	373.38	sample size*		2.43	1.9446	2.8685	990
			G. origin	New World	−1.139	−2.8862	0.8414	990
				Old/New World	−1.0646	−2.8333	0.7777	990
				Old World	−1.1396	−2.971	0.5435	990
			Strain	B956	−0.7993	−4.5012	3.0522	821
				Hu04	−0.1061	−2.1368	1.5552	990
				Ita98	−0.471	−2.9586	1.8072	814
				NY99	0.1747	−2.1999	2.2732	990

of the manuscript. O.R.-C. participated in data analysis. B.R. drafted the manuscript. G.S. coordinated the study and helped draft the manuscript. All authors gave final approval for publication.

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Capítulo 2

Life-histories of birds drive their survival and mortality by West Nile Virus

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ABSTRACT

Life-history theory and ecology have been used to recognize factors shaping the outcome of diseases and to identify and characterize the host species that are important for zoonotic pathogens. It is known that fast-paced species (highly reproductive species) are less protected against pathogens than slow-paced species (slowly reproductive species). Similarly, host ecology like habitat use also drives their level of pathogens exposition. To understand the role of these drivers in nature, we use the West Nile Virus (WNV) as a model of study. The WNV is a vector-borne pathogen that has impacted bird populations globally. From a macroecological approach, we tested the relationship between life history and bird's ecology, with survival and mortality rates by WNV. We performed a worldwide meta-analysis, using the survival and mortality rates as operator variables. Generalized Linear Mixed-Effects Models were used to test the relationship between survival and mortality rates with birds ecology and life-history traits. We identified 239 species tested for WNV antibodies and 106 species were found dead. Our results for survival rates coincide with the life-history theory. This suggests that slow paced species have more serological prevalence and therefore more chances to survive to WNV infection. Mortality was negatively related to bird's incubation period. Our results suggest that some life-history traits play a crucial role in disease dynamics identifying key host species, and this information may use in risk models to prevent an outbreak. In addition, this methodology can be applied into different host species and zoonotic pathogens.

Key words: West Nile Virus, birds, life-history, ecology, serological prevalence, mortality, macroecology

INTRODUCTION

Understand the factors that drive host-parasite relationship is crucial to prevent and mitigate disease risk. For many infectious diseases, the host-parasite link is widely diverse due to the variability associated with hosts and parasites traits. This variability can be understood exploring life-history and ecological features of host species (Previtali et al. 2012; Huang et al. 2013; Maxwell et al. 2013).

The life-history theory provides a key concept, *the pace of life*. This theory explains that all species live in a trade-off between reproduction and self-maintenance. Due to this trade-off, species can be classified into fast paced or slow paced (Gustafsson et al. 1995). Fast-paced species (FPS) have low adult survival and high reproductive rate, with low offspring investment, and short developmental times. Whereas slow-paced species (SPS) have high adult survival, slow reproductive rates, and invest more in immune defenses and self-maintenance (Saether 1988; Promislow and Harvey 1990).

From this approach, life-history theory predicts that FPS would be less protected against pathogens than SPS species. This may occur because life-history traits like body mass and/or incubation period are associated with the immune system (Norris & Evans 1999; Pap et al. 2015). For example, the proliferation and diversification of the cells cycles in the humoral immunity (B and T cells) are carried out during the chicken period of incubation (Pap et al 2015).

It has suggested that FPS can invest more heavily on innate immunity (heterophils, granulocytes, among others) than in adaptive immunity (cell-mediated and humoral components) to defend against pathogens. On the contrary, SPS invert more in adaptive immunity which retains the memory of pathogens encounters from the past, has faster responses to subsequent exposures, and is highly specific for a given pathogen (Norris and Evans 2000; Lee 2006; Pap et al. 2015).

However, our understanding of the relation between life-history traits and disease risk remains unclear. Some laboratory research data have shown an important relationship between life-history traits and infectious diseases metrics such as reservoir competence or host survival (Johnson et al. 2012; Huang et al. 2013; Ostfeld et al. 2014). Nevertheless, the associations between life-histories and pathogens cannot be entirely understood in laboratory conditions, because the ecological context that drives disease dynamics is neglected (Durand et al. 2017).

Host ecology, including the habitat, social system, human associations, and diet has the potential to increase host exposure to pathogens and thus the selective pressures

on immune defenses (Ezenwa et al. 2006; Garamszegi and Møller 2007; Chevalier et al. 2009). Few studies have tested the relationship between life-history and disease metrics using wild birds' data (Figuerola et al. 2008; Chevalier et al. 2009; Roche et al. 2015).

To elucidate the association between pathogens prevalence, life-history traits and host ecology different pathogens have been tested. One interesting model to evaluate this theory is the West Nile Virus (WNV) and bird populations as hosts. WNV is a mosquito-borne Flavivirus that has impacted bird populations around the globe since 1999 (Weissenbock et al. 2003; George et al. 2015). The diverse life-history and ecological characteristics bird species worldwide allow us to test the life-history theory from a multi-host scenario approach (Marra et al. 2004).

Here, we tested the relation between two disease metrics, serological prevalence and mortality, and bird life-history and ecological traits. We considered worldwide field data in a meta-analysis to identify if fast or slow species are more susceptible to survive or die to WNV infection. We hypothesized that SLP species would record more serological prevalence and lower mortality; on the contrary, FLP species will have more mortality and lower survival.

MATERIALS AND METHODS

Survival and mortality data

A literature review was undertaken. The search was conducted in the scientific databases ISI Web of Science and PubMed, using the following key words: "West Nile Virus", "Flavivirus", and "birds", and it ranged from 1959 to 2017. The review included scientific reports of birds found dead or alive, that were tested for WNV.

Our search was not limited by year of publication, country or journal. The reference lists of relevant papers found were also contemplated to expand our database. Additionally, we searched for published records of health institutions of the United States (Centers for Disease Control and Prevention, CDC 2015), Canada (Canadian Wildlife Health Cooperative, CCWHC 2009-2014), and Mexico (Centro Nacional de Programas Preventivos y Control de Enfermedades, CENAPRECE 2003-2004).

We excluded reviews and experimental studies, and we only considered localities where WNV circulation was corroborated. The negative reports, and species not recognized by birdlife international were eliminated. If the same species were tested

separately between sampling seasons and localities within a single study they were treated as independent observations using a different identifier. We took into account observations with more than three animals tested.

A database was constructed for each bird species, the number of birds tested and the number of WNV positive birds. We recorded each bird species tested, study, year or location as one observation. Our operator variables were: a) survival (serological prevalence), which refers to the presence of WNV antibodies in birds, that indicates the previous contact with WNV (Figuerola et al. 2008), and b) mortality, that suggests that birds replicated the virus, developed a multi-organ failure, and died by WNV infection (Gamino & Höfle 2013). These data show the virus incidence in bird mortality.

Life-history and ecological traits data

The modulators variables were life-history and ecological traits. Body mass was considered as a modulator variable because it impacts life-histories and the pace of life (Stearns, 1992, Lee 2006; Ostfeld et al. 2014). Incubation time and clutch size have been associated with immune status (i.e. Lymphocyte proliferation), and reproduction respectively (Lee 2006). We obtained information at species level on mean body mass (gr), clutch size (number of eggs per clutch), and incubation period (days). These life-history traits were chosen because they correlate with immune traits and are available in the literature (Table 1). Data of different magnitudes like body mass (gr) and incubation period (days) may cluster and separate from each other, generating spurious partitions. Thus, we avoided this problem by log-transformed body mass, incubation time and clutch size (Arriero and Moller 2008; Huang et al. 2013; Ostfeld et al. 2014).

Additionally, we considered some bird ecological traits that affect host exposure to WNV like the migratory status and habitat type (Figuerola et al. 2008) (Table 1). Life-history and ecological traits were obtained from the Handbook of the Birds of the World (Del Hoyo et al. 2017, https://www.hbw.com/), and in All about birds (www.allaboutbirds.org).

Statistical analysis

We evaluated the relationship between life-history and ecological variables on two operator variables: survival and mortality of birds associated with WNV. These

variables were analyzed independently. We carried out a meta-analysis using the *metafor* package in R (Viechtbauer 2010).

The variances and differences in the sample size of serological prevalence and mortality among studies were estimated using the *escalc* function in the *metafor* package. We measure the heterogeneity with the statistics *Q* and I^2 (amount of variation between-study in relation to the total variance) (Kamiya et al. 2014). The possible publication bias was tested visually using funnel plots and using rank correlation tests for asymmetry. We used the trim and fill method if a significant level of asymmetry was detected (Harrison 2011; Kamiya et al. 2014) (Appendix 2).

The meta-analysis was treated as a "random effect model". This takes into account that data represent a random subset of potentially more, not yet available for differences between studies according to the country, location, year and month where the birds were tested. This model considers that the data are a random subset of potentially more, not yet available data. The model assumes that the effect size can change between studies and incorporate the variability inter-studies and intra-studies (Jung and Threlfall 2018).

Also, we applied Generalized Linear Mixed-Effects Models (GLMM) using the *rma.mv* function in the *metafor* package. GLMM is a more versatile analysis of correlation because the error distribution of the dependent variable and the function linking predictors to it can be adjusted to the characteristics of our data (Figuerola et al. 2008). We used an information-theoretic approach to compare the effect of the modulators, with corrected Akaike Information Criterion (AIC) to assess model fit (Table 2). Ecological traits were considerate as random variables.

RESULTS AND DISCUSSION

Through a meta-analysis, the relation between life-history and ecological traits of bird species with two disease metrics, survival, and mortality due to WNV was tested. This study is the first macroecological approach at a global scale to test this relation using field data.

We constructed a database including 99 reports from 29 countries (Appendix 1). We identify 804 observations of birds serologically positive to WNV captured alive, and 293 observations of birds positive to WNV found dead.

These data together included a total of 156 815 individuals tested. The database included information from 294 bird species belonging to 21 orders such as: songbirds (Passeriformes), doves (Columbiformes), hawks (Accipitriformes), flamingos (Phoenicopteriformes), pelicans (Pelecaniformes), ducks (Anseriformes), gulls (Charadriiformes), owls (Strigiformes), turkeys (Galliformes), (Psittaciformes), hummingbirds (Caprimulgiformes), facons (Falconiformes), vultures (Cathartiformes), storks (Ciconiformes), cuckoos (Cuculiformes), woodpeckers (Piciformes), bobos (Suliformes), coots (Gruiformes), kingfishers (Coraciiformes), Turacos (Musophagiformes) and penguins (Sphenisciformes) (Figure 1).

