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Detecting the impacts of humidity, rainfall, temperature, and season on chikungunya, dengue and Zika viruses in *Aedes albopictus* mosquitoes from selected sites in Cebu city, Philippines

Frances Edillo[1*], Rhoniel Ryan Ymbong[1], Anthoddiemn Olin Navarro[1,2], Maureen Mathilde Cabahug[1] and Kristilynn Saavedra[1]

Abstract

Background *Aedes albopictus* is the secondary vector for dengue virus (DENV) in the Philippines, and also harbors chikungunya (CHIKV) and Zika (ZIKV) viruses. This study aimed to determine the minimum infection rates (MIRs) of CHIKV, DENV serotypes, and ZIKV in *Ae. albopictus* collected from selected two-site categories by altitude (highland [H] and lowland [L] sites) in Cebu city, Philippines during the wet (WS) and dry seasons (DS) of 2021–2022, and to explore the relationships between these arboviral MIRs and the local weather.

Methods The viral RNA extracts in pooled and reared adult *Ae. albopictus* collected during the DS and WS from two-site categories were subjected to RT-PCR to amplify and detect gene loci specific for CHIKV, DENV-1 to DENV-4, and ZIKV and analyzed with the weather data.

Results The range of CHIKV MIRs was higher in the WS (13.61–107.38 infected individuals per 1,000 mosquitoes) than in the DS (13.22–44.12), but was similar between the two-site categories. Rainfall (RF) influenced the CHIKV MIR. The MIR ranges of both DENV-2 (WS: H = 0, L = 0; DS: H = 0–5.92; L = 0–2.6) and DENV-4 (WS: H = 0, L = 0–2.90; DS: H = 2.96–6.13, L = 0–15.63) differed by season but not between the two-site categories. Relative humidity (RH), RF, and temperature did not influence DENVs' MIRs. The MIR range of ZIKV was similar in both seasons (WS: 11.36–40.27; DS: 0–46.15) and two-site categories (H = 0–90.91, L = 0–55.56). RH and temperature influenced ZIKV MIR.

Conclusions RF influenced CHIKV MIR in *Ae. albopictus*, whereas RH and temperature influenced that of ZIKV. Season influenced the MIRs of CHIKV and DENVs but not in ZIKV. *Ae. albopictus* were co-infected with CHIKV, DENVs, and ZIKV in both highland and lowland sites in Cebu city. Recommendations include all-year-round implementation of the Philippine Department of Health's *4S* enhanced strategy and installation of water pipelines in rural highlands for vector and disease control. Our findings are relevant to protect public health in the tropics in this climate change.

*Correspondence:
Frances Edillo
feedillo@usc.edu.ph
Full list of author information is available at the end of the article

Keywords Chikungunya, Dengue, Zika, Asian tiger mosquitoes, Arboviral diseases

Background

While *Aedes aegypti* (Linnaeus) is the main vector of arboviruses such as chikungunya (CHIKV), dengue (DENV), and Zika (ZIKV), *Aedes albopictus* (Skuse), the Asian tiger mosquito, plays also a role in its prevalent arboviral transmission to humans [1–4] throughout the tropics and subtropics [5–8]. Dengue fever is the most common global arboviral infection; 3.6 billion people reside in dengue-endemic areas [9]. Chikungunya reached its global significance in the 21st century [10], whereas Zika caused 7.6 million cases globally in 2016 [11]. In the Philippines, chikungunya infections (600 cases; 0 death) were 545% higher from January 1 to December 31, 2022 than in the same period in 2021. Of which, 127 cases occurred in Central Visayas, where Cebu city belongs [12]. The Philippine dengue surveillance (61,170 cases; 216 deaths; case fatality rate [CFR]=0.4%) ranked second and first in the number of cases and deaths, respectively, in the Western Pacific Region in 2021 [13]. Of which, 2,604 cases and 17 deaths (CFR=0.7%) occurred in Central Visayas during the same period of 2021 [12]. The same trend in 2022 was reported in the Philippines (205, 679 cases; 672 deaths; CFR=0.3%) [14]; of which 17,894 dengue cases (104 deaths; CFR=0.6%) were reported in Central Visayas [12]. Meanwhile, the first 17 Zika cases were reported in the Philippines in 2016 without travel history from an affected country a month prior to their onset of illness [15]. No Zika case was reported since 2016, but because *Ae. aegypti* and *Ae. albopictus* are found in the country, CHIKV and ZIKV might have been circulating around.

Most studies revealed that temperatures influence large epidemics of mosquito-borne arboviral diseases [16–18]. In Latin America, temperature and its range, rainfall (RF), and population size are the important predictors of Zika transmission [19]. In Brazil, RF predicts ZIKV and CHIKV with a lead time of three weeks each time [20]. In Mexico, the dynamics of dengue, chikungunya, and Zika are strongly associated with seasonal climatological variability and transmission potential of these arboviral pathogens [21]. In Asia, only a few studies explored the relationship between arboviral mosquito-borne diseases and the weather. In India, Shil et al. [22] observed a strong positive association between the occurrence of CHIKV cases and RF variations. In Japan, Furuya's model [23] predicts that if the daily mean temperature rises from 28 to 30 °C, the median arboviral reproduction number (R_0) increases by 18% for dengue, 4.3% for chikungunya, and 11.1% for Zika. However, most studies monitored only CHIKV and ZIKV in *Aedes* such as in Asia-Pacific [24], Hindu-Kush Himalayan region [25], Singapore [26], Sri Lanka [27], and Thailand [7]. In the Philippines, only Balingit et al. [28] and Edillo et al. [29] reported DENVs in *Ae. aegypti,* the primary dengue mosquito vector, in Tarlac and Cebu cities, Philippines, respectively. However, no study, to our knowledge, has determined the influence of the weather to the infections of CHIKV, DENVs, and ZIKV in the Philippine *Ae. albopictus*, the secondary dengue mosquito vector, in this current climate change.

The National Dengue Prevention and Control Program of the Philippine Department of Health (DOH) [30] includes the following components: (1) surveillance through the Philippine Integrated Disease Surveillance and Response, (2) case management and diagnosis, (3) integrated vector management, (4) outbreak response, (5) health promotion and advocacy, and (6) research. The enhanced *4 S* strategy [12] has been the main focus for dengue prevention and control, where "*4S*" stands for the following: (1) seek and destroy mosquito-breeding sites, (2) seek early consultation if one develops dengue-associated symptoms, (3) employ self-protection measures such as wearing long pants and long-sleeved shirts, and (4) say "no" to indiscriminate fogging, and implement fogging only during outbreaks in hotspots. Climate change affects the distribution of *Ae. aegypti* and *Ae. albopictus*, which allows them to spread new pathogens to initially-unaffected populations in the mountains [31]. The latter calls for urgent actions on vector control strategies [32], including source reduction of vectors, to manage the spread of these arboviral diseases, and to protect public health [33–35] considering the lack of effective vaccines available.

