Tracking West Nile virus dynamics using viral loads from trapped mosquitoes

Punya Alahakoon[*], Ian Marchinton[2], Joseph R. Fauver [†], James A. Hay[‡]

[1]Pandemic Sciences Institute, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom.
[2]College of Public Health, Epidemiology Department, University of Nebraska Medical Center, United States.

Abstract

West Nile virus (WNV) persists in an enzootic cycle between birds and mosquitoes. Human infections are incidental and usually sub-clinical with some cases of neuroinvasive disease. Mitigation relies on surveillance to guide decision making, including RT-qPCR testing pools of mosquitoes. Cycle threshold (Ct) values from these pools—semi-quantitative proxies for viral load—are binarised to estimate WNV prevalence in mosquitoes as a proxy for human risk. We showed that Ct value variation in Colorado and Nebraska (2022-2024) cannot be explained by laboratory factors, suggesting a role for biological and epidemiological mechanisms. We developed a multiscale model linking pooled Ct values to mosquito viral kinetics, bird-to-mosquito transmission, and seasonal force of infection. A novel method estimating WNV prevalence using pooled Ct values outperformed existing approaches at high prevalence. We demonstrate the importance of treating environmental viral load samples as quantitative, as binarising masks key information for arbovirus surveillance and risk mitigation.

1 Introduction

West Nile disease is a vector-borne disease caused by infection with West Nile virus (WNV) (Fesce et al., 2023). It is maintained in an enzootic cycle between mosquitoes and birds (Fesce et al., 2023; Pauli et al., 2013), though humans can become incidental hosts (Vogels, Hartemink, & Koenraadt, 2017). Since WNV was first isolated in 1937 in Uganda (Johnston & Conly, 2000), the pathogen has circulated globally, including southern Europe, Africa, Asia, the Middle East and Australia (Pauli et al., 2013). Due to climate change and human-induced activities such as land-use, travel, and urbanisation, there has also been evidence of changes in the ranges of mosquito species in different regions for vector-borne diseases, including WNV (Dekoninck et al., 2011; Kraemer et al., 2019; Lyberger et al., 2025; Mordecai et al., 2019). Before its introduction to the United States (US) in 1999, the severity of the disease among humans was considered low or moderate (Pauli et al., 2013). Since its introduction, there have been

[*]punya.alahakoon@ndm.ox.ac.uk
[†]jfauver@unmc.edu
[‡]james.hay@ndm.ox.ac.uk

more than 60,000 clinical cases of WNV disease reported in the US, more than 30,000 of which resulted in neuroinvasive disease, and nearly 3,000 deaths (CDC, 2025). Despite its severity, human vaccines or specific treatments are not yet available. (CDC, 2024b; Ulbert, 2019). As a result, disease mitigation depends on reducing human exposure to infectious mosquitoes through vector control and public health messaging (Bekele, Uwishema, Nazir, Kaushik, & Wellington, 2023; CDC, 2024b; Nasci & Mutebi, 2019). Therefore, these disease-mitigation interventions must be informed by improved immunological, epidemiological, and environmental parameter estimates of human risk.

Arboviral surveillance comprises both epidemiological and environmental approaches (CDC, 2024a; Cella, Cunha, Alcantara, Lourenço, & Giovanetti, 2025). While epidemiological surveillance is essential to quantify the human disease burden, it is insufficient to predict outbreaks by itself, as human cases are primarily subclinical, cases generally occur during peak transmission periods, and significant lags exist between diagnosis and exposure. Therefore, it is crucial to identify the prevalence of the virus in non-human populations through environmental surveillance as a first indicator of human risk (CDC, 2024a). Although there are various approaches to environmental surveillance, such as bird surveillance and monitoring weather and climatic factors, surveillance in mosquitoes remains the most reliable indicator of human risk during actionable time frames, as diseases can typically be detected in mosquitoes weeks earlier than human cases (Barker, 2019). Vector-based WNV surveillance involves collecting vector species mosquitoes at regular intervals (e.g., weekly, monthly) and testing them for the presence of WNV using molecular approaches (CDC, 2024a). Data gathered through this process enables the estimation of indicators that establish quantifiable thresholds such as vector abundance, infection rate, and vector index for implementing vector control efforts (CDC, 2024a). Consequently, this study focuses on vector-based surveillance and explores strategies to enhance its effectiveness in quantifying WNV within mosquito populations, as well as its implications for the risk of human transmission.

Mosquito-based surveillance largely relies on detecting WNV RNA in pools of trapped mosquitoes using reverse transcription quantitative polymerase chain reaction (RT-qPCR). Although these tests indicate the amount of virus in a pool of mosquitoes as cycle threshold (Ct) values, which are inversely correlated with the quantity of viral RNA, pool statuses are generally analysed as positive or negative for WNV. Consequently, mosquito-based surveillance indicators are impacted by the binarisation of these data, which results in statistical information loss (Cohen, 1983; MacCallum, Zhang, Preacher, & Rucker, 2002). However, it has been shown that using Ct values from pooled test data can improve prevalence estimation for SARS-CoV-2 infection, particularly in resource-constrained settings (Cleary et al., 2021). In addition, recent work has shown that Ct values from virologic RT-qPCR testing vary over the course of an epidemic, reflecting the interaction of epidemic dynamics and within-host kinetics (Hay et al., 2021). In the case of acute respiratory virus infections, this arises because infections are more recent on average during a growing epidemic, which coincides with typically higher viral load, whereas infections are less recent during a declining epidemic, coinciding with typically lower viral load.

Thus, for SARS-CoV-2, the distribution of Ct values can be used to infer epidemic dynamics with some advantages over estimates using tests as only positive or negative (Hay et al., 2021).

WNV dynamics are well-characterized in mosquitoes through laboratory experiments (Moudy, Meola, Morin, Ebel, & Kramer, 2007; Turell et al., 2005; Vogels, Fros, Göertz, Pijlman, & Koenraadt, 2016). These studies demonstrate that WNV titers in mosquitoes grow steadily following ingestion of an infectious bloodmeal, midgut infection, and viral dissemination. Importantly, mosquitoes are unable to clear viral infection and therefore maintain high viral titers throughout their lives (*FAQ: West Nile Virus*, 2013; Lee, Webster, Madzokere, Stephenson, & Herrero, 2019). WNV natural history in mosquito populations then suggest viral quantities sampled during cross-sectional entomological surveillance can provide insights into epidemic growth, prevalence in mosquito populations, and age-structure of infected mosquitoes, all crucial indicators of vector-borne disease risk. How the interaction between epidemic dynamics, within-host kinetics and sampling strategy determines the distribution of observed Ct values from entomological surveillance data for arboviruses such as WNV has not yet been explored.

Here, we analysed WNV mosquito surveillance data from Nebraska and Colorado, two high-incidence states with robust mosquito surveillance programs in the US. These weekly data consisted of pooled RT-qPCR test results and consisted of binary (positive/ negative) results as well as pooled Ct values. We sought to propose biological mechanisms which might explain observed variation in pooled Ct values. In particular, we observed a bimodality in the observed Ct value distributions, where the majority of the Ct values were low (high viral loads), but high Ct values (low viral loads) were still observable, which are unlikely to be explained by dilution or laboratory effects. To understand mechanisms which might explain observed variation in WNV Ct values over time, we developed a multi-scale agent-based model that integrated population-level dynamics and viral load kinetics of mosquitoes and birds. We implemented a synthetic sampling strategy to mimic mosquito surveillance and the RT-qPCR testing process, generating pooled Ct values consistent with surveillance data from Nebraska and Colorado. Through the generation of data that explained biological mechanisms, we investigated if pooled Ct values can serve as a reliable data source for accurately estimating mosquito prevalence in a population.

2 Results

2.1 Substantial variation in WNV quantities from entomological surveillance data

We analysed entomological trap data tested for West Nile virus (WNV) using RT-qPCR (Lanciotti et al., 2000) collected through routine environmental surveillance in Nebraska (statewide) from weeks 23-37 in 2022 to 2024, and Colorado (Larimer County) from weeks 23-37 in 2022 and 2023. In Nebraska, pools either combined *Culex pipiens/restuans/salinarius* or *Culex tarsalis*, whereas in Colorado, pools contained either only *Culex tarsalis* or *Culex pipiens*. For the purpose of measuring WNV prevalence, we combined data from all pools regardless of species. Ultimately, our data comprises of Ct values from thousands of pools of mosquitoes over 3 trapping seasons across two localities. Figure 1 presents

the pooled Ct values (panel (A)) obtained from these species and the corresponding positive/negative RT-qPCR result (panel (B)). See Supplementary Material S10 for a visualisation and distribution of Ct values of other species and to potential relationships between Ct values and other factors.

We observed significant variability in Ct values within the limit of detection (Figure 1). Further experiments and context confirm that this variation is likely genuine as opposed to a laboratory artefact. First, the variation we observe is consistent across five trapping seasons and two states. The surveillance data presented in this study come from two independent laboratories, one of which is a certified state public health laboratory. Both laboratories implement rigorous controls, including no template controls in both extractions and RT-qPCRs.

Second, the variation in Ct values is dramatic, ranging from 10's to 10's of millions of copies of WNV RNA in individual pools (Figure S4). Although pooling samples would not reduce the amount of target nucleic acid in a sample, we tested whether our Ct value data may be biased by the pooling of mosquitoes by reducing the sensitivity of the RT-qPCR assay. We hypothesised that if pooling had a strong effect on WNV Ct values, we would systematically observe higher Ct values and more false negatives in pools with more mosquitoes as the total amount of WNV RNA from a positive mosquito body would be diluted with additional WNV-negative mosquito tissue. To test whether there was an effect of pool size on Ct values, we conducted a correlation test between pool size and Ct value and found no association (Pearson correlation coefficient = 0.04, p-value = 0.31, Figure S16). A pool size of 50 is the most common pool size in our dataset and the Ct values from the Nebraska data ranged from 19.4-39.2 in pools of 50 mosquitoes, and 16.8-39.4 in pools of 25 or fewer mosquitoes (Figure S16). These results indicate that pooling mosquitoes is unlikely to reduce the sensitivity of RT-qPCR assays to detect WNV RNA.

Finally, to determine the reproducibility of these data, a subset of 143 WNV-positive pools from Nebraska were retested in a separate research laboratory at University of Nebraska Medical Center (UNMC). The average Ct value difference between samples tested in both labs was -2.26, or a ≈10-fold difference in measured RNA quantity, with Ct values consistently being lower in the independent laboratory (Figure S17). A total of 14 samples tested negative at the UNMC laboratory, and the average Ct value of these samples was 38.24. Ct values from the UNMC laboratory were consistently lower, however the reduction was consistent across the entire range of Ct values, highlighting the reproducibility of WNV CT values from mosquito pools. Taken together, the Ct values produced from routine WNV surveillance represent genuine variability in WNV RNA from mosquitoes.

