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Research Article

Revisiting the Impact of Temperature on Survival of Anopheles stephensi and Aedes aegypti and Implications on Extrinsic Incubation Period

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I N F O

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A B S T R A C T

Background: Vector-borne diseases are climate-sensitive as vectors are poikilothermic. Among climatic factors, temperature is of prime importance as it affects vectors' development and pathogen transmission as well. Therefore, the present study was undertaken to understand the impact of constant variable temperatures, and indoor versus outdoor temperatures on the survival of *An. stephensi* and *Ae. aegypti* and its implication on the transmission of malaria and dengue respectively.

Method: Two to three days old laboratory-bred *An. stephensi* and *Ae. Aaegypti* female mosquitoes were kept individually in environmental chambers at different temperatures ranging from 32-42 °C and relative humidity i.e. 65-75 ± 5%. Control experiment was set up at 26 °C and 65-75% RH. Kaplan-Meier method was employed for estimation of survival probabilities and log-rank (Mantel-Cox test) for comparison, and Chi-square was determined. The daily recorded temperature was used to calculate extrinsic incubation periods of malaria parasites and dengue virus using Indirect Moshkovsky's and Oganov-Rayevsky methods, respectively.

Results: The Kaplan Meier plots of adult survival revealed that the overall survival of exposed groups significantly decreased with increasing temperature in both the vectors. The median days of survival were found higher in *Ae. aegypti* than *An. stephensi*. EIP was shorter in dengue as compared to malaria parasites. Indoor temperature was found to be more conducive for both the pathogens' transmission. *Ae. aegypti* appears more sturdy in terms of thermal tolerance.

Conclusion: The potential increase in the faster rate of development of dengue at a higher temperature indicates that with a projected rise in temperatures due to climate change, the transmission of dengue would expand temporally. Further prospective studies are needed in real-time monitoring of temperature and RH in field conditions, vis-a-vis survival of vectors for refinement of the projected scenario of vectors' survival and/ or disease transmission.

Keywords: *Anopheles Stephensi, Aedes Aegypti*, Extrinsic Incubation

Introduction

Vector-borne diseases (VBDs) are of considerable public health importance as they result in million cases annually with 700000 deaths.[1] Of six major VBDs in India, malaria and dengue are of major concern with 186532 and 44585 annual morbidity respectively in the year 2020.[2] Both infectious diseases are transmitted by climate-sensitive mosquito vectors. Being poikilothermic, mosquitoes' life stages, survival, and development of pathogens in their body for completion of the transmission cycle are affected by climatic conditions.[3,4,5] Climate change poses a further threat as the hitherto disease-free areas, particularly malaria and dengue in the present context,[6] may also be having suitability for transmission by the year 2030s.[7] The scientific basis of the vulnerability of new areas for VBDs in the wake of climate change is basically related to the developmental time of mosquito vectors, their survival, and the extrinsic incubation period for the development of pathogens.[8,9]

Vector-temperature interactions have been studied in most of the aspects of vectors' life cycle describing intricate host immune and metabolic disruptions, affecting survival, and subsequent transmission of diseases. These interactions influence the physiology of the vector,[10] resulting in intertwined metabolism,[11,12] which makes it an intriguing theme for investigating the mechanisms of vector survival under the influence of temperature. It has been reported that at a lower degree of temperature, mosquitoes' developmental rate is slower with an enhanced life span,[13] while at a higher degree, the growth rate is faster. On a few occasions, stunted growth/ development and even diapause have also been reported during unfavourable temperature conditions.[14]

Anopheles stephensi (Liston 1902) and *Aedes aegypti* (Linnaeus in Hasselquist, 1762) are the primary vectors of malaria and dengue fever in urban India. The global distribution of Anopheles and Aedes is strongly influenced by climatic factors primarily temperature. The optimal range of temperatures for development, longevity, and fecundity of the above-mentioned vectors are known to some extent but are not well-defined. As per the scientific evidence available so far, 38°C and 39°C are considered the upper-temperature threshold for the survival of *An. stephensi*[15] and *Ae. aegypti*[6] but simultaneously its impact on transmission is not defined. Moreover, the extrinsic incubation period of the malaria parasite and dengue virus also gets reduced with higher temperature, resulting in a higher number of gonotrophic cycles.[16,17]

In the context of climate change,[18] its impact on the spread of these two important vectors and understanding how the vector survival is dependent on the temperature,[10] is imperative, for decoding the rate of transmission potential. In order to provide further evidence of the impact of temperature on the development of mosquito vectors, and its impact on the Extrinsic Incubation Period, the present study was undertaken on *An. stephensi* and *Ae. aegypti*.

