EcoHealth
https://doi.org/10.1007/s10393-019-01393-8

EcoHealth

Original Contribution

Linking Bird and Mosquito Data to Assess Spatiotemporal West Nile Virus Risk in Humans

Benoit Talbot,[1] Merlin Caron-Lévesque,[1] Mark Ardis,[2] Roman Kryuchkov,[1]
and Manisha A. Kulkarni[1]

[1]*School of Epidemiology and Public Health, University of Ottawa, Room 217A, 600 Peter Morand Crescent, Ottawa, ON K1G 5Z3, Canada*
[2]*GDG Environnement, Trois-Rivières, QC, Canada*

Abstract: West Nile virus (WNV; family Flaviviridae) causes a disease in humans that may develop into a deadly neuroinvasive disease. In North America, several peridomestic bird species can develop sufficient viremia to infect blood-feeding mosquito vectors without succumbing to the virus. Mosquito species from the genus *Culex*, *Aedes* and *Ochlerotatus* display variable host preferences, ranging between birds and mammals, including humans, and may bridge transmission among avian hosts and contribute to spill-over transmission to humans. In this study, we aimed to test the effect of density of three mosquito species and two avian species on WNV mosquito infection rates and investigated the link between spatiotemporal clusters of high mosquito infection rates and clusters of human WNV cases. We based our study around the city of Ottawa, Canada, between the year 2007 and 2014. We found a large effect size of density of two mosquito species on mosquito infection rates. We also found spatiotemporal overlap between a cluster of high mosquito infection rates and a cluster of human WNV cases. Our study is innovative because it suggests a role of avian and mosquito densities on mosquito infection rates and, in turn, on hotspots of human WNV cases.

Keywords: *Aedes vexans*, American robin, *Culex pipiens/restuans*, Disease ecology, Epidemiology, House finch, *Ochlerotatus japonicus*

INTRODUCTION AND PURPOSE

West Nile virus (WNV; family Flaviviridae) was first discovered in the West Nile district of Uganda (Hughes et al. 1940; Reisen 2013; Chancey et al. 2015). Infection by this arbovirus typically reaches high viremia in passerine bird species, and transmits among avian hosts via mosquito vectors (Kilpatrick et al. 2006; Marini et al. 2017). A large number of species, including humans, may be incidental hosts of the virus, meaning viremia never reaches potential for transmission (Artsob et al. 2006). In humans, infection by the virus is usually mild or asymptomatic, but in 20% of infections may develop into a febrile illness referred to as 'West Nile fever,' and in about 1 in 150 infections, may develop into a deadly neuroinvasive disease (Gubler 2007; Levi 2013). Since its discovery, WNV has historically been observed in Africa, Asia, and periodically in southern Europe (Chancey et al. 2015). Additionally, overwintering persistence of the virus is suggested to have led to invasion

---

Electronic supplementary material: The online version of this article (https://doi.org/10.1007/s10393-019-01393-8) contains supplementary material, which is available to authorized users.

Correspondence to: Benoit Talbot, e-mail: benoit.talbot@uottawa.ca

and endemism in temperate areas around the world, including Russia, USA and Canada (TSentr gigieny i ėpidemiologii v Respublike Tatarstan 2013; Reisen 2013). Since first detected in New York, USA, in 1999, WNV has become endemic in North America and has spread across the continental USA and Canada (Reisen 2013; Chancey et al. 2015). It has also been observed in tropical and subtropical areas of the Americas, notably in Guatemala, Argentina, México and Dominican Republic (Komar et al. 2003; Morales-Betoulle et al. 2013; Chaves et al. 2016; Diaz et al. 2016).

In North America, several peridomestic bird species such as American robins, house finches and house sparrows can develop sufficient viremia to infect blood-feeding mosquito vectors without succumbing to the virus (van der Meulen et al. 2005; Kilpatrick et al. 2006; Reisen et al. 2013; VanDalen et al. 2013), making them potent reservoirs of the virus. However, other birds such as crows and raptors tend to rapidly succumb to WNV (Foppa et al. 2011; Reisen et al. 2013; Smith et al. 2018). Levels of viremia in several small mammal species, such as cottontail rabbits, eastern chipmunks and fox squirrels (van der Meulen et al. 2005), and even ectotherms, such as alligators (Burkett-Cadena et al. 2008), are also reportedly sufficient to contribute to enzootic WNV transmission, thereby expanding the pool of potential reservoirs in some regions. Mosquito species from the genus *Culex* are recognized as the most important vectors maintaining the enzootic cycle of WNV among avian hosts in North America (Andreadis 2012; Ganser and Wisely 2013). Members of these species are usually ornithophilic in their choice of hosts and often feed on the main avian reservoirs of WNV (Farajollahi et al. 2011). However, *Culex* species also frequently feed on humans, bridging transmission between avian and human hosts. Mosquitoes from several other genera, including *Aedes*, *Ochlerotatus* and *Anopheles* are usually mammalophilic, but in some cases highly opportunistic in their choice of hosts. These species potentially bridge transmission from avian and mammalian WNV reservoirs to human hosts (Burkett-Cadena et al. 2008; Greenberg et al. 2013; Ganser and Wisely 2013; Kaufman and Fonseca 2014).

In 2002, the first human case of WNV was observed in Canada, in the province of Ontario (Drebot et al. 2003). Epidemics of WNV throughout the province and the country in 2003 and 2007 have coincided with a longer than usual transmission season for WNV (Reisen 2013). In these years, temperatures reached the threshold for replication of the virus earlier in the spring and later in the fall, and prolonged the breeding season of its main mosquito vectors (Reisen et al. 2006; Yoo et al. 2016). Multi-month droughts with infrequent but abundant rain downpours possibly contributed to the magnitude of epidemics, through an increase in mosquito populations and aggregation of bird reservoirs around left-over water sources (Epstein and Defilippo 2001). In Ontario, analysis of surveillance data from 2002 to 2013 from 29 public health units found that higher than usual winter and late summer minimum temperatures were predictive of higher WNV incidence rates in a given year, including in the major epidemic experienced in the province in 2012 (Mallya et al. 2018). Weekly average temperatures and cumulative precipitation were also found to be moderate to strong predictors of human WNV cases in seven southern Ontario public health units during this period (Giordano et al. 2017). *Culex pipiens/restuans* are recognized as the main WNV vector species in Ontario, and have been under surveillance by Public Health Ontario (PHO) since 2002 (Public Health Ontario 2013), for detection of WNV-positive mosquito pools, which may be used to predict WNV transmission hotspots. An additional seven mosquito species (*Aedes vexans*, *Culex salinarius*, *Anopheles punctipennis*, *Ochlerotatus japonicus*, *Ochlerotatus trivittatus*, *Ochlerotatus triseriatus* and *Ochlerotatus hendersoni*) are considered competent vectors of WNV in the area and are also targeted by the PHO (Public Health Ontario 2012).

