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Modelling the temperature dependent extrinsic incubation period of West Nile Virus using Bayesian time delay models

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s u m m a r y

West Nile Virus (WNV) is a mosquito-borne pathogen that primarily infects birds. Infections can spillover to humans and cause a spectrum of clinical symptoms, including WNV neuroinvasive disease. The extrinsic incubation period (EIP) is the time taken for a mosquito to become infectious following the ingestion of an infected blood meal. Characterising how the EIP varies with temperature is an essential part of predicting the impact and transmission dynamics of WNV. We re-analyse existing experimental data using Bayesian time delay models, allowing us to account for variation in how quickly individual mosquitoes developed disseminated WNV infections. In these experiments, cohorts of *Culex pipiens* mosquitoes were infected with WNV and kept under different temperature conditions, being checked for disseminated infection at defined timepoints. We find that EIPs are best described with a Weibull distribution and become shorter log-linearly with temperature. Under 18°C, less than 1% of infected *Cx. pipiens* had a disseminated infection after 5 days, compared to 9.73% (95% CrI: 7.97 to 11.54) at 25°C and 42.20% (95% CrI: 38.32 to 46.60) at 30°C. In the hottest experimental temperature treatment (32°C), the EIP_{50} was estimated at 3.78 days (CrI: 3.42 to 4.15) compared to over 100 days in the coolest treatment (15°C). The variance of EIPs was found to be much larger at lower temperatures than higher temperatures, highlighting the importance of characterising the time delay distribution associated with the EIP. We additionally demonstrate a competitive advantage of WNV strain WN02 over NY99, where the former infects mosquitoes more quickly at colder temperatures than the latter. This research contributes crucial parameters to the WNV literature, providing essential insights for modellers and those planning interventions.

Introduction

Vector-borne diseases (VBD) are responsible for a substantial burden of mortality and morbidity worldwide. West Nile Virus (WNV) is a mosquito-borne pathogen that primarily infects birds[1]; however, infections can spillover into humans and other mammals. Mammals are dead-end hosts with no prospect of onward transmission, although they can experience symptoms of infection. In humans, approximately 26% of cases are asymptomatic and most cases present with mild,[2] self-limiting flu-like symptoms. However, a small share of WNV cases (< 1%) develop into WNV neuroinvasive disease (WNV NID). The risk of WNV NID is age dependent,[3] with intensive care and long-term disability potential outcomes. WNV NID has a high case mortality rate of 10%.[4] Regrettably, there is no currently licenced human vaccine or specific treatment of WNV.[5] The primary ornithophilic vector for WNV is *Culex pipiens*,[6,7] which has a broad geographic distribution including Northern Africa, North America, Europe and Asia.

WNV was first isolated in West Nile district of Uganda[8] and is now commonly found in Africa, Europe, the Middle East, North America and West Asia.[9] WNV is spreading throughout Europe, with introductions moving further north in recent years including detections in Germany[3] and the Netherlands.[10] WNV was introduced to the Americas in 1999, with a likely Israeli origin.[11,12] Following crow die offs and severe cases of WNV in New York, the pathogen has spread across the Americas.[13] Preceding 2002, the WNV strain NY99 was dominant until the swift expansion of a new strain, WN02.[14] Due to its increasing global prominence, improving our understanding of key epidemiological parameters is essential to understand the risk posed by WNV.

The extrinsic incubation period (EIP) is the length of time it takes for a mosquito to become infectious after taking an infected blood meal. For the mosquito to transmit, the virus must infect the midgut before disseminating to secondary tissues and finally reaching the salivary glands. The EIP is temperature sensitive because higher temperatures lead to faster rates of viral replication and – as mosquitoes are poikilotherms – their internal body temperature is largely determined by the ambient environmental temperature. The specific relationship between EIP and temperature varies between viruses.[15,16]