Materials and Method

Vectors and Survival Experiments

Two-three day old unfed adult females of *Anopheles stephensi* and *Aedes aegypti* were taken from the Institute's insectary for laboratory experiments. The mosquitoes were held in fine cloth cages of 15x15 cm size and 5% glucose and resins were provided as a source of food. Adult Survival Experiments were carried out at different temperatures i.e., 32°C, 36°C, 38°C, 40°C, 42°C and relative humidity i.e. 65-75 ± 5%. The survival time right from putting the mosquito inside the rearing chamber until death was recorded as the hours/ number of days of survival. In control, the mosquito vectors were put at 26°C ± 1°C temperature and 70 ± 5% RH. The test replicates (three; n = 50) and control (n = 50) were put under controlled conditions of temperature and RH with 12:12 hours light-dark photoperiod in Percival Incubators, USA.

Statistical Analysis

A standard non-parametric method, i.e. survival analyses, was performed using Kaplan-Meier analysis. The Kaplan-Meier analysis estimates the conditional probabilities of survival at each time to event point and provides a detailed representation of survival data for each individual. Further, the comparison between different temperature exposures was done by using the log-rank (Mantel-Cox test). The mean and median survival time (with 95% confidence intervals) was calculated for each group to compare survival times and overall Chi-square was determined. All the statistical analyses were carried out using SPSS 26 software (IBM) and data formatting was done using MS Excel.

Daily Temperature Data

The hourly data on temperature for the period of Oct-2017 to Dec-18 at 6-hour intervals was recorded by installing HOBO data loggers (MicroDaq, Contoocook, NH, USA) at Okhla, Delhi. The mean daily temperature was computed from hourly data for the calculation of Extrinsic Incubation Period in respect of both the vectors.

Extrinsic Incubation Period

The Extrinsic Incubation Period (EIP) for the development of malaria parasite was determined using Moshkovsky's Method[19] wherein the reported sum of heat in degree-days required for completing a sporogonic cycle are 105°C and 111°C for *P.vivax*, and *P. falciparum* respectively. The time period in terms of the number of days is equivalent to the time required to obtain the sum of heat by a given species. Similarly, the extrinsic incubation period of dengue

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virus was based on variation in daily temperature and was calculated as per McLean et al.[20]

$n(T) = 97.177\ e^{(-0.795\ T)}$

Where n (T) is the EIP in days and T is the temperature in degree Celsius.

Further, the daily development rate of dengue virus based on daily temperature variation was also estimated as a percentage using the equation: 1 x 100/EIP as per Oganov-Rayevsky method (WHO manual II).

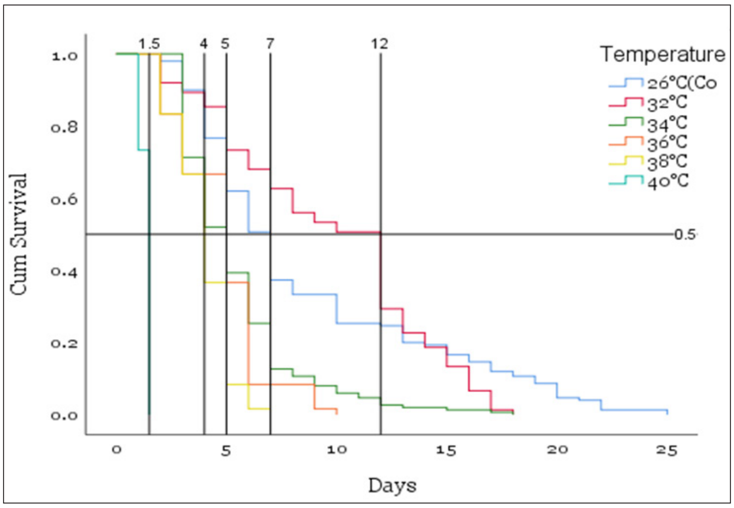
Disease Data: For identification of dengue and malaria transmission patterns, data on the monthly incidence of malaria and dengue from 2012 to 2017 were collected from the South Delhi Municipal Corporation (SDMC) of Delhi.