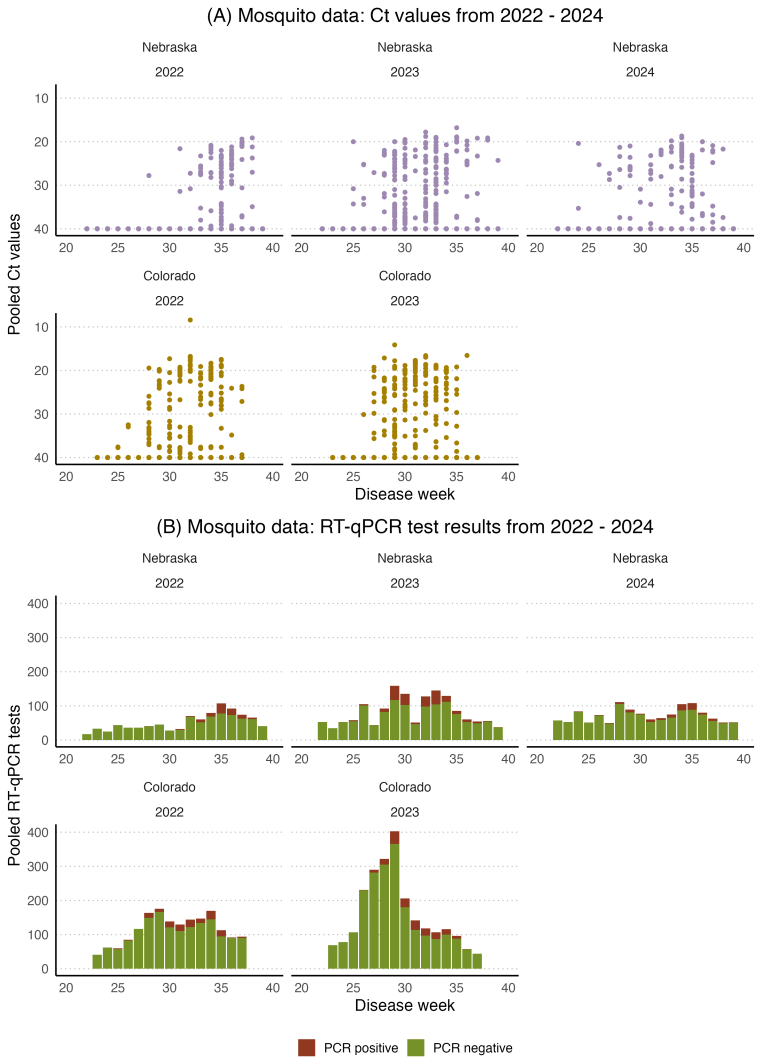

Figure 1: Observed data: Calculated pooled Ct value vs. disease week from 2022 to 2024. Pooled Ct values presented here are calculated by using species *Culex pipiens/restuans/salinarius* and *Culex tarsalis*.

After ruling out laboratory effects, we were interested in investigating potential mechanisms which might explain observed variation in pooled Ct values. Viral loads in infected mosquitoes are driven by multiple factors, including infectious dose, temperature, virus genotype, and time point of collection during the extrinsic incubation period (EIP) (Kilpatrick, Meola, Moudy, & Kramer, 2008; Moudy et al., 2007; Richards, Mores, Lord, & Tabachnick, 2007; Shocket et al., 2020). However, we would expect host-seeking mosquitoes infected with WNV to have high viral loads (low Ct values) due to viral replication during the length of time between bloodmeals. Given the substantial variability observed in Ct values from host-seeking mosquitoes (Figure 1), we hypothesised that this variation may be due to biological and epidemiological factors, such as variability in the viral kinetics in mosquitoes and mosquitoes being captured at different times post-infection. Accordingly, we developed an agent-based model that captured these mechanisms, enabling us to explore and compare these hypotheses with the observed data to those generated by our model.

Our agent-based model, as outlined in Section 5 and illustrated in Figure 2, was used to simulate population-level and viral load dynamics of mosquitoes and birds. Overall transmission dynamics were based on the Ross-McDonald model(Ross, 1911), describing the transmission cycle between bird hosts and mosquito vectors (Figure 2 (A), (B) & (D). We embedded within-host models of virus kinetics for mosquitoes and birds describing how WNV viral loads change over time since infection, and vary between individuals (Figure 2 (C)). The model also incorporates host seeking behaviour and key environmental factors such as seasonality and overwintering. Using this model, we simulated the trapping and pooling process to generate Ct values from pools of up to 50 mosquitoes to match the real-world surveillance data (Figure 2 (E); further details are in Section 5 and the Supplementary Material). The outputs of the model are simulated pooled mosquito Ct values for WNV, collected weekly over the course of multiple seasons and reflecting underlying WNV infection prevalence in the mosquito population (Figure 2 (F)). We parameterised our agent-based model to recreate WNV prevalence patterns observed in the Colorado and Nebraska surveillance data (Supplementary Material).

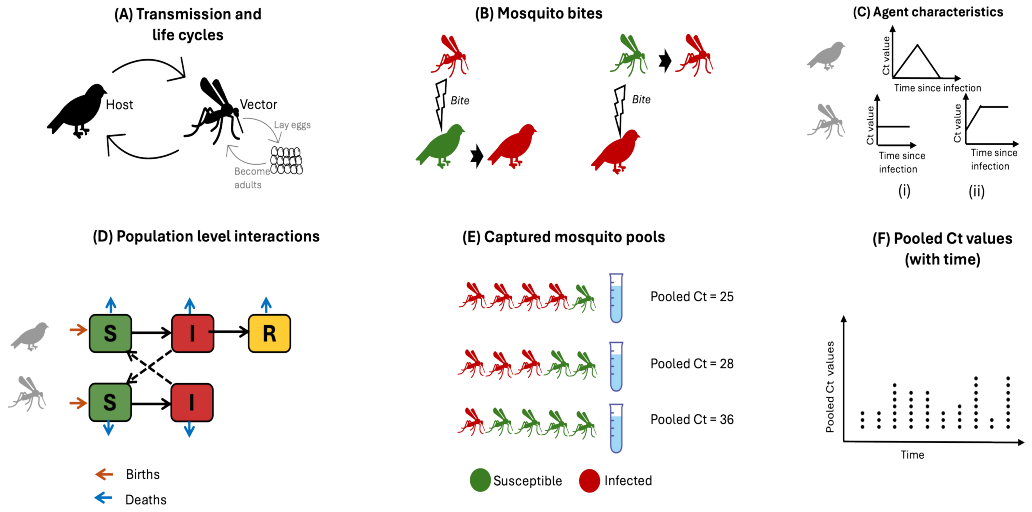

Figure 2: Overall structure of the agent-based model.
Panel (A): Transmission cycle of WNV between birds (hosts) and mosquitoes (vectors). Female mosquitoes lay eggs, which hatch after an average of 8 days, before becoming female adult mosquitoes that contribute to the transmission cycle.
Panel (B): Transmission occurs with some probability when an infected female mosquito bites a susceptible bird, or a susceptible female mosquito bites an infected bird.
Panel (C): Within-host viral kinetics model. Infected birds follow an acute viral load trajectory with rising and clearance phases, initiating from the RT-qPCR limit of detection. Female mosquitoes may follow one of two different viral kinetics models: the mosquito's initial viral load is proportional to the infector bird's viral load at the time of bite and either leads to a non-replicating viral trajectory (static viral load) (i) or a replication-competent infection (dynamic viral load) (ii).
Panel (D): Overall population-level dynamics of the agent-based model. If bitten by an infected mosquito, susceptible birds (S) can become infectious (I). Once infectious, they will eventually recover (R) with lifelong immunity following their infectious period. Susceptible mosquitoes (S), if they bite infectious birds, will become infectious (I) for the rest of their lifespan. Births and deaths are possible in all these states for both birds and mosquitoes.
Panel (E): Simulation of routine mosquito trapping and pooled testing accounting for blood meal-seeking behaviour. For each pool, a combined pooled Ct value is calculated as the mean viral load of captured WNV-positive mosquitoes, diluted by the contribution of WNV-negative mosquitoes.
Panel (F): Example population-level Ct dynamics from the simulation.

2.2 WNV population-level viral loads can be explained by lack of viral replication in mosquitoes

As shown in Figure 2(C), we have assumed that the mosquito's initial viral load is proportional to the infector bird's viral load at the time of bite, and this may lead to a mosquito infection with a viral kinetics curve of non-replicating virus (static viral load) or of replicating virus (dynamic viral load). Under this assumption, we hypothesised that when a susceptible mosquito bites an infected bird, it may ingest a certain percentage of the viral load from the bird. Following virus exposure, the mosquito may either maintain this viral load until its death or capture (non-productive infection), or experience an increase in viral load with time and remain at the peak level until its death or capture as observed in laboratory studies (productive infection). High Ct value samples might arise from mosquitoes harbouring low levels of virus in their bodies without active replication occurring, for example, by ingesting non-replicating viral material from the blood of infected birds. This would have implications for interpreting positive pools, as it would suggest that not all positive pools reflect actively infected mosquitoes.

To test this, as well as to identify which proportion of the host virus is being ingested by a mosquito and to identify the most likely post-infection trajectory (non-productive vs. productive infections), we systematically varied two model parameters: the proportion of viral load ingested and the probability of each mosquito's post-infection trajectory (static vs. dynamic viral loads). We conducted simulations with different combinations of these values, and for each combination of the simulated data, we repeated the mosquito capturing process and sampled pooled Ct values. Finally, we compared the density distributions of the pooled Ct values that fell within the limit of detection and compared them to the observations in Nebraska and Colorado from 2022 to 2024.

We observed that the distributions of the Ct values changed significantly when both the probability of the mosquitoes initiating either a productive or non-productive infection, and the amount of viral inheritance changed (Supplementary Material S6). Notably, we observed that when the proportion viral load inherited from birds was set to 100% and the probability of a productive or non-productive infection in the mosquitoes was 0.5, pooled Ct values of the captured mosquitoes better aligned with the observed data from Nebraska and Colorado, capturing a bimodal distribution of low Ct values (from productively infected mosquitoes) and high Ct values (from non-productively infected mosquitoes) (Figure 3 (B), (C) & (D); Supplementary Material S6, Figure S10). Consequently, hereafter, our analysis is carried out based on the assumption that 100% of the viral load is ingested from the bird to the mosquito at the time of a bite and that there is an equally likely chance that the post-infection trajectory of the mosquitoes is static or dynamic.

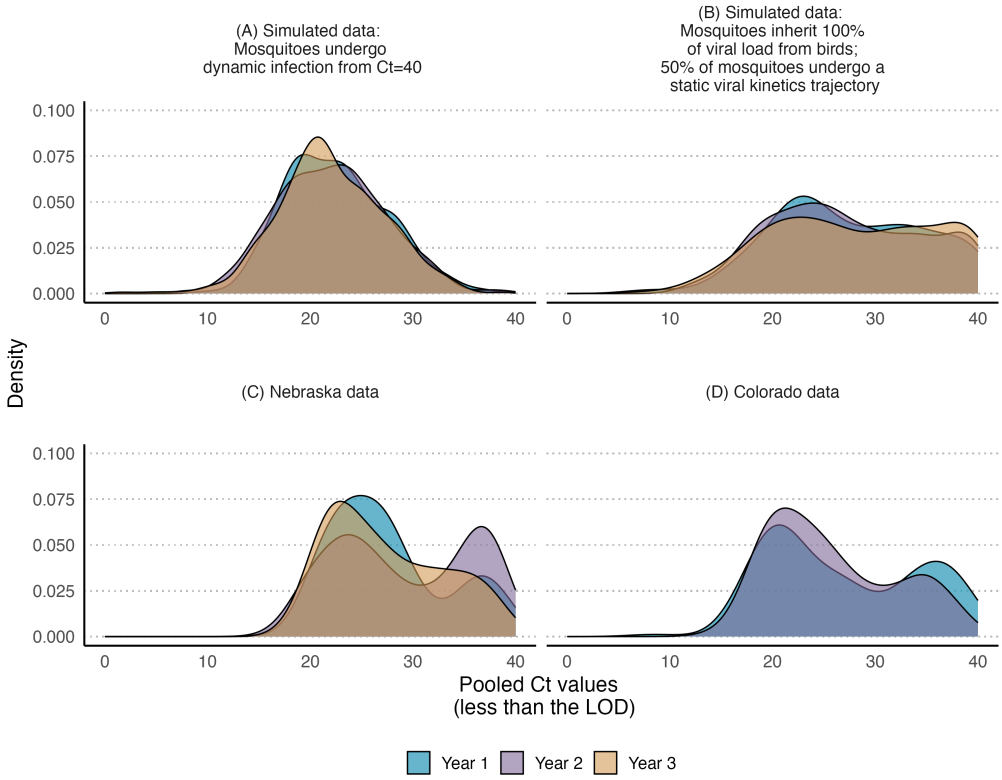

Figure 3: Distributions of the pooled Ct values within the limit of detection. The top left panel (Panel (A)) illustrates the simulated Ct values by assuming all mosquitoes undergo dynamic infection from Ct=40. The top right panel (Panel (B)) shows simulated Ct values assuming mosquitoes inherit 100% of viral load from birds, and 50% of mosquitoes undergo a static viral kinetics trajectory. The bottom left and right panels (Panel (C)) illustrate the density of pooled Ct values from Nebraska and Colorado states over three years, respectively.

