Analysis Of Entomological Surveillance Data To Predict West Nile Virus Cases In Connecticut

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Analysis of Entomological Surveillance Data to Predict West Nile Virus Cases in Connecticut

Lena Marie Tayo
Yale School of Public Health, Epidemiology of Microbial Diseases
Master of Public Health, 2019

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I gratefully acknowledge the Connecticut Agricultural Experiment Station (CAES) and Dr. Philip Armstrong for providing all mosquito surveillance and human cases data.

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ABSTRACT

**Introduction:** Since 1999, West Nile Virus (WNV) has been identified in a wide variety of mosquito species. Seasonal epidemics occur in the summer in all regions of the United States, with varying degrees of intensity and spread. In Connecticut, the state mosquito surveillance system for WNV has been conducted since 2000, collecting entomological and site data, and acting as an early warning system for potential human disease risk.

**Aims:** Re-analysis of the epidemiology of WNV in Connecticut after years of established endemicity will be conducted using recent mosquito surveillance and human case data. A predictive model will also be developed to determine which entomological surveillance indicators are best for predicting human cases.

**Results and Analysis:** The following were performed: 1) re-analysis of WNV epidemiology, 2) analysis of the predictive model, and 3) analysis of average seasonal lag between peak *Culex pipiens* abundance, significant predictive model indicators, and yearly human cases. Overall, the epidemiology of WNV in Connecticut has not differed significantly from earlier assessments. Only weekly Minimum Infection Rate (MIR) of bird-biting mosquitoes was a significant predictor of human WNV risk. Average lag between peak in human cases and peak in MIR of bird-biting mosquitoes suggested that peak MIR may indicate potential peak in human cases with a lag of 2 weeks (±3.13 weeks).

**Conclusions:** Entomological surveillance indicators, primarily MIR of bird-biting mosquitoes, is useful for seasonal prediction of human disease risk. Other factors important for WNV transmission, such as land use, climate, and human sociological factors, should also be included in future models to improve WNV human risk modeling.
INTRODUCTION

West Nile Virus (WNV), part of the *Flaviviridae* virus family, was first identified in the United States (U.S.) in 1999 with an outbreak of the disease in New York City, New York (Colpitts et al. 2012). WNV is detected in all regions of the U.S. and is associated with specific mosquito species, as well as ecological and climatic risk factors (Andreadis et al. 2012; CDC 2018). In humans, WNV often manifests asymptomatically (about 80% of WNV infections) (Lindsey et al. 2010) and sometimes symptomatically with fever (Andreadis et al. 2012). About 1% go on to develop neuro-invasive disease. Among people age 70 and older, mortality with these symptoms is estimated to be 15-29% (Lindsey et al. 2010; Colpitts et al 2012). Currently, no vaccine is available and treatment is primarily supportive (Colpitts et al. 2012). In Connecticut, located in the Northeast region of the U.S. (“U.S. Northeast”), the WNV surveillance system is operated by the Connecticut Agricultural Experiment Station (CAES). Following the first detection of WNV in Connecticut in 2000, mosquito collection has been conducted each year from June to October using 91 trap sites across the state (Liu et al. 2009; Andreadis et al. 2004). Most trap sites are concentrated along the shoreline and in urban and residential areas. This system provides early warning of human disease risk by tracking viral activity and infection prevalence among mosquitoes.

WNV is maintained in a primarily bird- mosquito enzootic cycle (Turell et al. 2005), with some transmission to dead end hosts such as humans, horses, and other mammals. The majority of species carrying WNV are well-known ornithophilic (bird-biting) species such as *Culex pipiens*, *Culex restuans*, and *Culiseta melanura* and generalist feeders such as *Culex salinarius* (Andreadis et al. 2012). Anthropophilic mosquitoes such as *Aedes*, *Anopheles*, and *Ochlerotatus*
mosquitoes can also carry the virus, but infection prevalence is much lower (Andreadis et al. 2004; Talbot et al. 2019). Most of these species are competent vectors of WNV (Turell et al. 2005; Sardelis et al. 2001). *Culex pipiens* and *Culex restuans* contribute to virus amplification in bird species during the summer, primarily the American robin, though house finches and sparrows are also commonly infected (Andreadis et al. 2004; Molaei et al. 2006; Kilpatrick et al. 2006). Persistence of WNV in the mosquito population between summer seasons has been attributed to overwintering, dispausing mosquitoes (Nasci et al. 2001; Andreadis et al. 2012; Talbot et al. 2019; Anderson et al. 2012). Mosquito surveillance studies show that virus amplification occurs early in the summer (June to mid/late-July) between mosquitoes and birds. This increase in abundance and infection prevalence among bird-biting mosquitoes usually precedes the first cases of human infection (illness onset dates of first human cases is usually in late-July) (Andreadis et al. 2012).