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The EIP is a crucial parameter for modelling dynamics of WNV transmission. A minimum requirement for transmission is that the mosquito must live at least as long as the EIP. This is because a second blood meal must be taken after the mosquito has become infectious to facilitate onward transmission. There is a dearth of high-quality data on the EIP of WNV (and other pathogens such as dengue) due to the challenges of working with live viruses, poor data reporting (e.g. failing to declare cohort sample sizes) and limitations in data analysis (e.g. failing to account for right or interval censoring). Indeed, very few experimental studies have analysed the effect of temperature on the EIP of WNV[17,18] with others analysing EIP in only one temperature condition.[19]

WNV dynamic transmission models (and derived metrics such as R_0) typically make simplifying assumptions about the EIP. The duration of the EIP is often represented as point estimates using summary statistics such as the EIP_{50} (the number of days post infection by which 50% of mosquitoes are infectious) or averages from lab and/or field experiments.[20] This ignores the considerable heterogeneity at the level of individual mosquitoes, where even under tightly controlled experimental conditions, variance in the time taken for mosquitoes to become infected is considerable. For instance, the EIP of dengue has been demonstrated to be log-normally distributed,[15] with significant variation in EIP, particularly at lower temperatures. Accounting for the variation in EIP on transmission potential of vector-borne diseases has been quantitatively demonstrated as a significant and overlooked confounder of transmission models, particularly at low temperatures.[21] Our understanding of the series of seemingly unlikely events (a relatively short-lived vector taking a blood meal during the viraemic phase of an infected host and surviving long enough to take another blood meal) is improved by characterising the distributed delays involved.

In this study, we reanalyse experimental data where adult mosquitoes were infected and maintained under different temperature regimes.[18] Cohorts of *Cx. pipiens* were infected with live virus and subsets tested for disseminated infection at set timepoints. We interpret the row-level observations associated with these data, accounting for the left and right censoring inherent in this experimental design. This contrasts with how these data are often interpreted, modelling proportions with binomial error. By comparing different distributions to model the delay between infection and dissemination, we characterise the distribution of WNV EIPs observed across the different temperature treatments.

Methods

Data and experimental design

This analysis used data from a laboratory study with permission from the authors.[18] The authors infected cohorts of lab reared *Cx. pipiens* mosquitoes with WNV (one of two strains, NY99 or WN02). A total of 2145 mosquitoes were held at multiple different temperatures (15°C, 18°C, 22°C, 32°C), with a target sample size of 25 mosquitoes removed without replacement at defined timepoints for each temperature treatment. A plaque assay on Vero cells[22] was used to determine if the mosquito was infected with WNV and if the virus had disseminated to the legs. The authors used the capillary assay on saliva secretions to test for infection in saliva. We chose to model the data on leg dissemination, as it is often a more reliable proxy of whether transmission is feasible through mosquito saliva.[23] Full details on precise experimental protocols can be found in the original publication.[18]

A challenge of this experimental setup is that the precise timing of dissemination could not be directly observed, as dissemination status was only determined at pre-defined timepoints using a plaque assay.[22] Therefore, data were censored in one of two ways. For mosquitoes in which dissemination had occurred, data were interval censored: dissemination could have occurred at any point from when the infectious blood meal was taken to the date on which they were sampled. For mosquitoes where dissemination had not occurred, data were right censored as dissemination occurred after the time of sampling, with no upper bound.

The data were collected as cohorts of mosquitoes (sampled at predefined timepoints t) that either did or did not have disseminated infection when tested. For mosquitoes that had disseminated infection at time t, we modelled an interval censored EIP (y) between the minimum possible time of zero days ($y_{min}=0$) and maximum possible number of days t ($y_{max}=t$). Those mosquitoes without disseminated infection at time t were modelled with only a minimum value of y ($y_{min}=t$), as only times prior to t could be ruled out.

Statistical modelling

We chose to compare four candidate distributions for the individual-level variation of WNV EIP in *Cx. pipiens*: the exponential, gamma, Weibull, and lognormal, based on Chan and Johansson.[15] All distributions have support for positive continuous values and have been used to model the EIP of VBD in other studies.[15,16] While the exponential is a special case of the Gamma distribution, an exponential EIP is often assumed in SEIR (Susceptible, Exposed, Infected, Recovered) models of vector-borne disease transmission. This makes it important to evaluate how suitable this specific distribution of EIPs is for the data.