Results

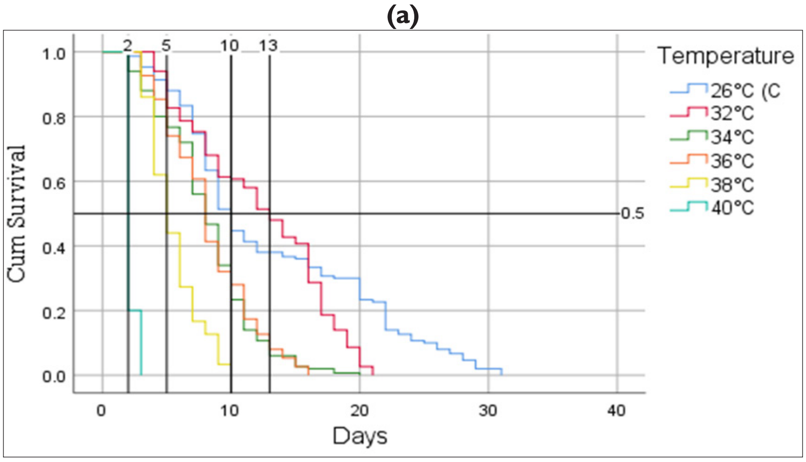
Vectors' Survival at different Temperatures

The Kaplan Meier plots of mosquitoes' survival revealed that the median number of days (number of days at which 50% of the exposed individuals survived) significantly decreased with increasing temperature in both the vectors (Figures 1a and 1b). The median days of survival at 32°, 34°, 36°, 38°, and 40° C temperatures for *Anopheles stephensi* were found to be 12 (± 0.32), 5 (± 0.25), 5 (± 0.41), 4 (± 0.21), 1.5 (± 0.0) days while in control it was 7 ± 0.32 (Figure 2a). On the other hand, in *Aedes aegypti*, the median survival days were 13 (± 0.8), 8 (± 0.37), 8 (± 0.20), 5 (± 0.22), 2 (± 0.0) days at 32°, 34°, 36°, 38°, and 40°C (Figure 2b). In control i.e. 26°C, median survival days were 10 (± 0.44). Results of survival experiments conducted at 42°C show survival only for 2-3 hours in respect of *Aedes aegypti*, while for *Anopheles stephensi*, it was only 30 minutes.

In respect of *An. stephensi* except at 32°C, other higher temperature affected the survival significantly ($p = 0.79$) (Table 1). The log rank (Mantel-Cox) analysis of the overall impact of temperature also shows that temperature also affected the survival of vector species significantly ($p < 0.001$) (Table 1). In other words, the result revealed that increasing temperature significantly ($p < 0.001$) reduces the survival of both the vector species (Table 1).



(a)



(b)