A number of studies have investigated the role of reservoir species (Reisen et al. 2013; VanDalen et al. 2013; Levine et al. 2017) and vector species (Epstein and Defilippo 2001; Reisen et al. 2006; Burkett-Cadena et al. 2008; Greenberg et al. 2013; Ganser and Wisely 2013; Mallya et al. 2018) on the WNV enzootic cycle. Other studies have looked at the role of vector species on WNV disease risk in human populations (Eisen and Eisen 2008; Winters et al. 2008). Given the complexity of WNV ecology, our practical understanding of enzootic WNV cycles and spill-over events to humans would benefit from integrated approaches combining data on reservoir and vector densities and infection rates, and human cases, across space and time.

In this study, we aimed to test the effect of density of the mosquito vector species and the avian reservoir species on WNV vector infection rates around the city of Ottawa, Canada, as an indicator of WNV transmission potential. We hypothesized that higher density of each of the vector and reservoir species may accentuate the WNV enzootic transmission cycle, contributing to higher vector infection rates. We also investigated the spatiotemporal association between clusters of WNV-positive mosquito catches and clusters of human WNV cases. We hypothesized that hotspots of WNV incidence in human populations would spatially overlap but temporally follow high localized WNV incidence in mosquito vectors.

METHODS

Data Sources

Mosquito Surveillance Data

We used data from mosquito surveillance activities conducted by GDG Environnement (Groupe de Gestion Environnement) for the City of Ottawa in 2007–2014. The municipal WNV surveillance program involves weekly collection of mosquitoes from 60 sites in Ottawa, Ontario, Canada (Fig. 1), during the WNV transmission season from June to September each year. Sites were chosen based on spatial representation of the city of Ottawa, high incidence of WNV-infected dead birds, high mosquito abundance and close proximity to large human populations. For each site, mosquitoes were collected using a light trap designed by Centers for Disease Control (CDC; Atlanta, GA, USA) baited with dry ice for CO_2 emissions. Mosquitoes were immediately stored on dry ice and transported to the laboratory for identification to the species and sex, according to several identification keys (Wood et al. 1979; Darsie and Ward 2005; Thielman and Hunter 2007). Females of seven mosquito species or species group (Table 1) that are considered competent vectors of WNV in the area by PHO (Public Health Ontario 2012) were pooled by species and trap-day (hereafter referred to as ‘catches’), with up to 50 individuals per catch. RNA from each catch was first extracted using RNeasy Mini Kit (Qiagen, Hilden, Germany). All RNA extracts were tested by quantitative Polymerase Chain Reaction (qPCR) with a TaqMan (Roche Molecular Diagnostics, Basel, Switzerland) generic probe, followed by a TaqMan envelope probe for confirmation of

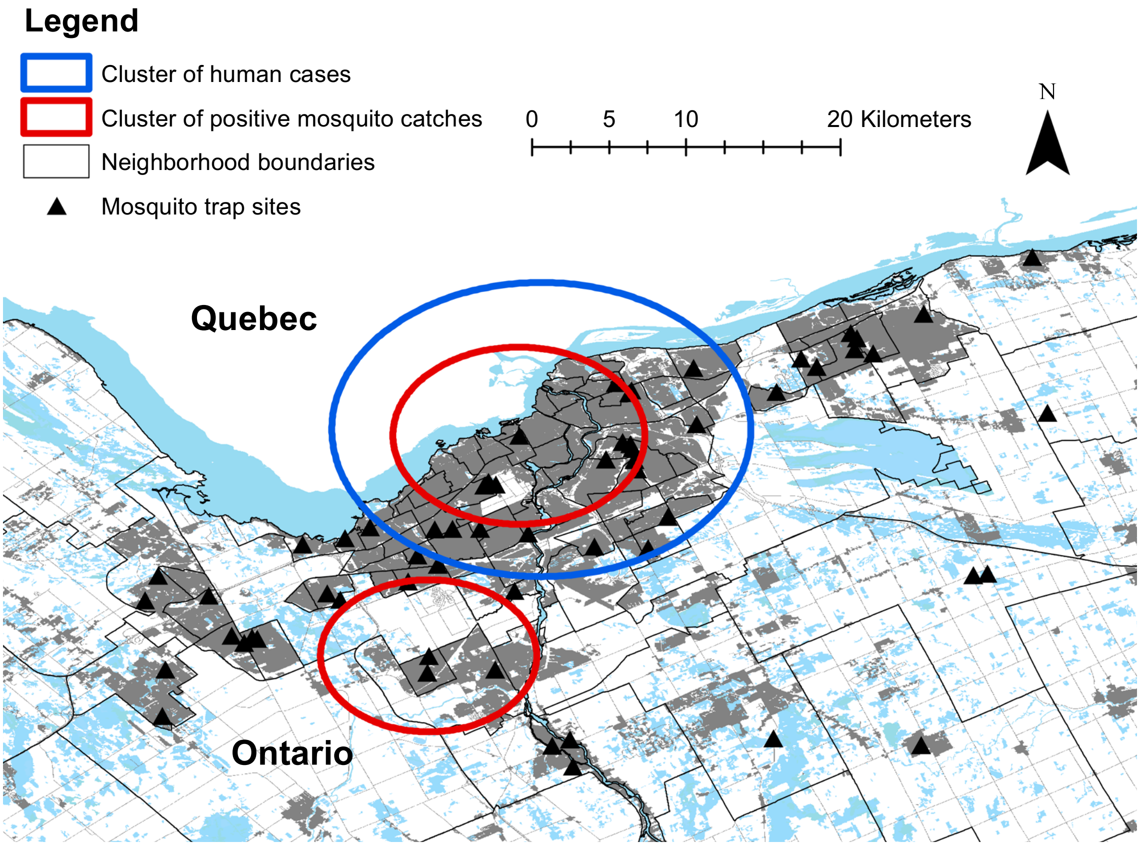

Figure 1. Map of mosquito trap sites (black triangles) for West Nile virus surveillance in the city of Ottawa, Canada. Built-up land is shown in dark gray shading and water bodies in lighter shading. Circles identify locations of space–time clusters of high WNV rates in mosquitoes and humans (as in Legend), detected using SaTScan 9.4 (Kulldorff 1997). Map was created using ArcGIS 10.5 (ESRI, Redlands, CA, USA).

Table 1. Average Abundance, and Total Number of WNV-Positive Catches of Each Mosquito Species Identified by the PHO as Main WNV Vector Species (Public Health Ontario 2012), in the City of Ottawa, Canada, 2007–2014.