For an observation of EIP within an interval $[y_{min}, y_{max}]$, the log- likelihood ($\log \mathbb{L}$) was modelled as:

$$\log \mathbb{L}(\theta) = \log(CDF(y_{max}; \theta) - CDF(y_{min}; \theta)),$$

where $CDF(t; \theta)$ is the cumulative distribution function of the chosen distribution with parameters θ. For right censored observations (where only y_{min} is known), the likelihood was calculated as: $\log \mathbb{L}(\theta) = \log(1 - CDF(y_{min}; \theta))$.

We compared three functions to explore the effect of temperature on the EIP, $f(T)$. A null model assumed no effect of temperature, a second assumed a log-linear effect of temperature, and the third a log-quadratic effect. These functions were implemented for each distribution as in[15] with details outlined in Table 1:

$$f = e^{\alpha},$$

$$f(T) = e^{\alpha+\beta_1 T},$$

$$f(T) = e^{\alpha+\beta_1 T+\beta_2 T^2}.$$

Statistical models were fit with the Bayesian model-building programming language "stan"[24] using Hamiltonian Monte Carlo (HMC) methods, interfaced through "R".[25] Results are presented

Table 1
Parametrisations of time delay EIP models. Note that there is no closed form expression for the median of the gamma distribution.

Distribution	PDF	Parameters	Parametrisation	EIP_{50}	Standard Deviation
Exponential	$\lambda e^{-\lambda y}$	$\lambda > 0$	$\lambda = \frac{1}{f(T)}$	$\frac{\log(2)}{\lambda}$	$\sqrt{\frac{1}{\lambda^2}}$
Gamma	$\frac{b^a}{\Gamma(a)} y^{a-1} e^{-by}$	$a > 0$ $b > 0$	$b = \frac{a}{f(T)}$	-	$\sqrt{\frac{a}{b^2}}$
Weibull	$\frac{a}{\phi}\left(\frac{y}{\phi}\right)^{a-1} e^{-\left(\frac{y}{\phi}\right)^a}$	$a > 0$ $\phi > 0$	$\phi = \frac{1}{f(T)}$	$\phi \log(2)^{\frac{1}{a}}$	$\sqrt{\phi^2\left[\Gamma\left(1+\frac{2}{a}\right) - \left(\Gamma\left(1+\frac{1}{a}\right)\right)^2\right]}$
Lognormal	$\frac{1}{\sqrt{2\pi}\sigma} \frac{1}{y} e^{-\frac{1}{2}\left(\frac{\log(y)-\mu}{\sigma}\right)^2}$	$\mu \in (-\infty, +\infty)$ $\sigma > 0$	$\mu = f(T)$	$\exp(\mu)$	$\sqrt{[\exp(\sigma^2) - 1]\exp(2\mu + \sigma^2)}$

with 95% credible intervals (CrIs). Models were compared using the leave one out cross validation information criterion scores (LOOIC).[26]

A second analysis was conducted to determine if the two tested strains, WN02 and NY99, had different thermal responses. Following the outcome from the model selection described above, we tested to see if including strain specific effects in the model was supported. We did so by comparing the set of interactions for each strain on the parameters of $f(T)$ using LOOIC.[26]

Results

Data

These data describe 2145 censored observations of WNV EIP in *Cx. pipiens* mosquitoes, split across 4 different temperature groups (15°C = 545; 18°C = 550; 22°C = 527; 32°C = 523). Of these observations, most were right censored (1686, where only a minimum EIP was observed) and the rest interval censored (459, where a maximum and minimum EIP were observed). Approximately half of the mosquitoes had disseminated infections with each strain of WNV used in the study (1079 mosquitoes with WN02 and 1066 with strain NY99).

Statistical modelling

We tested the pairwise combinations of exponential, gamma, Weibull, lognormal with an invariant, log-linear and log-quadratic response to temperature. A Weibull distribution with a log-linear temperature dependent response had the lowest LOOIC with a score of 1451 (Fig. 1A). The next best model was the gamma distribution with a log-linear response (1458), which was within the standard error of the estimated LOOIC for the Weibull (56). This is unsurprising given the similarity between the two distributions. There was no support for a quadratic response which suggests that – at least between the temperature ranges tested in this study (15 to 32°C) – the temperature response was monotonic. This does not preclude a more complex relationship between EIP and temperature at thermal extremes, just that it is not evident in these data. Parameter values (median values with 95% credible intervals) are reported in Table 2.