Figure 1.Kaplan Meier Survival Plots of a) *An. stephensi* b) *Ae. aegypti*

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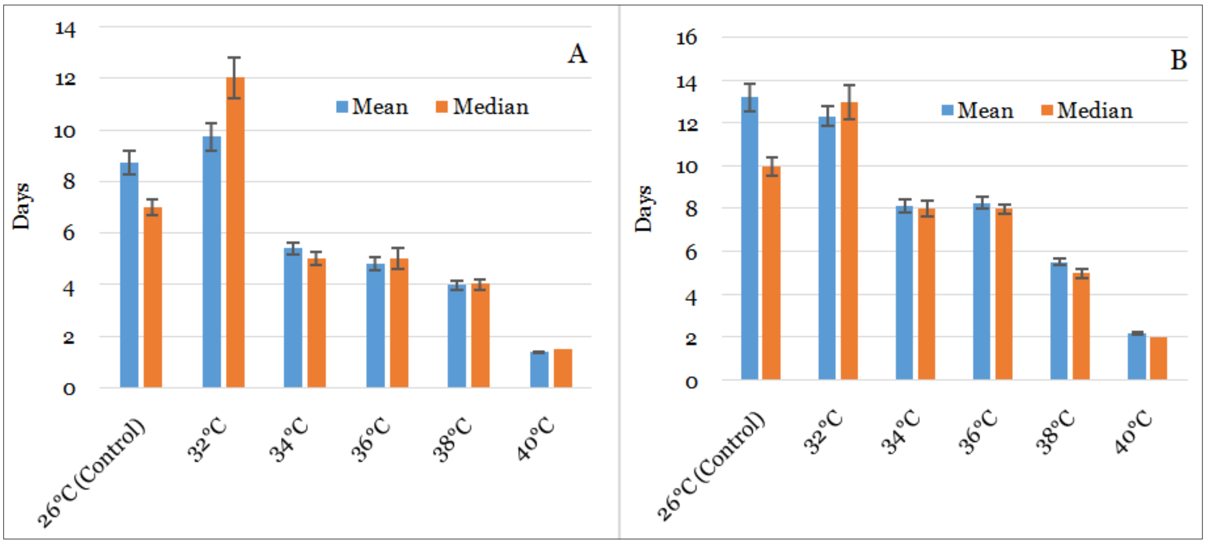


Figure 2.Mean and Median Survival Time of Adults (a) *Anopheles stephensi* and (b) *Aedes aegypti* at different Temperature Exposures. Mean is the estimation of the lowest to the largest survival time and Median survival defines the time point at which the survivorship curve crosses 0.5, or at which 50% of the sample is expected to survive.

Table 1.Log Rank (Mantel-Cox) Groupwise Comparison of Survival of Control with every 2°C Rise in Temperature Exposure and Overall Effect of Increasing Temperature on Survival of Adult *Anopheles stephensi* and *Aedes aegypti*

Log Rank (Mantel-Cox) 26°C (Control)	32°C		34°C		36°C		38°C		40°C		Overall	
	Chi-Square	Sig.	Chi-Square	Sig.	Chi-Square	Sig.	Chi-Square	Sig.	Chi-Square	Sig.	Chi-Square	Sig.
Anopheles stephensi	0.07	0.79	42.55	0.00	35.25	0.00	72.68	0.00	287.31	0.00	619.33	< 0.001
Aedes aegypti	9.14	0.00	47.14	0.00	47.84	0.00	142.35	0.00	312.20	0.00	1097.12	< 0.001

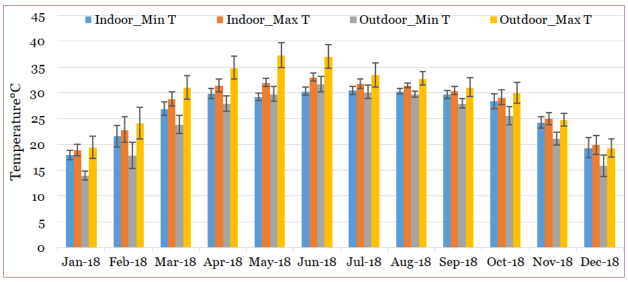


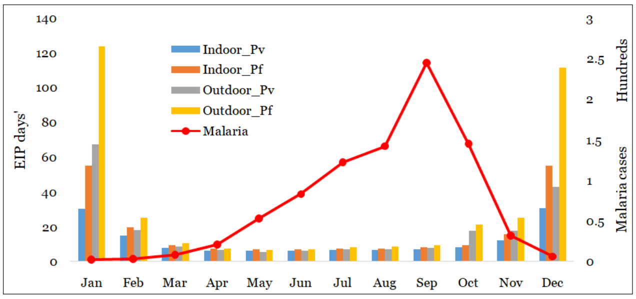
Figure 3.Monthly Recorded Indoor (a) and Outdoor (b) Temperature from January to December 2018 in Okhla, Delhi