Species	Average trap abundance (SD)	Total number of positive catches	Total number of catches tested
Culex pipiens/restuans	8.69 (18.57)	74	2370
Culex salinarius	< 0.01 (0.03)	0	1
Aedes vexans	24.78 (40.32)	1	1860
Ochlerotatus japonicus	1.21 (3.50)	0	635
Ochlerotatus triseriatus/hendersoni	0.56 (1.88)	0	109
Ochlerotatus trivittatus	2.58 (8.63)	0	89
Anopheles punctipennis	2.45 (5.73)	0	193
Total	40.28 (48.75)	75	5257

positive results, using primers from Lanciotti et al. (2000). Results with a Cycle Threshold (CT) values lower than 30 are considered positive and higher than 37 are considered negative. For results with CT values between 30 and 37, a re-extraction of RNA is performed followed by tests using both TaqMan generic and envelope probes.

Avian Data

Many bird species are known to develop chronic WNV infections and may survive high viremia (Kilpatrick et al. 2006; Reisen et al. 2013), allowing transmission by mosquito vectors and perpetuation of the enzootic WNV cycle. We obtained city-wide data on the estimated density of two bird species that are known as highly competent reservoirs of WNV: the American robin (*Turdus migratorius*) and the house finch (*Haemorhous mexicanus*) from Ottawa Bird Count (OBC; Carleton University, Ottawa, ON, Canada). Unfortunately, data for other competent WNV reservoir bird species in the same area were not available at the time of the study. Volunteers of OBC recorded counts of a number of bird species in locations across the study area, along with day of observation and observer identity, from 2007 onwards. Bird density is interpolated across the study area using ordinary kriging and expressed as number of birds per hectare of land, corrected for observer identity and day of observation, available at a resolution of 200 meters. We used the last updated file available in April 2016. Using ArcGIS 10.5 (ESRI, Redlands, CA, USA), we averaged American robin and house finch density at 100-m radius buffers around our study's mosquito collection sites. The choice of 100-m radius, or 200-m diameter, for buffers reflects the resolution of the data.

Human WNV Surveillance Data

We obtained WNV human case surveillance data from Ottawa Public Health, the local public health unit, for the period of 2007–2014 (Fig. 2). Cases of confirmed or probable human WNV are reported to PHO and recorded in the integrated public health information system (iPHIS; Ontario Ministry of Health and Long-Term Care 2017). We collected data for cases where Ottawa was identified as primary exposure location. We geocoded the location of residence of each patient and linked each case to their neighborhood using a boundary shapefile using ArcGIS. We also extracted data on the date of onset of the disease. We calculated WNV incidence rates for each neighborhood in the study area using population data from the 2011 Census, available from Statistics Canada (Statistics Canada 2016).

Statistical Analysis

Descriptive Analysis

We calculated the Minimum Infection Rate (MIR) for each trap-day, which is the number of detected positive catches in a given trap-day, divided by the number of analyzed mosquitoes in the same trap-day, multiplied by 1000 (20). We averaged mosquito MIR, and we averaged daily mean temperature and summed daily total precipitation, obtained from Environment and Natural Resources Canada in the Ottawa city center (Environment and Natural Resources Canada 2018), for each month from 2007 to 2014. We set an average mosquito MIR of 0 for October to May of each year.

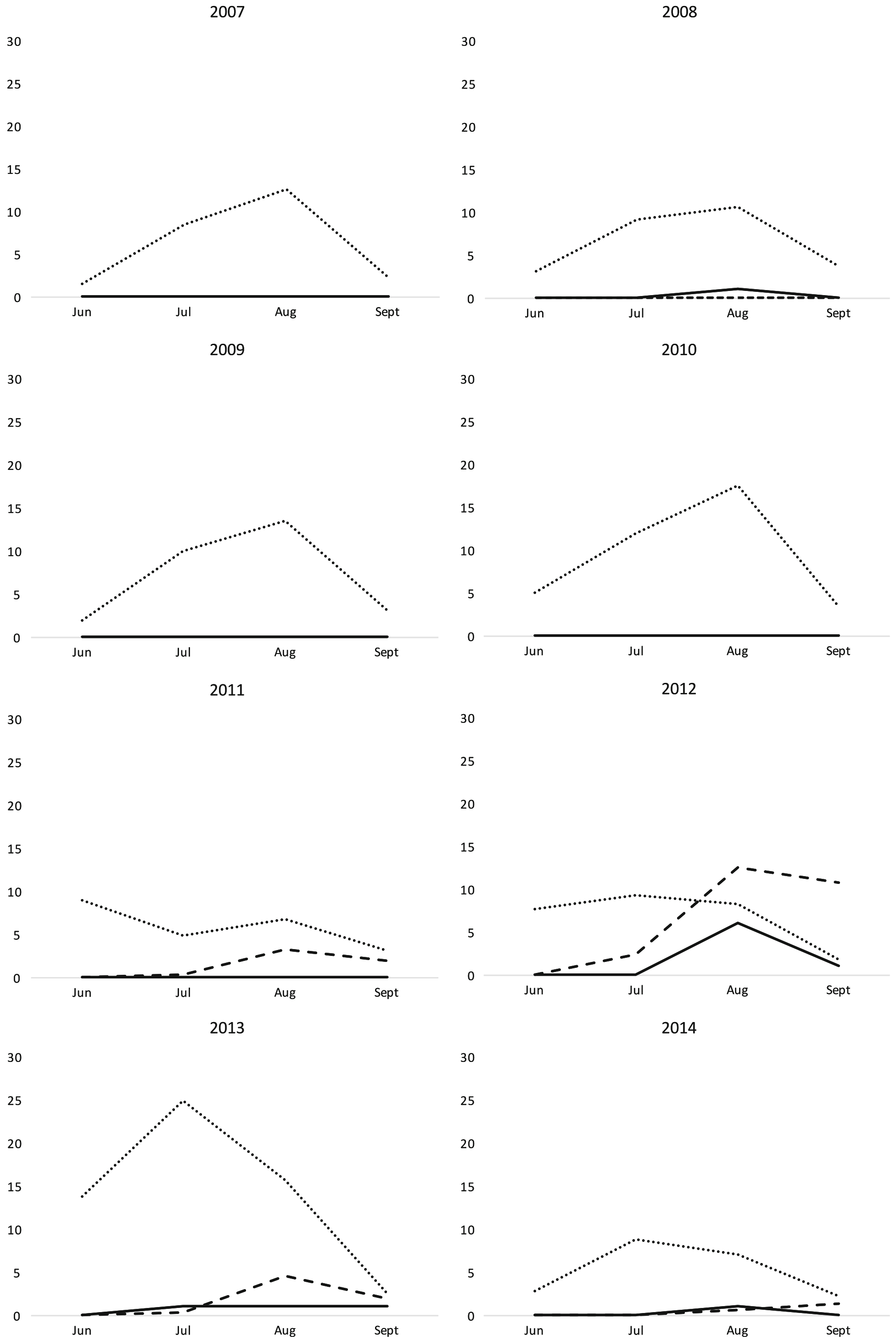

Figure 2. Mean monthly *Culex pipiens/restuans* abundance (dotted lines), minimum infection rates (dashed lines), and human WNV cases (solid lines), for each year of the study in the city of Ottawa, Canada, 2007–2014.