For both variables (survival and mortality), we observed a high heterogeneity: survival (Q df = 784 = 17054.2151, p-val < .0001, I2=99.43%), and mortality (Q df = 292= 2932.1510, p-val < .0001, I2=99.51%). A significant asymmetry was observed in the funnel plots for survival (Rank-test: Kendall's tau=0.3531, P =<0.0001) (Figure 2a), and for mortality (Rank-test: Kendall's tau=0.1587, P =<0.0001) (Figure 2b). For both variables, the results can be indicative of publication bias. Once the potential effects of publication bias were accounted, the trim and fill method were applied. The relation between host body mass and survival remained positive (Zr =, 95% CI=, P <, I 2 =%). Furthermore, the size effect remained roughly similar, after controlling the effect of the dataset identity (Zr =0.262, 95% CI=0.197–0.328, P <0.001, I 2 =61.24%).

The meta-analysis showed that the most important variable that explains the survival of birds to WNV was the incubation period (estimate= 0.178, p<0.0001). In addition, body mass had a positive and significant effect in this variable (estimate= 0.012, p<0.0001) (Table 3). Both variables suggest that large-bodied species with long incubation period have high survival before an exposition to this virus. These results agree with laboratory approaches, which found that serological prevalence was higher in moderately large-bodied species such as doves and quails which survive WNV experimental infection (Wheeler et al. 2009).

Neutralizing antibodies, which are part of the adaptive immunity, limit the spread of the infection by containing the viremia temporarily. It has shown that a decrease or dysfunction of cytotoxic T lymphocytes (precursors of antibodies), increase the mortality due to WNV (Diamond et al. 2003). Besides body mass has been associated with WNV serological prevalence due to another mechanism associated with mosquito

bites, in this sense, larger birds release more CO^2, and are thus more attractive for mosquitoes (Figuerola et al. 2008; Durand et al. 2017).

We observed negative and significant relation between survival and clutch size (estimate= -0.032, p=<0.0001) (Table 3). This result coincides with the theory which suggests that increasing reproductive effort progressively reduced humoral immunocompetence (Norris and Evans 2000). At the opposite, slow-paced species with smaller clutch size tend to invest more in immunity than in reproduction (Sheldon and Verhulst 1996; Lee et al. 2008; Pap et al. 2015).

In relation to mortality, we observed that the incubation period had a high and significant effect on this variable (estimate=0.46 p<0.001). This result was expected because during this period occurs the proliferation and diversification of the cells cycles in the humoral immunity, therefore better protection against the pathogens (Lee 2006; Pap et al. 2015). Some studies have suggested that there are many potential causes of positive correlations between an immune parameter (mortality, immune system failure), and the life-history traits. If the two processes do not share the same resources or if they are not limiting, trade-offs might not be found (Lee 2006).

Associated with above, we found a negative effect between mortality, body mass and clutch size (estimate= -0.078 p=<.0001; estimate= -0.05 p=<.0001 respectively). Many potential causes have been suggested for the lack of correlation. First, if the two processes, reproduction and self-maintenance, do not share important resources, or if resources are not limiting. A large number of interrelated mechanisms composed the immune system, and several immune tests are required to measure this association effectively (Norris and Evans 2000; Lee 2006).

Second, our results could reflect bias since dead individuals of large-bodied species can be seen more easily. The number of dead birds collected in an area is affected by body size, color, climate, land use, among others (Ward et al. 2006). Smaller species may be more susceptible to die, but their carcasses are less likely to be found and are often scavenged or deteriorate faster (Marra et al 2004). Third, bird mortality by WNV is associated with a complex interplay of variables such as hosts, vectors, viral dose, and strain (Figuerola et al. 2008; Pérez-Ramírez et al. 2014); and our study suffers from a limited number of data, particularly the virus strain, which represents a crucial factor that determines the outcome of the disease (Lim et al. 2015).

In general, we identified a wide gap in the knowledge of bird life-history traits like fledging period and longevity. This lack of data limits our analysis to find and test other

indicators that characterize host species of WNV and other pathogens. Multi-host pathogen like WNV is a good example to understand the host-parasite relationship. Nevertheless, these results are highly dependent on the data quality of host species (number tested) and their parasites (genotypes, strains).

CONCLUSIONS

Meta-analysis is a helpful tool to identify biological indicators such as life-history and ecological traits, which can contribute to characterize host species of infectious pathogens. The relation between these characteristics has been little studied, mainly using field data and also considering susceptible species rarely studied. Our results are relevant to increase our capacity to prevent infectious diseases. This is necessary to have a better understanding of the impact of pathogens in wildlife conservation and public health.

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Author contributions

MJTG, GEGP and CPS, designed research; MJTG, GEGP and CPS performed research; MJTG and GEGP analyzed data; MJTG, GEGP, CPS and GSA wrote the paper.

Competing interests

We declare we have no competing interests.

Ethical statement

Table 1. Life history and ecology traits

LIFE HISTORY TRAITS			
Trait	**Ecophysiological functions**	**Classification**	**References**
Body mass	Body mass was considered as a predictor variable because it impacts life histories, and it is involved in shaping a species immune defenses	-	Stearns 1992, Lee 2006; Ostfeld et al. 2014
Clutch size	Clutch size has been associated with immune status (i.e. Lymphocyte proliferation) (Lee 2006).	-	
Incubation time	In vertebrates lymphocyte proliferation and diversification is mostly restricted to the developmental period (incubation time) and requires substantial energy and nutrients	-	Lee 2006; Pap et al. 2015
ECOLOGICAL TRAITS			
Migratory behavior	Bird migration can cause more significant exposure to WNV in birds; also migration haven been associated with immune changes.	Resident or migratory	Hawley and Altizer 2011; Durand et al. 2017
Habitat type	Exposition to mosquitoes bites	Terrestrial aquatic (marshes, wetlands)	Pap et al. 2015

Table 2. Mixed models description for survival and mortality

MODEL	DESCRIPTION
model 1	survival ~ body mass + (1 |habitat+ 2| migration)
model 2	survival ~ body mass + clutch size+(1 |habitat+ 2| migration)
model 3	survival ~ body mass + clutch size+ incubation period + (1 |habitat+ 2| migration)
model 4	mortality ~ body mass + (1 |habitat+ 2| migration)
model 5	mortality ~ body mass + clutch size+(1 |habitat+ 2| migration)
model 6	mortality ~ body mass + clutch size+ incubation period + (1 |habitat+ 2| migration)

Table 3. Mixed models explaining the survival and mortality between species in relation to life history traits

SURVIVAL							
moderator variable	**estimate**	**se**	**z val**	**p value**	**ci.lb**	**ci.ub**	**AIC**
body mass	0.012	0.001	6.46	<.0001	0.008	0.016	
clutch size	-0.032	0.005	-6.46	<.0001	-0.042	-0.022	
incubation period	0.178	0.01	16.39	<.0001	0.15	0.191	
MORTALITY							
moderator variable	**estimate**	**se**	**z val**	**p value**	**ci.lb**	**ci.ub**	**AIC**
body mass	-0.078	0.002	-26.78	<.0001	-0.083	-0.072	
clutch size	-0.05	0.009	-5.5	<.0001	-0.068	-0.032	
incubation period	0.46	0.021	21.6	<.0001	0.425	0.51	

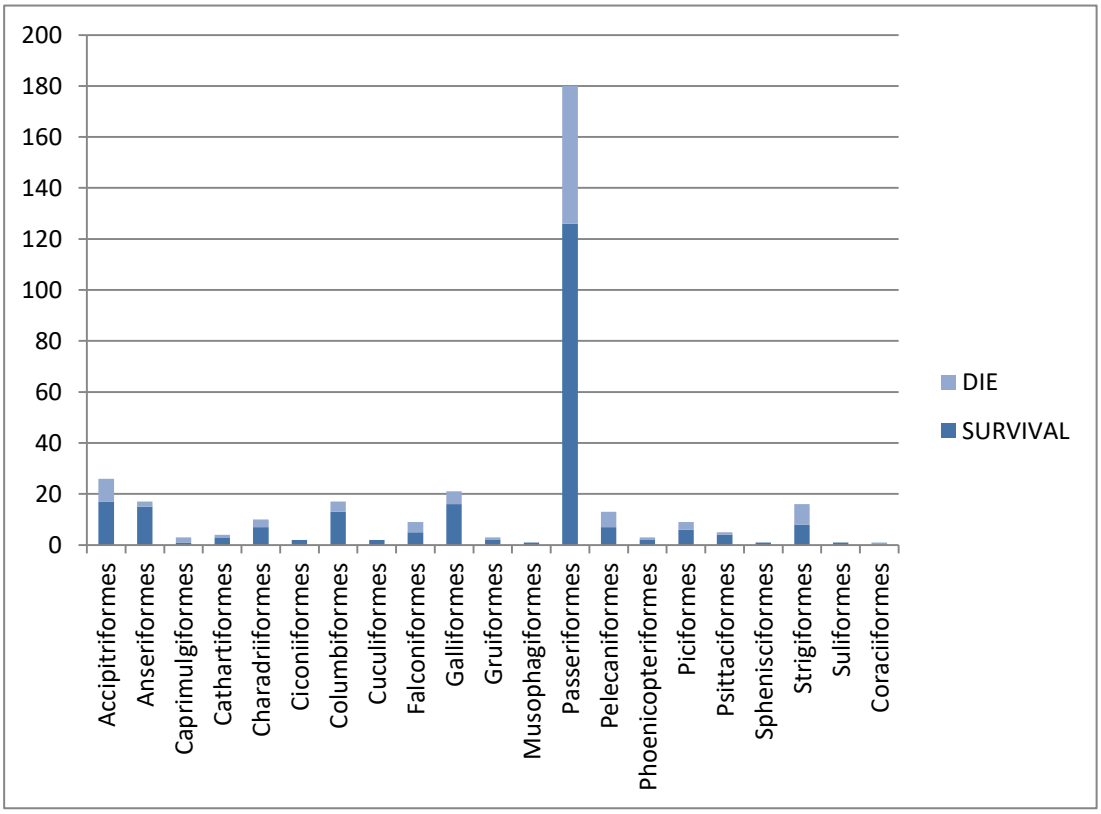
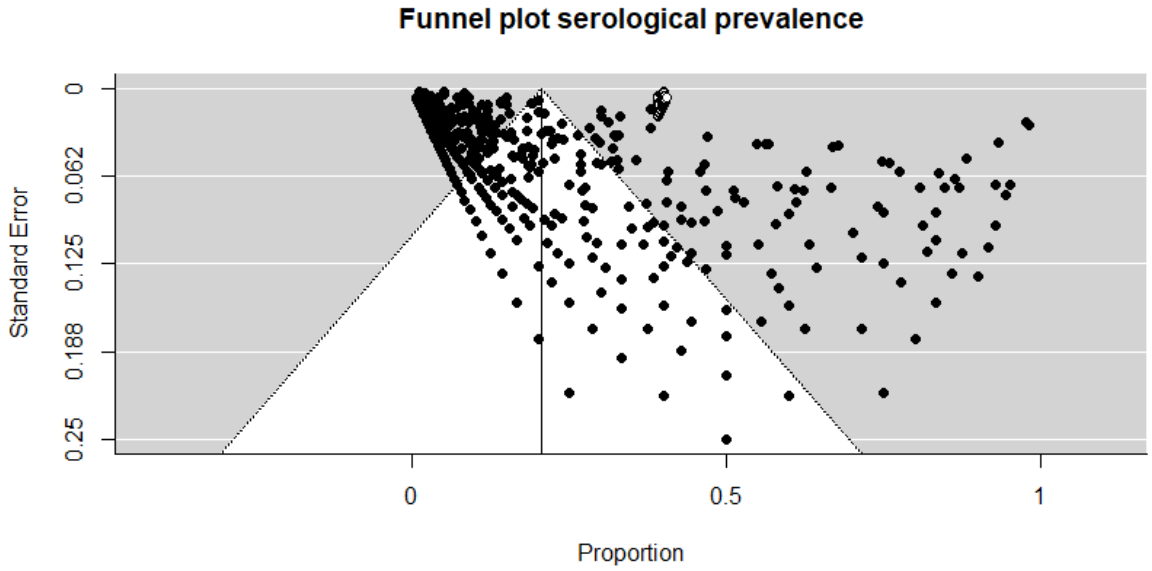