Prevalent Temperature during the Study Period in Delhi

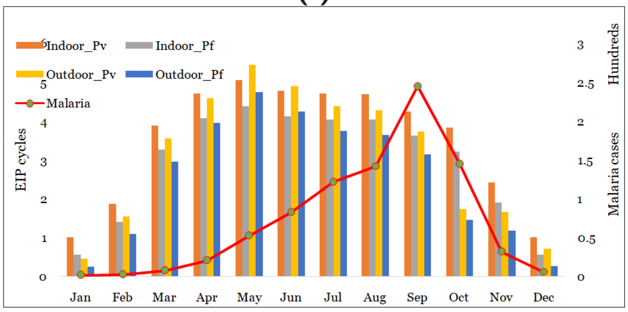
The monthly maximum/ minimum temperature recorded from January to December 2018 in Okhla, Delhi, ranged from 33.01-18.89°C/ 30.46-17.95°C indoor and 37.31-19.31°C/ 31.72-13.97°C outdoor (Figure 3). It was found that maximum temperature was more outdoors as compared to indoors while the case was the opposite in minimum temperature. Further, fluctuations were more pronounced in outdoor temperature than indoors suggesting that minimum temperatures indoors are more conducive (30.46-17.95°C) than outdoors for vector survival and pathogen transmission.

Extrinsic Incubation Period (EIP) of Malaria Parasites and Dengue Virus

The daily recorded temperature was used to calculate the number of monthly possible EIPs of Plasmodium parasites i.e. *Plasmodium vivax* and *Plasmodium falciparum*, and dengue virus. Theoretically, for *P. vivax*, the smallest duration of EIP was calculated as 5 and 6 days with outdoor and indoor temperatures during the summer month. On the other hand, for *P. falciparum*, EIP was found to be 6 and 7 days (Figure 4A). In the winter months, i.e. January and December, EIP was longest i.e. 30 and 67 days for *P. vivax* with indoor and outdoor temperatures while 55 and 123 days for *P. falciparum*. In terms of the possible number of EIP cycles, it was calculated as 1/ < 1 and less than one for *Plasmodium vivax* and *Plasmodium falciparum* during January and December months. However, during the span of one year, the possible EIP cycles are 42/35 indoor and 37/30 outdoor EIPs for *Plasmodium vivax/ falciparum* (Figure 4b).

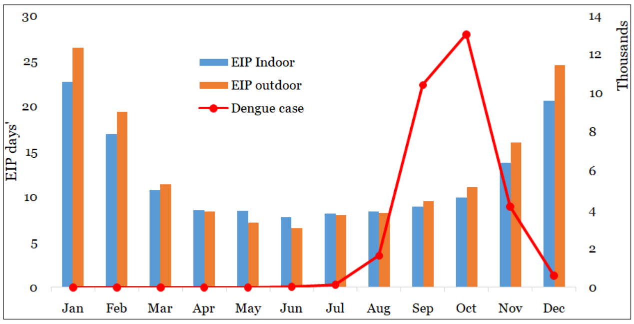


(a)

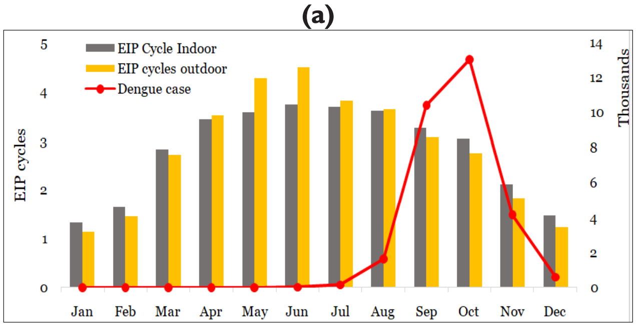


(b)

Figure 4(a).Number of Days required to Complete One EIP Cycle of Malaria and (b) Number of Monthly Possible EIPs Cycles of Plasmodium Parasite in Outdoor and Indoor Temperature Conditions



(a)



(b)

Figure 5(a).Number of Days required to Complete One EIP Cycle (b) Number of Monthly Possible Infectious Cycle of Dengue Virus in Mosquito Vector in Outdoor and Indoor Temperature Conditions

Similarly, the duration of completion of EIP for dengue virus was found to range from 6.5 to 26.42 days at outdoor temperatures while 7.7 to 22.6 days at indoor temperatures (Figure 5 a). From April to September months, EIP was found below 10 days while in extreme winter months, it reached a maximum of 26.4 days. During the span of one year, the total number of possible dengue EIP cycles were found to be 33 and 34 in indoor and outdoor temperatures.