We first tested the association between monthly mosquito MIR and weather data. Since both mean temperature and total precipitation were found to affect mosquito MIR in the region (Giordano et al. 2017; Mallya et al. 2018), we wanted to test the association between monthly mosquito MIR and both these predictors, in a 'partial' cross-correlation. Therefore, we first ran a linear model between monthly total precipitation and monthly mean temperature, using the 'lm' function in R 3.5.1. We then computed a time series for both monthly MIR and residuals of the linear model between monthly total precipitation and monthly mean temperature, which is analogous to the interaction term between the two predictors, using the 'ts' function in R 3.5.1. Finally, we ran a cross-correlation between both time series, using the 'ccf' function in R 3.5.1.

We also compared averaged monthly *Cx. pipiens/restuans* abundance, targeted by PHO as the top vector of WNV in the area (31), averaged monthly mosquito MIR and total monthly human WNV cases, for each month during which mosquitoes were captured (June to September) for 2007–2014.

Mixed-Effects Modeling Analysis

To determine factors affecting MIR of mosquito catches, we used a mixed-effects modeling approach. To this end, we used the 'glmer' function from the 'lme4' package in R 3.5.1. We assessed variable importance using model averaging (Symonds and Moussalli 2011). In all models, we treated MIR from each trap-day as individual data points. We rounded the MIR data to remove decimals, to allow statistical modeling using count data. We used the Poisson modeling family, due to the zero-inflated positive nature of the response data. We included site identifier as random factor to correct for multiple sampling at each site. We also included the interaction between monthly mean temperature and monthly total precipitation, from the previous month, as random-effects variable to correct for temporal fluctuations in mosquito populations, which are the strongest drivers of adult mosquito abundance and arbovirus risk in the region (Yoo et al. 2016; Giordano et al. 2017). We calculated a marginal and conditional pseudo-R^2 for each model, following the method of Johnson (2014), using the 'r.squaredGLMM' function from the 'MuMIn' package (Barton 2013) in R 3.5.1, to measure the strength of the effect of fixed-effects variables excluding versus including random-effects variables, respectively.

As the initial step in model averaging, we ran univariate models including each of the following fixed-effects variables: number of sampled mosquito individuals from three out of seven main WNV vector species (those for which WNV-positive catches were detected each year from 2007 and 2013 in Ontario: *Cx. pipiens/restuans*, *Oc. japonicus* and *Ae. vexans*; Giordano et al. 2017) at the site, and predicted density of American robins and house finches (two of the main avian reservoirs of WNV in the area; Reisen et al. 2013) in 100-m radius buffers around the site. We then ran bivariate and trivariate models of each possible combination of fixed-effects variables, a full model including all fixed-effects variables, and a null model including no fixed-effects variable. We averaged all models to obtain corrected Akaike Information Criterion values and importance of each fixed-effects variable, and weights of the most informative models, using the 'model.avg' function from the 'MuMIn' package (Barton 2013) in R 3.5.1.

Prior to these analyses, we tested for correlation among pairs of predictors, to detect potential collinearity effects on the results. We also tested for spatial autocorrelation of data points, with the 'Moran.I' function in the 'ape' package and a matrix of inverse spatial distances as 'weights,' in R 3.5.1. Finally, we standardized all numeric variables for more meaningful comparisons among predictors and to remove dispersion issues in the models.

Cluster Detection

We identified spatiotemporal clusters with high rates of WNV-infected mosquito catches and human WNV cases using Kulldorf's spatial scan statistic with SaTScan 9.4 software (Kulldorff 1997). We aggregated the data for each week (7 days) of the study. We performed a Likelihood ratio test to identify clusters with significantly higher relative risk than expected, defined as the observed versus expected number of cases. For the mosquito data, we treated individual mosquito catches as data points, assigning a value of 1 for catches testing positive for WNV and 0 for catches testing negative, and applying geographic coordinates of the trap-site in which each catch was collected. We used the Bernoulli model, and specified 10% of the population at risk, which is recommended by the author when only specific sites in the study area were sampled, as opposed to the whole area (Kulldorff 1997). We kept other parameters at default values. For human WNV data, we used a Poisson model with the neighborhood of residence and date of each WNV human case, and population estimates of neighborhoods of the city of Ottawa from the 2011 Census, available from Statistics Canada (Statistics Canada 2016). We kept other parameters at default values. We imported cluster data from both analyses to ArcGIS 10.5 (ESRI, Redlands, CA, USA) for visualization.

Ethical Approval

We obtained ethical approval for this study from the Ottawa Public Health Research Ethics Board (protocol no.

226-16) and the University of Ottawa Science & Health Sciences Research Ethics Board (file no. H06-16-22). Access to anonymized iPHIS data was granted through a data sharing agreement with Ottawa Public Health.

RESULTS

Mosquito Surveillance

We obtained data on a total of 178,772 mosquitoes, of which 115,082 were identified as one of seven vector species identified by the PHO (See Table 1 for average abundance among trap-days across the city of Ottawa from 2007 to 2014). A total of 61,581 adult female mosquitoes were collected comprising 5257 mosquito catches (average of 12 female mosquitoes per catch). Monthly average abundance of *Cx. pipiens/restuans* ranged from 1 to 26 (Fig. 2) across summer months (June to September). Across study sites, average abundance of *Cx. pipiens/restuans*, *Ochlerotatus japonicus* and *Aedes vexans* ranged from 0 to 40, 0 to 13 and 0 to 144, respectively (Table S1).

A total of 75 mosquito catches tested WNV-positive; 74 of which were *Cx. pipiens/restuans* catches, and a single catch was comprised of *Ae. vexans*. Minimum infection rate (MIR) for a given trap-day ranged from 0 to 333, and monthly average MIR ranged from 0 to 5, except for August and September 2012, where it reached 17 and 15, respectively (Fig. 2).

Avian Data

Predicted density per hectare varied between 2.0 and 3.0 for American robins (*Turdus migratorius*), and between 0.3 and 1.1 for house finches (*Haemorhous mexicanus*), across study sites (Table S1). Total predicted density ranged from 2 to 4 birds per hectare, when considering the two species together.