Epidemic transmission to humans is still unclear. Although anthropophilic mosquitoes have been collected and tested positive for WNV (Andreadis et al. 2004), a definitive bridge vector(s) has not been identified (Rochlin et al. 2009). *Culex pipiens* is unanimously considered an important WNV vector in Connecticut (Andreadis et al. 2001; Andreadis et al. 2012) but its strong ornithophilic background often excludes it as a strong bridge vector candidate (Andreadis et al. 2012; Turell et al. 2005). The WNV cycle in the U.S. Northeast is unique in that it lacks a vector that is shown to be capable of high to moderate enzootic and epizootic transmission (Andreadis et al. 2012). In most other regions of the U.S., *Culex quinquefasciatus* and *Culex tarsalis* have been implicated as important vectors for amplification among birds (and other wild hosts) and have been shown to have some propensity to bite humans (Andreadis et al. 2012). *Culex tarsalis* has the ability to switch from primarily bird-biting in the early summer months to
mammalian-biting later in the season, making this species both a strong amplification vector in birds and a bridge vector to humans (Turell et al. 2005). Neither of these vectors are found in the U.S. Northeast. *Culex salinarius* is a generalist feeder and has been strongly indicated as a possible WNV bridge vector (Molaei et al. 2006; Andreadis et al. 2012). This species tends to occupy coastal locations and is usually abundant in August and September (Andreadis et al. 2012). *Aedes* vectors are notoriously aggressive human biters; some species are found in Connecticut with considerable abundance (Andreadis et al. 2004). However, their low preference for biting birds makes them a weak bridge vector candidate, and their role in the WNV transmission cycle is still unclear (Andreadis et al. 2004).

The exact enzootic and epidemic transmission mechanisms among mosquitoes, birds, and humans are complex and many factors need to be considered when analyzing human disease risk and predicting human cases. Predictive modeling of human WNV disease risk in Connecticut has been conducted in the past using mosquito surveillance, avian host, land cover, and climate data (Liu et al. 2009). However, mosquito surveillance data in the model proposed by Liu (2009) were limited and entomological factors, such as Minimum Infection Rates (MIRs) and total number of WNV positive pools, were not included. Mosquito abundance (number of mosquitoes), WNV isolations, and MIR are commonly used to describe and analyze mosquito distribution and activity, as well as prevalence of virus infection. Analysis based on host feeding preference of vectors (bird-biting compared to mammal/human-biting) has also yet to be fully explored in Connecticut. Since WNV has been detected in Connecticut for almost 20 years, the virus has had time to establish itself. Therefore, it is also important to analyze recent mosquito surveillance data to determine not only which indicators collected each season are most important for predicting human disease, but to also analyze whether any shifts in the virus’
epidemiology have occurred. Additionally, based on summary data from past seasons, a lag has been observed between the peak abundance of mosquitoes and peak of human cases. This lag has not been recently or fully characterized. This current thesis will analyze mosquito surveillance data collected over 12 years to compare trends to earlier assessments and assess the use of entomological data to predict human disease risk.
MATERIALS AND METHODS

Study Area

Mosquito collection and human case data were limited to three counties that have been areas of high WNV focus in the past—Fairfield County, Hartford County, and New Haven County. Mosquito collections and reported cases most often came from these areas (Andreadis et al. 2004). Fairfield County and New Haven County lie along the urban/suburban corridor, which ends near the lower end of Hartford county (Andreadis et al, 2004). Fairfield County and New Haven County include the majority of the towns along the Long Island Sound, as well as many of the high population cities and towns. Hartford county is located inland and includes Hartford, the capital of Connecticut. These three counties were chosen due to their similarities. Approximately 2/3 of all trap sites were placed in these three counties (See Appendix A for map of counties and trapping sites). These three counties contain most of the large cities in Connecticut (Bridgeport in Fairfield County, New Haven in New Haven County, and Hartford in Hartford County) and have the highest total populations and population densities in the state (Statistical Atlas 2017). Fairfield is the largest county by population with a population size of 949,921 people and the highest population density (1,467 people per mi²). The three counties were considered predominately urban and suburban, with some rural areas. Fairfield County had the highest median household income ($89,773) (U.S. Census Bureau 2017). Median household income for New Haven County was $64,872 and for Hartford County was $69,936.

Mosquito Surveillance Data
The Connecticut Agricultural Experiment Station (CAES) provided all mosquito collection data, including location of trap sites (site code, town, county), abundance per trap night, number of WNV isolations per trap night, trap type, and date of collection. Although data were available for 2001 to 2017, only data collected from 2006 to 2017 were used for analysis. Analyses on earlier data has been conducted previously and extensively (Andreadis et al. 2001; Andreadis et al. 2004). Two types of traps were used for mosquito collection: 1) the CO2-baited CDC Light trap (light trap) and 2) a Gravid Mosquito Trap (gravid trap). The light trap attracts most host-seeking mosquitoes, whereas the gravid trap was designed to attract already blood-fed females, primarily *Cx. pipiens* and *Cx. restuans* (Andreadis et al. 2004). In 2006, the bait formula for gravid traps was changed to the current formula.