The model predictions are compared to the data in Fig. 1B. The effect of temperature is acute; at 22°C, just over 10% (10.52%, 95% CrI: 8.91 to 12.21) of *Cx. pipiens* were had a disseminated infection after 10 days, compared to over 95% at 32°C (95.97%, 95% CrI: 92.94 to 97.96). The full temperature dependent response is shown in Fig. 1C, with the temperature dependent EIP_{50} marked as a black contour (the number of days following infection where 50% of mosquitoes are estimated to be infected). In our models, the EIP_{50} is the median of the Weibull distribution.

In Fig. 2A we show how the EIP_{50} varies with temperature, alongside the standard deviation. This was calculated using the formulas given in Table 1; note that for the given parametrisation of the Weibull, the median decreases with increases in $f(T)$. As the Weibull assumes an increasing variance with the mean, it can capture the increased role that variation in EIPs plays at lower temperatures, with far more conserved EIPs when temperatures are warmer. The EIP_{50} falls from 110.67 days (95% CrI: 94.62 to134.50) at 15°C to 5.60 days (95% CrI: 5.04 to 6.25) at 30°C. The standard deviation falls from 81.40 (95% CrI: 63.20 to 108.98) at 15°C to 4.10 (95% CrI: 3.53 to 4.82) at 30°C. Illustrative examples of the Weibull density function are provided in Fig. 2B to demonstrate the extent to which the distribution of EIPs changes with temperature.

We compared model structures for $f(T)$ where the strains were described by the same log-linear response, one where each strain was modelled with a different intercept but the same slope, $f(T) = \exp(^{NY99} + T$, and one where both parameters were allowed to vary, $f(T) = \exp\left(\begin{bmatrix}\alpha \\ \alpha_{WN02}\end{bmatrix} \begin{bmatrix}\beta_{NY99} \\ \beta_{WN02}\end{bmatrix} + {}^{NY99} T\right)$. Model selection narrowly favoured a response where both the intercept and slope of the log-linear thermal response differed between strains, with a LOOIC of 1433 (SE: 57.48), compared to 1442 (SE: 57.01) for a model where only the intercept varied. The parameters for this model are given in Table 2.

Fig. 3 shows the effect of strain on the EIP for the selected model. Panel 3A shows that NY99 is characterised by longer EIPs at lower temperatures, but that the difference between strains is negated at the highest temperatures. In Fig. 3B, we show the fitted cumulative probability to the data from each temperature treatment; the model fit to the data in the highest temperature treatment shows the importance of the interaction term on the slope so that the model can equalise EIPs. It is likely that if there were more experimental temperature treatments between 22 and 32°C there would be clearer statistical support for the strain specific slope. Fig. 3C summarises the difference in the cumulative probability of infection for WN02 compared to NY99, with cool colours showing parity between strains and hotter colours an advantage for WN02.

Discussion

Models of vector-borne diseases often have to make simplifying assumptions about parameters and processes due to a lack of experimental, semi-field or field data examining crucial components of the transmission cycle.[20] This can lead to inaccurate model outputs and conclusions.[21] This is true for an important component of transmission dynamics, the extrinsic incubation period (EIP), where point estimates from summary statistics, or averages from lab and / or field experiments are used in models.[20] We addressed this by characterising the relationship between temperature and the distribution of EIPs for West Nile Virus (WNV), a virus with a substantial public health burden.