We hypothesized that weather conditions might influence CHIKV, DENVs, and ZIKV in *Ae. albopictus* from selected highland and lowland sites in Cebu city, Philippines during the wet (WS) and dry seasons (DS). Thus, this study aimed: (1) to determine the presence and calculate the minimum infection rates (MIRs) of CHIKV, DENV-1 to DENV-4, and ZIKV in *Ae. albopictus* collected from selected two-site categories by altitude (highland and lowland sites) in Cebu city during the 2021–2022 WS and DS, and (2) to explore the relationships among the arboviral MIRs with the local weather in the study sites. *Ae. albopictus* was chosen in this study for the following reasons: (1) there has been no study in the Philippines, to our knowledge, that reported the impacts of weather, season, and altitude to the arboviral infections in this mosquito species. (2) Arboviral monitoring in *Ae. albopictus* is a relevant strategy for tracking their activities and for implementing direct control actions [28, 30, 36] against associated diseases and vectors as

Table 1 Coordinates and elevation of the six highland and lowland sites in Cebu city

Study Sites (Code number)	Coordinates	Elevation (m ASL)
Highlands		
Taptap (S1)	N 10.42619°; E 123.8442°	719
Babag– 1 (S2)	N 10.36876°; E 123.8601°	617
Babag– 2 (S3)	N 10.37282°; E 123.8459°	405
Lowlands		
Bacayan (S4)	N 10.38690°; E 123.91910°	83
Pit-os (S5)	N 10.40160°; E 123.91910°	83
Talamban (S6)	N 10.35373°; E 123.91264°	65

influenced by the weather. However, the local Philippine DOH focuses in monitoring the number of suspected arboviral mosquito-borne diseases in humans and not in mosquito vectors, and community participation of the enhanced *4S* strategy. (3) Also, *Ae. albopictus* is a good sentinel species in DENV prevalence during the inter-epidemic period [37] in this climate change.

Methods

Study sites

The study sites in Cebu city, Philippines were selected *barangays* (i.e., smallest government units) based on: (1) altitude (highland vs. lowland), (2) presence of local dengue cases, (3) vegetation cover, and (4) in-between site distance of more than 500 m (Table 1; Fig. 1). Highland sites had elevations between 400 and 700 m above sea level (m ASL); lowland sites, between 60 and 80 m ASL.

Mosquito collections

Aedes eggs and sub-adults were collected bimonthly during the DS (March– May 2021 and 2022) and WS (June– November 2021; February and June 2022) using the modified ovicidal/larvicidal (O/L) traps or ovitraps (Department of Science and Technology, Manila). Briefly,

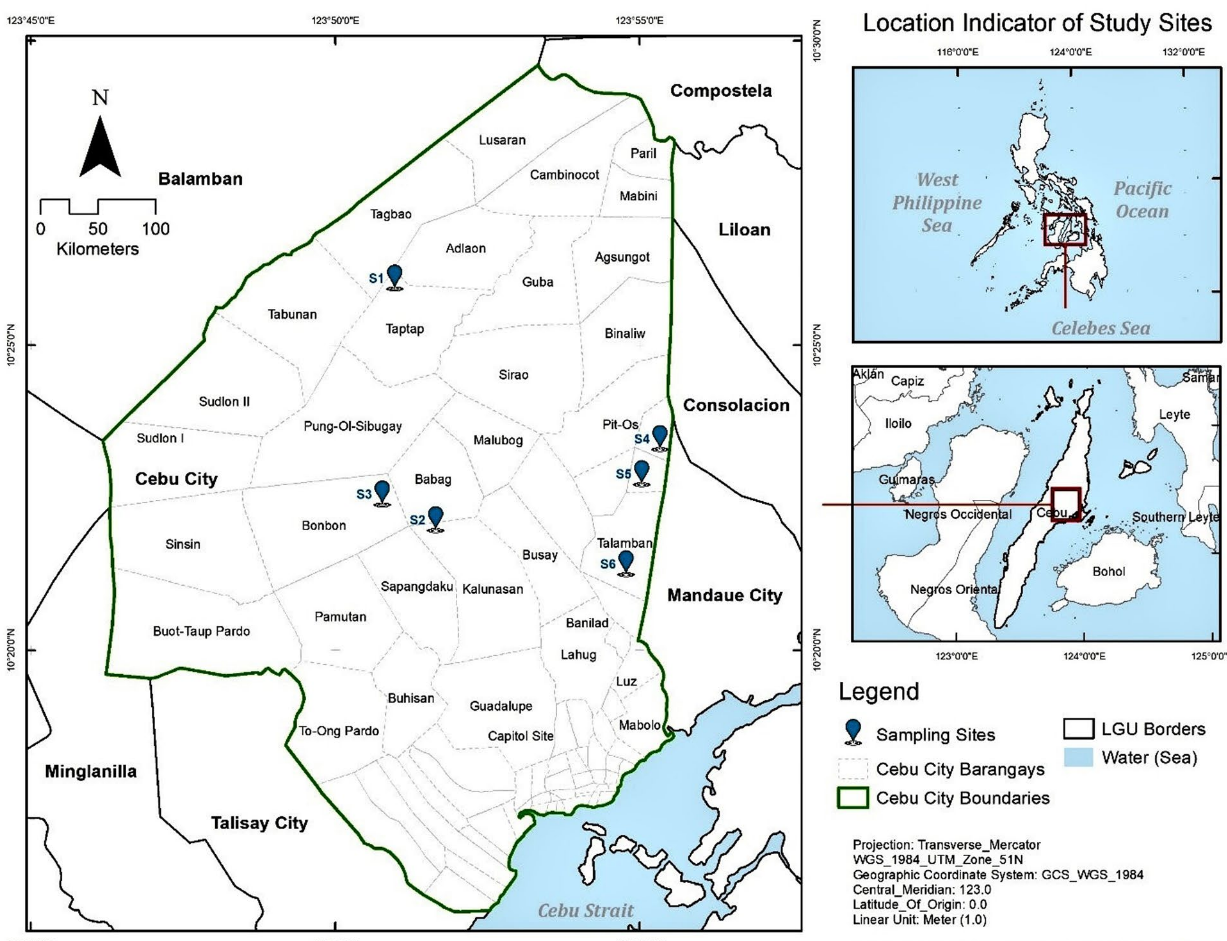

Fig. 1 Map of the six highland (S1: Taptap, S2: Babag 1 and S3: Babag 2) and lowland (S4: Pit-os, S5: Bacayan, S6: Talamban) sites in Cebu city, Cebu, Philippines

the ovitrap consisted of a black plastic container, filled halfway with water, and a filter paper-wrapped wooden panel. The original O/L pellets were discarded to allow the oviposited *Aedes* eggs and sub-adults to survive [34, 35].