2.3 Simulated population-level WNV viral loads in mosquitoes are varied but not correlated with time-varying epidemic dynamics

We used our agent-based model to simulate population-level and within-host-level viral load dynamics of birds and mosquitoes over multiple WNV seasons (Figure 4). We ran the simulation four times, and each simulation was run for twenty-six years, with the first year excluded as a burn-in period due to the atypical dynamics expected following initial WNV introduction into a fully susceptible population (Di Giallonardo et al., 2015; Petersen, 2019). This resulted in 100 one-year WNV epidemics. Full simulation dynamics are shown in Supplementary Material S7. The model generated peak WNV infection prevalence of 3-4% in mosquitoes and peak infection incidence of 0.17-0.36% in week 29-36, coinciding with the summer months in the US (Supplementary Material S7). As a post-processing step from the simulations, we emulated the mosquito trapping process and calculated the pooled Ct values, as detailed in Section 5. Mosquito capture was simulated based on the data observed in Nebraska (Supplementary Material) and therefore, the simulated pooled Ct values were calculated in weeks 22 to 39.

Unlike in many acute viral infections in humans, where viral load initially increases but then decreases as the infection is cleared (Hay et al., 2022; Kissler et al., 2021, 2023), productively infected mosquitoes

quickly reach and maintain a high WNV viral load once infected until death. This is reflected in the population Ct value distribution, which does not change over time, both assuming perfect individual-level testing and through trapping and pooled testing (Figure 4C&D). For time variation in the population-level mean Ct value to arise, two conditions must be met (Hay et al., 2021). First, there must be variation in the time-since-infection distribution over time, which is guaranteed in an epidemic with a growth and decline phase; the average time-since-infection is lower in a growing epidemic than a declining one. Second, the expected Ct value must vary over time-since-infection, such that recently infected individuals are distinguishable (on average) from individuals further into their infection based on their viral load. Although the first condition is met here, the variation in viral load over time-since-infection is small for WNV infection in mosquitoes, and thus the population-level Ct distribution is static. Furthermore, for a WNV-positive mosquito to be captured in the pool, it must have already obtained one blood meal from an infected bird and be seeking its next blood meal, which introduces a delay between infection and being captured. This shifts the time-since-infection distribution of captured mosquitoes beyond the initial viral growth phase into the period of constant, high viral load. Thus, pooled Ct values for WNV reflect individual-level variation in mosquito set-point viral loads, but do not vary depending on the phase of the epidemic.

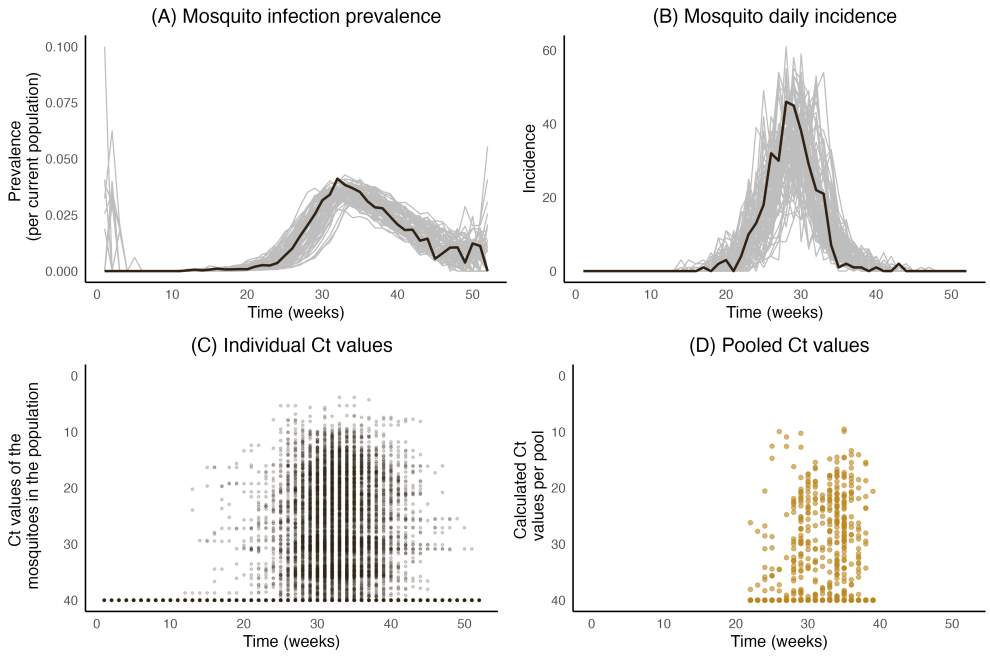

Figure 4: Simulated population-level WNV infection dynamics in mosquitoes. Each line represents one year of simulated data, and the bold black line highlights the simulated trajectory used for Panel (C).
Panel (A): Weekly mosquito infection prevalence per capita.
Panel (B): Weekly mosquito infection incidence per capita.
Panel (C): Individual-level Ct values of the female mosquito population from one year of a single simulation. Each point represents a Ct value from a single randomly selected mosquito, representing perfect observations of the entire population at all times.
Panel (D): Calculated pooled Ct values from the mosquito population based on the individual-level Ct values in (C)

2.4 Calculating WNV infection prevalence using pooled Ct values

Next, using the calculated pooled Ct values from the simulated captured mosquitoes, we developed a method to estimate WNV infection prevalence in mosquitoes. Based on a previous method developed for SARS-CoV-2 prevalence estimation, the method uses the expected distribution of Ct values for pools of a given size, a given number of positives, dilution effects, measurement error and variation in viral loads from infected mosquitoes(Cleary et al., 2021). We fitted smoothed kernel density curves to our simulated viral loads to generate empirical distributions of Ct values for each combination of pool size and number of positive mosquitoes. Our approach uses these density curves to construct a likelihood function for the population prevalence, conditional on the Ct values from multiple pooled test results. Using a maximum likelihood estimation method, we estimated the prevalence per week and the corresponding confidence intervals. This prevalence estimation process is explained in more detail in Section 5.4 and Supplementary Material S8.

To estimate the prevalence, we used the calculated pooled Ct values presented in Figure 4 based on the simulated data (illustrated again in Panel (A) of Figure 5). Using these pooled Ct values, we compared the estimates of the prevalence of WNV infection based on our method (Panel (B) of Figure 5) and the existing gold standard method, treating the pooled test results as positive or negative to estimate the prevalence (Panel (C) of Figure 5), with the true prevalence of WNV infection from the simulation. We binarised the Ct values based on whether or not the values were below the limit of detection (Panel (B) of Figure 5), noting that it is possible, but unlikely, for an infected mosquito to have a Ct value greater than the limit of detection.

We carried out prevalence estimation based on the binarised (positive/ negative to WNV) using the CDC *PooledInfRate* R package (Biggerstaff, 2025). This package is based on the work by Hepworth and Biggerstaff (2017), which presents a biased corrected estimator applied to the Maximum Likelihood Estimator (MLE) for the proportions by pooled testing. Here, the MLE is constructed based on the assumption that individuals in the pools follow independent and identically distributed Bernoulli distributions, and once tested, the pooled tests follow a Binomial distribution.

Overall, we observed that both estimation methods yielded similar results (see Supplementary Material for prevalence estimation for multiple simulations) and were able to estimate the mosquito prevalence when sufficient data were available. We observed that under both estimation methods, although the point estimates were sometimes not accurate—likely due to sampling variation where the sample is a poor representation of the population prevalence—the 95% confidence intervals contained the true prevalence values.

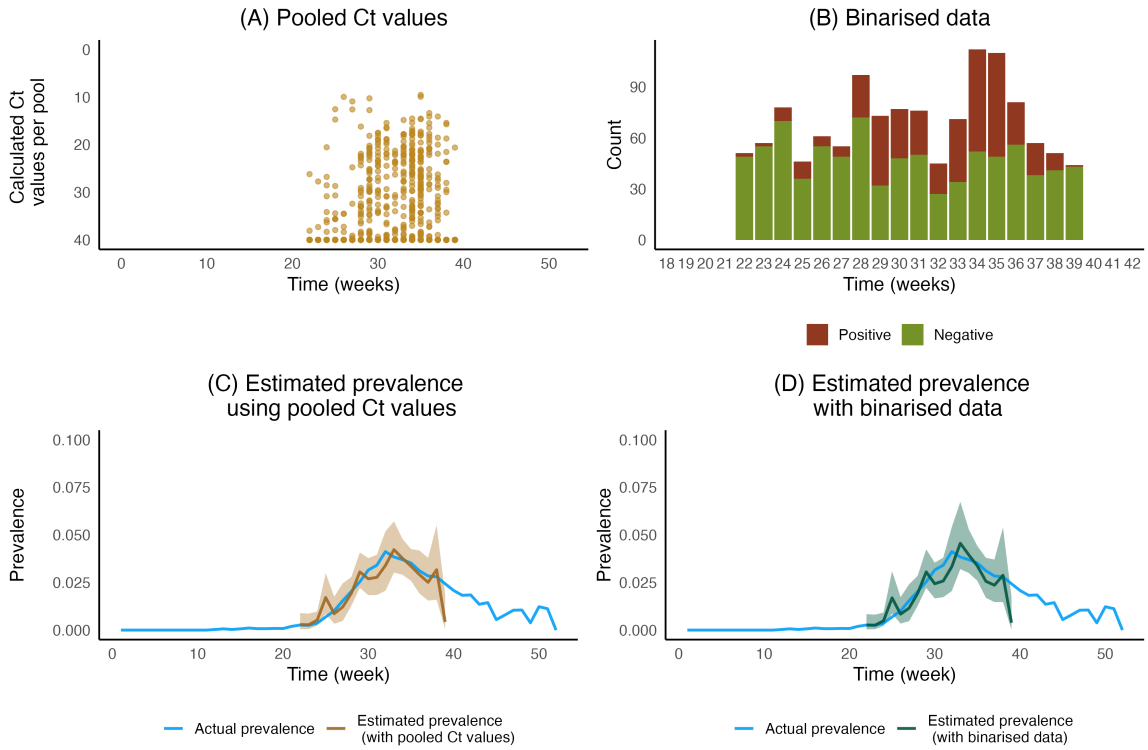

Figure 5: **Panel (A)**: Weekly pooled Ct values from the captured mosquitoes (using the sampling procedure used in the agent-based model). Each dot represents the Ct value calculated per pool.
Panel (B): Binarised data based on the pooled Ct values in Panel (A). Binarisation was based on whether or not the pooled Ct values were within the limit of detection.
Panel (C): Estimated weekly WNV infection prevalence among mosquitoes using the pooled Ct values (beige line). The beige ribbon shows the 95% confidence interval.
Panel (D): The estimated weekly WNV prevalence among mosquitoes using the binarised data with the *PooledInfRate* R package (green line). The green ribbon shows the 95% confidence interval. These values are compared with the true prevalence from the simulation (blue line).