Figure 1. Number of species tested by disease metric



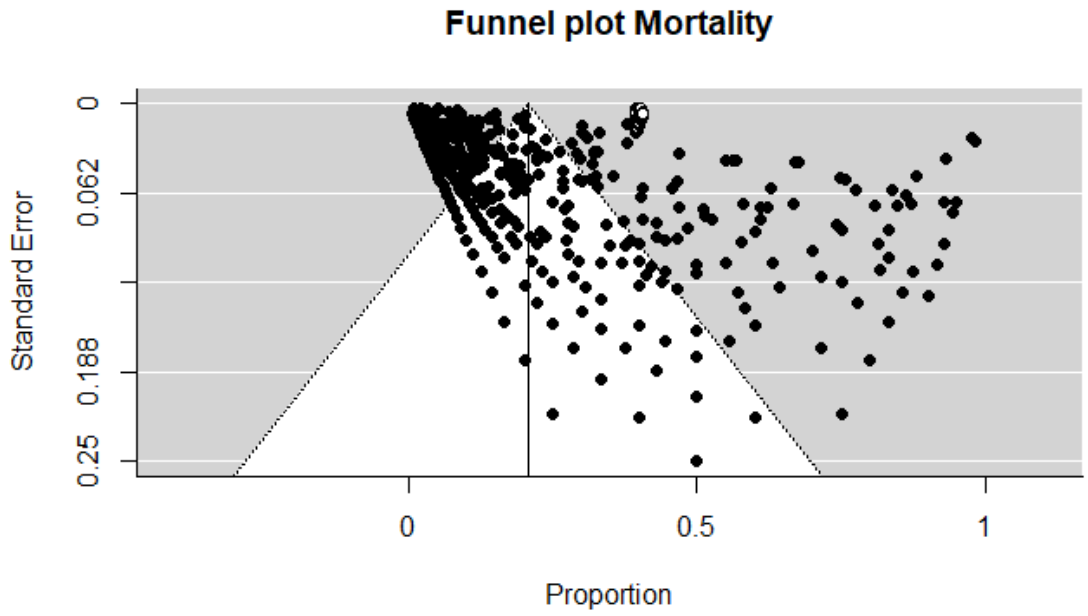


Figure 2. Funnel plot for serological prevalence and mortality

Appendix 1. Database

Capítulo 3

Wild bird mortality due to West Nile Virus: Insights for conservation in a multi-host, multi-vector complex system

Running head: Bird mortality by multi-vectors of WNV

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ABSTRACT

Infectious diseases, their vectors and reservoirs, have rarely been incorporated into conservation strategies worldwide, even though disease outbreaks are events that may diminish populations, reduce biodiversity, and interrupt ecosystem processes and services. An example of such an infectious disease is West Nile Virus (WNV), a vector-borne pathogen important for wild bird conservation. Modelling and predicting these events is a crucial prerequisite for prevention and impact biodiversity reduction. Here we used a spatial data mining framework to identify and infer interactions in the WNV transmission cycle between potential mosquito vectors and bird hosts, concentrating on bird mortality risk due to WNV infection in the United States (U.S.), thereby contributing to conservation by relating Important Bird and Biodiversity Areas (IBAs) with bird mortality data. Initial analysis showed strong relationships between avian mortality and the *Culex* genus. We identified 220 IBAs in the far west, where *Culex* mosquitoes occur and therefore which present a particular risk of bird mortality. We suggest that three potential mechanisms can explain associations between certain mosquito genera and bird mortality: 1) host-feeding preferences of mosquitoes; 2) vector and host competence, and 3) evolutionary dynamics between WNV strains and mosquitoes. The inclusion of systematic epidemiological surveillance of birds and mosquitoes in natural protected areas and the implementation of risk modelling using spatial data mining approaches are required for conservation strategies following the EcoHealth/One Health paradigm.

Keywords: West Nile Virus, avian mortality, Important Bird and Biodiversity Areas, mosquito vectors, spatial data mining.

1. INTRODUCTION

Biodiversity loss leads to a reduction of vital ecosystem processes and services, herein defined as those processes of ecosystems and species that benefit humans (Daily et al., 2009). An important class of events that can severely disturb ecosystem processes and services are epizootic outbreaks that cause mortality in some wildlife populations and communities (DeFries et al., 2004; Coutts and Hahn, 2015). Such events are mostly caused by emerging or re-emerging pathogens (Buttke et al., 2015). Thus, the development and application of different modelling approaches for infectious diseases can increase our ability to predict and understand outbreaks that compromise the integrity of wildlife and the ecosystem services they provide (Webster et al., 2015).

Wild birds are fundamental in many vital ecosystem processes and services, such as pollination, pest control, seed dispersal, human feeding and cultural services (Wenny et al., 2011; Donázar et al., 2016). Hence, it is concerning, that in the United States (U.S.) bird populations have decreased due to outbreaks of West Nile Virus (WNV), an important vector-borne pathogen that has had a severe impact on both bird conservation and human health (LaDeau et al., 2007; George et al., 2014).

WNV is a Flavivirus with a transmission cycle that involves mainly birds and mosquitoes, and in some cases mammals (Padgett et al., 2007; Kramer et al., 2008; Calistri et al., 2010). Since it was introduced into the Americas in 1999, a broad range of strains has spread and diversified across a diverse set of mosquito vectors and vertebrate hosts (Di Giallonardo et al., 2016). Currently, it is widely distributed across the whole continent (Kramer et al., 2007).

Diverse studies have determined that WNV has caused significant mortality in a wide range of wild bird species in the U.S. (LaDeau et al., 2007; Wheeler et al., 2009; George et al., 2015), with some of these studies reporting severe histological lesions on WNV-positive dead birds (Gibbs et al., 2005; Ernest et al., 2010). However, measuring mortality and population decline of wild birds is difficult, and the effects of WNV on them may well have been underestimated (Ward et al., 2006).

WNV-associated mortality has been observed in more than 300 species of wild bird (CDC, 2016), with the most dramatic example being the American crow (*Corvus brachyrhynchos*) where it has been suggested that their populations have decreased by up to 45% since the arrival of WNV in the U.S. (LaDeau et al., 2007). It has also been estimated that up to 30 and 15 million individuals, respectively, of the red-eye

vireo (*Vireo olivaceus*) and the warbling vireo (*Vireo gilvus*) have died since the emergence of WNV in this country (George et al., 2015).

In the case of WNV vectors, particular attention has been focused on the role of *Culex* and *Aedes* mosquitoes as competent vectors (Sardelis et al. 2001; Kilpatrick et al., 2010). Nevertheless, the virus has been detected in many other mosquito genera, such as *Anopheles, Coquillettidia, Culiseta, Deinocerites, Mansonia, Orthopodomyia, Psorophora and Uranotaenia* (CDC, 2016). There are many uncertainties that severely limit our understanding of the WNV transmission cycle and its impact on bird populations (Kilpatrick et al., 2010), such as the relative competence of the different vectors, their feeding preferences and with which WNV strains they are associated.

Given the importance of WNV to conservation and health, and the many uncertainties that exist concerning its transmission cycle, it is imperative to generate novel and innovative approaches that allow us to better predict and understand both the transmission cycle and the risk of an outbreak of an infectious zoonotic pathogen, or other pathogens threatening wildlife (Stephens et al. 2016). In the last decade, climate-based species distribution models have emerged as an important tool with which to study zoonotic diseases and have been used to predict potential distributions of vectors, hosts or pathogens (e.g. Garza et al., 2014; Levine et al., 2007; Pigott et al., 2014). However, these models are limited, as they only identify suitable climate areas for particular vectors or hosts. They are unable to identify actual or potential biotic interactions among vectors, hosts and pathogens.

The question of whether biotic interactions may leave imprints on species ranges at regional scales, and therefore whether eco-geographic analysis conducted at coarse scales can reflect ecological interactions at a local level, is associated with a long running debate (Pearson and Dawson, 2003; Gotelli et al., 2010). Recently, however, a spatial data mining framework, which quantifies the degree of species co-distribution at the regional scale has been successfully used to infer and later to discover unknown hosts for the pathogen *Leishmania* (Stephens et al., 2009, 2016). This provides clear evidence that, at least in certain cases analysis at coarse geographic scales can provide information on ecological interactions at a local scale.