Each highland and lowland site had 10 modified O/L traps, placed in locations with thick vegetation cover. The traps were left for 10–15 days. The filter papers with oviposited *Aedes* eggs (referred to as "egg papers" henceforth) were collected, dried, placed in a clean and dry container and stored in a dark place. Sub-adults were collected from the traps or other water-filled containers within the vicinity by scooping with nets or aspirating with Pasteur pipettes.

Rearing of collected *Aedes* eggs and sub-adults

Field-collected *Aedes* egg papers and sub-adults were transported to the University of San Carlos - Mosquito Research Laboratory (USC-MRL), Biology Department, Talamban campus, Cebu city, Philippines. *Aedes* egg hatching was performed by submerging the egg papers in 10% ascorbic acid solution [38]. *Aedes* larvae and pupae were transferred to a clean plastic container filled halfway with distilled water (DW) and covered with a fine-mesh cloth. They were fed daily with a dash (~0.02 g) of fish food (Sakura; All Aquariums Co., Ltd., Thailand). The DW in the larval plastic container was changed regularly to prevent the formation of bacterial scum until adult emergence. Adult *Ae. albopictus* samples were identified [39] and transferred to a separate container. They were fed with 10% sucrose solution for five days, individually transferred to a microcentrifuge tube, and stored in a -80 °C ultralow freezer until further processing.

Sample size

The sample size was calculated to consider the difference between binomial proportions (i.e., detection or non-detection) [40] of CHIKV, DENVs, and ZIKV in *Ae. albopictus* collected from highland and lowland sites of Cebu city during the DS and WS. Neyman allocation was used as an adjunct for generating a sample size for each site (i.e., apportioned from computed total sample size) with an allocated fixed budget [29]. At the significance level (α) of 0.05, 80% power (β=0.2), probabilities p1=0.10 and p2=0.30 (based from the detection of Guedes et al. [41] and Medeiros et al. [36], respectively) and 20% attrition rate, four pools (i.e., a pool ≤ 30 adults) of *Ae. albopictus* collected from each site monthly for DS and three pools collected from each site monthly for WS were considered sufficient to warrant rejecting the hypothesis of no difference. A total of 288 pools of *Ae. albopictus* were prepared for both the DS (*n*=144 pools) and the WS (*n*=144 pools).

RNA extraction

RNA extraction of adult *Ae. albopictus* was performed using the RNeasy mini kit (Qiagen, Germany) following the manufacturer's protocol. The quality of RNA extraction was determined by gel electrophoresis. RNA extracts of *Ae. albopictus* were expected to produce 18s and 28s ribosomal RNA bands at 1,000 and 1,500 base pairs (bp), respectively [42]. All extracted sample pools were composed of either male or female, or mixed adults.

Detection of CHIKV, DENV, and ZIKV

Reverse transcription-polymerase chain reaction (RT-PCR)

One-step RT-PCR was performed to detect these arboviruses in pooled *Ae. albopictus* samples using Qiagen kit (Germany; Cat No. 210212). Primer sequences are listed in Additional file 1: Table S1.

CHIKV PCR

CHIKV PCR reaction was composed of 2.5 µL 5x RT-PCR buffer, 0.5 µL deoxynucleotide triphosphate (dNTP) mix (10 µM), 0.5 µL enzyme mix, 0.25 µL 6 K/E1 OA (10 µM) and 1 µL 6 K/E1 OB (10 µM) primers, 1.25 µL RNA template, and 6.5 µL RNAse-free water (RFW). The RT-PCR profile was set at 50˚C RT for 30 min, 95˚C incubation for 10 min, 40 cycles of 95˚C denaturation for 30 s, 53˚C annealing for 30 s, 72˚C extension for 30 s, and 72˚C final extension for 10 min.

Nested PCR of CHIKV

CHIKV nested PCR was done to further amplify the inner region of 6 K/E1 gene. A nested PCR concoction (10 µL) was prepared: 5 µL 2x PCR mastermix, 0.5 µL of both inner 6 K/E1 IA and 6 K/E1 IB primers, 1 µL cDNA template and 3 µL RFW.The PCR profile was set at 95˚C incubation for 10 min, 35 cycles of 95˚C denaturation for 30 s, 55˚C annealing for 30 s, 72˚C extension for 30 s, and 72˚C final extension for 10 min. CHIKV outer and inner primers used in nested RT-PCR were based on Laras et al. [43].

DENV PCR

The DENV PCR reaction contained 2.5 µL 5x buffer with 1.5 mM MgCl2, 0.5 µL dNTPs (10 Mm), 0.5 µL RT- PCR enzyme mix, 0.75 µL D1 (10 µM) and 0.75 µL D2 (10 µM) primers, 2 µL RNA sample, and 5.5 µL RFW. The PCR profile was set at 50 °C RT for 30 min, 95 °C incubation for 15 min; 35 cycles of 94 °C denaturation for 30 s, 55 °C annealing for 1 min, 72 °C extension for 1 min, and final extension at 72 °C for 10 min. After RT-PCR, a second PCR was done using similar PCR reaction and conditions to visualize distinct bands in gel electrophoresis.

Table 2 Positive control profiles of the three arboviruses isolated from the local *Aedes* species

Arbovirus	Expected band size (bp)	Aedes species	Site of Origin	Date of Collection
CHIKV	200 (49)	*Ae. albopictus*	Talamban (S6)	August 2021
DENV (consensus)	511 (50)	*Ae. aegypti*	Liloan, Cebu	August 2021
DENV-1	482 (50)	*Ae. aegypti*	Liloan, Cebu	August 2021
DENV-2	119 (50)	*Ae. albopictus*	Talamban (S6)	November 2021
DENV-3	290 (50)	*Ae. aegypti*	Liloan, Cebu	October 2021
DENV-4	392 (50)	*Ae. aegypti*	Liloan, Cebu	November 2021
ZIKV	192 (51)	*Ae. albopictus*	Taptap (S1)	August 2021

Serotyping of DENVs

Products from the DENV RT-PCR were used for DENV serotyping using HotStarTaq master mix kit (Qiagen, Germany; Cat. No. 203446) with PCR profile of 95 °C incubation for 15 min, 25 cycles of 94 °C denaturation for 30 s, 55 °C annealing for 1 min, 72 °C extension for 1 min, and final extension at 72 °C for 10 min.The DENV serotyping PCR reaction was made up of 12.5 µL 2x HotStarTaq master mix, 1 µL 25 mM MgCl2, 0.5 µL dNTPs (10 Mm), 1.5 µL cDNA template, 6.375 µL NFW, and 0.625 µL of each of the following primers (10 µM) such as D1, D2, TS1, TS2, TS3, and TS4. DENV PCR and serotyping were based on Lanciotti et al. [44].