2.4.1 Potential effects of pool size and number of pools on the accuracy of prevalence estimation

While both the *PooledInfRate* R package based on binarised data and our method of using pooled Ct values estimated the prevalence generally well, rather than depending on the estimation performance over a single epidemic trajectory, we were interested in understanding the overall power and bias, accounting for pool size, number of pools, and the true prevalence. To study this, we conducted an analysis using the simulated data from the agent-based model, simulating pooled Ct values and binarised results with assumed prevalence values ranging from 0.001 to 0.8 for different pool and sample size combinations (Supplementary Material S9). Figure 6 plots the estimated prevalence against the true prevalence using simulations based on datasets of 100 pools. We observed a significant contrast between the two methods to estimate the prevalence at different levels. The *PooledInfRate* package could not estimate the prevalence effectively as the actual prevalence and pool size increased. Specifically, with 50 mosquitoes per pool, we observed that the *PooledInfRate* failed to estimate the prevalence beyond 0.155. In contrast, using pooled Ct values allowed prevalence to be estimated consistently, regardless of the

actual prevalence in the mosquito population. This is because with large pool sizes, a method treating pools as positive or negative fails at higher prevalence, as almost all pools are expected to include at least one infected mosquito, whereas the Ct value-based method is still able to distinguish between higher and lower prevalences even if all pools are positive. In both cases, the accuracy of the prevalence estimates improved as the number of pools increased.

We also investigated the impact of pool size, number of pools, and inference method on accuracy (difference between the MLE estimate and true prevalence) and coverage probability (whether the true prevalence is within the 95% confidence interval) (Supplementary Material S9). We found that the Ct-based method was unbiased at all pool sizes and number of pools, and became more accurate both with increasing number of pools (up to 500 pools) and with larger pools of up to 200 mosquitoes (Figure S13). Furthermore, we found that coverage was very high for both the Ct-based and binary-based estimates for smaller pools, though coverage of the binary-based estimates degraded as pool size increased, particularly with a large number of pools (Figure S14) when the true prevalence increased.

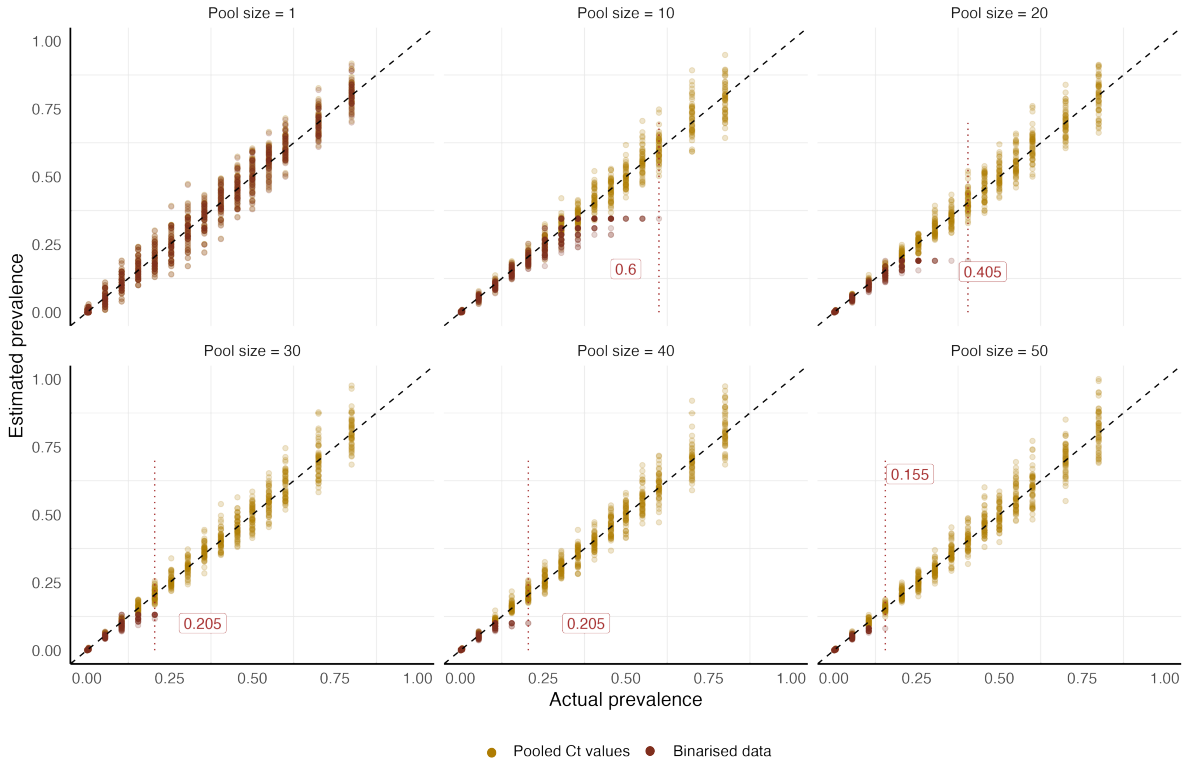

Figure 6: Comparison of prevalence estimates based on the Pooled Ct values and the *PooledInfRate* R package with varying pool sizes. The beige colour dots present maximum likelihood prevalence estimates calculated from pooled Ct values from a different simulation. The estimated prevalence calculated using the binarised data is in dark red. Each panel presents the estimates based on 100 pools containing up to 50 mosquitoes (pool size). The black dashed line represents perfect estimates. Labels along with the red dotted lines illustrate the maximum prevalence level at which the *PooledInfRate* R package returns accurate estimates.

3 Estimating WNV prevalence in the mosquito population based on RT-qPCR testing data from Nebraska and Colorado

Our model suggests that observed pooled Ct values for WNV in Nebraska and Colorado can be explained by mosquitoes inheriting initial viral loads from the infected birds, coupled with a 50% chance of of initiating a productive infection. Therefore, using the simulated pooled Ct values under this model (Supplementary Material S9), we estimated the mosquito prevalence for Colorado and Nebraska from 2022 to 2024, comparing our method using Ct value distributions with estimates based on binary pooled status (Figure 7). Overall, we observed that under both estimation methods, peak WNV infection prevalence remained below 0.014 [(0.009,0.022) 95% confidence intervals] for Nebraska and 0.013 [(0.008, 0.02) 95% confidence intervals] for Colorado in years 2022 and 2023 respectively. Furthermore, we noticed that the general prevalence estimates calculated using the pooled Ct values and those estimated using the binarised data from the *PooledInfRate* R package remained similar, but the 95% confidence intervals estimated using the binarised data on some weeks were slightly wider than those estimated using pooled Ct values.

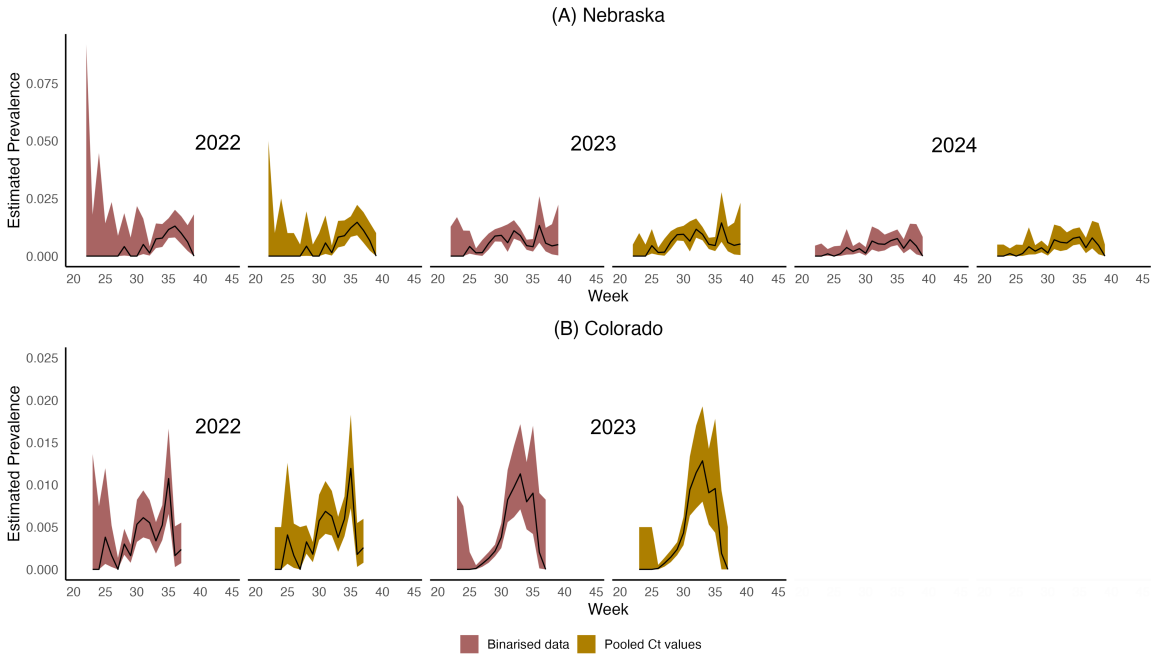

Figure 7: Prevalence estimation per year for Nebraska and Colorado from 2022-2024 using pooled Ct values (beige) and binarised data (red). Black lines show mean estimates. Beige and red ribbons are 95% confidence intervals.

4 Discussion

In this study, we used WNV Ct value data generated from routine entomological surveillance to model complex vector/virus interactions and improve estimates of WNV prevalence in mosquito populations. We developed an agent-based model capturing transmission dynamics between mosquito vectors and

bird hosts, heterogenous individual-level viral kinetics, and a realistic model for trapping and pooling mosquitoes. Based on comparison between our simulated data with real-world surveillance data from Nebraska and Colorado, we suggest that variation in WNV Ct values from mosquito pools might be explained by detecting a mixture of productively infected mosquitoes with high viral load and mosquitoes harbouring low-levels of non-productive viral material. Furthermore, because the model was able to reproduce realistic pooled Ct value distributions, we were able to develop a new method for estimating WNV infection prevalence from mosquito pools using Ct values rather than binary status.

Initial observations from entomological surveillance of five transmission seasons in Nebraska and Colorado revealed substantial variation in Ct value data produced from pools of mosquitoes that test positive for the presence of WNV RNA by RT-qPCR. We were able to determine that laboratory artefacts or dilution effects due to pooling were unlikely to account for the variation observed. Thus, we concluded that the observed Ct values are the result of biological processes. Unlike for SARS-CoV-2 infection in humans, where individuals experience a viral growth phase before generally clearing the virus (Puhach, Meyer, & Eckerle, 2023), WNV viral loads in infected mosquitoes rapidly reach and remain at high levels following infection (Dohm, O'Guinn, & Turell, 2002; Fesce et al., 2023; Reisen, Fang, & Martinez, 2006; Vogels et al., 2016), and thus the population-level Ct value distribution is not correlated with the epidemic growth rate. Nonetheless, we showed that population-level viral loads still reflect the interaction of epidemic dynamics and within-host viral kinetics; binarising pooled virologic surveillance data masks this information, which may be important for correct interpretation.