Therefore, such a spatial data mining approach represents a useful tool for the inference and study of complex ecological interactions, allowing for the formulation of new hypotheses and directing studies towards sensitive but poorly studied systems. For example, it has predicted possible mammal hosts of Zika virus (ZIKV) and identified risk zones using incidence data of ZIKV in humans (González-Salazar et al.,

2017). This approach and perspective have implications for the decision-making processes regarding both wildlife conservation and public health.

Many emerging and re-emerging infectious diseases are embedded in natural systems, wherein multiple species of host and vector occur (Roche et al., 2013). Hence, community ecology and disease ecology approaches must be considered together. Thus, analysis of co-occurrence patterns of vectors, hosts, and pathogens is required in order to develop appropriate strategies for host conservation and health (Buttke et al., 2015).

Due to the importance of safeguarding the integrity of bird populations, in this study, we used a validated spatial data mining approach to identify and infer interactions in the transmission cycle between mosquito genera and bird species with evidence of mortality due to WNV infection. Using this perspective, we identify high-risk zones situated in Important Bird and Biodiversity Areas (IBAs). We also identify those bird species that are of special concern, as they provide ecosystem services or are considered emblematic species (flagships).

2. METHODS

2.1. Data collection

2.1.1. Disease databases

We carried out a bibliographical search to identify mosquito genus positives to WNV, as well as bird mortality records associate to WNV in the U.S. For mosquito, published records were obtained from the ISI Web of Knowledge using as keywords: “mosquito genus” AND “West Nile Virus” OR “Flavivirus”. Additionally, we considered published records from the Centers for Disease Control and Prevention (CDC, 2016). We included in our database mosquito species and diagnostic test (Appendix 1).

For birds, published records of bird mortality by WNV were obtained from the ISI Web of Knowledge using as keywords: “West Nile Virus”, “Flavivirus” AND “birds”. Information from the CDC was also included. We considered only information at the species level, and literature reviews and laboratory studies were excluded (Appendix 2). We defined an independent observation as a record for a bird or mosquito species on a particular sampling site, season and year.

Studies that consider bird mortality by an infectious disease have a wide range of biases that should be taken into consideration, such as: i) sampling is not random; and ii) detection of dead birds is influenced by the degree of urbanisation, body size, plumage, density and abundance of carcasses the presence of scavengers; the weather; and habitat characteristics (Bernard et al., 2001; Ward et al., 2006).

2.1.2. Geographical databases

The mosquito geographical database was obtained from the electronic databases VectorMap and Global Biodiversity Information Facility (GBIF). This contained 8,671 unique presence records for 10 genera: *Aedes* (6,166), *Anopheles* (433), *Coquillettidia* (303), *Culex* (870), *Culiseta* (491), *Deinocerites* (2), *Mansonia* (46), *Orthopodomyia* (42), *Psorophora* (236), and *Uranotaenia* (82).

For birds, we built a geographic database of collection points for 404 land bird species distributed throughout the U.S. These species comprise 50% of the total of avifauna of the U.S. belonging to 11 orders: Accipitriformes (hawks, 22 spp.), Apodiformes (hummingbirds, 18 spp.), Caprimulgiformes (nightjars, 7 spp.), Columbiformes (doves, 8 spp.), Coraciiformes (kingfishers, 3 spp.), Cuculiformes (cuckoos, 6 spp.), Falconiformes (falcons, 9 spp.), Galliformes (pheasants, 16 spp.), Passeriformes (song birds, 274 spp.), Piciformes (woodpeckers, 23 spp.), and Strigiformes (owls, 18 spp.). The bird dataset contained 693,381 unique georeferenced localities for the U.S.

2.2. Data analysis

2.2.1. Inferring ecological interactions using spatial data

We used a spatial data mining framework to identify potential mosquito-bird interactions based on the degree of co-occurrence between taxa, as proposed in Stephens et al. (2009). The explicit model for predicting possible interactions was created using presence records of mosquitoes and birds in the U.S. The first step was to determine the co-occurrence of taxa in our study region (continental region of U.S.). To obtain an adequate spatial representation of the coexistence relationship among the species, we sampled the geographical region with a regular grid that divided the space into regular spatial cells, x_α. Here, we used a uniform grid of rectangular cells of 25 km x 25 km size and then counted co-occurrences within each x_α of mosquito genus, B_i, and each bird species, I_k. To quantify the relationship between taxa, and determine which co-distributions $P(B_i \mid I_k)$ shows a statistically significant correlation relative to the null hypothesis, $P(B_i)$, we applied the following binomial test as a statistical significance indicator:

$$\varepsilon(B_i \mid I_k) = \frac{N_{I_j}(P(B_i \mid I_k) - P(B_i))}{(N_{I_j} P(B_i)(1 - P(B_i)))^{1/2}}$$

which measures the statistical dependence of B_i on I_k relative to the null hypothesis that the distribution of B_i is independent of I_k and randomly distributed over the grid, i.e., $P(B_i) = N_{Bi}/N$, where N_{Bi} is the number of grid cells with point collections of taxon B_i and N is the total number of cells of the grid. When the binomial distribution may be approximated by a normal distribution, values of $|\varepsilon| > 1.96$ correspond to a higher than 95% confidence that the co-occurrences occur at a rate inconsistent with the null hypothesis (for more details see Stephens et al., 2009). As ε increases monotonically with the frequency of co-occurrence, we interpret a statistically significant positive correlation as inferring a potential mosquito-bird interaction. The final result is a list of mosquito-bird pairs ranked by a range of positive to negative values of epsilon. Thus, this list can be interpreted as a group of birds with a significant co-distribution with a given mosquito (positive ε values), and a group of birds that are mainly not present where a given mosquito is present (negative ε values).

2.2.2. Predictive risk map

We may also determine the ecological niche of different mosquito species or genera by calculating $P(B|\boldsymbol{I})$, where B is the presence of the mosquito and $\boldsymbol{I} = (I_1, I_2,...,I_N)$ is a vector where each I_k represents presence/no presence of the bird species I_i. Higher/lower values of P (B|**I**) correspond to conditions of niche/anti-niche for the corresponding mosquito taxon. Using Bayes theorem and the Naive Bayes approximation (Stephens et al. 2009) a score function, S (**I**), which is a monotonic function of P(B|**I**) can be used explicitly:

$$S(\boldsymbol{I}) = \sum_{i=1}^{N} s(I_i) + ln\frac{P(B)}{P(B')}$$

where $s(I_i) = \ln(P(I_i|B)/P(I_i|B'))$ is the contribution to the presence of the mosquito B from the bird species I_i and B' is the set complement of B, i.e., $P(B') = (N - N(B))/N$, the set of cells without a presence of B. S(**I**) is a measure of the propensity to find the mosquito taxon B. It can be calculated for any spatial cell and a consequent risk map plotted.

This risk map was overlapped with the Important Bird and Biodiversity Areas map (IBAs) in the U.S. in the National Audubon Society to determine the degree of coincidence with bird conservation areas. We assigned risk score values to each genus

of mosquito associated with bird mortality and applied them to 2199 recognised IBAs. We then ranked the IBAs by risk score value and selected the top decile associated with the highest probability of mosquito presence (i.e., 220 IBAs for each genus). Those IBAs with a high probability of the presence of mortality-associated mosquito genera that could potentially present high bird mortality were identified.

We searched the bird identities available to us by each IBA from the National Audubon Society (2017). Finally, we categorised the species list based on its global conservation status in the IUCN Red list, federal conservation status in the U.S. Fish and Wildlife Act List if the species are considered umbrella or flagship species, and on the ecosystem services that these species provide.

2.2.3. Statistical analysis

The above analysis identifies potential biotic interactions between mosquito and bird species. Using epsilon as a statistical measure of the degree of co-occurrence between bird species and mosquito genera we can then examine the relation between bird mortality and the presence of a given mosquito genus. We ranked all bird species using epsilon values of each mosquito genus. These lists were then divided into deciles, with the top decile being the 10% of bird species with the most statistically significant geographic overlap with the given mosquito genus. For each decile, we then counted the number of bird species in that decile which exhibited mortality and regressed, for each mosquito genus, the percentage of bird species with mortality versus the average epsilon value for that decile.

The study was carried out at the mosquito genus level due to the high heterogeneity in the sample number among the mosquito species in the database. We also start from the fact that there is a greater biological and ecological similarity between species of the same genus (Gaunt et al. 2001).

3. RESULTS

We recorded 1,338 mosquito observations (as with birds an independent observation was considered to be a record for a particular sampling site and year) from 1999 to 2012, corresponding to 31 states. We found 116 mosquito species positive for WNV belonging to 10 genera: *Aedes* (22), *Culex* (14), *Anopheles* (6), *Psorophora* (4), *Culiseta* (3), *Uranotaenia* (2), *Deinocerites* (1), *Mansonia* (1), *Orthopodomyia* (1), and *Coquillettidia* (1) (see Figure 1 and Appendix 1).

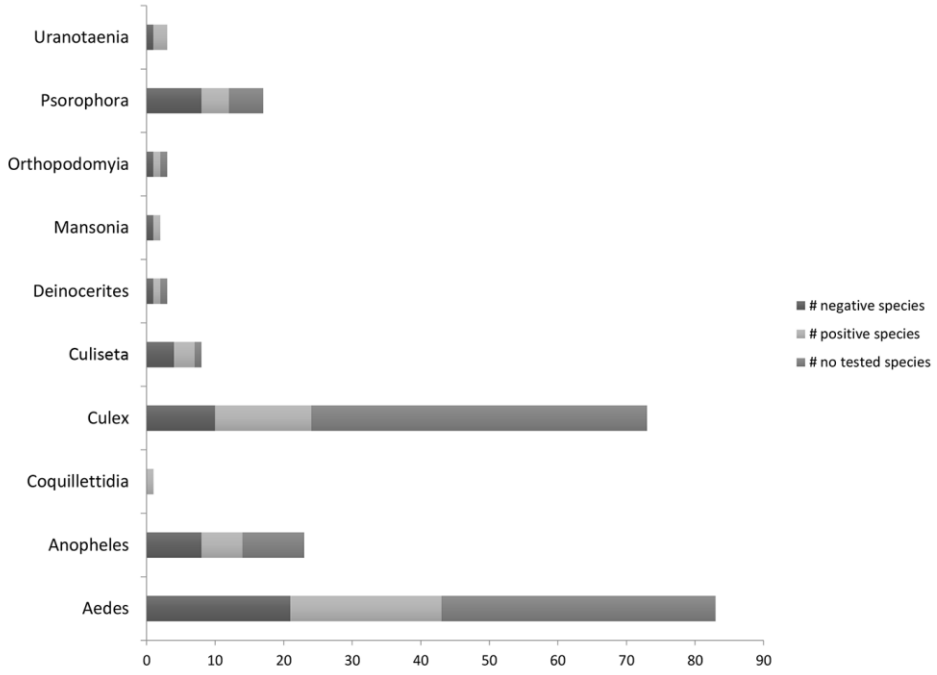

Figure 1. Number of mosquito species tested for WNV present in the U.S.