We found that WNV infections disseminated more rapidly in *Cx. pipiens* at higher temperatures: at 22°C, just over 10% (10.52%, 95% CrI: 8.914 to 12.211) were infectious after 10 days, compared to over 95% at 32°C (95.97%, 95% CrI: 92.94 to 97.96). Within the

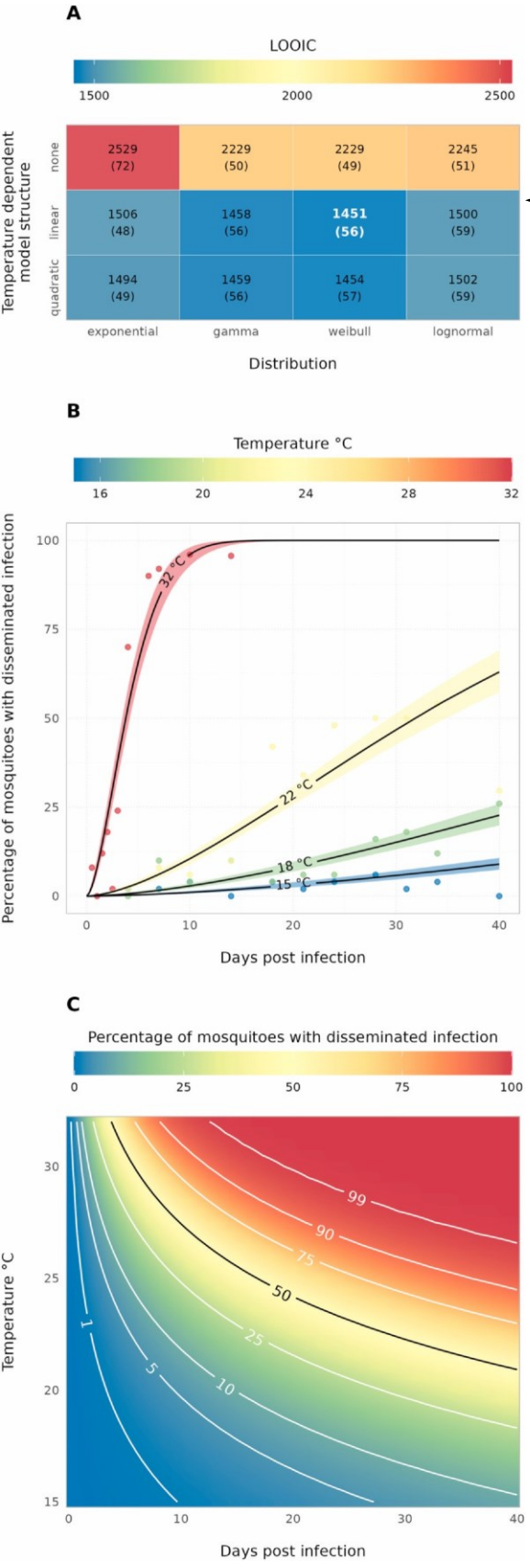

Fig. 1. A compares the leave one out cross-validation information criterion (LOOIC) for models using different distributions to describe extrinsic incubation periods (columns) using different assumptions about the shape of the temperature dependent relationship (rows). A Weibull distribution using a log-linear relationship with temperature was the preferred model. **B** shows how the fitted Weibull cumulative density function fits raw proportion data at each temperature treatment in the study. **C** shows the estimated percentage of mosquitoes with a disseminated infection through time and across temperatures. The median (50% of mosquitoes with disseminated infection, or EIP_{50}) is marked as a black contour.

temperature range examined (15 to 32°C), the relationship between temperature and WNV EIP is positive and log-linear. We suggest that future empirical studies should examine the impact of higher temperatures on WNV EIP, so that temperature dependent models of EIP can be applied across a broader temperature range (additional temperature treatments between 22 and 32°C would also be beneficial). Fortunately, the data presented in this paper cover a relevant range of temperatures with respect to both viral replication and *Culex* lifespans.[27,28]