In this system, our results suggest that some low-viral load pools may contain mosquitoes that are incapable of transmitting the virus. This could be explained by a variety of factors, including mosquitoes being refractory to infection due to tissue escape barriers (Carpenter & Clem, 2023; Franz, Kantor, Passarelli, & Clem, 2015; Johnson et al., 2024), relative infectiousness of viremic birds (Vaughan, Newman, & Turell, 2022), or timing of mosquito collection in relation to the EIP. The ladder is unlikely to account for the observed variation, however, as all Ct value data presented were generated from host-seeking mosquitoes collected via CDC Light Traps, meaning that WNV-positive mosquitoes were exposed to viraemic blood meals days or weeks prior to their collection. The low viral amounts detected in mosquito pools may then be the result of the virus failing to initiate a productive infection, potentially due to virus and vector factors, implying that mosquitoes with high Ct values may not be able to transmit WNV. Our results highlight the importance of considering variation in the individual-level virus/mosquito dynamics.

Multi-scale modelling techniques (Dada & Mendes, 2011) allow propagating individual-level biology and variation into population-level dynamics—this approach underpins the methodological aspect of this study. WNV infection in mosquitoes is a complex process where, upon ingestion of an infectious bloodmeal, the virus must initiate an infection in the epithelial cells of the mosquito midgut, replicate, and disseminate through the haemocoel prior to infecting salivary glands to perpetuate transmission (Rückert & Ebel, 2018). Laboratory studies show WNV infection and growth rate in mosquitoes are not

uniform, and the ability of mosquitoes to transmit virus is influenced by multiple factors (Kilpatrick et al., 2008), including temperature(Shocket et al., 2020), host viremia (Komar et al., 2003), and genotype of the virus (Moudy et al., 2007) and vector (Vogels et al., 2016). These factors result in heterogeneous mosquito infection kinetics, which is captured by our agent-based model. Furthermore, our data suggest that there is substantial variation in WNV infection kinetics, and therefore, potentially rates of infectiousness, between mosquitoes exposed to WNV. From our model, we found that the bimodal distribution of Ct values could be explained by a significant proportion of RT-qPCR positive mosquitoes having low levels of WNV RNA without necessarily being productively infected. The low levels of virus are contrary to what we would expect given the time since infection and when these mosquitoes are caught (host-seeking traps). These insights allowed us to develop a synthetic surveillance system derived from our agent-based model, whilst offering a better understanding of transmission dynamics in mosquito populations.

Using this synthetic surveillance system, we evaluated the existing methodology as well as our method of using pooled Ct values to estimate the prevalence. We showed that pooled Ct values can be used to estimate WNV prevalence in a mosquito population, at all prevalence levels, regardless of the pool size or the number of pools. The existing binary data only method implemented in the *PooledInfRate* R package and our method using pooled Ct values to estimate mosquito prevalence when the true prevalence is low (less than 0.155, with 100 pools of size 50), performed similarly in accurately estimating underlying infection prevalence. We further observed that both methods yielded similar prevalence estimates for the Nebraska and Colorado data. However, the Ct-based method performed well across all prevalence levels, particularly when both the pool size and the number of pools are high. The Ct-based approach may therefore be more suitable in settings where pool sizes are large (e.g., more than 50 mosquitoes per pool), or where underlying prevalence might exceed 10%, which can occur during outbreak years (Fauver et al., 2016; Kretschmer, 2023; Martinez et al., 2017).Furthermore, we found that when using pooled Ct values, increasing both pool size and the number of pools improves accuracy (see Figure S13). In contrast, when using binarised data, the pool size does influence estimate accuracy. As shown in Figure 6 and Figure S14, the maximum identifiable prevalence with binarised data decreases as the pool size increases. Additionally, in cases where all the pools are positive, the binary data only method cannot distinguish between different prevalence levels, whereas the use of pooled Ct values will continue to correctly estimate the prevalence, as there is still information stored as a form of viral material in the pooled Ct value. Overall, our method remains robust across both high and low mosquito abundance periods (summer and winter), adapting effectively to varying pool numbers and sizes, unlike the binary-only approach.

Throughout this study, we based our inferences on synthetic Ct value distributions generated by our agent-based model, which leads to a number of limitations. While our estimates aligned well with those derived from binary data, we parametrised the viral kinetic models for both birds and mosquitoes using values from the literature (Pérez-Ramírez, Llorente, & Jiménez-Clavero, 2014), and more robust results

would require data from deliberate infection and pooling experiments. For instance, experimentally deriving the distribution of Ct values for k positives in a pool of n would provide more realistic Ct value distributions than simulation alone. Our model also simplified the system by treating all mosquito and bird species interchangeably, assuming a single vector and a single host species. In reality, variation in viral kinetics between different species likely affect viral load kinetics, especially in regions where multiple vectors play a role in driving transmission. We attempted to parametrise the ecological aspects of the agent-based model using the literature. Still, we note that some parameters, particularly relating to overwintering, are not well defined. Finally, in this study, we did not conduct any formal model fitting to the data using our agent-based model. Our main objective of this study was not to formally fit the multi-scale agent-based model to the data, but rather to understand how individual-level viral kinetics in mosquitoes are reflected in pooled surveillance data, and to demonstrate the use of pooled Ct values in estimating WNV infection prevalence. However, it would be helpful to conduct formal model fitting, for example using an emulator (Iskauskas et al., 2024), and then replicate the experiment to test the viral inheritance hypothesis to further solidify our results.

There is a robust literature regarding the vector competence—the ability of a mosquito to transmit a pathogen—of WNV in relevant vector species; however, no studies have explicitly made the connection between Ct values, replication kinetics and infectiousness in mosquitoes. Exposing cohorts of mosquitoes to a range of viremias or allowing mosquitoes to feed on birds through a course of infection and taking daily measurements of virus replication, dissemination, and infection potential would provide high-resolution insights into viral kinetics in mosquitoes and the relationship between infectiousness and Ct values. In our model, a key future direction for WNV surveillance is to better interpret the distribution of Ct values as a mixture of both infected and infectious mosquitoes. Population-level modelling, as we have done here, is a key step towards providing plausible mechanisms and frameworks for interpreting this variability, but deliberate laboratory-based infection experiments in mosquitoes to better understand the relationship between Ct values and infectivity will be necessary to confirm its impact on transmission dynamics.

WNV transmission, like other arboviruses, is heterogenous in time and space, making timely entomological surveillance an invaluable tool for understanding transmission risk and informing control(Barker, 2019). Increasing WNV prevalence in mosquito populations is a reliable indicator of increased human risk in the near future and is often used to initiate WNV control mechanisms such as spraying insecticides or asking the public to modify behaviours (Fauver et al., 2016; Kilpatrick & Pape, 2013; Kwan et al., 2012; Winters et al., 2008). Robust estimates of mosquito prevalence across a range of surveillance approaches and epidemiological scenarios are therefore crucial to inform public health policy. As arbovirus surveillance is routinely done via RT-qPCR, Ct values produced from these assays represent an untapped dataset to better understand transmission dynamics and inform human risk that comes at no additional cost to public health entities. This study represents the first effort to understand and model Ct value dynamics observed in WNV entomological surveillance and use these data to produce

more refined estimates of WNV prevalence in mosquito populations. The results of this study indicate that viral kinetics in natural mosquito populations are not uniform and binary interpretations of WNV surveillance likely miscalculate mosquito infection rates and, therefore, estimates of human risk.

Although our main focus here is on WNV due to the robust, routine entomological surveillance systems that exist in the US, these ideas may be generalisable for surveillance of other arboviruses such as chikungunya, dengue, and Zika virus (dos Reis et al., 2019; Vairo et al., 2018) that use semi-quantitative viral load data, particularly through pooled testing. Our approach might be generalisable for other data sources, particularly next-generation surveillance approaches such as metagenomics, which allows sequencing microbial communities from environmental samples (Bragg & Tyson, 2014). As these methods allow for quantification of the viral load contained in a sample (Bergner et al., 2025; Bonsall et al., 2015; Goh et al., 2019), it may be possible to extend our method as a tool to identify transmission processes within wildlife populations for multiple pathogens simultaneously.

Arboviruses are a persistent and growing threat to global public health. Due to climate change, globalisation, and human activities, the distribution of arbovirus vectors continues to expand (Kraemer et al., 2019). For example, warmer temperatures in Europe have seen local outbreaks of dengue, chikungunya, WNV, yellow fever, and malaria, and it is predicted that they will continue to spread throughout Europe (Liu-Helmersson, Rocklöv, Sewe, & Brännström, 2019; Watts et al., 2021). Furthermore, as we have discussed and illustrated, there is still a large gap in the literature in understanding WNV (and other vector-borne diseases) ecology and biology. Vector-borne diseases are part of the complex ecological processes, and they are highly sensitive to environmental changes, non-human reservoirs, and human and non-human activities (Mack et al., 2008). Therefore, an interdisciplinary and multi scale approach to understand the interaction of between-and within-host biology of vector-borne diseases, environmental, and ecological processes is necessary to improve surveillance and optimise public health interventions.

5 Materials and Methods

5.1 Mosquito Collections and WNV Ct value Data

For both Colorado and Nebraska, CO_2-baited CDC light traps are set at multiple sites to collect host-seeking mosquitoes. Traps are set weekly during peak WNV transmission season from epidemiological weeks 22-39 (Nebraska) and weeks 23-37 (Colorado). Traps are placed for a single night, and collected mosquitoes are stored on dry ice, sorted by species, and placed into pools of up to 50 individual mosquitoes prior to testing for WNV RNA via RT-qPCR (Lanciotti et al., 2000). Colorado mosquito surveillance data was provided by Colorado State University and generated as previously described (Fauver et al., 2016). Nebraska mosquito surveillance data was provided by the Nebraska Public Health Laboratory (NPHL). Mosquito pools were tested for WNV, St. Louis Encephalitis virus (SLE), and Western Equine Encephalitis virus (WEEV) via a trioplex RT-qPCR assay as previously described (A. C. Brault, Fang, & Reisen, 2015). Notably, the same primer/probe sequences are used to detect

WNV RNA in both the Colorado and Nebraska datasets. Following RT-qPCR, pools were reported as either positive or negative depending on the Ct value cut-off used (<40), alongside associated Ct values. Summary statistics from mosquito surveillance data for the Colorado and Nebraska datasets are reported in Supplementary Tables S2 and S3.

5.2 Modeling WNV Dynamics

We developed a mathematical model of WNV transmission between mosquitoes and birds and WNV viral dynamics, including within-host viral kinetics and simulated entomological surveillance to address our two study aims:

1. Explore potential mechanisms which might explain the time-varying signal in Ct values from pooled mosquito RT-qPCR testing.
2. Generate a synthetic dataset with which to develop and evaluate a novel estimator for WNV prevalence using Ct values from pools rather than binary positive/negative status.

We developed an agent-based model of mosquitoes and birds, inspired by the classic Ross-McDonald model(Ross, 1911), summarised in Figure 2. The model has three major components: 1) population-level interactions between *Culex* female mosquitoes and birds, 2) within-host viral dynamics of the mosquitoes and birds, and 3) routine weekly surveillance by capturing and pooling mosquitoes. All chosen parameter values are based on the literature, or, where necessary, based on informed assumptions (Supplementary Material S2).

5.2.1 Population-level interactions between mosquitoes and birds, and other dynamics

In our model, we assumed that birds may become susceptible (S_H), infectious (I_H), or recover (R_H) with lifelong immunity following infection. We also model births and deaths of birds over the simulation, though these rates are slow relative to the rapid dynamics of the mosquito population.