Our bird database consisted of 650 observations collected from 1999 to 2015 from 11 states. The data included 245 bird species from 13 orders: Passeriformes (138), Accipitriformes (20), Psittaciformes (17), Strigiformes (17), Galliformes, (15), Piciformes (11), Columbiformes (9), Caprimiulgiformes (8), Falconiformes (5), and Casuariformes (1) (Appendix 2).

3.1. Interaction between mosquitoes v/s bird mortality

We found four mosquito genera that were significantly associated with bird mortality: *Culex* (R^2=0.74, *P*= 0.0013), *Anopheles* (R^2=0.73, *P*= 0.002*), Coquillettidia* (R^2=0.50, *P*= 0.023*),* and *Culiseta (*R^2=0.71, *P*= 0.002*)*. Conversely, the remaining six genera did not show significant association with bird mortality: *Aedes* (R^2=0.03, *P*= 0.65), *Uranotaenia* (R^2=0.0006, *P*=0.9), *Orthopodomyia* (R^2=0.008, *P*= 0.80), *Mansonia* (R^2=0.086, *P*= 0.41), *Psorophora* (R^2=0.16, *P*= 0.26), and *Deinocerites* (R^2=0.09, *P*= 0.40).

The role of *Culex* mosquitoes in WNV transmission has been well documented (Turrel et al. 2005; Reisen et al. 2006; Kilpatrick et al., 2010) consequently, their relationship with bird mortality might be expected. However, for *Anopheles*, *Coquillettidia* and *Culiseta* this result is somewhat unexpected as their role as being associated with WNV transmission has been dismissed (Goodard et al. 2002; Andreadis et al. 2004).

To account for this potential discrepancy, we explored the possibility that there exist possible confounding variables intermediating between these mosquitos' genera and bird mortality (Stephens et al., 2017). Particularly, we focused on the effect of the co-distribution with *Culex* mosquitoes as being a possible confounder.

To do this we quantified the degree of co-occurrence between birds and each mosquito genus significantly associated with bird mortality, conditioned on the presence or no presence of *Culex*. For instance, we calculated two new epsilon values for birds and *Anopheles* using *P* (*Anopheles= presence*, *Culex= no presence*), and *P*(*Anopheles= presence*, *Culex= presence*) as the null hypothesis. We built two new bird lists ranked by epsilon values for each mosquito genus, which were then divided into deciles to count the number of bird species in each decile which exhibited mortality. We performed a linear regression between the percentage of bird species with mortality versus the average epsilon value by decile. These analyses were carried out for the *Anopheles*, *Coquillettidia* and *Culiseta* genera.

We found that significant association among mosquito genera and bird mortality was conserved when Culex is present: *Anopheles* (R^2=, 0.89 P< 0.0001)*, Coquillettidia* (R^2= 0.62, P= 0.007)*,* and *Culiseta* (R^2= 0.79, P= 0.0006)*;* however, this relationship is not maintained when Culex is not present: *Anopheles* (R^2= 0.11, P= 0.34)*, Coquillettidia* (R^2= 0.17, P= 0.24)*,* and *Culiseta* (R^2= 0.34, P= 0.06; Table 1). This clearly shows the role of *Culex* as a confounder and we conclude that the role of these other genera in bird mortality can be discarded. However, effects at the community level should not be neglected, and they will be addressed in future field work.

Table 1. Correlation between *Anopheles, Coquilletidia and Culiseta* with the presence and not presence of *Culex* genus

	Culex= present			*Culex*= not present		
Genus	***R^2***	***ß coefficient***	***P***	***R^2***	***ß coefficient***	***P***
Anopheles	0.89	1.66	0.00004	0.11	0.61	0.34
Coquillettidia	0.66	1.21	0.007	0.17	0.83	0.33
Culiseta	0.79	1.12	0.0006	0.34	1.18	0.08

3.2. Risk map and the Important Bird and Biodiversity Areas (IBAs) with a high risk of bird mortality

Although areas of high-risk were found both on the West and the East coast of the U.S. (Figure 2), the most significant number of IBAs with high-risk of mosquito presence were located in the western U.S.

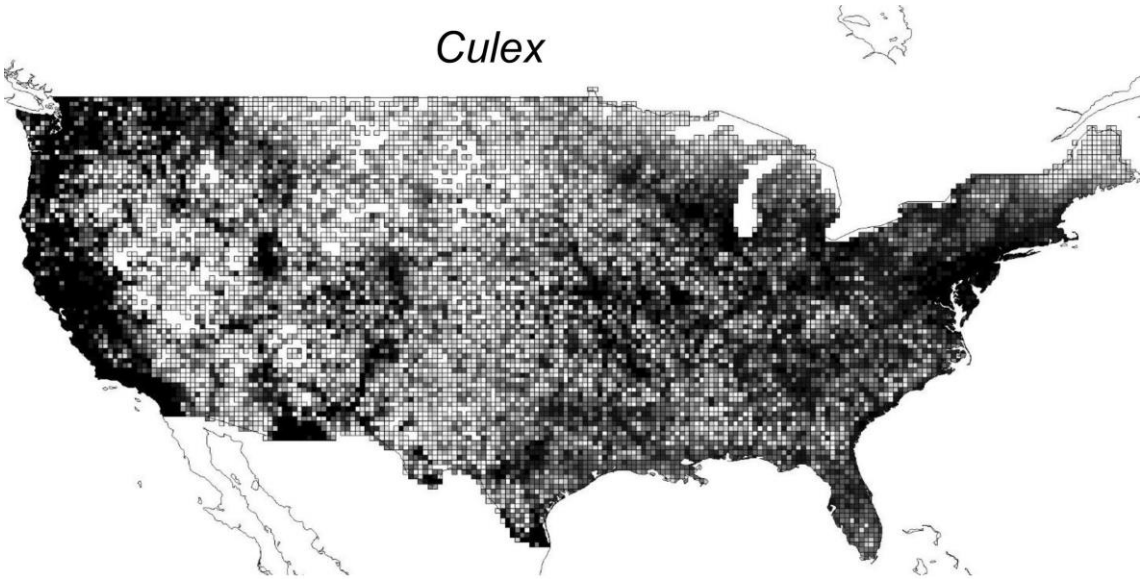

Figure 2. High-risk areas (dark zones) based on the degree of co-occurrence between birds and the *Culex* mosquito genus

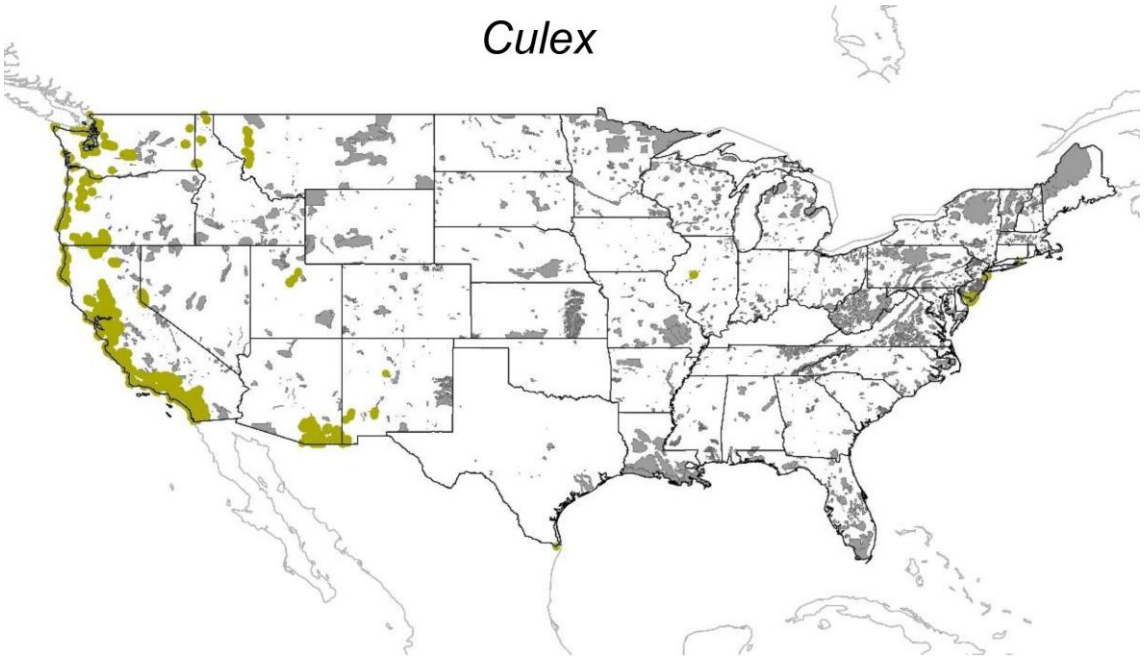

Figure 3. IBAs with high-risk of bird mortality (olive green color) based on the probability of *Culex* mosquito presence. The gray polygons show the IBAs without significant risk.

We identified 220 IBAs in the United Sates (14.6%) with a high probability for the presence of the *Culex* genus, located in 12 states: California, Oregon, New Mexico, Idaho, Montana, Nevada, Utah, Arizona, New York, Texas, New Jersey and Washington (Figure 3). Bolinas Lagoon had the highest score (65.12), followed by Corte Madera Marshes (65.12) and San Mateo/Monterrey, CA (63.96) (Appendix 3). Of these 220 IBAs, 47 are categorised as A1 (Globally threatened species), and 17 as A4i (Congregations), both considered as a global status by BirdLife. Additionally, we found 1 IBAs Sub-Global priority, the Cape May National Wildlife Refuge - Great Cedar Swamp Division (Appendix 3).