ZIKV PCR

ZIKV PCR reaction was made of 2.5 µL 5x RT-PCR buffer, 0.5 µL dNTP mix (10 µM), 0.5 µL enzyme mix, 0.625 µL ZIKV (10 µM) forward and reverse primers, 2 µL RNA template, and 5.75 µL RFW. The RT-PCR profile was set at 50˚C RT for 30 min, 95˚C incubation for 10 min, 40 cycles of 95˚C denaturation for 30 s, 57˚C annealing for 30 s, 72˚C elongation for 30 s, and 72˚C final elongation for 10 min. The protocol was based on Balm et al. [45] with modifications.

PCR product purification and sequencing of positive controls

Positive controls for CHIKV and ZIKV amplicons were isolated from local *Ae. albopictus*, whereas those for DENV-1 to DENV-4 were isolated from *Ae. aegypti* or *Ae. albopictus* (Table 2). They were subjected to the aforementioned RNA extraction and RT-PCR protocols. For confirmation of arboviral amplicons, they were electrophoresed on 1% agarose gel at 200 VDC for 20 min.

Non-specific amplifications were observed among the PCR products of positive controls, thus, gel excision and extraction of the bands corresponding to expected band size of each arbovirus (Table 2) were done using QIAquick PCR and gel cleanup kit (Qiagen, Germany; Cat. No. 28704) following the manufacturer's protocol. Another PCR run was done to further amplify the desired DNA fragment using Type-it microsatellite PCR kit (Qiagen, Germany). A PCR reaction (20 µL) was made of 10 µL 2x PCR mastermix, 1 µL (10 µM) each of the forward and reverse primers, 4 µL DNA template, and 4 µL RFW. The PCR profile for CHIKV positive control was set at 95˚C incubation for 10 min, 35 cycles of 95˚C denaturation for 30 s, 53˚C annealing for 30 s, 72˚C extension for 30 s, and 72˚C final extension for 10 min. For DENVs and ZIKV positive controls, the PCR profile was similar except annealing temperatures (i.e., 55˚C for DENV and 57˚C for ZIKV).

Purified PCR products for CHIKV, DENV-1 to DENV-4, and ZIKV positive controls were brought to the Philippine Genome Center, University of the Philippines - Diliman, Quezon city for capillary sequencing. Their results were processed using DNA Baser Assembler v4, and were trimmed and cleaned using BioEdit v7.2 [46]. The final sequences were subjected to the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (NCBI) [47] for identity verification.

Table 3 Categorized ranks for weather data (mean monthly relative humidity [RH], monthly total rainfall [RF] and mean monthly temperature) utilized in Kruskal-Wallis statistical tests

Weather Condition	Low	Moderate	High
RH	74–76%	77–78%	79–80%
RF	< 120 mm	120–150 mm	> 150 mm
Temperature	27 °C	28 °C	29 °C

Weather data

The mean monthly relative humidity (RH), monthly total rainfall (RF), and mean monthly temperature nearest (11.8– 32.4 km) to the study sites throughout the study period were obtained from the local satellite office of the Philippine Atmospheric Geophysical and Astronomical Services Administration (PAG-ASA), Cebu city.

Data and statistical analyses

The monthly MIRs of CHIKV, DENV-1 to DENV-4, and ZIKV were computed for each site by dividing the total number of positive pools by the total number of individual mosquito samples, multiplied by 1,000 [48]. Because of non-normality of data distributions, the dependent variables (arboviral MIRs) and the independent variables (season, altitude, and weather data) were subjected to Kruskal-Wallis (K-W) statistical tests with arbitrarily categorized ranks for weather data (Table 3) by using SPSS Advanced Professional (v28). Spearman's rank coefficient

Table 4 MIRs of CHIKV, DENV and ZIKV with number of positive pools in *Ae. albopictus* by season (dry and wet) and two-site categories by altitude (highland and lowland) in Cebu city, Philippines

Season and Sites	n[a]	MIRs (w/ number of positive pools[b])					
		CHIKV	DENV-1	DENV-2	DENV-3	DENV-4	ZIKV
Dry Season							
Highland							
Taptap (S1)	358	33.52 (12)	0.00 (0)	0.00 (0)	2.79 (1)	5.59 (2)	27.93 (10)
Babag 1 (S2)	326	18.40 (6)	3.07 (1)	3.07 (1)	6.13 (2)	6.13 (2)	36.82 (12)
Babag 2 (S3)	338	32.54 (11)	2.96 (1)	5.92 (2)	8.88 (3)	2.96 (1)	17.75 (6)
Lowland							
Bacayan (S4)	384	15.63 (6)	7.81 (3)	2.60 (1)	13.02 (5)	15.63 (6)	31.25 (12)
Pit-os (S5)	319	34.48 (11)	0.00 (0)	0.00 (0)	9.40 (3)	3.13 (1)	18.81 (6)
Talamban (S6)	274	47.45 (13)	7.30 (2)	0.00 (0)	3.65 (1)	0.00 (0)	18.25 (5)
Wet Season							
Highland							
Taptap (S1)	313	51.12 (16)	0.00 (0)	0.00 (0)	12.78 (4)	0.00 (0)	31.95 (10)
Babag 1 (S2)	312	48.08 (15)	3.21 (1)	0.00 (0)	0.00 (0)	0.00 (0)	32.05 (10)
Babag 2 (S3)	335	47.76 (16)	2.99 (1)	0.00 (0)	0.00 (0)	0.00 (0)	17.91 (6)
Lowland							
Bacayan (S4)	475	40.00 (19)	4.21 (2)	0.00 (0)	4.21 (2)	0.00 (0)	27.37 (13)
Pit-os (S5)	345	57.97 (20)	8.70 (3)	0.00 (0)	2.90 (1)	2.90 (1)	20.29 (7)
Talamban (S6)	311	51.45 (16)	3.22 (1)	0.00 (0)	6.43 (2)	0.00 (0)	25.72 (8)

[a]n = total number of individuals

[b]number of positive pools out of 24 pools per site

of correlations were performed between the MIRs of CHIKV and ZIKV, and the weather with significant K-W test results to assess the correlation. Pearson's product-moment correlation tests were performed between the MIRs of DENVs and the dengue cases. Two-tailed Student's t-tests were determined for the statistical differences between seasonal weather data.