Seasonality of Malaria and Dengue in Delhi

Based on the analysis of disease data from 2012 to 2017, it was observed that a noticeable number of malaria and dengue cases start occurring in the month of May and July respectively (Figure 4). Also, the transmission period of malaria and dengue was found to coincide with each other commencing from July and declining by the month of December.

The rise, as well as fall of an almost similar rate, was observed in malaria transmission during the pre and post-monsoon period i.e. June/ July and October/ November respectively. During the peak transmission i.e. June-November months, the estimated number of possible EIP cycles for *P. vivax* and *P. falciparum* were 24/20 and 21/17 in indoor and outdoor temperatures respectively. The monthly possible number of cycles was found to vary between 1-4 (6-25 days long) in indoor and outdoor temperatures for both parasites (Figure 4b).

As compared to malaria, the magnitude of dengue transmission has been recorded higher in post-monsoon months, i.e. September to November. After November, a sudden drop in cases has been noticed (Figure 5). Based on the calculated EIP, approximately 11-12 cycles of dengue infection can be completed during the peak transmission period i.e. August to November. During this period, monthly 2-3 EIP cycles of 8-15 days' duration are possible, indicating high suitability for transmission while only one cycle (i.e. 20-24 days) is possible in the month of January and December.

Discussion and Conclusion

In the present study, we analysed the implication of higher temperature on the survival of dengue and malaria vectors and related the same with possible transmission cycles and the occurrence of cases in Delhi. Our study substantiates the impact of temperature on the survival of adult *An. stephensi*[21] and *A. aegypti*.[22] The survival of *An. stephensi* was found 12 days at 32˚C, thereafter, the survival reduced to 5 or < 5 days indicating that 32˚C is the upper threshold for the possibility of transmission of malaria. On the other hand, *Ae. aegypti* could survive up to 8 days at 36˚C indicating the sturdiness of the vector species as well as the possibility of transmission of dengue at a higher temperature. A temperature of 42˚C can be considered

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as the upper threshold of vector survival of *An. stephensi* and *Ae. aegypti* as the mosquitoes could survive for only 30 minutes and 3 hours respectively.

Based on the generated data of temperature in Okhla, Delhi, it was estimated that during extremes of temperatures, EIP is either too short (5-6 days) or too long (123 days). From May to June, though EIP is the shortest, the increase in malaria transmission is not possible owing to unsuitable higher temperatures up to 37°C, which does not help the vector mosquito to survive long enough to complete sporogony.[23] Similarly, during December-February, even one EIP per month is not possible indicating the unsuitability of temperature for malaria transmission.[24]

In the context of dengue, possible EIP ranged from 6.5 to 26.42 days. EIP cycles were 1-2 from October to February months indicating low/ no transmission months. With the projected rise in temperature, it is expected that transmission of dengue in Delhi would extend from December to February months and malaria during November and February as well. The outbreaks of dengue in colder states like Uttarakhand were reported for the first time in Lal Kuan[25]and Himachal Pradesh also witnessed such an outbreak in 2019.[26] The projected scenario of malaria has already been made which should be taken into consideration for early response to the threat of malaria in the Himalayan region.[27]

The present study also highlights that indoor temperature is more conducive for transmission of both malaria and dengue as the temperature indoors during winter months is more than that outdoors and vice versa, increasing the suitability of transmission.

Further, the knowledge of the ecological niche of vector species is also to be viewed in the context of indoor temperature and container materials.[28] The dynamics of the biology of mosquito vectors being complex, warrants further prospective studies in real-time monitoring of temperature and RH in field conditions, vis-a-vis survival of vectors for refinement of the projected scenario of vectors' survival and disease transmission.

Authors' Contribution

PS performed the experiments, analysed the data and wrote the manuscript. VP analysed the data and wrote the manuscript. RCD conceived and designed the study, and wrote and edited the final manuscript.

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Competing Interest: None

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