Within the IBAs with a global priority, we recorded some bird species with some conservation risk status based on the IUCN red list; these are: Critically endangered (CR) - one species, the California condor (*Gymnogyps californianus*); Endangered (E) - 6 species, such as the Tricolored blackbird (*Agelaius tricolor*), Marbled murrelet (*Brachyramphus marmoratus*), or Ashy Storm-petrel (*Oceanodroma homochroa*), Thick-billed Parrot (*Rhynchopsitta pachyrhyncha*), Saltmarsh Sharp-tailed Sparrow (*Ammospiza caudacuta*); Near threatened (NT) - 21 species as the Olive-sided flycatcher (*Contopus cooperi*), Spotted owl (*Strix occidentalis*), and Black rail (*Laterallus jamaicensis*), among others; Vulnerable (VU) - 10 species, the Long-tailed

duck (*Clangula hyemalis*), Black-legged Kittiwake (*Rissa tridactyla*). In addition, we recorded two species on the U.S. Fish and Wildlife Act list - the California condor and the Marbled murrelet (Appendix 3).

Species that are exposed to higher mortality risk through their distribution within the high-risk IBAs are the Tricolored blackbird (32 IBAs) Loggerhead shrike (*Lanius ludovicianus,* 31), Northern harrier (*Circus hudsonius*, 30). Some flagship species present in the high-risk IBAs were: the Golden eagle (*Aquila chrysaetos*), Northern harrier *(Circus cyaneus)* and Spotted owl among others (Appendix 4). The ecosystem services with the highest risk were the pest control associated with insectivorous birds and scavengers. We also found 193 bird species with previous WNV-associated mortality and 263 tested for WNV in previous studies (Appendix 4).

IBAs with the highest number of globally threatened species were: Sandy Hook/ Gateway National Recreation Area (11), Big Sur (7) and Olympic Continental Shelf (6). We also recorded 174 IBAs with a State category (B1, B3, D1, D3, D4i, D4ii, D4iii, D4iv, D4v and D5). Cape May Intracoastal Waterway had the highest number of global and state criteria (9) (Appendix 3).

4. DISCUSSION

In this paper, we have proposed a novel and interesting approach to wildlife conservation studies that accounts for the potential impact of disease vectors on wildlife species. This approach has been successfully used to analyse other emerging diseases and biodiversity (Stephens et al., 2009; González-Salazar et al., 2017). The methodology is based on the idea that ecological interactions can be inferred from the location of species as a function of space and time (Stephens et al., 2016). Hence, if an ecological factor, e.g. a species or climate condition, has an effect on the range of a target species, then this should manifest itself in a correlation between both distributions.

In other words, the “interaction” between two species can be inferred from their co-occurrence data. If they co-occur more/less than would be expected relative to the null hypothesis that they are randomly distributed, then this implies a potential interaction, the precise nature of which must be further studied. Here, using WNV as a model, we considered the co-occurrence between distinct mosquito genera and land bird species.

Our results are consistent with the fact that only *Culex* spp. are important transmission vectors of WNV that are also associated with significant bird mortality. This might be expected since it has been identified previously as a primary vector (Sardelis et al., 2001; Kilpatrick et al., 2010). The apparent significant correlation among *Anopheles*, *Coquillettidia* and *Culiseta* mosquitoes with bird mortality was determined to be due to the influence of *Culex* presence as a confounder. Therefore, we conclude that these genera alone do not represent a significant threat for bird mortality. However, an open question remains on the potential threat and effects of these mosquitos at the community level (Roche et al. 2013).

The significant associations found between certain mosquito genus and bird mortality entail at least three different potential mechanisms, which can be linked in a causal chain beyond the particular fact that if a specific mosquito taxon is responsible for passing a particularly pathogenic strain of WNV, then it must, perforce, co-occur spatially with the infected bird species.

First, bird mortality can be influenced by mosquitoes' feeding preferences. Positive results observed may be partially attributed to the fact that the *Culex* genus has highly ornithophilic preferences (Hassan et al., 2003; Molaei et al., 2007). On the contrary, the negative relationship between bird mortality and *Aedes* and *Psorophora* coincides with their mammal preferences, and also with the fact that they are a less competent vector to WNV (Apperson et al., 2004).

Second, mosquito and bird species have different degrees of competence to WNV. Vector competence involves a higher viral replication on mosquito cells and therefore a higher amount of viral particles inoculated in the host, higher viremia and higher mortality risk (Ciota et al., 2013). Also, bird mortality may be driven by the species composition (competent, alternative and/or incompetent host) and the relative abundance in communities (Roche et al., 2013; Reisen et al., 2005). With this rationale, we found 220 IBAs that are habitats of *Culex* mosquitoes and therefore associated with significant bird mortality (Figure 3). Thus, we suggest that competent bird species that co-occur with competent mosquitoes will have higher mortality suggesting that these 220 IBAs may be at higher risk for WNV epizootics.

Third, these mosquito genera may be associated with a particular WNV virulent strain that causes bird mortality. The evolutionary dynamics between WNV and distinct mosquito genera are different. These produce genetic changes on virus replication and

virulence, and both are correlated with host viremia and therefore mortality (Ebel et al., 2004; Ciota et al., 2013; Grubaugh & Ebel 2016).

Based on our results, we identified high risk zones for WNV where it might be useful to increase surveillance for mosquitoes and bird mortality. We saw that mortality might be occurring in natural protected areas that are currently not being monitored. This could occur, for example, because these ecosystems are far from urban zones (Ward et al., 2006). Although our co-occurrence approach between vectors (mosquitoes) and host (birds) has been proposed as a tool for bird conservation, it can also be applied to other vector-borne diseases that affect birds, such as St. Louis Encephalitis Virus (SLEV) and Lyme disease.

Particular bird species associated with high mortality risk were identified due to their high degree of co-occurrence with Culex mosquitoes. We identified 22 bird species that are at high risk but that have not previously been sampled for WNV, and for which its effects on these bird populations remains unknown (Appendix 4).

Possible limitations of our work are that we did not take into consideration in our analysis are: 1) mortality is a measure that has changed over time. A study found that mortality on the American crow and the fish crow (*Corvus ossifragus*) declined at a rate of 1.5 % and 1.1% respectively every year (Reed et al., 2009); 2) bird species are not identical in their susceptibility to infection with WNV and subsequent death. We have assumed that mortality is relatively homogeneous across different bird species.

However, some species are resistant to WNV mortality, such as the American robin *(Turdus migratorious),* while others, such as the American crow, are more susceptible and show high mortality rates (Grubaugh et al., 2015). Also, differences can be seen within the same species. For instance, in an experiment, 25 American robins were inoculated with WNV, which generated different responses to the same treatments. While some were resistant to the infection and did not develop the disease, others died after inoculation with the lowest dose of WNV (VanDalen et al., 2013).

Infectious diseases have not previously been explicitly considered as a threat in bird conservation plans. Therefore, our results may be useful to: i) propose where to implement systematic epidemiological surveillance of birds and mosquitoes for WNV in natural protected areas; ii) identify conservation areas with high risk of vector-borne diseases, such as SLEV; iii) infer and propose risk areas based on co-occurrence between mosquitoes and bird species; and iv) propose conservation strategies for endangered endemic, umbrella and specialist species.

Conservation actions require the understanding of host-pathogen interactions. The uses of available epidemiological information, and the implementation of modelling approaches such as shown here, are needed for conservation strategies following the EcoHealth / One Health paradigm.

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COMPETING INTERESTS

We declare we have no competing interests.

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CAPÍTULO 4

Is it the West Nile Virus circulating in wild birds in the North of Mexico?

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ABSTRACT

The West Nile Virus (WNV) is a zoonotic pathogen associated with high mortality in wild birds and humans. In Mexico, its activity has been recorded since 2003 in all the country. Here, we monitored WNV activity in wild birds of Bahia de Kino, Sonora, Mexico. Between 2015 and 2016 cloacal swab samples were tested using a reverse transcription-Polymerase Chain Reaction (rt-PCR) specific for WNV. We sampled 988 individuals belonging to 74 bird species. Passeriformes was the dominant order and the better-sampled species was the White-crowned Sparrow (*Zonotrichia leucophris*). We tested a subgroup of 773 cloacal samples and all were negative to WNV. Possible explanations of lack of the molecular evidence in our study were: i) bird species tested did not be viremic at time of sampling, ii) our sample size was not enough to detect the virus, and iii) we could not sample other groups that belong to the bird community with more chance to be positive. We considered relevant to continue molecular and serological monitoring of WNV at the study area due to is an essential zone for wild bird's conservation.

Key words

Birds, West Nile Virus, Bahía de Kino, Molecular prevalence

1. INTRODUCTION

Surveillance for zoonotic pathogens in wildlife is a research priority for conservation and human health (Calzolari et al., 2012; Alba et al., 2014). Surveillance systems are helpful to record pathogens activity, and they allow early detection of infection in the wildlife and humans in order to prevent and manage specific interventions (Langevin et al., 2005; Angelini et al. 2011).

Among the most important viruses associated with high mortality in humans and birds in a wide range of geographic zones is the West Nile Virus (WNV; family Flaviviridae, genus Flavivirus). It is maintained in cycles between ornithophilic mosquitoes and bird populations (Bakonyi et al., 2013; George et al., 2015).

Some species of Passeriformes, Charadriiformes, and Strigiformes orders are considered competent reservoirs for WNV (species highly efficient to transmit the virus) (Komar et al., 2003; Kilpatrick et al., 2006), and migratory birds have the potential to maintain, transport, and disperse the virus into new areas (Reed and Medical, 2005; Owen et al., 2006).

In México, monitoring WNV activity is an interesting issue for several situations (Deardorff et al., 2006). First, Mexico has a high diversity of bird species. It has approximately 1150 species representing 11% compared to the world total (Navarro-Sigüenza et al., 2014). Second, through Mexico pass three routes of migratory birds, and it has suggested that migratory birds may carry the virus from the southeastern United States into Mexico (Peterson et al., 2004; Deardorff et al., 2006). Third, the low WNV incidence in humans and birds has been explained by the cross-protective immunity from another flavivirus such as Dengue and St. Louis encephalitis viruses (Rodríguez et al., 2010).

WNV activity in the country has been reported since 2003 in wild birds, mosquitoes, humans and horses (Blitvich et al., 2003; Loroño-Pino et al., 2003; Farfan-Ale et al., 2004; Chaves et al., 2016). Some studies have suggested that migratory birds can carry the WNV between California and Arizona to northern Mexico (Deardorff et al., 2006). Bahía Kino in the state of Sonora is a biologically rich coastal region located on the Pacific migratory route (Ramsar 2013). Their rich biodiversity is attributed to a juxtaposition of several ecosystems: marine, intertidal, estuarine, and desert, and the heterogeneity of these habitats provide a high diversity of breeding, migrant, and wintering bird species (Fleischner and Riegner, 1981).