Mosquitoes were collected every 10 days from trap sites starting in the beginning of the WNV season (beginning of June) to mid-October. Trap frequency increased to twice a week when WNV and EEEV were detected in a given location. Collected mosquitoes were transported back to the lab in the Connecticut Agricultural Experiment Station. In the lab, mosquitoes were placed on chill tables and identified by species (Andreadis et al. 2001; Andreadis et al. 2004). Each species was then pooled into groups of up to 50 mosquitoes and these pools were then tested for the presence of WNV using cell culture technique (Andreadis et al. 2004).

Only mosquito species that tested positive for WNV from 2006 to 2017 were included in the analysis. These species were then organized into groups according to host-biting preference. Species acknowledged to be primarily ornithophilic (preferring to feed on avian hosts) were placed in one group (“bird-biting” group) and species known to be generalist (without a particular preference between avian or human, but lean more towards biting humans) or anthropophilic (preferring to feed on mammalian, including human, hosts) were placed in
another group (“mammal-biting” group). Weekly total abundance, total number WNV isolations, and Minimum Infection Rates (MIRs) were calculated for 1) all mosquitoes, 2) bird-biting mosquitoes, and 3) mammal-biting mosquitoes (Lord and Bustamante 2010; Gu et al. 2008). MIR was calculated using the Biggerstaff method (Biggerstaff 2003; Bartlett-Healy et al. 2008). When sorting these species into bird-biting and mammal-biting groups, three species were excluded (Culex territans, Psorophora ferox, Uranotaenia sapphirina). Past literature indicated that these species were not part of the enzootic or epidemic cycle and primarily fed on non-avian and non-mammalian hosts, such as reptiles and amphibians (Andreadis et al. 2004; Turell et al. 2005; Bartlett-Healy et al. 2008; Ferguson et al. 2013).

Human Case Data

Human WNV cases have been investigated and collected by the Connecticut Department of Public Health (CT DPH). Data collected from 2000 to 2018 were available, but only 2006 to 2017 data were used. The dataset included the town of residence, date of illness onset, age, gender, and list of symptoms. Only cases reported between 2006 and 2017 were used in analysis to align with mosquito surveillance data. The data were further limited to locally reported cases (cases reported in Connecticut). Total case count after exclusions was 81 cases (total number of reported cases between 2006 to 2017 without exclusions was 87).

Statistical Analysis

Weekly and yearly case counts were calculated and combined with entomological variables to create the final, complete dataset. Microsoft Excel was used for all data clean-up and organization. SAS version 9.4 was used for some data clean-up, calculations, and analysis.
Descriptive analysis was conducted on the dataset. A predictive model was developed to assess which entomological factors were the best indicators for human risk. A multivariable Poisson-distributed regression was used because of the skewed distribution. Independent variables included in the model were as follows: total abundance and weekly MIR for all mosquitoes, total abundance and weekly MIR for mammal-biting species, and total abundance and weekly MIR for bird-biting species. Non-significant variables were removed in a step-by-step process until only significant indicators remained (significance was measured by significance level \( p = 0.05 \)). Total WNV isolations for all groups were also calculated. All correlation tests between independent variables were conducted using Spearman correlation test. A high Spearman correlation coefficient \( r_s \) indicated a strong degree of association and collinearity between predictors. If this occurred, only one predictor was used for subsequent analyses. The seasonal lag observed between the peak in human cases and peak in entomological variables was also analyzed. The difference between peak in human cases and all other entomological variables was calculated and the average was taken to determine average length of the lag between peaks.
RESULTS

Mosquito Collection and Testing for West Nile Virus

Between 2006—2017, 22 mosquito species tested positive for WNV among all mosquitoes trapped in Connecticut in Fairfield, New Haven, and Hartford Counties. Among these species, 1,435,002 mosquitoes were trapped and 1,532 WNV isolations were recorded. *Culex pipiens* had the highest abundance of mosquitoes among the 12 years (222,082), followed by *Ochlerotatus canadensis* (208,131), *Coquillettidia perturbans* (189,024), and *Aedes vexans* (167,046) (Table 1). *Culex pipiens* also had the highest number of WNV isolations (1,173), followed by *Cx. restuans* (193), *Cx. salinarius* (98), and *Cs. melanura* (24) (Table 1). Two species, *Anopheles crucians* and *Anopheles walkeri*, had at least one WNV isolation, but were collected outside Fairfield, New Haven and Hartford Counties. *Culiseta melanura* and *An. punctipennis* tested positive for WNV alone, or co-infected with EEEV or Highlands J (HJ).