Table 5 Kruskal-Wallis statistical test results for the minimum infection rates (MIR) of chikungunya (CHIKV), dengue (DENV), and Zika (ZIKV) in *Ae. albopictus* when grouped by season (dry and wet) and by two-site categories by altitude (highland and lowland) in Cebu city, Philippines

Category	Kruskal-Wallis H	df	*P*-value
By season			
CHIKV	14.814	1	0.000*
DENV-1	0.119	1	0.730
DENV-2	4.097	1	0.043*
DENV-3	2.894	1	0.093
DENV-4	11.928	1	0.001*
ZIKV	1.377	1	0.241
By altitude			
CHIKV	0.007	1	0.932
DENV-1	1.960	1	0.162
DENV-2	0.341	1	0.559
DENV-3	0.018	1	0.892
DENV-4	0.108	1	0.743
ZIKV	0.000	1	0.993

*Statistically significant

Results

Isolation of CHIKV, DENVs, and ZIKV in *Ae. albopictus* as positive controls

The positive controls isolated from the local *Ae. albopictus* formed the expected gel bands consistently at 200 bp for CHIKV [43], 511 bp for DENV [44], and192 bp for ZIKV [45] (Table 2). The NCBI BLAST results of amplicon nucleotide sequences of these bands yielded 100% identity matches. Likewise, the positive controls with their expected gel bands for DENV-1 (482 bp), DENV-3 (290 bp), and DENV-4 (392 bp) isolated from the local *Ae. aegypti*, and for DENV-2 (119 bp) isolated from the local *Ae. albopictus* were formed consistent with Lanciotti et al. [44].

MIRs of CHIKV in *Ae. albopictus* by season and altitude

Table 4 (Additional file 2: Dataset 1) shows that during the DS, the highest MIR of CHIKV (47.45 per 1,000 mosquitoes in the wild; from here onward MIR refers to an estimate for every 1,000 *Ae. albopictus* in the wild) was recorded in the lowland Talamban site. The lowland Bacayan had the lowest MIR (15.63) in the DS and in the entire sampling period; likewise it had also the lowest MIR (40.00) in the WS. The lowland Pit-os had the highest MIR (57.97) of CHIKV during the WS and in the entire sampling period.

K-W test results (Table 5) showed that seasonal MIRs of CHIKV in *Ae. albopictus* differed (K-W H-test, $H=14.814$, $df=1$, $P<0.0001$), with the mean MIR ranks at 30.69 for the DS and at 51.35 for the WS, but did not differ (K-W H-test, $H=0.007$, $df=1$, $P=0.932$) in the two-site categories by altitude. The mean MIR ranks of CHIKV MIR were 42.27 for highland and 42.73 for lowland. Residents in highland sites stored water in artificial containers for domestic use that served as potential breeding sites for *Aedes* owing to the lack of installed water pipelines by the local government.

MIRs of DENVs in *Ae. albopictus* by season and altitude

During the WS (Table 4; Additional file 2: Dataset 1), only DENV-1 (MIR range: 0–3.21) and DENV-3 (0–12.78) were detected in the highland sites, while DENV-1 (3.22–8.70), DENV-3 (2.90–6.43), and DENV-4 (0–2.90) were detected in the lowland sites. Results suggest that during the WS, *Ae. albopictus* in highland sites might have harbored more DENV-3, while that of the lowland might have harbored more DENV-1. Also, the absence of DENV-2 in *Ae. albopictus* in both two-site categories in the WS suggests that *Ae. aegypti*, the primary dengue mosquito vector, might have maintained DENV-2. During the DS (Table 4), *Ae. albopictus* in both two-site categories had the four DENV serotypes. Results suggest that *Ae. albopictus* played an important role in DENV maintenance during the DS when breeding sites were scarce.

K-W test results (Table 5) revealed that seasonal MIRs of DENV-2 (K-W H-test, $H=4.097$, $df=1$, $P=0.043$) and DENV-4 (K-W H-test, $H=11.928$, $df=1$, $P=0.001$) differed, however, all DENV serotypes when grouped in two-site categories by altitude did not differ (K-W H-test, $P>0.05$). Results suggest that *Ae. albopictus* circulated all DENV serotypes in both highland and lowland sites, and their residents were exposed to risks of contracting dengue. Moreover, the seasonality of DENV-2 and DENV-4 in *Ae. albopictus* across sites may suggest that local weather might have strongly influenced their viral replications.

Table 6 Weather data (mean monthly relative humidity [RH] and temperature, and monthly total rainfall [RF]) in Cebu city, Philippines during the dry (March - May 2021; March– May 2022) and wet (June– November 2021; February and June 2022) seasons

Season	Month	RH (%)	RF (mm)	Temperature (ºC)
Dry Season 2021	March	77.00	90.20	28.35
	April	74.00	52.00	28.60
	May	78.00	120.60	29.40
Mean		76.33	87.60	28.78
Wet Season 2021	June	80.00	226.20	28.75
	July	75.00	130.50	29.30
	August	78.00	143.60	28.96
	September	80.00	124.60	28.60
	October	79.00	203.00	28.44
	November	79.00	146.50	28.76
Mean		78.50	162.40	28.80
Dry Season 2022	March	79.00	102.40	28.31
	April	80.00	368.20	27.84
	May	78.00	69.80	29.12
Mean		79.00	180.13	28.42
Wet Season 2022	February	79.00	60.10	27.80
	June	75.00	179.30	28.62
Mean		77.00	119.70	28.21

MIRs of DENVs in *Ae. albopictus* and dengue cases in Cebu city

While the combined MIRs of all DENV serotypes in *Ae. albopictus* were high during the DS (March to May 2021 and 2022) and decreased during the start of the WS (July 2021 and June 2022), dengue cases in Cebu city (Additional file 3: Table S2) gradually increased from March 2021 until June 2022 (Fig. 4). Results showed a low, negative, and insignificant correlation (Pearson's correlation coefficient, $r_{(14)} = -0.131$, $P=0.654$), implying a weak association between the MIRs of DENVs in *Ae. albopictus* and reported dengue cases. Results suggest that the local *Ae. albopictus* maintained DENVs more frequently during the DS, and transmitted them to humans during the WS.

MIRs of ZIKV in *Ae. albopictus* by season and altitude

During the DS, the highest MIR (36.81) of ZIKV in *Ae. albopictus* was recorded in Babag 1 highland site, which was also the highest MIR in the entire sampling period (Table 4; Additional file 2: Dataset 1). The lowest MIR (17.75) of ZIKV was recorded in Babag 2, which was also the lowest MIR in the entire study period. During the WS, the highland Taptap site had the highest MIR (31.95) of ZIKV, while that of Babag 2 was lowest (17.91).

The seasonal mean MIRs of ZIKV in *Ae. albopictus* did not differ (K-W H-test, $H=1.377$, $df=1$, $P=0.241$) with 45.14 and 38.99 for the WS and the DS, respectively. Likewise, the mean MIRs of ZIKV were similar (K-W H-test, $H=0.000$, $df=1$, $P=0.993$) between the two-site categories by altitude (Table 5).