Due to the biological and epidemiological importance of this area, here we explored the WNV activity in wild birds of Bahia de Kino, Sonora, Mexico as a possibility of WNV introduction through the California border by migratory birds.

2. METHODS

2.1. Study site

Our survey area was Bahia de Kino located on the Gulf of California in the State of Sonora, Mexico. The region has two recognized seasons a warm season in June-October with 29.6 ºC and a cold season in December-April with 17.3 ºC. The average annual precipitation is 122 mm (Ramsar 2013).

Wild birds were captured at three study sites i) Halophytic vegetation, near to the Estero Santa Cruz a wetland of international importance (Ramsar site) (28º49´4´´N, 111º50´33´´W). ii) Mesquite localized on the Sonoran desert characterized by Cardon *(Pachycereus pringlei),* Senita *(Lophocereus schottii),* Organpipe *(Stenocereus thurberi),* and Saguaro *(Carnegia gigantea),* Torchwood *(Bursera microphylla),* and Ocotillo (*Fouquiera splendens*) (28º50´25´´N, 111º49´28´´W). iii) Mangrove localized in two points: Estuario Santa Rosa and the Paraiso both in the Ramsar site "Canal del infiernillo y esteros del territorio Comcaac". These sites are characterized by the presence of the Black Mangrove *(Avicennia germinans),* Red Mangrove *(Rhizophora mangle),* White Mangrove *(Laguncularia racemosa)* and xeroriparian habitats (28º57´ 56´´N, 112º 8´8´´W) (Figure 1).

2.2. Bird sampling

In October 2015 and January and September 2016, birds were captured using ten mist nets run from sunrise for approximately six hours and checked hourly. Birds were sampled seven days per habitat per season covering proximally 3780 net-hours. Captured birds were held in cloth bags until they were processed. Date of capture, species, ring number, age, and weight were recorded for each and were identified according to field guides and migratory or resident status (Howell & Webb 1995; Sibley 2014). Recaptures of previously banded birds were noted, and then they were released alive. All bird species were standardized based on the American Ornithologist Union (AOU) checklist.

Cloacal swabs were taken (sterile polyester swabs Deltalab) and placed in 1.5ml cryo-tubes containing 200 microliters of RNA-later (AMBION, Inc., Austin, Texas). Samples were kept refrigerated during the transport to the laboratory, where they were stored at -80°C. All fieldwork was carried out under the permit number FAUT-025, Folio SGPA/DGVS/01610/16, issued by the Secretaria de Ganadería, Desarrollo Rural y Pesca (SAGARPA).

2.3. Laboratory Diagnostic

RNA extractions were conducted at the Genética del desarrollo y fisiología molecular Laboratory of Instituto de Biotecnología, UNAM. Total RNA was extracted from 100 µl of the supernatant of the cloacal swabs with TRIzol Reagent (AMBION, Inc., Austin, Texas) and according to manufacturer's instructions. Synthesis of cDNA was achieved at the One Health Institute Laboratory of California Davis. cDNA synthesis was conducted with the SuperScript III First-Strand Synthesis System (Invitrogen, Carlsbad, CA) and using random hexamers according to the manufacturers' instructions.

A WNV specific qPCR modified from Lanciotti and collaborators (2000) was performed. Two µl of cDNA were added to an 18 µl reaction mix. This assay targets an 88bp fragment of the non-coding 3' region of the WNV genome. The products of the qPCR were analyzed by electrophoresis on 1.5% TAE agarose gels.

3. RESULTS

We sampled 998 individuals of 7 orders, 52 genera, and 75 species in the three seasons (Table 1). Passeriformes was the dominant order with 66 species. The better sampled-species were the White-crowned Sparrow *(Zonotrichia leucophris,* n=196), Orange-crowned Warbler (*Oreothlypis celata*, n=165*)* and Brewer´s Sparrow *(Spizella breweri,* n=75*).* The habitat with the highest species richness in the three seasons was the Mezquital (n=45). We recorded 44 resident and 31 migratory species.

We tested a subgroup of 773 cloacal swabs; all the samples tested were negative for WNV (Table 1).

4. DISCUSSION

We monitored the WNV activity in wild birds of Bahía Kino, Sonora, and we found negative results. Possible explanations of the lack of the molecular evidence in our study were i) bird species did not be viremic at time of sampling, ii) our sample size was not enough considering the birds populations size, iii) we could not sample others groups that belong to bird community (i.e. Charadriiformes, Strigiformes or Accipitriformes). These groups also are recognized as highly susceptible to WNV infection (Komar *et al.*, 2003; Nemeth *et al.*, 2006).

Despite our negative results, we suggest that our study site is crucial to monitoring WNV activity, due to important species for WNV amplification were tested as the English sparrow, Orange-crowned Warbler and Northen cardinal (*Cardinalis cardinalis*). These species are considered highly competent reservoirs for WNV (Langevin *et al.*, 2005; Wheeler *et al.*, 2010; McKee *et al.*, 2015). Also, we captured alternative reservoirs as the Common Ground-Dove (*Columbina passerina)*, Mourning Dove (*Zenaida macroura*) and Northern Mockingbird (*Mimus polyglottos*) (Kilpatrick *et al.*, 2006).

As well, in the area lives a high diversity of birds did not sampled, but important to the WNV transmission. Such as the Ring-billed Gull (*Larus delawarensis*) classified as a competent reservoir and Black-crowned Night-heron (*Nycticorax nycticorax*) considered moderately competent (Kilpatrick *et al.*, 2006)(Table 2).

Therefore, we considered relevant to continue molecular monitoring of WNV in the birds of the study area. In addition, an integrated monitoring system that includes birds, humans, mosquitoes, and other mammals are required. We recommend reach the sampling of highly sensitive birds groups as aquatic birds and raptors.

5. CONCLUSIONS

In this study, we did not identify the presence of WNV in cloacal swabs of wild species. However, it does not exclude the circulation of this virus at Bahía de Kino, Sonora. Factors as the high bird species diversity, the presence of competent reservoirs, environmental conditions and existence of important wetlands are crucial for WNV activity surveillance.

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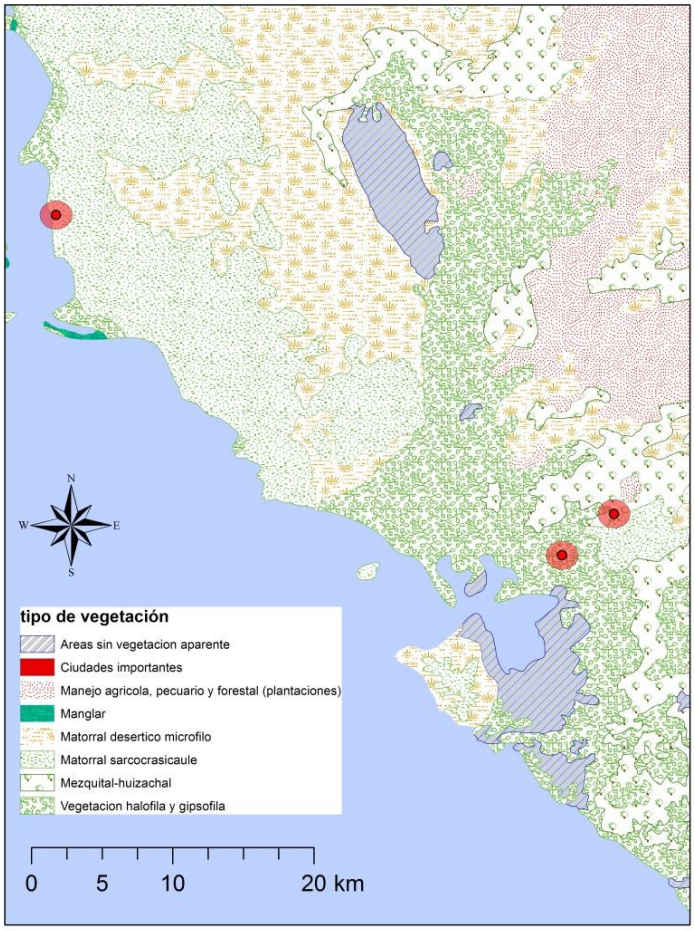

Figure 1. Sampling zones at Bahía de Kino

Table 1. Bird species tested for WNV

NO	ORDER	FAMILY	SCIENTIFIC NAME	COMMON NAME
1	Passeriformes	Emberizidae	Ammodramus bairdii	Baird's Sparrow
2	Passeriformes	Emberizidae	Ammodramus savannarum	Grasshopper sparrow
3	Passeriformes	Emberizidae	Amphispiza bilineata	Black-throated Sparrow
4	Caprimulgiformes	Trochilidae	Archilochus alexandri	Black-chinned Hummingbird
5	Passeriformes	Remizidae	Auriparus flaviceps	Verdin
6	Passeriformes	Emberizidae	Calamospiza melanocorys	Lark Bunting
7	Galliformes	Odontophoridae	Callipepla gambelii	Gambel's Quail
8	Passeriformes	Troglodytidae	Campylorhynchus brunneicapillus	Cactus Wren
9	Passeriformes	Parulidae	Cardenilla pusilla	Wilson's Warbler
10	Passeriformes	Cardinalidae	Cardinalis cardinalis	Northern Cardinal
11	Passeriformes	Cardinalidae	Cardinalis sinuatus	Pyrrhuloxia
12	Passeriformes	Turdidae	Catharus guttatus	Hermit Thrush
13	Passeriformes	Turdidae	Catharus sp.	
14	Passeriformes	Turdidae	Catharus ustulatus	Swainson's Thrush
15	Passeriformes	Emberizidae	Chondestes grammacus	Lark Sparrow
16	Caprimulgiformes	Caprimulgidae	Chordeiles acutipennis	Lesser Nighthawk
17	Piciformes	Picidae	Colaptes chrysoides	Gilded Flicker
18	Columbiformes	Columbidae	Columbina passerina	Common Ground-Dove
19	Columbiformes	Columbidae	Columbina talpacoti	Ruddy Ground-Dove
20	Passeriformes	Mimidae	Dumetella carolinensis	Gray Catbird
21	Passeriformes	Tyrannidae	Empidonax sp.	Flycatcher
22	Passeriformes	Parulidae	Geothlypis trichas	Common Yellowthroat
23	Passeriformes	Fringillidae	Haemorhous mexicanus	House Finch
24	Piciformes	Fringillidae	Haemorhous purpureus	Purple Finch
25	Passeriformes	Icteridae	Icterus cucullatus	Hooded Oriole
26	Passeriformes	Icteridae	Icterus pustulatus	Streak-backed oriole
27	Passeriformes	Emberizidae	Junco hyemalis	
28	Passeriformes	Laniidae	Lanius ludovicianus	Loggerhead Shrike
29	Columbiformes	Columbidae	Leptotila verreauxi	White-tipped dove
30	Coraciiformes	Alcedinidae	Megaceryle alcyon	Belted Kingfisher
31	Strigiformes	Strigidae	Megascops trichopsis	Whiskered Screech-Owl
32	Piciformes	Picidae	Melanerpes uropygialis	Gila Woodpecker
33	Passeriformes	Emberizidae	Melospiza lincolnii	Lincoln´s Sparrow
34	Passeriformes	Mimidae	Mimus polyglottos	Northern Mockingbird
35	Passeriformes	Parulidae	Mniotilta varia	Black-and-white Warbler
36	Passeriformes	Tyrannidae	Myiarchus cinerascens	Ash-throated Flycatcher
37	Passeriformes	Tyrannidae	Myiarchus nuttingi	Nutting´s Flycatcher
38	Passeriformes	Tyrannidae	Myiarchus tyrannulus	Brown-crested Flycatcher
39	Passeriformes	Parulidae	Oreothlypis celata	Orange-crowned Warbler
40	Passeriformes	Parulidae	Oreothlypis luciae	Lucy's Warbler
41	Passeriformes	Parulidae	Oreothlypis peregrina	Tennessee Warbler
42	Passeriformes	Passeridae	Passer domesticus	House Sparrow