We examined which of the exponential, gamma, Weibull, or log- normal distributions was most appropriate to describe the individual-level variation of WNV EIP. These were the same distributions as examined by Chan *et al.* when fitting models of yellow fever EIPs[16] and dengue EIPs.[15] Our results show that WNV EIPs in *Cx. pipiens* are best described by the Weibull distribution. In the literature it has been demonstrated that yellow fever EIPs are also Weibull distributed,[16] but dengue EIPs are log-normally distributed.[15] Furthermore, we found that the exponential distribution was the least appropriate for modelling EIPs, as was the case for dengue and yellow fever.[15,16] In some cases (such as a simple ordinary differential equation models), the dwell time in model compartments is exponentially distributed. As we demonstrate, this assumption would misrepresent the true timing of WNV dissemination in *Cx. pipiens.* This is because the exponential distribution assumes that the highest probability density falls on the very shortest EIPs that are not empirically overserved or indeed probable. The consequences for not using the best delay distribution for EIPs in epidemiological models is substantial, particularly at lower temperatures.[21] Thus, it is important to characterise the distribution of EIPs for different viruses, which requires row-level empirical data and appropriate analyses. These data are not currently available for multiple prominent, emerging arboviruses (for example, Usutu and chikungunya), highlighting the necessity for further empirical studies.

Our work emphasises the importance of modelling individual variation in the temperature dependent distribution of WNV EIP, especially when modelling WNV in cooler areas. Regions with lower temperatures are of interest to modellers due to the Northward geographic expansion of WNV, with reports increasing in Europe.[3,10] We found that at lower temperatures, where the EIP is longer, our model captures the empirically observed increased variation of WNV EIPs. Using fixed parameters such as the EIP_{50}/ mean EIP can underestimate the proportion of mosquitoes infectious, as shorter EIPs are ignored at these lower temperatures unless the correct delay distribution is applied. This is because empirically observed EIPs that are substantially shorter than the median or mean value are ignored. With a relatively short-lived vector (such as mosquitoes), capturing rapidly disseminated infections is very consequential for transmission.[21] By not fully accounting for the correct distribution of WNV EIPs, researchers could underestimate WNV transmission risk in cooler areas.

Our results suggest that WN02 may have a competitive advantage over NY99 up to approximately 32 °C, due to having a shorter median EIP under these conditions. An advantage in EIP

Table 2
Model coefficients for the two strains combined, as well as WN02 and NY99 separately (median with 95% credible intervals).

Strain	α	β	σ
Both strains combined	−8.39 (95% CrIs: −8.77 to −8.05)	0.21 (95% CrI: 0.20 to 0.23)	1.58 (95% CrI: 1.44 to 1.72)
WN02	−7.94 (95% CrIs: −8.37 to −7.53)	0.20 (95% CrI: 0.18 to 0.22)	1.59 (95% CrI: 1.45 to 1.73)

NY99	−8.94 (95% CrIs: −9.48 to −8.45)	0.23 (95% CrI: 0.21 to 0.25)

duration across this broad temperature range likely has a large epidemiological effect, as *Cx. pipiens* lives longer at lower and intermediate temperatures.[27] The comparative probability of transmission for WN02 is therefore increased because the shorter EIPs associated with this strain coincide with temperatures where *Cx. pipiens* lifespans are typically longer. Indeed, at the temperatures where the strains are comparable (~32°C) *Cx. pipiens* lifespans are short.[27] This evidence supports the hypothesis that the shorter EIP of WN02 has contributed to the strain outcompeting NY99.[18,29] It is important to note that beyond the hottest / coldest temperature treatments we have no data; it may be the case that the strains remain comparable beyond 32°C, or that the advantage WN02 is not present below 15°C. We therefore recommend that the model coefficients are not extrapolated beyond the range explored in this study.

The strains examined in this study exist in an unusual context, with the WNV strains in the Americas sharing a single common Israeli ancestor that was introduced relatively recently. It has subsequently diversified in isolation from the rest of the world.[13] As WNV must be vectored between birds, we would expect that intercontinental transmission events are primarily contingent on migratory bird movements, with the introduction to the USA from abroad an exception, not a rule. In this research we have demonstrated that key epidemiological differences can exist between strains of WNV. Given that the strains being transmitted in Western Europe are clustered separately from strains in Israel (and therefore the Americas),[30] we suggest that experiments examining the properties of specific, locally circulating strains are important for epidemiological models to reflect local patterns of transmission.