The bird-biting group had the most WNV isolations (1,391), whereas the mammal-biting group had the highest number of mosquitoes collected (1,012,066). Among the mammal-biting group, 167,046 *Cx. salinarius* mosquitoes and 145,393 *Ae. vexans* mosquitoes were collected. These species also had the most WNV isolations among this group (98 isolations and 9 isolations, respectively). Among the bird-biting group, *Cx. pipiens* and *Cx. restuans* had the highest number of mosquitoes collected and the highest WNV isolations (Table 1).
Table 1 — Total specimens trapped and tested in Hartford county, New Haven county, and Fairfield county, 2006-2017

<table>
<thead>
<tr>
<th>Mosquito Species</th>
<th>Total Abundance</th>
<th>Total WNV isolations (% of all WNV isolations)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bird-Biting Species</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Culex pipiens</em></td>
<td>222,082</td>
<td>1173 (76.7)</td>
</tr>
<tr>
<td><em>Culex restuans</em></td>
<td>73,185</td>
<td>193 (12.6)</td>
</tr>
<tr>
<td><em>Culiseta melanura</em></td>
<td>43,571</td>
<td>24 (1.6)</td>
</tr>
<tr>
<td><em>Culiseta morsitans</em></td>
<td>585</td>
<td>1 (0.07)</td>
</tr>
<tr>
<td>Total</td>
<td>339,423</td>
<td>1,391</td>
</tr>
<tr>
<td><strong>Mammal-Biting Species</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aedes albopictus</em></td>
<td>3,381</td>
<td>1 (0.07)</td>
</tr>
<tr>
<td><em>Aedes cinereus</em></td>
<td>61,712</td>
<td>3 (0.2)</td>
</tr>
<tr>
<td><em>Aedes vexans</em></td>
<td>167,046</td>
<td>9 (0.6)</td>
</tr>
<tr>
<td><em>Anopheles crucians</em></td>
<td>705</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td><em>Anopheles punctipennis</em></td>
<td>21,650</td>
<td>2 (0.1)</td>
</tr>
<tr>
<td><em>Anopheles quadrimaculatus</em></td>
<td>5,929</td>
<td>1 (0.07)</td>
</tr>
<tr>
<td><em>Anopheles walkeri</em></td>
<td>13,645</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td><em>Coquillettidia perturbans</em></td>
<td>189,024</td>
<td>6 (0.4)</td>
</tr>
<tr>
<td><em>Culex salinarius</em></td>
<td>145,393</td>
<td>98 (6.4)</td>
</tr>
<tr>
<td><em>Ochlerotatus canadensis</em></td>
<td>208,131</td>
<td>2 (0.1)</td>
</tr>
<tr>
<td><em>Ochlerotatus japonicus</em></td>
<td>18,825</td>
<td>7 (0.5)</td>
</tr>
<tr>
<td><em>Ochlerotatus stimulans</em></td>
<td>7,324</td>
<td>1 (0.07)</td>
</tr>
<tr>
<td><em>Ochlerotatus taeniorhynchus</em></td>
<td>96,624</td>
<td>4 (0.3)</td>
</tr>
<tr>
<td><em>Ochlerotatus triseriatus</em></td>
<td>9,867</td>
<td>2 (0.1)</td>
</tr>
<tr>
<td><em>Ochlerotatus trivittatus</em></td>
<td>62,810</td>
<td>2 (0.1)</td>
</tr>
<tr>
<td>Total</td>
<td>1,012,066</td>
<td>138</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Culex territans</em></td>
<td>1,026</td>
<td>1 (0.07)</td>
</tr>
<tr>
<td><em>Psorophora ferox</em></td>
<td>56,661</td>
<td>1 (0.07)</td>
</tr>
<tr>
<td><em>Uranotaenia sapphirina</em></td>
<td>25,826</td>
<td>1 (0.07)</td>
</tr>
</tbody>
</table>
**Table 2**—Summary of yearly human cases, bird-biting group, and mammal-biting group*

<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Human cases</td>
<td>9 (11.1)</td>
<td>4 (4.9)</td>
<td>7 (8.6)</td>
<td>0 (0)</td>
<td>8 (9.9)</td>
<td>9 (11.1)</td>
<td>20 (24.7)</td>
<td>4 (4.9)</td>
<td>7 (8.6)</td>
<td>9 (11.1)</td>
<td>1 (1.2)</td>
<td>3 (3.7)</td>
<td>81</td>
</tr>
<tr>
<td>Bird-Biting Group†</td>
<td>147 (10.6)</td>
<td>66 (4.7)</td>
<td>173 (12.4)</td>
<td>31 (2.2)</td>
<td>186 (13.4)</td>
<td>140 (10.1)</td>
<td>208 (15.0)</td>
<td>66 (4.7)</td>
<td>57 (4.1)</td>
<td>117 (8.4)</td>
<td>114 (8.2)</td>
<td>86 (6.2)</td>
<td>1,391 (91.0)</td>
</tr>
<tr>
<td>Mammal-Biting Group†</td>
<td>15 (10.9)</td>
<td>3 (2.2)</td>
<td>13 (9.4)</td>
<td>1 (0.7)</td>
<td>21 (15.2)</td>
<td>15 (10.9)</td>
<td>12 (8.7)</td>
<td>5 (3.6)</td>
<td>5 (3.6)</td>
<td>29 (21.0)</td>
<td>5 (3.6)</td>
<td>14 (10.1)</td>
<td>138 (9.0)</td>
</tr>
</tbody>
</table>