43	Passeriformes	Cardinalidae	Passerina amoena	Lazuli Bunting
44	Passeriformes	Cardinalidae	Passerina cyanea	Indigo Bunting
45	Passeriformes	Emberizidae	Peucaea carpalis	Rufous-winged sparrow
46	Passeriformes	Emberizidae	Peucaea cassinii	Cassin's Sparrow
47	Passeriformes	Ptiliogonatidae	Phainopepla nitens	Phanopepla
48	Passeriformes	Cardinalidae	Pheucticus ludovicianus	Rose-breasted Grosbeak
49	Passeriformes	Emberizidae	Pipilo chlorurus	Green-tailed Towhee
50	Passeriformes	Polioptilidae	Polioptila caerulea	Blue-gray Gnatcatcher
51	Passeriformes	Polioptilidae	Polioptila californica	California Gnatcatcher
52	Passeriformes	Polioptilidae	Polioptila melanura	Black-tailed Gnatcatcher
53	Passeriformes	Hirundinidae	Progne subis	Purple Martin
54	Passeriformes	Icteridae	Quiscalus mexicanus	Great-tailed Grackle
55	Passeriformes	Troglodytidae	Salpinctes obsoletus	Rock Wren
56	Passeriformes	Tyrannidae	Sayornis saya	Say´s Phoebe
57	Passeriformes	Parulidae	Seiurus noveboracensis	Northern Waterthrush
58	Passeriformes	Parulidae	Setophaga coronata	Yellow-rumped Warbler
59	Passeriformes	Parulidae	Setophaga petechia	Yellow Warbler
60	Passeriformes	Cardinalidae	Spiza americana	Dickcissel
61	Passeriformes	Emberizidae	Spizella atrogularis	Black-chinned Sparrow
62	Passeriformes	Emberizidae	Spizella breweri	Brewer´s Sparrow
63	Passeriformes	Emberizidae	Spizella pallida	Clay-colored Sparrow
64	Columbiformes	Columbidae	Streptopelia decaocto	Eurasian Collared-Dove
65	Passeriformes	Mimidae	Toxostoma bendirei	Bendire's Thrasher
66	Passeriformes	Mimidae	Toxostoma crissale	Crissal Thrasher
67	Columbiformes	Troglodytidae	Troglodytes aedon	House Wren
68	Passeriformes	Tyrannidae	Tyrannus melancholicus	Tirano Pirirí
69	Passeriformes	Vireonidae	Vireo bellii	Bell´s Vireo
70	Passeriformes	Vireonidae	Vireo cassinii	Cassin´s Vireo
71	Passeriformes	Vireonidae	Vireo gilvus	
72	Passeriformes	Vireonidae	Vireo plumbeus	Plumbeous Vireo
73	Passeriformes	Vireonidae	Vireo vicinior	Gray vireo
74	Columbiformes	Columbidae	Zenaida asiatica	White-winged Dove
75	Columbiformes	Columbidae	Zenaida macroura	Mourning Dove
76	Passeriformes	Emberizidae	Zonotrichia leucophrys	White-crowned Sparrow

Table 2. Bird species present in the Kino Bay presumed to be important in WNV transmission

NO	SPECIES	SANTA CRUZ LAGOON	CANAL DEL INFIERNILLO Y ESTEROS DEL TERRITORIO COMCAAC
1	*Accipiter cooperii*	X	X
2	*Anas clypeata*	-	X
3	*Anas crecca*	-	X
4	*Aquila chrysaetos*	-	X
5	*Ardea herodias*	X	X
6	*Asio flammeus*	-	X
7	*Asio otus*	-	X
8	*Athene cunicularia*	-	X
9	*Aythya affinis*	-	X
10	*Aythya collaris*	-	X
11	*Aythya valisineria*	-	X
12	*Bubo virginianus*	-	XI
13	*Bubulcus ibis*	XI	-
14	*Bucephala albeola*	-	X
15	*Buteo jamaicensis*	-	XI
16	*Butorides virescens*	X	-
17	*Calidris mauri*	-	X
18	*Calypte costae*	-	X
19	*Cathartes aura*	-	X
20	*Charadrius vociferus*	X	-
21	*Circus cyaneus*	-	X
22	*Coragyps atratus*	-	X
23	*Corvus corax*	-	X
24	*Egretta caerulea*	X	X
25	*Egretta thula*	X	X
26	*Egretta tricolor*	X	-
27	*Falco mexicanus*	-	X
28	*Falco peregrinus*	X	X
29	*Falco sparverius*	-	XI
30	*Gavia immer*	-	X
31	*Haematopus palliatus*	X	-
32	*Ixobrychus exilis*	X	-
33	*Larus argentatus*	-	X
34	*Larus delawarensis*	-	X (C)
35	*Melanerpes lewis*	-	X
36	*Melospiza lincolnii*	-	X
37	*Mergus serrator*	-	X
38	*Molothrus aeneus*	-	X
39	*Nyctanassa violacea*	-	X
40	*Nycticorax nycticorax*	X (M)	X (M)
41	*Pandion haliaetus*	X	X

42	*Passerculus sandwichensis*	-	X
43	*Passerina amoena*	-	X
44	*Pelecanus occidentalis*	-	X
45	*Phalacrocorax auritus*	-	X
46	*Pheucticus ludovicianus*	-	X
47	*Progne subis*	-	X
48	*Rallus longirostris*	X	-
49	*Selasphorus rufus*	-	X
50	*Setophaga nigrescens*	-	X
51	*Sterna antillarum*	X	-

X Evidence of contact, (C) Competent reservoir, (M) Moderately competent and (I) Incompetent

DISCUCIÓN GENERAL

Utilizando al VON como modelo de estudio, en este trabajo se utilizaron perspectivas macroecológicas (escala global y regional) y un estudio de campo (escala local) con el objetivo de evaluar las dinámicas de transmisión del virus en aves y mosquitos. Para lo cual se identificaron y caracterizaron a las especies hospederas y se aplicó este conocimiento en modelos geográficos con el fin de proponer áreas prioritarias para la conservación de las aves.

La aproximación macroecológica en el capítulo uno permitió identificar y proponer especies potenciales de aves competentes para el VON. Asimismo, se identificaron especies y grupos altamente susceptibles a este virus. Finalmente se analizaron simultáneamente factores asociados a la susceptibilidad encontrando que la filogenia de las aves, el tamaño de muestra y el tiempo-espacio afectan las tres métricas analizadas (prevalencia serológica y molecular y mortalidad).

En el capítulo dos se corroboró a través del estudio mundial que las especies con vida lenta invierten más en inmunidad con respecto a las de vida rápida que priorizan la reproducción. Además, se observó que aún existe un gran desconocimiento en las historias de vida de las especies de aves; lo que limitó la oportunidad de probar otras variables predictoras como la longevidad, número de nidadas o el periodo de volantón.

A través de una escala regional (Estados Unidos), en el capítulo tres se encontró que a través de la co-ocurrencia geográfica entre aves y mosquitos del género *Culex* se puede inferir la mortalidad de las aves por VON. Con base en dichos resultados se propusieron 220 IBAs en riesgo por mortalidad por este virus, algunas de las cuales están consideradas como prioridad global debido a que son hábitats de especies de aves en alguna categoría de riesgo con base en la lista roja de la Unión para la Conservación de la Naturaleza (IUCN).

El estudio a nivel local en Bahía Kino fue el primer trabajo en monitorear al VON en esta zona. Sin embargo, a pesar de encontrar resultados negativos a través de métodos moleculares es necesario realizar diagnósticos serológicos para determinar si las aves de esta localidad han estado en contacto en algún momento con este virus. Es importante continuar con el monitoreo de VON en esta zona debido a que en ella se encuentran dos sitios Ramsar y en dónde habitan especies reconocidas como reservorios competentes del VON.

El estudio de las dinámicas de transmisión entre patógenos zoonóticos, sus hospederos y en algunos casos sus vectores debe de ser una prioridad mundial por sus implicaciones en conservación, salud y economía. Las enfermedades infecciosas son sistemas ecológicos con una alta complejidad que necesitan ser estudiadas bajo diferentes aproximaciones. Con ello se puede tener un mayor entendimiento de su funcionamiento y por consiguiente de su prevención, control y mitigación.

CONCLUSIONES

El VON es un patógeno zoonótico altamente complejo que no puede ser comprendido a través de una sola aproximación. Se requiere una integración de todo el conocimiento generado para poder detectar patrones, sesgos y vacíos en la información. Así como proponer nuevas hipótesis y herramientas analíticas. Lo cual ayudará también a tener un mejor entendimiento de las dinámicas de transmisión de otros patógenos zoonóticos permitiendo prevenir y/o mitigar sus efectos en la conservación de la fauna silvestre y en la salud humana y animal.

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