Human WNV Surveillance

A total of twelve WNV cases were recorded in the city of Ottawa between 2007 and 2016, with half (50%) occurring in 2012 (Fig. 2), and all identified Ottawa as primary exposure location. Mean annual WNV incidence in Ottawa ranged from 0.00 to 0.64 per 100,000 population.

Descriptive Analysis

We found the largest cross-correlation coefficient ($r = 0.36$) between residuals of a linear model between monthly mean temperature and monthly total precipitation, and monthly mosquito MIR, at a lag of 1 month. This suggests weather conditions 1 month prior are the most important in predicting mosquito MIR. We also graphically observed averaged monthly abundance of *Cx. pipiens/restuans* to peak around a month before or the same month as averaged monthly MIR and total monthly WNV human cases, between 2011 and 2014 (Fig. 2). Averaged monthly MIR and monthly WNV human cases were almost always null from 2007 and 2010, with the exception of August 2008 were one human WNV case was recorded.

Mixed-Effects Modeling Analysis

Prior model development, we combined two predictors that displayed high correlation ($r = 0.54$), namely predicted density of the two reservoir bird species, into one variable to remove significant collinearity effects in the models. After combining the two variables, $r < 0.25$ for all pairs of predictor variables. Residuals of the full model (incorporating interaction between mean temperature and total precipitation at a lag of 1 month, and site identifier, as random-effects variables, and abundance of *Cx. pipiens/restuans*, *Oc. japonicus* and *Ae. vexans*, and sum of predicted density of American robins and house finches, as fixed-effects variables) did not display significant spatial autocorrelation (Moran's I $P = 0.42$.

When examining factors affecting mosquito MIR, four models had a ΔAICc < 2 (Table 2): the full model including all predictors, a model including all predictors except predicted density of reservoir bird species, a model including all predictors except abundance of *Oc. japonicus*, and a model including only abundance of *Cx. pipiens/restuans* and abundance of *Ae. vexans*. Additionally, variance explained by the interaction between total precipitation and mean temperature was approximately 14 times higher than that of site identifier (Table 2). Marginal pseudo-R^2 values were about a fifth of conditional pseudo-R^2 values for the three models with the highest ΔAICc value (Table 2), signifying a modest but substantial effect of mosquito and bird densities compared to weather and spatial location data. Two fixed-effects variables, abundance of *Cx. pipiens/restuans* and abundance of *Ae. vexans*, had a relative importance of 1.00, across all 16 models

Table 2. Model Selection Parameters, Model-Averaged Akaike Weights (Weight), Variance (σ^2) Explained by Random-Effects Variables, and Marginal and Conditional Pseudo-R^2 (Johnson 2014) of Top Generalized Linear Mixed-Effects Models Explaining Minimum Infection Rates of Mosquitoes Collected Around the City of Ottawa from 2007 to 2014.

Models	1 + 2 + 3 + 4	1 + 2 + 3	1 + 3 + 4	1 + 3
Degrees of freedom	7	6	6	5
Log-likelihood	− 8609.5	− 8610.5	− 8610.7	− 8611.7
AICc	17,233.0	17,233.1	17,233.4	17,233.4
ΔAICc	0.00	0.07	0.39	0.46
Weight	0.28	0.27	0.23	0.22
σ^2 for precipitation: temperature	121.9	122.0	121.5	121.6
σ^2 for site identifier	8.5	9.2	8.4	9.1
Marginal pseudo-R^2	0.002	0.002	0.002	0.001
Conditional pseudo-R^2	0.011	0.011	0.011	0.010

1 = abundance of *Cx. pipiens/restuans*, 2 = abundance of *Oc. japonicus*, 3 = abundance of *Ae. vexans*, 4 = predicted density of reservoir bird species.

Table 3. Model-Averaged Coefficients of Four Predictor Variables Explaining Minimum Infection Rates of Mosquitoes Collected Around the City of Ottawa from 2007 to 2014, from a Total of 16 Mixed-Effects Models.

Explanatory variables	Estimate	SE	Relative variable importance	*P*
Abundance of *Cx. pipiens/restuans*	0.16	0.02	1.00	< 0.001
Abundance of *Oc. japonicus*	− 0.02	0.01	0.55	0.132
Abundance of *Ae. vexans*	0.19	0.04	1.00	< 0.001
Predicted density of reservoir bird species	0.46	0.32	0.51	0.148

(Table 3), signifying large effect size of the two variables on MIR. Abundance of *Oc. ochlerotatus* and predicted density of the two reservoir bird species had a relative importance of 0.55 and 0.51, respectively, across all 16 models (Table 3). The lower variable importance of predicted density of the two reservoir bird species likely stems from the large standard error of its estimated effect across models, despite having the largest model-averaged estimated effect on MIR among predictors (Table 3). These results suggest an effect of predicted bird density that may be dependent on other variables included in the model, such as abundance of two WNV mosquito vectors, namely *Cx. pipiens/restuans* and *Ae. vexans*. These results suggest an interaction between bird and mosquito densities is likely the best predictor of mosquito MIR. Unfortunately, we could not test the effects of interaction terms of predictors together with predictors in the same models, due to multi-collinearity issues. On the other hand, lower variable importance of abundance of *Oc. japonicus* likely stems from its relatively low estimate, indicating a weak effect of the variable on MIR (Table 3). All variables had a positive relationship with MIR, except abundance of *Oc. japonicus* which had a negative relationship.

Spatiotemporal Cluster Detection

We identified a total of seven clusters in the study area based on WNV-positive and negative mosquito catches; all had more WNV-positive trap-days than expected (Table 4), and two were statistically significant ($P < 0.05$; Table 4). We found a total of one cluster in the study area based on human WNV cases, with significantly more observed than expected number of cases ($P < 0.05$; Table 4). It is spatially concentric, but larger, than one of the statistically significant clusters detected using mosquito catch data (Fig. 1). The two clusters detected using mosquito catch data temporally overlap with the epidemiological cluster detected using human case data, but start fifty-three

Table 4. Characteristics (Start and End Date, Observed and Expected Number of Cases, Relative Risk (RR), Likelihood Ratio (LLR) and *P* Value) of Detected Space–Time Clusters of High West Nile Virus (WNV) Rates in the City of Ottawa, Canada, 2007–2014.

Dataset	Start	End	Observed	Expected	RR	LLR	*P*
Mosquito positive WNV catches	17/7/2012	17/9/2012	14	0.3	52	46	< 0.01
	02/8/2011	27/8/2012	15	0.9	21	32	< 0.01
	31/7/2012	19/8/2013	6	0.7	9	8	0.58
	24/7/2012	30/7/2012	2	< 0.1	69	8	0.54
	14/8/2012	27/8/2012	3	0.1	21	7	0.80
	04/9/2012	10/9/2012	2	0.1	35	6	1.00
	24/7/2012	09/9/2013	4	0.4	10	6	0.94
Human WNV cases	07/8/2012	03/9/2012	7	0.6	280	29	< 0.01

(little more than a year) and 3 three weeks before its start date, respectively, and end within 3 weeks around its end date (Table 4).