Description of the seasonal weather data

Both the seasonal mean monthly RH (DS 2021=76.33%; WS 2021=78.50%; DS 2022=79%; WS 2022=77%) and mean monthly temperatures (DS 2021=28.78 °C; WS 2021=28.80 °C; DS 2022=28.42 °C; WS 2022=28.21 °C) were comparable in both WS and DS of 2021 and 2022 sampling years (Table 6). By Student's t-test, the seasonal monthly RH (t-test, $t_{(11)}=2.201$, $P=0.687$) and temperatures (t-test, $t_{(9)}=2.262$, $P=0.862$) did not differ. Likewise, the seasonal mean monthly total RF did not differ (t-test, $t_{(6)}=2.447$, $P=0.739$) in the WS and DS of 2021 and 2022. Expectedly, the mean monthly total RF was higher in the WS of 2021 (162.40 mm) than that of the DS of the same year (87.60 mm), however, the mean monthly total RF in the DS (2022) (180.13 mm) was higher than that

(79.80 mm) of the WS (2022) that apparently could be attributed to climate change.

Relationship between the MIRs of CHIKV in *Ae. albopictus* and the weather

Figure 2 shows the monthly CHIKV's MIRs plotted against monthly readings of RH, rainfall, and temperature in Cebu city. CHIKV's MIRs in *Ae. albopictus* differed (K-W H-test, $H=8.537$, $df=2$, $P=0.014$; Table 7) between rainfall categories (Table 3). The highest mean MIR (52.38) occurred in the moderate RF category (120.1–150.0 mm), followed by high RF category (>150.0 mm) with mean MIR of 40.42, and lowest at the low RF category (<120.0 mm) with mean MIR of 34.28. Spearman's correlation test results revealed a weak positive but insignificant correlation (Spearman's correlation coefficient, $rs=0.16$, $P=0.573$) between the MIR of CHIKV and RF, implying that RF did not positively affect CHIKV. Moreover, CHIKV's MIRs in *Ae. albopictus* did not differ (K-W H-test, $H=3.560$, $df=2$, $P=0.169$) (Table 7) as influenced by temperature (Table 3) with mean MIRs of 32.04, 45.88, and 39.33 for low (27 °C), moderate (28 °C), and high (29 °C) temperature categories, respectively. Likewise, CHIKV's MIRs did not differ (K-W H-test, $H=4.489$, $df=2$, $P=0.106$; Table 7) as influenced by RH with mean MIRs of 42.19 (low), 34.19 (moderate), and 47.36 (high) RH categories.

Relationship between the MIRs of ZIKV in *Ae. albopictus* and the weather

Figure 2 shows the monthly ZIKV's MIR plotted against monthly readings of RH, rainfall, and temperature in Cebu city. ZIKV's MIR in *Ae. albopictus* differed between categorized ranges (Table 3) for RH (K-W H-test, $H=18.106$, $df=2$, $P<0.0001$) (Table 7) and temperatures (K-W H-test, $H=7.067$, $df=2$, $P=0.029$) (Table 7) but not for RF (K-W H-test, $H=1.630$, $df=2$, $P=0.443$) (Table 7). ZIKV's MIR was highest in the high RH category (79–80%) with a mean MIR of 53.42, followed by the low monthly RH category (74–76%) with a mean MIR of 34.22, and lowest at moderate monthly RH (77–78%) with a mean MIR of 29.60. A significant positive Spearman's correlation (Spearman's correlation coefficient, $rs=0.65$, $P=0.012$) between the MIR of ZIKV and RH was observed. On the other hand, ZIKV's MIR was highest in the low temperature category (27 °C) with a mean MIR of 52.58, followed by moderate (28 °C), and high (29 °C) categories with mean MIRs of 44.27 and 30.47, respectively. A negative and insignificant correlation (Spearman's correlation coefficient, rs = -0.44, $P=0.117$) was observed between the MIR of ZIKV and temperature.

Relationship between the MIRs of DENVs in *Ae. albopictus* and weather

Figure 3 shows the monthly MIRs of DENVs plotted against monthly readings of RH, rainfall, and temperature in Cebu city. K-W test results showed no differences (K-W H-test, $P>0.05$) (Table 7) in all DENV serotypes across RH, rainfall, and temperature.

Discussion

This study is the first report in detecting the impacts of Philippine weather (i.e., RH, RF, and temperature) and seasonal (i.e., WS and DS) conditions on the MIRs of CHIKV, four DENV serotypes, and ZIKV in *Ae. albopictus*, the secondary dengue mosquito vector in the country, collected from selected highlands and lowlands in Cebu city, Philippines during the WS and DS (2021–2022).

CHIKV in *Ae. albopictus*

First, the seasonal MIRs of CHIKV in *Ae. albopictus* differed but not in two-site categories by altitude (Table 5). The former had higher MIR in the WS (2021–2022), suggesting higher CHIKV prevalence during the WS as supported by the significant difference (Table 7) of CHIKV's MIRs in *Ae. albopictus* as influenced by RF. Results were consistent with reported positive effect of RF on CHIKV transmission [20, 49–51]. However, similar MIRs of CHIKV in *Ae. albopictus* in both Cebu city's highland and lowland sites (Table 5) indicate that CHIKV naturally circulated in both two-site categories by altitude. Hence, residents were exposed to risks of contracting CHIKV as evidenced by reported cases described above. Results were consistent in Albanian infestation by CHIKV-infected *Ae. albopictus* at high altitudes [52].

Second, a significant difference of CHIKV's MIR in *Ae. albopictus* was observed in RF with a weak positive yet insignificant correlation (Spearman's correlation coefficient, $rs=0.16$, $P=0.573$), but not with the RH and temperature (Table 7). The mean monthly total RF (180.13 mm) during the DS (2022) was higher than that (162.4 mm) of the WS (2022), and huge difference of mean monthly total RF between the DS of 2021 (87.6 mm) and 2022 (180.13 mm) (Table 6). Increased precipitation supports the maintenance of breeding sites contributing to mosquitoes' survival [53]. Currently, the correlation between CHIKV's prevalence in *Ae. albopictus* and RF is consistent with the reports in Thailand [49, 51] and Kenya [50]. CHIKV is maintained throughout all seasons in Kenya but the abundance of CHIKV-infected mosquitoes is more evident during increased RF (~250 mm) [50]. Heavy RF with a three-week interval predicts the onset of CHIKV outbreaks [20]. Although, this study did not observe significant relationship between CHIKV's MIR in *Ae. albopictus* and mean