* Format for table is # (%)
† Number of WNV isolations

**Table 3**—Summary of human cases, bird-biting group, and mammal-biting group, by county*

<table>
<thead>
<tr>
<th></th>
<th>Fairfield County</th>
<th>New Haven County</th>
<th>Hartford County</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human cases</td>
<td>52 (62.7%) ±</td>
<td>23 (27.7%)</td>
<td>6 (7.2%)</td>
</tr>
<tr>
<td>Bird-Biting Group†</td>
<td>879 (63.2%)</td>
<td>397 (28.5%)</td>
<td>115 (8.3%)</td>
</tr>
<tr>
<td>Mammal-Biting Group†</td>
<td>60 (43.5%)</td>
<td>64 (46.4%)</td>
<td>14 (10.1%)</td>
</tr>
</tbody>
</table>

* Format for table is # (%)
† Number of WNV isolations
± Percentage is in proportion to total cases from all counties

**Summary of Human WNV Case Data**

Among human cases, 81 total cases were reported between 2006 to 2017 in Fairfield, New Haven, and Hartford Counties. The highest number of cases (20 cases) was reported in 2012 and the lowest number of cases (0 cases) was reported in 2009 (Table 2). August had the highest total number of cases (44 total cases across all years), whereas June generally had almost no human cases. In 2016 and 2017 (the two years with the lowest human case counts), the majority of cases reported illness onset in August. Most cases were reported from Fairfield.
County (62.7%), New Haven County (27.7%), and Hartford County (7.2%) (Table 3). For all other counties, only one or no cases were reported.

Summary of Spatial and Temporal Distribution of Mosquito Data

The majority of WNV isolations (>50%) was collected in Fairfield County for most years. When comparing by host preference, 91% of WNV isolations were among the bird-biting group (Table 3). Among the mammal-biting group, most isolations were collected in New Haven County (46.4%) and Fairfield County (43.5%). This was similar for bird-biting vectors, although 63.2% of all WNV isolations were collected in Fairfield County (Table 3). The least number occurred in 2009, with only 1 WNV isolation (0.7%) collected from Cx. salinarius (Table 2). Most WNV isolations for the mammal-biting group were collected in 2015 (29, 21.0%). Despite having the most cases, the mammal-biting group did not have a lot of WNV isolations in 2012 (12, 8.7%).

Overall, 2009 had the lowest number of WNV isolations for the three counties (32 total pools, 2% of all pools) and had no human cases. On the other hand, 2012 had the most WNV isolations from the bird-biting group (208, 15.0% of total pools) but not for the mammal-biting group. Among both groups, most WNV isolations were collected in August (860, 56.1%), July (380, 24.8%) and September (268, 17.5%).