DISCUSSION

As predicted, we found a positive effect of abundance of two of three vector species, namely *Cx. pipiens/restuans*, and *Ae. vexans*, and density of two avian reservoir species, on minimum infection rates (MIR) of mosquito catches. Also, as predicted, we observed space and time overlap between hotspots of WNV-infected mosquito catches and human WNV cases.

Almost all detected WNV-positive catches were *Cx. pipiens/restuans*, with a single one being *Ae. vexans*. This is not unexpected, since *Cx. pipiens/restuans* are targeted for testing, but it is consistent with data from other regions of the province. Most WNV-positive mosquito catches were detected in 2012, which corresponds to the year with half of all WNV cases across the study period. Being primarily ornithophilic in its choice of host (Farajollahi et al. 2011), but also feeding on humans frequently (Hamer et al. 2009), *Cx. pipiens/restuans* is typically the vector species most frequently in contact with the main reservoirs of WNV in northeastern regions of North America, and one of the main contributors of spill-over to humans (Hamer et al. 2008, 2009). Results from our modeling analysis suggest higher abundance of *Cx. pipiens/restuans* is linked with higher MIR in mosquito catches. A possible explanation is that higher vector abundance results in higher rates of enzootic transmission among avian reservoir species and, consequently, higher probability for a mosquito to feed from a viremic reservoir (Artsob et al. 2006; Ganser and Wisely 2013).

Our results also suggest abundance of *Ae. vexans* may potentially play a role in the enzootic cycle of WNV, being associated with higher MIR in mosquito catches. On the other hand, the species is linked only to a very small fraction of all positive catches, which suggests a limited role of *Ae. vexans* in WNV transmission in the present study. Being primarily mammalophilic (Apperson et al. 2004; Molaei and Andreadis 2006; Shepard et al. 2016), this species may come in contact more frequently than *Cx. pipiens/restuans* with secondary WNV reservoirs, such as small mammals (van der Meulen et al. 2005). Members of the species also feed sporadically on avian hosts, and may potentially bridge transmission among different types of reservoirs, while also contributing to the main enzootic WNV cycle in avian reservoirs (Apperson et al. 2004; Molaei and Andreadis 2006; Shepard et al. 2016). Finally, *Ae vexans* is thought to contribute to the spill-over of WNV to livestock and humans, being highly opportunistic in its choice of hosts (Ganser and Wisely 2013).

We found density of American robins and house finches to be associated with MIR in mosquito catches. American robins and house finches are known to develop chronic WNV infections and may survive high viremia (Reisen et al. 2013; VanDalen et al. 2013), allowing transmission by mosquito vectors and perpetuation of the enzootic WNV cycle. Therefore, higher densities of those two species will result in higher probability for a mosquito to feed from a viremic reservoir (Artsob et al. 2006; Ganser and Wisely 2013). Data from a variety of other potential WNV reservoir species, such as the common grackle and the house sparrow (Kilpatrick et al. 2007; Reisen 2013), could potentially increase the reliability of our modeling approach.

We detected hotspots of WNV infection in mosquitoes and humans that overlapped in a highly urbanized area of the City of Ottawa. Another hotspot of WNV infection in mosquitoes was in a less densely populated area, and did not overlap with a hotspot of WNV in humans. Several studies found a link between human demographic data such as population density, housing density, age of housing and land use on *Cx. pipiens* populations (Trawinski and MacKay 2008; Ozdenerol et al. 2013) and on WNV risk in human populations (Ruiz et al. 2004). Clearly, more studies looking at associations of human demography and land use to WNV risk to human populations are needed to understand factors affecting immediate WNV risk in cities across the world.

Our study suffers from a limited dataset of human cases. Cases of infection by WNV in humans are usually unreported due to only a small proportion of cases involving severe symptoms (Gubler 2007). A larger human case dataset, possibly combined with a serosurvey of antibodies specific to WNV and a map of common routes employed by people who have been infected, would provide higher resolution in detection of epidemiological hotspots around the city of Ottawa. Although no nuisance control program was in place in the city of Ottawa at the time of the study, a larvicide program targeting *Cx. pipiens/restuans* at all roadside catch basins and ditches, and storm water management ponds, was in place during the whole time of the study. We do not expect heterogeneity in the effects of this program among study sites on mosquito abundance. However, studying the effects of these programs in a similar approach as this current study would be an interesting future step. Another limitation is the poor resolution of our bird observation data and the low number of bird species from which data was available. Incorporating additional known reservoir species, such as the house sparrow (Kilpatrick et al. 2006), and higher resolution of observation data, would lead to higher statistical power in determining which avian species are most important in the enzootic cycle of WNV in the city of Ottawa, and potentially in northeastern North America. Additionally, omission of demographic, land cover and land use variables, which potentially have a direct effect on mosquito infection rates, may limit the meaningfulness of results from our modeling approach.

CONCLUSION

Our study specifically examined the impact of vector species density on mosquito infection rates, providing a more nuanced understanding of the factors contributing to WNV transmission among mosquito populations. It is innovative in that it suggests a role of avian and vector densities on vector infection rates, and in turn, on hotspots of human WNV cases. Such integrated approaches are crucial for our understanding of the epidemiology of WNV in Canada and worldwide.

ACKNOWLEDGEMENTS

We thank Canadian Institutes of Health Research for funding of the project. We thank employees of GDG Environment for their mosquito abundance and WNV test data, volunteers of Ontario Bird Count for access to their spatially interpolated bird density data, employees of Ottawa Public Health for their human WNV case data, and employees of Environment and Natural Resources Canada for meteorological data. We thank Monir Taha for their useful comments on an earlier version of the manuscript. We thank all members from the INSIGHT lab for their suggestions and comments on the analyses and the initial draft of the manuscript.