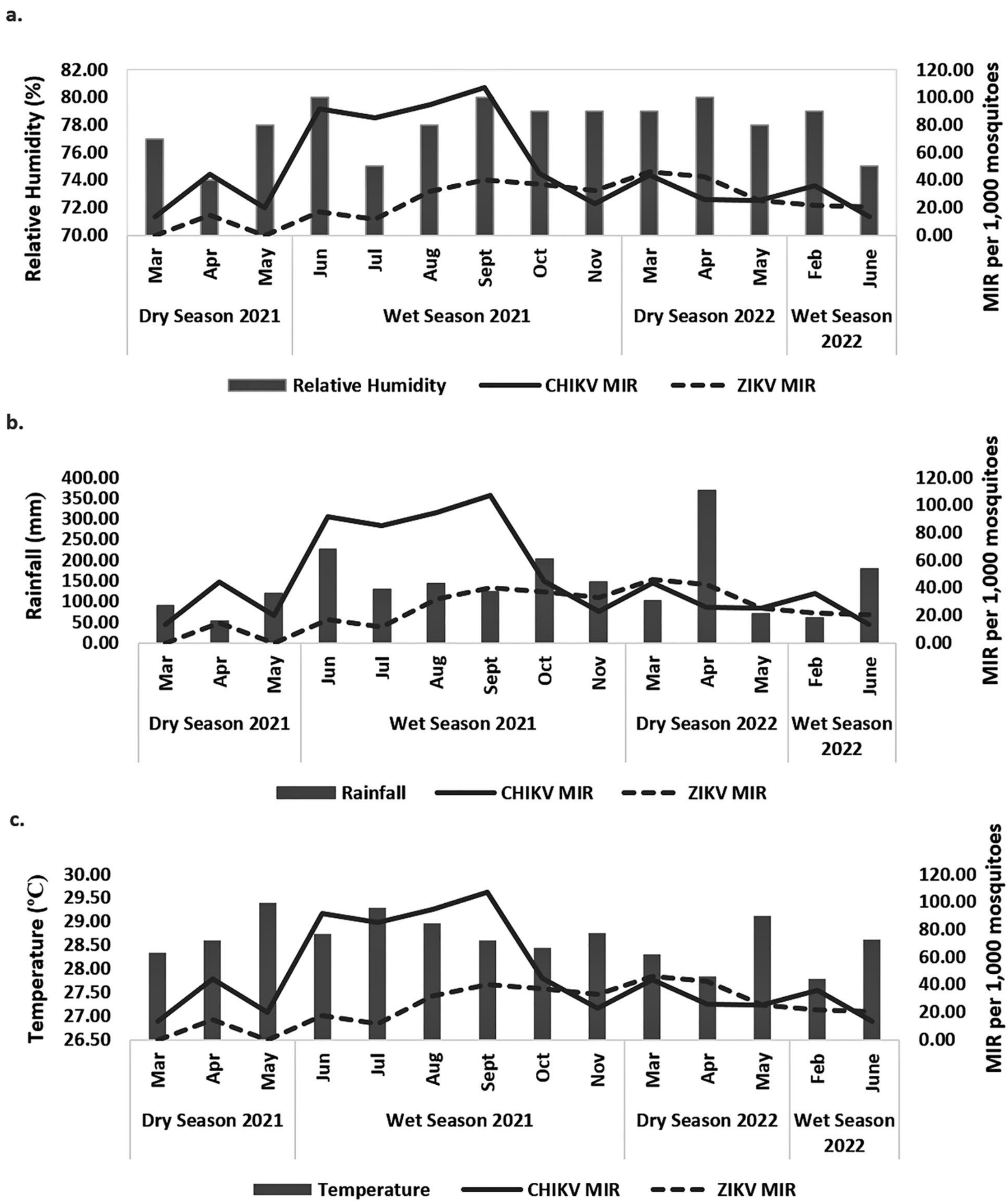

Fig. 2 Monthly CHIKV and ZIKV MIR in *Ae. albopictus* with monthly mean relative humidity (%)(**a**), monthly total rainfall (mm)(**b**), and monthly mean temperature (°C)(**c**) in Cebu city, Philippines during the dry (March - May 2021; March– May 2022) and wet (June– November 2021; February and June 2022) seasons. CHIKV, chikungunya; ZIKV, Zika; MIR, minimum infection rate

Table 7 Kruskal-Wallis statistical test results between the minimum infection rates (MIRs) of chikungunya (CHIKV), dengue (DENV), and Zika (ZIKV) viruses in *Ae. albopictus* and weather data (mean monthly relative humidity [RH] and temperature, and monthly total rainfall [RF]) in Cebu city during the dry (March - May 2021; March– May 2022) and wet (June– November 2021; February and June 2022) seasons

Category	Kruskal-Wallis H	df	*P*-value
By RH			
CHIKV	4.489	2	0.106
DENV-1	0.885	2	0.244
DENV-2	3.073	2	0.215
DENV-3	4.087	2	0.130
DENV-4	2.622	2	0.270
ZIKV	18.106	2	0.000*
By RF			
CHIKV	8.537	2	0.014*
DENV-1	1.422	2	0.491
DENV-2	5.531	2	0.063
DENV-3	0.382	2	0.826
DENV-4	1.503	2	0.472
ZIKV	1.630	2	0.443
By temperature			
CHIKV	3.560	2	0.169
DENV-1	3.426	2	0.180
DENV-2	1.707	2	0.426
DENV-3	0.279	2	0.870
DENV-4	4.815	2	0.090
ZIKV	7.067	2	0.029*

*Statistically significant

monthly temperature nearest to the study sites, Mercier et al. [52] reported that *Ae. albopictus* only transmits CHIKV at 28 °C.

DENVs in *Ae. albopictus*

Currently, *Ae. albopictus* carried all four DENV serotypes (Table 4), like *Ae. aegypti*, consistent with other studies [54–58], although its odds of having DENV-infected saliva is lower than that of *Ae. aegypti* [58]. Interestingly, seasonal MIRs of DENV-2 and DENV-4 in *Ae. albopictus* differed (Table 5), suggesting a specific vector-virus interaction. However, the lack of MIR differences of the four DENV serotypes in *Ae. albopictus* from two-site categories by altitude (Table 5), implies their expanded distribution to highlands as affected by the changing climate [31]. Among the highland sites, Taptap had the highest altitude (719 m ASL), where householders stored water for domestic use in artificial containers [34, 35]. This practice increased the breeding sites in highlands, and may amplify the risks of contracting mosquito-borne diseases. The presence of *Ae. albopictus* in both Cebu city's lowland (urban) and highlight (rural) sites elucidates their ability to spread these arboviruses [37]. With expanded distribution of *Ae. albopictus* to highlands [59], current vector control programs should be extended there as well.

Currently, the MIRs of DENV-1 and DENV-3 in *Ae. albopictus* were similar with those of *Ae. aegypti* [60], whereas those of DENV-2 and DENV-4 were different. In Brazil, DENV-1 and DENV-3 are most common in *Aedes* and in humans [61]. In Singapore, DENV-1 is most common in both *Ae. aegypti* and *Ae. albopictus* [62]. These studies corroborate with the higher MIRs of DENV-1 and DENV-3 in *Ae. albopictus* from lowland and highland, respectively (Table 4).