We modelled aspects of the mosquito life cycle that directly influence the transmission cycle of WNV between birds and mosquitoes. In our model, mosquitoes are initially susceptible (S_M) after birth and can become infected and infectious (I_M) after biting an infected bird. Once a mosquito becomes infectious, it stays infected and infectious until its death (D_M) or until it is captured. We assume that although there might be evidence for vertical transmission (Baqar, Hayes, Murphy, & Watts, 1993; Fechter-Leggett, Nelms, Barker, & Reisen, 2012), it is negligible. We consider only one mosquito species in the population with characteristics similar to *Culex pipiens* or *Culex tarsalis*. Transmission was assumed to occur with some probability following a bite between infected mosquitoes and susceptible birds and vice versa. Once a mosquito bites and takes a blood meal, we assume that it does not seek blood meals for data, and the number of days are Poisson distributed with rate 4 day^{-1}. In addition to infection states, we consider the two major stages of the mosquito life cycle: egg and adult. We assume that female mosquitoes lay eggs which hatch into adult female mosquitoes after an average of 8 days (see

Figure 2). Male mosquitoes are not included in the model, as they do not contribute to the transmission cycle and are not represented in the observed data.

Mosquito biting, birth and death rates were assumed to be temperature dependent, which we proxied by varying these rates over the year with a sinusoidal function (see Supplementary Material S2) (Marini et al., 2018; Moschini, Bisanzio, & Pugliese, 2017; Reisen & Wheeler, 2019). Furthermore, we assume that the adult mosquitoes will always either overwinter or actively contribute to transmission, depending on the environment's temperature. Assumed parameter values are shown in the Supplementary Material.

5.3 Within-host viral kinetics of birds and mosquitoes

The model incorporates an explicit viral kinetics—or Ct dynamics—framework for infected birds and mosquitoes. For birds, following infection, log viral load is assumed to increase linearly from the limit of detection immediately following infection until it reaches a peak a few days later (approximately 2.5 days). We then assume that the virus is cleared at a constant rate (on the log scale) over time. See Supplementary Material S3 for further details.

In contrast, we assume that mosquitoes can inherit the viral load of the bird they were infected by. In this model, the initial viral load of the infected mosquito is proportional to the viral load of the infected bird at the time of the bite. This reflects the possibility that the infectious dose in the infectee from a bite depends on the virus abundance in the infector. Once infected, mosquitoes can follow two possible fates: 1) the mosquito ingests detectable viral material from the bird but is not productively infected, and viral load remains static at the inherited value; 2) the mosquito becomes infected, and the viral kinetics model is initiated from the inherited viral load and reach the peak within days, and the number of days are normally distributed with mean 5 and standard deviation 0.1 (see Supplementary Material S3). For mosquitoes, infection is considered lifelong, with viral load remaining at its peak until the mosquito's death.

Motivated by previous models of other pathogens (e.g., Russell et al. (2024)), we assume that each individual's model parameters are sampled from a normal distribution (see Supplementary Material S3 for the parameter values), ensuring individual-level variability in viral kinetics. See the Supplementary Material S3 for an illustration of the viral load dynamics of the birds and mosquitoes, the corresponding Ct dynamics models, and details about the parameters and their construction within a hierarchical structure in the Ct dynamics model.

5.4 Routine weekly surveillance through pooled RT-PCR testing of captured mosquitoes

We modelled weekly surveillance of WNV prevalence in trapped mosquitoes as a post-processing step, aiming to replicate routine entomological surveillance. After randomly sampling mosquitoes for each pool based on Nebraska data (see Supplementary Material S4 for further details), we calculate the pooled Ct value based on the methodology outlined by V. Brault, Mallein, and Rupprecht (2021). Here,

the Ct values of each pool were transformed to the corresponding viral load and the average of the viral loads in the pool (assuming that negative mosquitoes contribution no viral load) were counted as the pooled viral load. Finally, this pooled viral load was transformed back to a Ct value, and we considered this value to be the pooled Ct value (see Supplementary Material S4 for further details). We repeated these calculations for all the pools each week.

5.5 Estimation of WNV Prevalence in the Mosquito Population

We determined whether WNV prevalence in a mosquito population could be accurately estimated using the pooled Ct values obtained each week. Additionally, we examined whether prevalence estimates could be improved by incorporating Ct values, as opposed to relying solely on binarised positive/negative test results.

We calculated WNV prevalence using methodologies based on Cleary et al. (2021) and Zenios and Wein (1998). We constructed a Maximum Likelihood Estimator (MLE) by taking into consideration the conditional probability density distribution of an observed pooled Ct value given the possible number of positive samples in the pool. Particular details on how we calculated these density distributions and the corresponding MLE estimator are detailed in Supplementary Material S8. Point estimates for prevalence and confidence intervals were estimated using the MLE function from the *bbmle* package in R (Bolker & R Development Core Team, 2007), or where the MLE function failed, a profile likelihood approach (Cole, Chu, & Greenland, 2014; Murphy, , & Van Der Vaart, 2000).

To compare the accuracy of the estimates, we compared the estimated time-dependent prevalence with the true WNV prevalence in the mosquito population. We compared our Ct-based estimates to those obtained using binarised test results with the R package *PooledInfRate*, developed by the CDC (Biggerstaff, 2025), to estimate the prevalence of WNV. See the Supplementary Material for further methodological details and relevant calculations.

6 Acknowledgements

We would like to acknowledge Greg Ebel and Tobias Koch from Colorado State University and Ryan Vincent and Matt Parker from the City of Fort Collins for providing WNV surveillance data from Colorado used in this study. We would also like to acknowledge personnel at the Nebraska Public Health Laboratories and the Nebraska Department of Health and Human Services for providing WNV surveillance data from Nebraska used in this study.

7 Funding

PA and JH are funded by a Wellcome Trust Early Career Award (grant 225001/Z/22/Z). This work was supported in part by start up funds from the UNMC VCR Office provided to JRF.

8 Availability of codes and related data

All the code and data required to reproduce the analyses are available online at `https://github.com/PunyaAlahakoon/west_nile_virus_abm.git`.

References

Baqar, S., Hayes, C. G., Murphy, J. R., & Watts, D. M. (1993, June). Vertical transmission of West Nile virus by Culex and Aedes species mosquitoes. *The American journal of tropical medicine and hygiene*, *48*(6), 757–762. Retrieved 2025-06-23, from https://doi.org/10.4269/ajtmh.1993.48.757 doi: 10.4269/ajtmh.1993.48.757

Barker, C. M. (2019, October). Models and Surveillance Systems to Detect and Predict West Nile Virus Outbreaks. *Journal of Medical Entomology*, *56*(6), 1508–1515. Retrieved 2025-06-16, from https://doi.org/10.1093/jme/tjz150 doi: 10.1093/jme/tjz150

Bekele, B. K., Uwishema, O., Nazir, A., Kaushik, I., & Wellington, J. (2023, January). Addressing the challenges of prevention and control of West Nile virus in Africa: A correspondence. *International Journal of Surgery (London, England)*, *109*(2), 186–188. Retrieved 2025-04-11, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10389590/ doi: 10.1097/JS9.0000000000000188

Bergner, L., Catalano, S., Nichols, J., Filipe, A. D. S., Cao, X., Mair, D., ... Raghwani, J. (2025, April). *Quantifying virus load and characterising virus diversity in wildlife samples with target enrichment sequencing.* bioRxiv. Retrieved 2025-06-13, from https://www.biorxiv.org/content/10.1101/2025.04.16.648874v1 (Pages: 2025.04.16.648874 Section: New Results) doi: 10.1101/2025.04.16.648874

Biggerstaff, B. J. (2025). *PooledInfRate: Estimation for Pooled or Group Testing.* Retrieved from https://github.com/CDCgov/PooledInfRate

Bolker, B., & R Development Core Team. (2007, February). *bbmle: Tools for General Maximum Likelihood Estimation.* Retrieved 2025-06-11, from https://CRAN.R-project.org/package=bbmle (Institution: Comprehensive R Archive Network Pages: 1.0.25.1) doi: 10.32614/CRAN.package.bbmle

Bonsall, D., Ansari, M. A., Ip, C., Trebes, A., Brown, A., Klenerman, P., ... Bowden, R. (2015, October). *ve-SEQ: Robust, unbiased enrichment for streamlined detection and whole-genome sequencing of HCV and other highly diverse pathogens.* F1000Research. Retrieved 2025-06-23, from https://f1000research.com/articles/4-1062 doi: 10.12688/f1000research.7111.1

Bragg, L., & Tyson, G. W. (2014). Metagenomics Using Next-Generation Sequencing. In I. T. Paulsen & A. J. Holmes (Eds.), *Environmental Microbiology: Methods and Protocols* (pp. 183–201). Totowa, NJ: Humana Press. Retrieved 2025-06-13, from https://doi.org/10.1007/978-1-62703-712-9_15 doi: 10.1007/978-1-62703-712-9_15

Brault, A. C., Fang, Y., & Reisen, W. K. (2015, May). Multiplex qRT-PCR for the Detection of Western Equine Encephalomyelitis, St. Louis Encephalitis, and West Nile Viral RNA in Mosquito Pools (Diptera: Culicidae). *Journal of Medical Entomology*, *52*(3), 491–499. Retrieved 2025-05-12, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4581483/ doi: 10.1093/jme/tjv021

Brault, V., Mallein, B., & Rupprecht, J.-F. (2021, March). Group testing as a strategy for COVID-19 epidemiological monitoring and community surveillance. *PLOS Computational Biology*, *17*(3), e1008726. Retrieved 2024-09-04, from https://journals.plos.org/ploscompbiol/article

?id=10.1371/journal.pcbi.1008726 (Publisher: Public Library of Science) doi: 10.1371/journal.pcbi.1008726

Carpenter, A., & Clem, R. J. (2023, February). Factors Affecting Arbovirus Midgut Escape in Mosquitoes. *Pathogens*, *12*(2), 220. Retrieved 2025-06-23, from https://www.mdpi.com/2076-0817/12/2/220 (Number: 2 Publisher: Multidisciplinary Digital Publishing Institute) doi: 10.3390/pathogens12020220

CDC. (2024a, August). *Guidelines for West Nile Virus Surveillance and Control.* Retrieved 2025-01-10, from https://www.cdc.gov/west-nile-virus/php/surveillance-and-control-guidelines/index.html

CDC. (2024b, May). *Treatment and Prevention of West Nile Virus Disease.* Retrieved 2025-04-11, from https://www.cdc.gov/west-nile-virus/hcp/treatment-prevention/index.html

CDC. (2025, June). *Historic Data (1999-2024).* Retrieved 2025-06-11, from https://www.cdc.gov/west-nile-virus/data-maps/historic-data.html

Cella, E., Cunha, M. V., Alcantara, L. C. J., Lourenço, J., & Giovanetti, M. (2025, April). Strengthening arbovirus surveillance: bridging gaps for global health preparedness. *Trends in Microbiology*. Retrieved 2025-05-12, from https://www.sciencedirect.com/science/article/pii/S0966842X25001064 doi: 10.1016/j.tim.2025.03.010

Cleary, B., Hay, J. A., Blumenstiel, B., Harden, M., Cipicchio, M., Bezney, J., ... Mina, M. J. (2021, April). Using viral load and epidemic dynamics to optimize pooled testing in resource-constrained settings. *Science Translational Medicine*, *13*(589), eabf1568. Retrieved 2024-05-15, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8099195/ doi: 10.1126/scitranslmed.abf1568

Cohen, J. (1983, June). The Cost of Dichotomization. *Applied Psychological Measurement*, *7*(3), 249–253. Retrieved 2025-04-11, from https://journals.sagepub.com/doi/10.1177/014662168300700301 doi: 10.1177/014662168300700301