General Seasonal Trends, 2006-2017

Average number of mosquitoes and average number of WNV isolations were calculated for the bird-biting group (Figure 1A) and the mammal-biting group (Figure 1B). Human cases often first appeared in June, increased from July to August, and dropped during September to
October. Peak number of human cases typically occurred in August. With respect to host preference of mosquitoes, among the bird-biting group (Figure 1A), abundance began to increase in June and peaked near the end of July or beginning of August. The highest average abundance of bird-biting mosquitoes was over 9,000 mosquitoes. From August to October, abundance steadily declined. For mosquitoes in the mammal-biting group, (Figure 1B), abundance steadily increased in June and peaked around the end of June. From July to October, abundance fell overall, though this trend was not consistent. Slight dips in abundance occurred in early-July and late-July/ early-August. The peak in average abundance for this group was almost 35,000 mosquitoes. Average number of WNV isolations peaked before human cases peaked. For the bird-biting group, highest average number of WNV isolations occurred in the beginning of August (average of 54 WNV isolations in this week), whereas human cases peaked in the end of August. For the mammal-biting group, the average number of WNV isolations was much lower compared to the bird-biting group (the highest average number of WNV isolations was 6 isolations in August). Peak in the number of WNV isolations occurred at approximately the same time as the bird-biting mosquitoes (Figure 1A and 1B), but a second peak also occurred just prior to the peak in human cases (Figure 1B).
Figure 1A — Seasonal trend of total human cases, abundance of bird-biting mosquitoes, and number of WNV isolations. Abundance and number of WNV isolations of bird-biting mosquitoes averaged over 2006-2017.
Figure 1B — Seasonal trend of total human cases, abundance of mammal-biting mosquitoes, and number of WNV isolations. Abundance and number of WNV isolations of mammal-biting mosquitoes averaged over 2006-2017.
Using a Poisson-distributed regression, model results of entomological variables and human cases were summarized in Table 4. Weekly WNV isolations and weekly MIRs for all mosquitoes, bird-biting mosquitoes, and mammal-biting mosquitoes were highly correlated \((r_s > 0.90, p < 0.001)\). Therefore, only weekly MIRs for all groups were used in the model. Based on this model, only weekly MIR of the bird-biting mosquitoes was a significant predictor for human cases \((p<0.001)\). Based on the Akaike information criterion (AIC) for each model, the first model, which includes all variables, had an AIC = 353.35. When the most significant variable was kept in the model (weekly MIR of the bird-biting group), AIC = 348.03. However, keeping weekly MIR of mammal-biting vectors in the model lowered AIC slightly \((\text{AIC} = 347.60)\). This may indicate that MIR of mammal-biting mosquitoes does improve the model despite not being significant at the 0.05 level. According to this model, MIR of the bird-biting group was significant and positively associated with human WNV disease risk. In contrast, MIR of the mammal-biting group was negatively associated with human disease risk.

### Table 4 — Multivariable regression model (using Poisson distribution) of entomological variables and human West Nile Virus cases

<table>
<thead>
<tr>
<th></th>
<th>Full Model</th>
<th>Reduced Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate (SE)</td>
<td>p</td>
</tr>
<tr>
<td><strong>Weekly abundance</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All species</td>
<td>0.0002 (0.260)</td>
<td>0.377</td>
</tr>
<tr>
<td>Bird-biting group</td>
<td>-0.0002 (0.0003)</td>
<td>0.355</td>
</tr>
<tr>
<td>Mammal-biting group</td>
<td>-0.0002 (0.0003)</td>
<td>0.334</td>
</tr>
<tr>
<td><strong>Weekly MIR</strong></td>
<td></td>
<td></td>
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<tr>
<td>All species</td>
<td>-0.077 (0.144)</td>
<td>0.545</td>
</tr>
<tr>
<td>Bird-biting group</td>
<td>0.155 (0.038)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mammal-biting group</td>
<td>-0.193 (0.262)</td>
<td>0.404</td>
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</table>
Based on results of the predictive model, the lag between peak in human cases and peak in MIR for *Culex pipiens*, as well as the peak in human cases and peak in MIR for the mammal-biting group was calculated. Amplification in bird hosts, an important factor for spillover to humans, was primarily driven by *Cx. pipiens*. Furthermore, the abundance and MIRs of *Cx. pipiens* were highly correlated with the abundance and MIRs of bird-biting mosquitoes (*r* > 0.90, *p* > 0.001). Therefore, only entomological factors for *Culex pipiens* were used to analyze seasonal lag.

The average lag and 95% Confidence Interval between the peak in human cases and peak of each of the variables is summarized in **Table 5**. The average lag between the peak in abundance of *Cx. pipiens* and peak in human cases was the longest compared to the other average seasonal lags (5.27 ±2.83 weeks). An average lag of about 2 weeks was calculated for both the peak in MIR for *Cx. pipiens* and the peak MIR for the mammal-biting group, with similar 95% CIs and standard deviations. Moreover, the 95% CIs included a difference of less than one week between peaks for the *Cx. pipiens* MIR and the mammal-biting group MIR. This indicated that peak in MIR sometimes occurred around the same week as the peak in human cases. Overall, the average lags suggested that for the mammal-biting group and the bird-biting group (particularly *Cx. pipiens*), prevalence of virus tended to build up and peak in mosquito species, regardless of host-feeding preference, before number of human cases reached its peak.

<table>
<thead>
<tr>
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<th>Mean Lag in weeks (Standard deviation)</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak abundance of <em>Culex pipiens</em></td>
<td>5.27 (±2.83)</td>
<td>3.37, 7.17</td>
</tr>
<tr>
<td>Peak MIR of <em>Culex pipiens</em></td>
<td>2.00 (±3.13)</td>
<td>-0.10, 4.10</td>
</tr>
<tr>
<td>Peak MIR of mammal-biting group</td>
<td>2.00 (±3.38)</td>
<td>-0.27, 4.27</td>
</tr>
</tbody>
</table>
DISCUSSION