REFERENCES

Andreadis TG (2012) The contribution of *Culex pipiens* complex mosquitoes to transmission and persistence of West Nile Virus in North America. *Journal of the American Mosquito Control Association* 28:137–151. https://doi.org/10.2987/8756-971X-28.4s.137

Apperson CS, Hassan HK, Harrison BA, Savage HM, Aspen SE, Farajollahi A, Crans W, Daniels TJ, Falco RC, Benedict M, Anderson M, McMillen L, Unnasch TR (2004) Host feeding patterns of established and potential mosquito vectors of West Nile Virus in the eastern United States. *Vector-Borne Zoonotic Diseases* 4:71–82. https://doi.org/10.1089/153036604773083013

Artsob H, Lindsay R, Drebot M (2006) Biodiversity-related aspects of West Nile Virus and its cycle in nature. *Biodiversity* 7:18–23. https://doi.org/10.1080/14888386.2006.9712790

Barton K (2013) *MuMIn: multi-model inference*, Krakow, Poland: Institute of Nature Conservation

Burkett-Cadena ND, Graham SP, Hassan HK, Guyer C, Eubanks MD, Katholi CR, Unnasch TR (2008) Blood feeding patterns of potential arbovirus vectors of the genus *Culex* targeting ectothermic hosts. *American Society of Tropical Medicine and Hygiene* 79:809–815

Chancey C, Grinev A, Volkova E, Rios M (2015) The global ecology and epidemiology of West Nile Virus. *BioMed Research International* 2015:1–20. https://doi.org/10.1155/2015/376230

Chaves A, Sotomayor-Bonilla J, Monge O, Ramírez A, Galindo F, Sarmiento-Silva RE, Gutiérrez-Espeleta GA, Suzán G (2016) West Nile Virus in resident birds from Yucatan, Mexico. *Journal of Wildlife Diseases* 52:159–163. https://doi.org/10.7589/2015-02-046

Darsie R, Ward R (2005) *Identification and geographical distribution of the mosquitoes of North America, North of Mexico, 2nd ed.*, Gainsville, FL, USA: University Press of Florida

Diaz LA, Quaglia AI, Konigheim BS, Boris AS, Aguilar JJ, Komar N, Contigiani MS (2016) Activity patterns of St. Louis Encephalitis and West Nile Viruses in free ranging birds during a human encephalitis outbreak in Argentina. *PLOS ONE* 11:e0161871. https://doi.org/10.1371/journal.pone.0161871

Drebot MA, Lindsay R, Barker IK, Buck PA, Fearon M, Hunter F, Sockett P, Artsob H (2003) West Nile virus surveillance and diagnostics: a Canadian perspective. *Canadian Journal of Infectious Diseases and Medical Microbiology* 14:105–114

Eisen RJ, Eisen L (2008) Spatial modeling of human risk of exposure to vector-borne pathogens based on epidemiological versus arthropod vector data. *Journal of Medical Entomology* 45:181–192. https://doi.org/10.1603/0022-2585(2008)45[181:SMOHRO]2.0.CO;2

Environment and Natural Resources Canada (2018) *Past weather and climate, historical data*, Ottawa: ON, Canada

Epstein PR, Defilippo C (2001) West Nile Virus and drought. *Global Change Human Health* 2:105–107. https://doi.org/10.1023/A:1015089901425

Farajollahi A, Fonseca DM, Kramer LD, Marm Kilpatrick A (2011) "Bird biting" mosquitoes and human disease: A review of the role of *Culex pipiens* complex mosquitoes in epidemiology. *Infection, Genetics and Evolution* 11:1577–1585. https://doi.org/10.1016/j.meegid.2011.08.013

Foppa IM, Beard RH, Mendenhall IH (2011) The impact of West Nile virus on the abundance of selected North American birds. *BMC Veterinary Research* 7:43. https://doi.org/10.1186/1746-6148-7-43

Ganser C, Wisely SM (2013) Patterns of spatio-temporal distribution, abundance, and diversity in a mosquito community from the eastern Smoky Hills of Kansas. *Journal of Vector Ecology* 38:229–236. https://doi.org/10.1111/j.1948-7134.2013.12035.x

Giordano BV, Kaur S, Hunter FF (2017) West Nile Virus in Ontario, Canada: a twelve-year analysis of human case prevalence, mosquito surveillance, and climate data. *PLoS ONE* 12:e0183568. https://doi.org/10.1371/journal.pone.0183568

Greenberg JA, Lujan DA, DiMenna MA, Wearing HJ, Hofkin BV (2013) Identification of blood meal sources in *Aedes vexans* and *Culex quinquefasciatus* in Bernalillo County, New Mexico. *Journal of Insect Science* 13:1–12. https://doi.org/10.1673/031.013.7501

Gubler DJ (2007) The continuing spread of West Nile Virus in the western hemisphere. *Clinical Infectious Diseases* 45:1039–1046. https://doi.org/10.1086/521911

Hamer GL, Kitron UD, Brawn JD, Loss SR, Ruiz MO, Goldberg TL, Walker ED (2008) *Culex pipiens* (Diptera: Culicidae): a bridge vector of West Nile Virus to humans. *Journal of Medical Entomology* 45:125–128. https://doi.org/10.1093/jmedent/45.1.125

Hamer GL, Kitron UD, Goldberg TL, Brawn JD, Loss SR, Ruiz MO, Hayes DB, Walker ED (2009) Host selection by *Culex pipiens* mosquitoes and West Nile Virus amplification. *American Society of Tropical Medicine and Hygiene* 80:268–278

Hughes TP, Paul JH, Smithburn KC, Burke AW (1940) A neurotropic virus isolated from the blood of a native of Uganda. *American Society of Tropical Medicine and Hygiene* s1-20:471–492. https://doi.org/10.4269/ajtmh.1940.s1-20.471

Johnson PCD (2014) Extension of Nakagawa & Schielzeth's R^2_{GLMM} to random slopes models. *Methods in Ecology and Evolution* 5:944–946. https://doi.org/10.1111/2041-210X.12225

Kaufman MG, Fonseca DM (2014) Invasion biology of *Aedes japonicus japonicus* (Diptera: Culicidae). *Annual Review of Entomology* 59:31–49. https://doi.org/10.1146/annurev-ento-011613-162012

Kilpatrick AM, Daszak P, Jones MJ, Marra PP, Kramer LD (2006) Host heterogeneity dominates West Nile Virus transmission. *Proceedings of Royal Society B: Biological Sciences* 273:2327–2333. https://doi.org/10.1098/rspb.2006.3575

Kilpatrick AM, LaDeau SL, Marra PP (2007) Ecology of West Nile Virus transmission and its impact on bird in the western hemisphere. *The Auk* 124:1121. https://doi.org/10.1642/0004-8038(2007)124[1121:EOWNVT]2.0.CO;2

Komar O, Robbins MB, Klenk K, Blitvich BJ, Marlenee NL, Burkhalter KL, Gubler DJ, Gonzálvez G, Peña CJ, Peterson AT, Komar N (2003) West Nile Virus transmission in resident birds, Dominican Republic. *Emerging Infectious Diseases* 9:1299–1302. https://doi.org/10.3201/eid0910.030222

Kulldorff M (1997) A spatial scan statistic. *Communications in Statistics Theory and Methods* 26:1481–1496. https://doi.org/10.1080/03610929708831995