Cole, S. R., Chu, H., & Greenland, S. (2014, January). Maximum Likelihood, Profile Likelihood, and Penalized Likelihood: A Primer. *American Journal of Epidemiology*, *179*(2), 252–260. Retrieved 2025-04-11, from https://academic.oup.com/aje/article-lookup/doi/10.1093/aje/kwt245 doi: 10.1093/aje/kwt245

Dada, J. O., & Mendes, P. (2011, February). Multi-scale modelling and simulation in systems biology. *Integrative Biology*, *3*(2), 86–96. Retrieved 2025-06-19, from https://doi.org/10.1039/c0ib00075b doi: 10.1039/c0ib00075b

Dekoninck, W., Hendrickx, F., Van Bortel, W., Versteirt, V., Coosemans, M., Damiens, D., ... Grootaert, P. (2011, July). Human-Induced Expanded Distribution of Anopheles plumbeus, Experimental Vector of West Nile Virus and a Potential Vector of Human Malaria in Belgium. *Journal of Medical Entomology*, *48*(4), 924–928. Retrieved 2025-05-12, from https://doi.org/10.1603/ME10235 doi: 10.1603/ME10235

Di Giallonardo, F., Geoghegan, J. L., Docherty, D. E., McLean, R. G., Zody, M. C., Qu, J., ... Holmes, E. C. (2015, December). Fluid Spatial Dynamics of West Nile Virus in the United States: Rapid Spread in a Permissive Host Environment. *Journal of Virology*, *90*(2), 862–872. Retrieved 2025-06-

16, from https://journals.asm.org/doi/full/10.1128/jvi.02305-15 (Publisher: American Society for Microbiology) doi: 10.1128/jvi.02305-15

Dohm, D. J., O'Guinn, M. L., & Turell, M. J. (2002, January). Effect of Environmental Temperature on the Ability of Culex pipiens (Diptera: Culicidae) to Transmit West Nile Virus. *Journal of Medical Entomology*, *39*(1), 221–225. Retrieved 2025-06-18, from https://doi.org/10.1603/0022-2585-39.1.221 doi: 10.1603/0022-2585-39.1.221

dos Reis, I. C., Gibson, G., Ayllón, T., de Medeiros Tavares, A., de Araújo, J. M. G., da Silva Monteiro, E., ... Alves Honório, N. (2019, September). Entomo-virological surveillance strategy for dengue, Zika and chikungunya arboviruses in field-caught *Aedes* mosquitoes in an endemic urban area of the Northeast of Brazil. *Acta Tropica*, *197*, 105061. Retrieved 2025-06-11, from https://www.sciencedirect.com/science/article/pii/S0001706X19304553 doi: 10.1016/j.actatropica.2019.105061

FAQ: West Nile Virus. (2013). Washington (DC): American Society for Microbiology. Retrieved 2025-06-12, from http://www.ncbi.nlm.nih.gov/books/NBK562891/

Fauver, J. R., Pecher, L., Schurich, J. A., Bolling, B. G., Calhoon, M., Grubaugh, N. D., ... Moore, C. G. (2016, March). Temporal and Spatial Variability of Entomological Risk Indices for West Nile Virus Infection in Northern Colorado: 2006–2013. *Journal of Medical Entomology*, *53*(2), 425–434. Retrieved 2025-06-19, from https://doi.org/10.1093/jme/tjv234 doi: 10.1093/jme/tjv234

Fechter-Leggett, E., Nelms, B. M., Barker, C. M., & Reisen, W. K. (2012). West Nile virus cluster analysis and vertical transmission in Culex pipiens complex mosquitoes in Sacramento and Yolo Counties, California, 2011. *Journal of Vector Ecology*, *37*(2), 442–449. Retrieved 2024-08-01, from https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1948-7134.2012.00248.x (_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1948-7134.2012.00248.x) doi: 10.1111/j.1948-7134.2012.00248.x

Fesce, E., Marini, G., Rosà, R., Lelli, D., Cerioli, M. P., Chiari, M., ... Ferrari, N. (2023, May). Understanding West Nile virus transmission: Mathematical modelling to quantify the most critical parameters to predict infection dynamics. *PLOS Neglected Tropical Diseases*, *17*(5), e0010252. Retrieved 2025-06-12, from https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0010252 (Publisher: Public Library of Science) doi: 10.1371/journal.pntd.0010252

Franz, A. W. E., Kantor, A. M., Passarelli, A. L., & Clem, R. J. (2015, July). Tissue Barriers to Arbovirus Infection in Mosquitoes. *Viruses*, *7*(7), 3741–3767. Retrieved 2025-06-23, from https://www.mdpi.com/1999-4915/7/7/2795 (Number: 7 Publisher: Multidisciplinary Digital Publishing Institute) doi: 10.3390/v7072795

Goh, C., Golubchik, T., Ansari, M. A., Cesare, M. d., Trebes, A., Elliott, I., ... Bowden, R. (2019, July). *Targeted metagenomic sequencing enhances the identification of pathogens associated with acute infection.* bioRxiv. Retrieved 2025-06-23, from https://www.biorxiv.org/content/10.1101/716902v1 (Pages: 716902 Section: New Results) doi: 10.1101/716902

Hay, J. A., Kennedy-Shaffer, L., Kanjilal, S., Lennon, N. J., Gabriel, S. B., Lipsitch, M., & Mina, M. J. (2021, June). Estimating epidemiologic dynamics from cross-sectional viral load distributions. *Science*, *373*(6552), eabh0635. Retrieved 2023-11-09, from https://www.science.org/doi/full/10.1126/science.abh0635 (Publisher: American Association for the Advancement of Science) doi: 10.1126/science.abh0635

Hay, J. A., Kissler, S. M., Fauver, J. R., Mack, C., Tai, C. G., Samant, R. M., ... Grad, Y. H. (2022, November). Quantifying the impact of immune history and variant on SARS-CoV-2 viral kinetics and infection rebound: A retrospective cohort study. *eLife*, *11*, e81849. Retrieved 2025-06-17, from https://doi.org/10.7554/eLife.81849 (Publisher: eLife Sciences Publications, Ltd) doi: 10.7554/eLife.81849

Hepworth, G., & Biggerstaff, B. J. (2017, December). Bias Correction in Estimating Proportions by Pooled Testing. *Journal of agricultural, biological, and environmental statistics*, *22*(4), 602–614. Retrieved 2025-06-04, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6325642/ doi: 10.1007/s13253-017-0297-2

Iskauskas, A., Vernon, I., Goldstein, M., Scarponi, D., McCreesh, N., McKinley, T. J., & White, R. G. (2024, June). Emulation and History Matching Using the hmer Package. *Journal of Statistical Software*, *109*, 1–48. Retrieved 2025-06-23, from https://doi.org/10.18637/jss.v109.i10 doi: 10.18637/jss.v109.i10

Johnson, R. M., Stopard, I. J., Byrne, H. M., Armstrong, P. M., Brackney, D. E., & Lambert, B. (2024, April). Investigating the dose-dependency of the midgut escape barrier using a mechanistic model of within-mosquito dengue virus population dynamics. *PLOS Pathogens*, *20*(4), e1011975. Retrieved 2025-06-23, from https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1011975 (Publisher: Public Library of Science) doi: 10.1371/journal.ppat.1011975

Johnston, B. L., & Conly, J. M. (2000). West Nile virus - Where did it come from and where might it go? *The Canadian Journal of Infectious Diseases*, *11*(4), 175–178. Retrieved 2024-09-03, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2094770/

Kilpatrick, A. M., Meola, M. A., Moudy, R. M., & Kramer, L. D. (2008, June). Temperature, Viral Genetics, and the Transmission of West Nile Virus by Culex pipiens Mosquitoes. *PLOS Pathogens*, *4*(6), e1000092. Retrieved 2025-06-18, from https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1000092 (Publisher: Public Library of Science) doi: 10.1371/journal.ppat.1000092

Kilpatrick, A. M., & Pape, W. J. (2013, September). Predicting Human West Nile Virus Infections With Mosquito Surveillance Data. *American Journal of Epidemiology*, *178*(5), 829–835. Retrieved 2025-06-19, from https://doi.org/10.1093/aje/kwt046 doi: 10.1093/aje/kwt046

Kissler, S. M., Fauver, J. R., Mack, C., Olesen, S. W., Tai, C., Shiue, K. Y., ... Grad, Y. H. (2021, July). Viral dynamics of acute SARS-CoV-2 infection and applications to diagnostic and public health strategies. *PLOS Biology*, *19*(7), e3001333. Retrieved 2025-06-17, from https://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.3001333 (Publisher: Public Library of Science) doi: 10.1371/journal.pbio.3001333

Kissler, S. M., Hay, J. A., Fauver, J. R., Mack, C., Tai, C. G., Anderson, D. J., … Grad, Y. H. (2023, October). Viral kinetics of sequential SARS-CoV-2 infections. *Nature Communications*, *14*(1), 6206. Retrieved 2025-06-17, from https://www.nature.com/articles/s41467-023-41941-z (Publisher: Nature Publishing Group) doi: 10.1038/s41467-023-41941-z

Komar, N., Langevin, S., Hinten, S., Nemeth, N., Edwards, E., Hettler, D., … Bunning, M. (2003, March). Experimental Infection of North American Birds with the New York 1999 Strain of West Nile Virus. *Emerging Infectious Diseases*, *9*(3), 311–322. Retrieved 2025-06-18, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2958552/ doi: 10.3201/eid0903.020628

Kraemer, M. U. G., Reiner, R. C., Brady, O. J., Messina, J. P., Gilbert, M., Pigott, D. M., … Golding, N. (2019, March). Past and future spread of the arbovirus vectors Aedes aegypti and Aedes albopictus. *Nature Microbiology*, *4*(5), 854–863. Retrieved 2025-05-12, from https://www.nature.com/articles/s41564-019-0376-y doi: 10.1038/s41564-019-0376-y

Kretschmer, M. (2023). Unprecedented Outbreak of West Nile Virus — Maricopa County, Arizona, 2021. *MMWR. Morbidity and Mortality Weekly Report*, *72*. Retrieved 2025-06-19, from https://www.cdc.gov/mmwr/volumes/72/wr/mm7217a1.htm doi: 10.15585/mmwr.mm7217a1

Kwan, J. L., Park, B. K., Carpenter, T. E., Ngo, V., Civen, R., & Reisen, W. K. (2012, August). Comparison of enzootic risk measures for predicting West Nile disease, Los Angeles, California, USA, 2004-2010. *Emerging Infectious Diseases*, *18*(8), 1298–1306. doi: 10.3201/eid1808.111558

Lanciotti, R. S., Kerst, A. J., Nasci, R. S., Godsey, M. S., Mitchell, C. J., Savage, H. M., … Roehrig, J. T. (2000, November). 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*(11), 4066–4071. doi: 10.1128/JCM.38.11.4066-4071.2000

Lee, W.-S., Webster, J. A., Madzokere, E. T., Stephenson, E. B., & Herrero, L. J. (2019, April). Mosquito antiviral defense mechanisms: a delicate balance between innate immunity and persistent viral infection. *Parasites & Vectors*, *12*(1), 165. Retrieved 2025-06-16, from https://doi.org/10.1186/s13071-019-3433-8 doi: 10.1186/s13071-019-3433-8

Liu-Helmersson, J., Rocklöv, J., Sewe, M., & Brännström, (2019, May). Climate change may enable *Aedes aegypti* infestation in major European cities by 2100. *Environmental Research*, *172*, 693–699. Retrieved 2025-06-13, from https://www.sciencedirect.com/science/article/pii/S0013935119301069 doi: 10.1016/j.envres.2019.02.026

Lyberger, K., Robinson, A. R., Couper, L., Delwel, I., Glidden, C., Qian, C., … Mordecai, E. A. (2025, March). *A systematic review of climate-change driven range shifts in mosquito vectors.* Retrieved 2025-05-12, from http://biorxiv.org/lookup/doi/10.1101/2025.03.25.645279 doi: 10.1101/2025.03.25.645279

MacCallum, R. C., Zhang, S., Preacher, K. J., & Rucker, D. D. (2002). On the practice of dichotomization of quantitative variables. *Psychological Methods*, *7*(1), 19–40. (Place: US Publisher: American Psychological Association) doi: 10.1037/1082-989X.7.1.19

Mack, A., Choffnes, E. R., Relman, D. A., Hamburg, M. A., Sparling, P. F., & Lemon, S. M. (2008). *Vector-borne diseases: understanding the environmental, human health, and ecological connections:*

workshop summary. National Academies Press.