It is important to note that these models were fit to data from a laboratory study, which limits the transferability of results. Laboratory conditions provide a much more constant environment than in nature: for instance, natural variation in temperature (e.g. diurnal temperature cycles) and other confounders are omitted and mosquitoes are confined to a uniform space. Furthermore, blood feeding occurs from an artificial membrane system, rather than live birds. The plaque assay used to detect disseminated infections in samples has been thoroughly validated for WNV, although imperfect performance may lead to imperfect detection.[22] Using lab strains of mosquitoes can also be a substantial confounder. However, laboratory studies also provide some key benefits: measurements of temperature are precise, and the effects of confounding variables are prevented.

The use of a lineage of *Cx. pipiens* in this experiment is sensible and appropriate. The species has a propensity for biting birds which maintains cycles of transmission in the WNV avian reservoir.[6] It is also considered the primary vector bridge vector from the avian resevoir to humans and other mammals in North America.[31] The role of vectors can, however, be geographically dependent. For instance, studies that examine the EIP in other bridge vectors, such as *Cx. modestus*, would be of value to assess the spillover risk to humans in Europe.[32] Indeed, understanding geographically localised patterns of the host selection functional response of *Culex* species (when faced with a choice of hosts) is essential for accurately describing transmission cycles.

To our knowledge, this research is the first use of time delay models to characterise the temperature dependent EIP of WNV. The research highlights the acute sensitivity of the WNV EIP to temperature and characterises the importance role of the variation in EIP across individual mosquitoes, particularly at low temperatures. With analyses often focused on the climatic "leading edge" of the pathogen, the role of variation in key epidemiological parameters becomes more important. We hope that the parameters estimated in this study will be useful for epidemiological modellers of WNV.