Lanciotti RS, Kerst AJ, Nasci RS, Godsey MS, Mitchell CJ, Savage HM, Komar N, Panella NA, Allen BC, Volpe KE, Davis BS, Roehrig JT (2000) Rapid detection of West Nile Virus from human clinical specimens, field-collected mosquitoes, and avian samples by a TaqMan reverse transcriptase-PCR assay. *Journal of Clinical Microbiology* 38:4066–4071

Levi ME (2013) West Nile Virus Infection in the Immunocompromised Patient. *Current Infectious Disease Reports* 15:478–485. https://doi.org/10.1007/s11908-013-0367-8

Levine RS, Hedeen DL, Hedeen MW, Hamer GL, Mead DG, Kitron UD (2017) Avian species diversity and transmission of West Nile Virus in Atlanta, Georgia. *Parasit Vectors* 10. https://doi.org/10.1186/s13071-017-1999-6

Mallya S, Sander B, Roy-Gagnon M-H, Taljaard M, Jolly A, Kulkarni MA (2018) Factors associated with human West Nile virus infection in Ontario: a generalized linear mixed modelling approach. *BMC Infectious Diseases* 18:1–9. https://doi.org/10.1186/s12879-018-3052-6

Marini G, Rosá R, Pugliese A, Heesterbeek H (2017) Exploring vector-borne infection ecology in multi-host communities: a case study of West Nile virus. *Journal of Theoretical Biology* 415:58–69. https://doi.org/10.1016/j.jtbi.2016.12.009

Molaei G, Andreadis TG (2006) Identification of avian- and mammalian-derived bloodmeals in *Aedes vexans* and *Culiseta melanura* (Diptera: Culicidae) and its implication for West Nile Virus transmission in Connecticut, U.S.A. *Journal of Medical Entomology* 43:1088–1093. https://doi.org/10.1603/0022-2585(2006)43[1088:IOAAMB]2.0.CO;2

Morales-Betoulle ME, Komar N, Panella NA, Alvarez D, López MR, Betoulle J-L, Sosa SM, Müller ML, Kilpatrick AM, Lanciotti RS, Johnson BW, Powers AM, Cordón-Rosales C, Arbovirus

Ecology Work Group (2013) West Nile virus ecology in a tropical ecosystem in Guatemala. *American Society of Tropical Medicine and Hygiene* 88:116–126. https://doi.org/10.4269/ajtmh.2012.12-0276

Ontario Ministry of Health and Long-Term Care (2017) *Infectious disease protocol, Appendix B: provincial case definitions for reportable diseases: West Nile Virus illness*, Toronto: ON, Canada

Ozdenerol E, Taff G, Akkus C (2013) Exploring the spatio-temporal dynamics of reservoir hosts, vectors, and human hosts of West Nile Virus: a review of the recent literature. *International Journal of Environmental Research and Public Health* 10:5399–5432. https://doi.org/10.3390/ijerph10115399

Public Health Ontario (2013) *Guide for public health units: considerations for adult mosquito control*, Toronto, ON, Canada: Queen's Printer for Ontario

Public Health Ontario (2012) *Vector-borne diseases: 2012 summary report*, Toronto, ON, Canada: Queen's Printer for Ontario

Reisen WK (2013) Ecology of West Nile Virus in North America. *Viruses* 5:2079–2105. https://doi.org/10.3390/v5092079

Reisen WK, Fang Y, Martinez VM (2006) Effects of temperature on the transmission of West Nile Virus by *Culex tarsalis* (Diptera: Culicidae). *Journal of Medical Entomology* 43:309–317. https://doi.org/10.1093/jmedent/43.2.309

Reisen WK, Padgett K, Fang Y, Woods L, Foss L, Anderson J, Kramer V (2013) Chronic infections of West Nile Virus detected in California dead birds. *Vector-Borne Zoonotic Diseases* 13:401–405. https://doi.org/10.1089/vbz.2012.1097

Ruiz MO, Tedesco C, McTighe TJ, Austin C, Kitron U (2004) Environmental and social determinants of human risk during a West Nile Virus outbreak in the greater Chicago area, 2002. *International Journal of Health Geographic* 3:8. https://doi.org/10.1186/1476-072X-3-8

Shepard JJ, Andreadis TG, Thomas MC, Molaei G (2016) Host associations of mosquitoes at Eastern Equine Encephalitis Virus foci in Connecticut, USA. *Parasit Vectors* 9. https://doi.org/10.1186/s13071-016-1765-1

Smith KA, Campbell GD, Pearl DL, Jardine CM, Salgado-Bierman F, Nemeth NM (2018) A retrospective summary of raptor mortality in Ontario, Canada (1991–2014), including the effects of West Nile Virus. *Journal of Wildlife Diseases* 54:261–271. https://doi.org/10.7589/2017-07-157

Statistics Canada (2016) *Health Profile*. Ottawa, ON, Canada

Symonds MRE, Moussalli A (2011) A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's Information Criterion. *Behavioral Ecology and Sociobiology* 65:13–21. https://doi.org/10.1007/s00265-010-1037-6

Thielman A, Hunter F (2007) A photographic key to the adult female mosquitoes (Diptera: Culicidae) of Canada. *Canadian Journal of Arthropod Identification* 4:1–116. https://doi.org/10.3752/cjai.2007.04

Trawinski PR, MacKay DS (2008) Meteorologically conditioned time-series predictions of West Nile Virus vector mosquitoes. *Vector-Borne Zoonotic Diseases* 8:505–522. https://doi.org/10.1089/vbz.2007.0202

TSentr gigieny i ėpidemiologii v Respublike Tatarstan (2013) *Likhoradka Zapadnogo Nila. Kazanskiĭ gosudarstvennyĭ meditsinskiĭ*, Kazan, Russia

van der Meulen KM, Pensaert MB, Nauwynck HJ (2005) West Nile Virus in the vertebrate world. *Archives of Virology* 150:637–657. https://doi.org/10.1007/s00705-004-0463-z

VanDalen KK, Hall JS, Clark L, McLean RG, Smeraski C (2013) West Nile Virus infection in American robins: new insights on dose response. *PLoS ONE* 8:e68537. https://doi.org/10.1371/journal.pone.0068537

Winters AM, Bolling BG, Beaty BJ, Blair CD, Eisen RJ, Meyer AM, Pape WJ, Moore CG, Eisen L (2008) Combining mosquito vector and human disease data for improved assessment of spatial West Nile Virus disease risk. *American Society of Tropical Medicine and Hygiene* 78:654–665

Wood DM, Dang PT, Ellis RA (1979) *The mosquitoes of Canada: Diptera*, Agriculture Canada, Hull, QC, Canada: Culicidae

Yoo E-H, Chen D, Diao C, Russell C (2016) The effects of weather and environmental factors on West Nile Virus mosquito abundance in Greater Toronto Area. *Earth Interaction* 20:1–22. https://doi.org/10.1175/EI-D-15-0003.1