Marini, G., Rosà, R., Pugliese, A., Rizzoli, A., Rizzo, C., Russo, F., ... Capelli, G. (2018, September). West Nile virus transmission and human infection risk in Veneto (Italy): a modelling analysis. *Scientific Reports*, *8*(1), 14005. Retrieved 2025-06-23, from https://www.nature.com/articles/s41598-018-32401-6 (Publisher: Nature Publishing Group) doi: 10.1038/s41598-018-32401-6

Martinez, D., Murray, K. O., Reyna, M., Arafat, R. R., Gorena, R., Shah, U. A., & Debboun, M. (2017, August). West Nile Virus Outbreak in Houston and Harris County, Texas, USA, 2014. *Emerging Infectious Diseases*, *23*(8), 1372–1376. Retrieved 2025-06-19, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5547786/ doi: 10.3201/eid2308.170384

Mordecai, E. A., Caldwell, J. M., Grossman, M. K., Lippi, C. A., Johnson, L. R., Neira, M., ... Villena, O. (2019). Thermal biology of mosquito-borne disease. *Ecology Letters*, *22*(10), 1690–1708. Retrieved 2025-05-12, from https://onlinelibrary.wiley.com/doi/abs/10.1111/ele.13335 (_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/ele.13335) doi: 10.1111/ele.13335

Moschini, P., Bisanzio, D., & Pugliese, A. (2017). A Seasonal Model for West Nile Virus. *Mathematical Modelling of Natural Phenomena*, *12*(2), 58–83. Retrieved 2025-06-23, from https://www.mmnp-journal.org/articles/mmnp/abs/2017/02/mmnp2017122p58/mmnp2017122p58.html (Number: 2 Publisher: EDP Sciences) doi: 10.1051/mmnp/201712205

Moudy, R. M., Meola, M. A., Morin, L.-L. L., Ebel, G. D., & Kramer, L. D. (2007, August). A newly emergent genotype of West Nile virus is transmitted earlier and more efficiently by Culex mosquitoes. *The American Journal of Tropical Medicine and Hygiene*, *77*(2), 365–370.

Murphy, S. A., , & Van Der Vaart, A. W. (2000, June). On Profile Likelihood. *Journal of the American Statistical Association*, *95*(450), 449–465. Retrieved 2025-04-11, from https://www.tandfonline.com/doi/abs/10.1080/01621459.2000.10474219 (Publisher: ASA Website _eprint: https://www.tandfonline.com/doi/pdf/10.1080/01621459.2000.10474219) doi: 10.1080/01621459.2000.10474219

Nasci, R. S., & Mutebi, J.-P. (2019, October). Reducing West Nile Virus Risk through Vector Management. *Journal of medical entomology*, *56*(6), 1516–1521. Retrieved 2025-04-11, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7092639/ doi: 10.1093/jme/tjz083

Pauli, G., Bauerfeind, U., Blümel, J., Burger, R., Drosten, C., Gröner, A., ... Willkommen, H. (2013, August). West Nile Virus. *Transfusion Medicine and Hemotherapy*, *40*(4), 265–284. Retrieved 2024-08-02, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3776406/ doi: 10.1159/000353698

Petersen, L. R. (2019, October). Epidemiology of West Nile Virus in the United States: Implications for Arbovirology and Public Health. *Journal of Medical Entomology*, *56*(6), 1456–1462. Retrieved 2025-06-16, from https://doi.org/10.1093/jme/tjz085 doi: 10.1093/jme/tjz085

Puhach, O., Meyer, B., & Eckerle, I. (2023). SARS-CoV-2 viral load and shedding kinetics. *Nature Reviews. Microbiology*, *21*(3), 147–161. Retrieved 2024-04-10, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9716513/ doi: 10.1038/s41579-022-00822-w

Pérez-Ramírez, E., Llorente, F., & Jiménez-Clavero, M. (2014, February). Experimental Infections of Wild Birds with West Nile Virus. *Viruses*, *6*(2), 752–781. Retrieved 2025-06-23, from https://www.mdpi.com/1999-4915/6/2/752 (Number: 2 Publisher: Multidisciplinary Digital Publishing Institute) doi: 10.3390/v6020752

Reisen, W. K., Fang, Y., & Martinez, V. M. (2006, March). Effects of Temperature on the Transmission of West Nile Virus by Culex tarsalis (Diptera: Culicidae). *Journal of Medical Entomology*, *43*(2), 309–317. Retrieved 2025-06-18, from https://doi.org/10.1093/jmedent/43.2.309 doi: 10.1093/jmedent/43.2.309

Reisen, W. K., & Wheeler, S. S. (2019, October). Overwintering of West Nile Virus in the United States. *Journal of Medical Entomology*, *56*(6), 1498–1507. Retrieved 2025-06-23, from https://doi.org/10.1093/jme/tjz070 doi: 10.1093/jme/tjz070

Richards, S. L., Mores, C. N., Lord, C. C., & Tabachnick, W. J. (2007). Impact of Extrinsic Incubation Temperature and Virus Exposure on Vector Competence of Culex pipiens quinquefasciatus Say (Diptera: Culicidae) for West Nile Virus. *Vector borne and zoonotic diseases (Larchmont, N.Y.)*, *7*(4), 629–636. Retrieved 2025-06-17, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2724990/ doi: 10.1089/vbz.2007.0101

Ross, R. (1911). *The prevention of malaria*. John Murray.

Russell, T. W., Townsley, H., Abbott, S., Hellewell, J., Carr, E. J., Chapman, L. A. C., … Kucharski, A. J. (2024, January). Combined analyses of within-host SARS-CoV-2 viral kinetics and information on past exposures to the virus in a human cohort identifies intrinsic differences of Omicron and Delta variants. *PLOS Biology*, *22*(1), e3002463. Retrieved 2024-03-19, from https://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.3002463 (Publisher: Public Library of Science) doi: 10.1371/journal.pbio.3002463

Rückert, C., & Ebel, G. D. (2018, April). How Do Virus–Mosquito Interactions Lead to Viral Emergence? *Trends in Parasitology*, *34*(4), 310–321. Retrieved 2025-06-18, from https://www.cell.com/trends/parasitology/abstract/S1471-4922(17)30306-9 (Publisher: Elsevier) doi: 10.1016/j.pt.2017.12.004

Shocket, M. S., Verwillow, A. B., Numazu, M. G., Slamani, H., Cohen, J. M., El Moustaid, F., … Mordecai, E. A. (2020, September). Transmission of West Nile and five other temperate mosquito-borne viruses peaks at temperatures between 23°C and 26°C. *eLife*, *9*, e58511. Retrieved 2025-06-18, from https://doi.org/10.7554/eLife.58511 (Publisher: eLife Sciences Publications, Ltd) doi: 10.7554/eLife.58511

Turell, M. J., Dohm, D. J., Sardelis, M. R., O'guinn, M. L., Andreadis, T. G., & Blow, J. A. (2005, January). An Update on the Potential of North American Mosquitoes (Diptera: Culicidae) to Transmit West Nile Virus. *Journal of Medical Entomology*, *42*(1), 57–62. Retrieved 2025-06-17, from https://doi.org/10.1093/jmedent/42.1.57 doi: 10.1093/jmedent/42.1.57

Ulbert, S. (2019, July). West Nile virus vaccines – current situation and future directions. *Human Vaccines & Immunotherapeutics*, *15*(10), 2337–2342. Retrieved 2025-04-11, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6816401/ doi: 10.1080/21645515.2019.1621149

Vairo, F., Di Pietrantonj, C., Pasqualini, C., Mammone, A., Lanini, S., Nicastri, E., … Ippolito, G. (2018, September). The Surveillance of Chikungunya Virus in a Temperate Climate: Challenges and Possible Solutions from the Experience of Lazio Region, Italy. *Viruses*, *10*(9), 501. Retrieved 2025-06-11, from https://www.mdpi.com/1999-4915/10/9/501 (Number: 9 Publisher: Multidisciplinary Digital Publishing Institute) doi: 10.3390/v10090501

Vaughan, J. A., Newman, R. A., & Turell, M. J. (2022, October). Bird species define the relationship between West Nile viremia and infectiousness to Culex pipiens mosquitoes. *PLoS Neglected Tropical Diseases*, *16*(10), e0010835. Retrieved 2025-06-23, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9578590/ doi: 10.1371/journal.pntd.0010835

Vogels, C. B. F., Fros, J. J., Göertz, G. P., Pijlman, G. P., & Koenraadt, C. J. M. (2016, July). Vector competence of northern European Culex pipiens biotypes and hybrids for West Nile virus is differentially affected by temperature. *Parasites & Vectors*, *9*(1), 393. Retrieved 2025-06-17, from https://doi.org/10.1186/s13071-016-1677-0 doi: 10.1186/s13071-016-1677-0

Vogels, C. B. F., Hartemink, N., & Koenraadt, C. J. M. (2017, July). Modelling West Nile virus transmission risk in Europe: effect of temperature and mosquito biotypes on the basic reproduction number. *Scientific Reports*, *7*(1), 5022. Retrieved 2024-11-21, from https://www.nature.com/articles/s41598-017-05185-4 (Publisher: Nature Publishing Group) doi: 10.1038/s41598-017-05185-4

Watts, N., Amann, M., Arnell, N., Ayeb-Karlsson, S., Beagley, J., Belesova, K., … Costello, A. (2021, January). The 2020 report of The Lancet Countdown on health and climate change: responding to converging crises. *The Lancet*, *397*(10269), 129–170. Retrieved 2025-06-13, from https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)32290-X/fulltext (Publisher: Elsevier) doi: 10.1016/S0140-6736(20)32290-X

Winters, A. M., Bolling, B. G., Beaty, B. J., Blair, C. D., Eisen, R. J., Meyer, A. M., … Eisen, L. (2008, April). Combining Mosquito Vector and Human Disease Data for Improved Assessment of Spatial West Nile Virus Disease Risk. Retrieved 2025-06-19, from https://www.ajtmh.org/view/journals/tpmd/78/4/article-p654.xml (Section: The American Journal of Tropical Medicine and Hygiene) doi: 10.4269/ajtmh.2008.78.654

Zenios, S. A., & Wein, L. M. (1998). Pooled testing for HIV prevalence estimation: exploiting the dilution effect. *Statistics in Medicine*, *17*(13), 1447–1467. Retrieved 2025-04-11, from https://onlinelibrary.wiley.com/doi/abs/10.1002/%28SICI%291097-0258%2819980715%2917%3A13%3C1447%3A%3AAID-SIM862%3E3.0.CO%3B2-K (_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/%28SICI%291097-0258%2819980715%2917%3A13%3C1447%3A%3AAID-SIM862%3E3.0.CO%3B2-K) doi: 10.1002/(SICI)1097-0258(19980715)17:13<1447::AID-SIM862>3.0.CO;2-K