According to descriptive analysis of surveillance data from 2006 to 2017, many of the characteristics of WNV in Connecticut supported the earlier assessments (Andreadis et al. 2004; Andreadis et al. 2012). In past analysis, yearly human cases tended to fluctuate, but generally increased in late July through to September and were highly associated with areas with high populations, such as urban and residential locations (Andreadis et al. 2012). In 2006 to 2017, most cases of human WNV illness were predominantly reported from Fairfield, New Haven, and Hartford Counties, which contain the highest total populations and population densities in the state. Similar to 1998—2003, among mosquito species, *Cx. pipiens* still had the highest abundance and highest WNV isolations among the bird-biting group, whereas *Cx. salinarius* had the highest abundance and highest WNV isolations among the mammal-biting group (Andreadis et al. 2004). *Culex salinarius* has been strongly indicated as a possible bridge vector (Molaei et al. 2006; Andreadis et al. 2012). This species tends to occupy coastal locations (Andreadis et al. 2012). This may explain its abundance since most of the mosquito data was collected from large coastal counties: Fairfield County and New Haven County.

In 2006-2017, abundance among the mammal-biting group reached its peak much earlier in the season, before human cases usually began to increase. Some findings from this descriptive analysis were slightly different compared to earlier years. First WNV isolations were usually reported in July (Andreadis et al. 2004). For 2006-2017, WNV has been detected as early as June, around the same time the first human illness onset date was reported. In 1998—2003, first human illness onset dates were generally reported 5-6 weeks after the first report of WNV isolations (Andreadis et al. 2004).
Based on the Poisson regression model, only the weekly MIR of bird-biting mosquitoes was significant. This suggested that the weekly MIR of bird-biting mosquitoes earlier in the season may be able to predict human disease risk later in the season. Additionally, based on calculation of the seasonal lags, peak MIR among *Cx. pipiens* had an average lag of about 2 weeks (±3.13 weeks) from when human cases peaked. Peak in MIR of mammal-biting vectors had a similar 2-week lag (±3.38 weeks) from peak in human cases. However, MIR of mammal-biting mosquitoes had a negative association with human disease risk. This suggested that an increase in MIR of mammal-biting mosquitoes earlier in the season may either not be useful in predicting human disease risk or may predict a decrease in risk.

MIR of the bird-biting group and the mammal-biting group peaked approximately 2 weeks before the peak in human cases. Incubation period for humans was generally 2-14 days (Colpitts et al. 2012). The average 2-week lags for bird-biting and mammal-biting groups coincided with the average 2-week incubation period for humans, the period between when someone is bit by an infectious mosquito and is infected and when symptoms begin to manifest. For mosquito species, the extrinsic incubation period with WNV ranges widely and can be less than 1 week (at high temperatures) or to 30 or more days post infection (Dohm et al. 2002; Anderson et al. 2008). The extrinsic incubation period is the period from when the mosquito is infected after a bloodmeal to when the mosquito becomes infectious and capable of transmitting the virus to a susceptible host. Based on the Dohm et al. and Anderson et al. studies, the longer the extrinsic incubation period, the higher the probability of transmission. One study reported that within 16-25 days, a 75-100% transmission to a susceptible host was attained (Anderson et al. 2008). The length of this extrinsic period, and also human host immune factors, may account
for the range in the average seasonal lags and for some of the uncertainty in predicting human disease risk.

Why was the MIR of the bird-biting group the most significant and a better predictor for human disease risk, whereas the MIR for the mammal-biting group was not? *Culex pipiens* is characterized as having high vectorial capacity for enzootic transmission and low vectorial capacity for epidemic transmission. Nevertheless, *Cx. pipiens* is significantly associated with human WNV disease risk, despite not usually biting humans (Liu et al. 2009; Talbot et al. 2019). *Culex pipiens* (and, to a lesser degree, *Cx. restuans*) made up the majority of the abundance and WNV isolations. Furthermore, *Cx. pipiens* and *Cx. restuans* have been reported to be associated with about 80% of WNV human infections, based on a risk assessment conducted in New York and New Jersey (Kilpatrick et al. 2005; Andreadis et al. 2012). Focusing control efforts on *Cx. pipiens* and *Cx. resutans* had been proposed previously (Kilpatrick et al. 2005). In the U.S. Northeast, *Cx. pipiens* had been shown to feed on humans occasionally; analysis of blood collected from trap-caught *Cx. pipiens* mosquitoes was mostly from birds, but the presence of human blood meals was reported as well (Andreadis et al. 2004; Molaei et al. 2006). Another possible explanation was that there may be a shift in *Cx. pipiens’* feeding preference from birds to humans later in the season, as proposed by Kilpatrick et al. (2006). Researchers have also proposed that some *Cx. pipiens*, particularly in urban areas, may be hybrid forms of the regular ornithophilic *Cx. pipiens* combined with a more anthropophilic form (*Cx. pipiens molestus*), suggesting that these hybrid forms are biting and transmitting WNV to humans (Andreadis et al. 2004; Kilpatrick et al. 2006; Farajollahi et al. 2011).