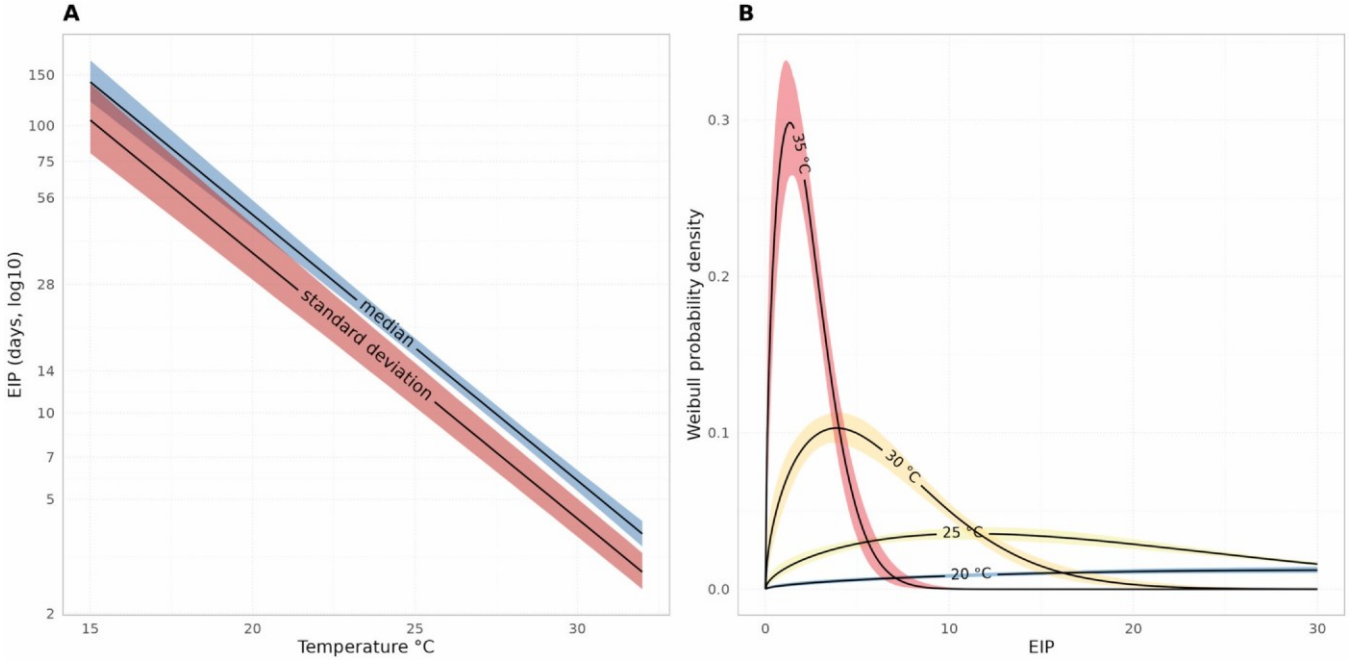

Fig. 2. A shows how the $\log_{10}$-scale temperature dependent EIP_{50} in blue and standard deviation of the EIP in red. **B** shows how this log-linear relationship with temperature changes the shape of the Weibull distribution for a range of temperatures.

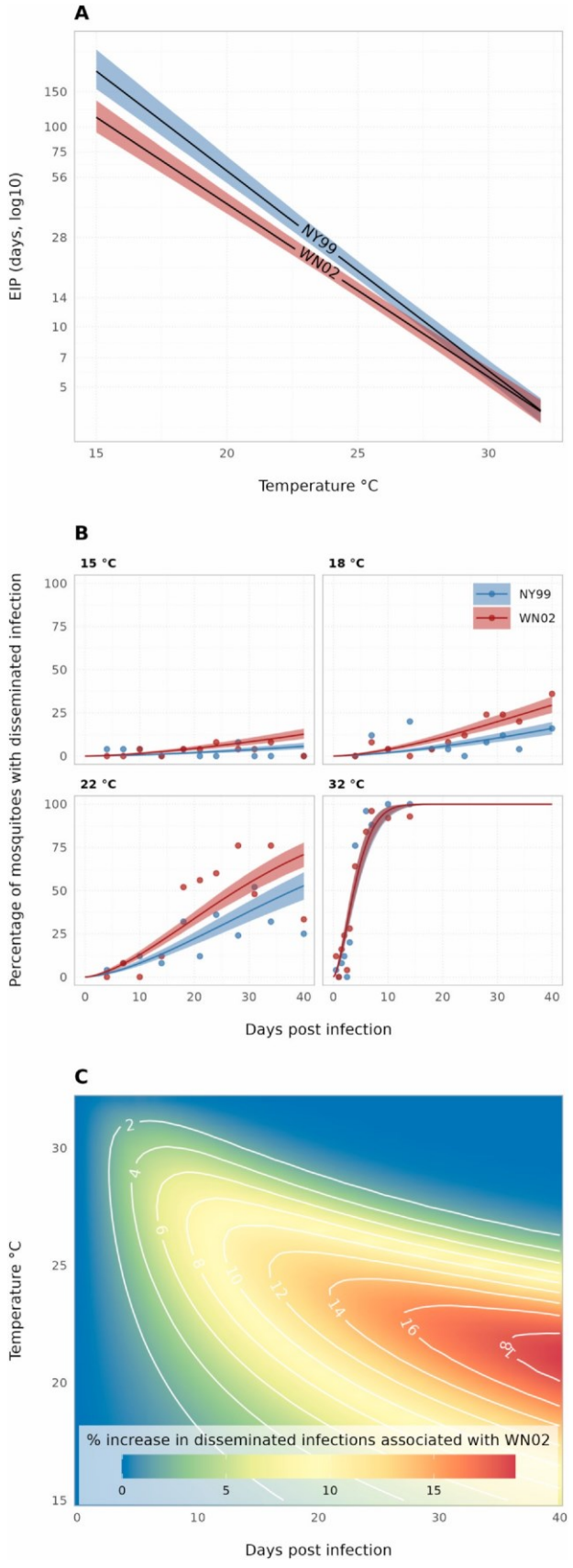

Fig. 3. A shows the predicted temperature dependent median (EIP_{50}) for the two strains. **B** shows the fit of the two-strain model to the raw proportions. **C** shows the increase in the share of mosquitoes with disseminated infections associated with WN02 compared to NY99.

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Author contributions

RSP and MV conceived the study. AMK provided the data used in the manuscript. LDK conducted the laboratory study on which this research is based. RSP wrote the statistical models and created the visualisations. MV and RSP drafted the original manuscript. All authors reviewed and contributed to the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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