Whereas the model did indicate a negative association between MIR for the mammal-biting group and human disease risk, the total WNV isolations were much lower and the
abundance much higher for mammal-biting mosquitoes compared to the bird-biting group. This disproportion may hide the true effect of mammal-biting mosquitoes on human disease risk. MIR for the mammal-biting group may have some power in predicting human cases in a given season, but this was not discerned with the methods used in this analysis. Other measurements of entomological risk necessitate further exploration to investigate the association between mammal-biting mosquitoes and human disease risk.

Other limitations in the current study are acknowledged. Temperature was not controlled in the predictive model. Temperature has a well-known effect on MIR, such as shortening extrinsic incubation; hotter seasonal temperatures also have the effect of extending the transmission season (Dohm et al. 2002; Turell et al. 2005; Talbot et al. 2019). Future modelling of human disease risk must take environmental temperature and overall changes in climate into consideration. Use of MIR to measure virus activity as a predictive indicator of human disease risk also has limitations. MIR values assume that only one mosquito is infected in each pool, and this assumption probably leads to an underestimation of the true infection prevalence during seasons of high transmission (Gu et al. 2008). Therefore, although MIRs provide a useful and simple method to measure virus infection prevalence, the underlying assumptions must be considered when interpreting these results. Additionally, mosquito identification was conducted manually and is therefore subject to some degree of human error—although this usually cannot be easily quantified.

Human WNV cases are widely acknowledged to be underreported (Brownstein et al. 2004; Petersen et al. 2013). Neuro-invasive WNV disease has been a national notifiable disease since 2001 (Lindsey et al. 2010), but the surveillance system for WNV disease in humans is passive and thus not all cases of neuro-invasive disease are tested for WNV. Additionally,
routine testing for WNV is not recommended if presenting with fever and most patients do not seek medical care, further leading to underestimation of true WNV disease prevalence (Petersen et al. 2013). Most human cases were reported from Fairfield, New Haven, and Hartford Counties, all of which have high populations compared to the rest of the state. Fairfield County, in particular, had the most WNV isolations from both the bird-biting and mammal-biting group and the most reported human cases. Fairfield County is an affluent, coastal county with a median household income that is higher than the state median ($73,781). The characteristics of each county may influence health-seeking behavior and, therefore, reporting of WNV illness and total case counts. Overall, analysis using human case data is often underpowered and discussion of results must keep these limitations of using human case data in mind. However, if active surveillance of human WNV illness becomes possible, the actual prevalence of WNV among human populations could be measured.

The type of trap used (gravid or light trap) has been shown to have an effect on the abundance of mosquitoes, as well as the number of WNV isolations and the MIR (Ginsberg et al. 2010; Andreadis et al. 2004). Gravid traps are designed to trap blood-fed female mosquitoes and, therefore, tend to collect more WNV-infected bird-biting species (Cx. pipiens and Cx. resutans in particular) compared to light traps (Andreadis et al. 2004; CAES 2019). Previous studies have looked at infection prevalence of mosquitoes caught in light traps compared to gravid traps. The MIR of Cx. pipiens caught in gravid traps was higher than for those caught in light traps (Ginsberg et al. 2010; Andreadis et al. 2004). The CAES surveillance system changed the bait used in gravid traps in 2006, and this did result in more Cx. pipiens caught in gravid traps and more WNV isolations. This may explain why WNV isolations among the mammal-biting mosquitoes were much lower than bird-biting mosquitoes. For future studies, analysis of
entomological data (abundance, number of WNV isolations, and MIR) among mosquito species collected only in light traps may be useful to assess associations among mammal- and bird-biting groups without the potential bias of trap type.

Currently, the information collected throughout each season is useful as an advanced warning system for when to initiate public advisories and increase preventative interventions to help protect against WNV illness. Based on the results of the analyses herein, entomological indicators are useful for predicting human disease risk, particularly MIR of bird-biting mosquitoes and approximately 2 weeks before expected peak in human cases. Including entomological indicators when modelling human risk in Connecticut may also be useful, but other factors have a strong impact, such as climate, land use/land cover, and population density of hosts; inclusion will greatly improve the ability to predict human cases during WNV seasons. Moreover, more active surveillance of human WNV cases will also increase the effectiveness of modeling and predicting disease risk for future studies.
REFERENCES


https://doi.org/10.1017/S0950268812001070


Appendix A Map references:

“Map of Connecticut with county lines” [https://mapchart.net/usa-counties.html](https://mapchart.net/usa-counties.html) (created and downloaded April 17, 2019).
APPENDIX A: Map of Counties and Mosquito Trapping Stations

Map of study area. Fairfield County, New Haven County, and Hartford County are highlighted in different colors. Blue dots mark trapping sites.