Moreover, *Ae. albopictus* showed higher MIRs of DENVs (mean = 13.33) during the DS, the inter-epidemic period, than during the WS (mean MIR = 8.85) (Table 4), suggesting the silent DENVs' circulation in mosquitoes on areas with low dengue cases in the DS [63]. In addition, DENV-2 was only detected during the DS regardless of altitude. Hence, *Ae. albopictus* is a good sentinel species in monitoring DENV prevalence during inter-epidemic period [37]. A low negative insignificant correlation (Pearson's correlation coefficient, $r_{(14)}$ = -0.131, $P = 0.654$) between the MIRs of DENV-infected *Ae. albopictus* and dengue cases (Fig. 4) could be attributed to the fact that this species is the secondary dengue vector in the Philippines.

Furthermore, temperature, RF, and RH did not affect the MIRs in DENV-infected *Ae. albopictus* between seasons and altitudes (Table 5). However, these weather predictors influence dengue occurrences and viral transmission in *Ae. aegypti* [35, 62, 64–68]. Generally, DENVs' mean extrinsic incubation periods (EIP) in vector mosquitoes are 15 days and 6.5 days at 25 and 30 °C, respectively [69]. DENV-2 decreases its EIP in *Ae. albopictus* as temperature increases, with infection rate and transmission efficiency at 31 °C [70]. Atmospheric temperatures (DS: 27.84 to 29.40 °C; WS: 27.80 to 29.30 °C) in Cebu city within the study period (Fig. 3c) were comparable with those of Xiao et al. [70]; *Ae. albopictus* has high infection rates in the head, salivary glands, and thorax-abdomen. DENV-infected *Ae. albopictus* in both lowland (urban) and highland (rural) sites indicates potentially high DENV transmission in both areas [71].

ZIKV in *Ae. albopictus*

Current results showed a significant difference (Table 7) of ZIKV's MIRs in *Ae. albopictus* as influenced by RH with positive significant correlation (Spearman's correlation coefficient, $rs = 0.65$, $P = 0.012$). Studies [72, 73] reported the correlation between ZIKV detection in *Ae. albopictus* and RH, particularly in its survival, fecundity, and egg-hatching. At 47–52% RH, this species' fecundity and longevity are better than other *Aedes* species [74]. *Ae. albopictus*' longevity is relatively better at higher RH (85%) than lower RH (35%) [73].

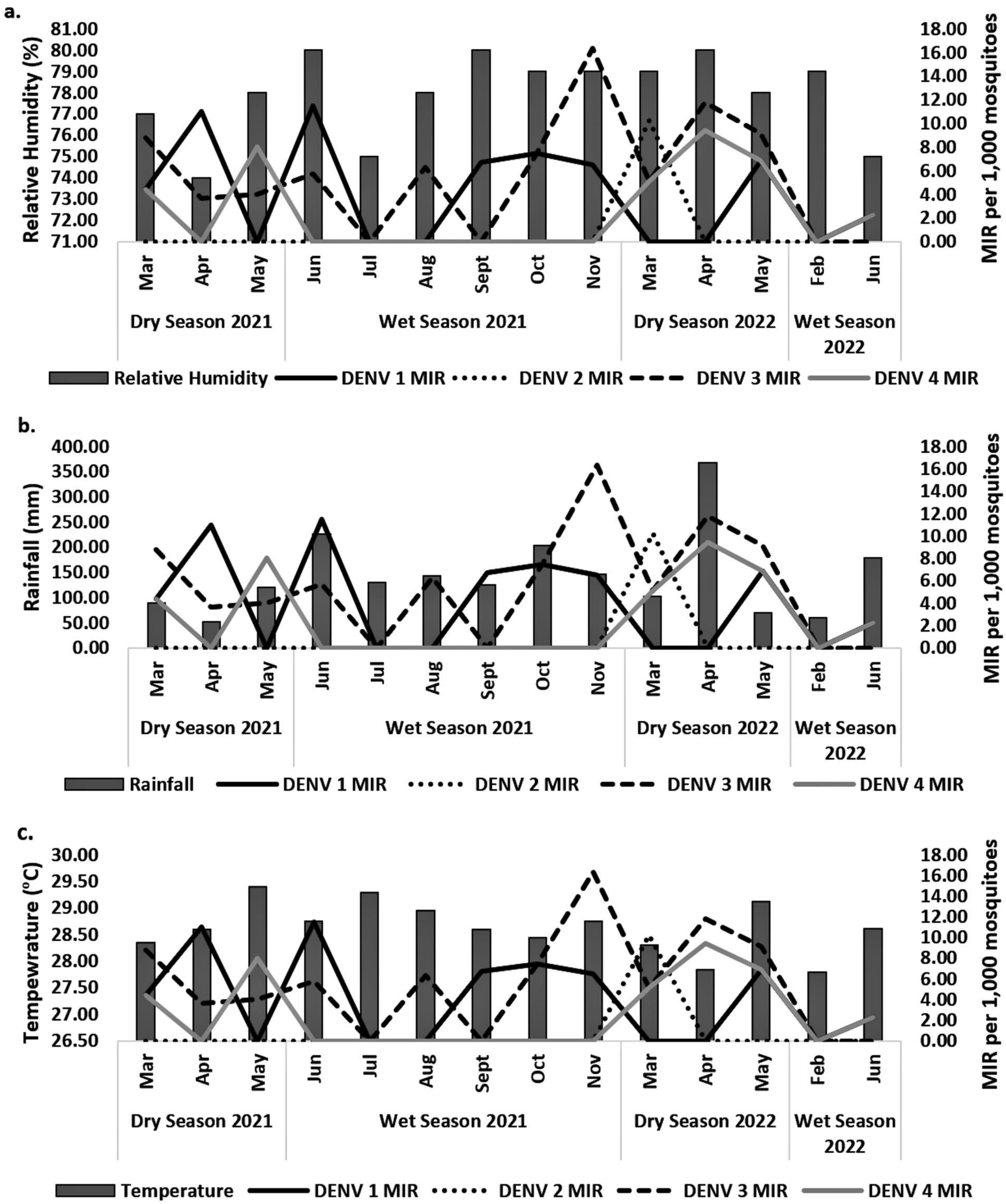

Fig. 3 Monthly DENV serotypes (1–4) MIR in *Ae. albopictus* with monthly mean relative humidity (%)(**a**), monthly total rainfall (mm)(**b**), and monthly mean temperature (°C)(**c**) in Cebu city, Philippines during the dry (March - May 2021; March– May 2022) and wet (June– November 2021; February and June 2022) seasons. DENV, Dengue; MIR, minimum infection rate

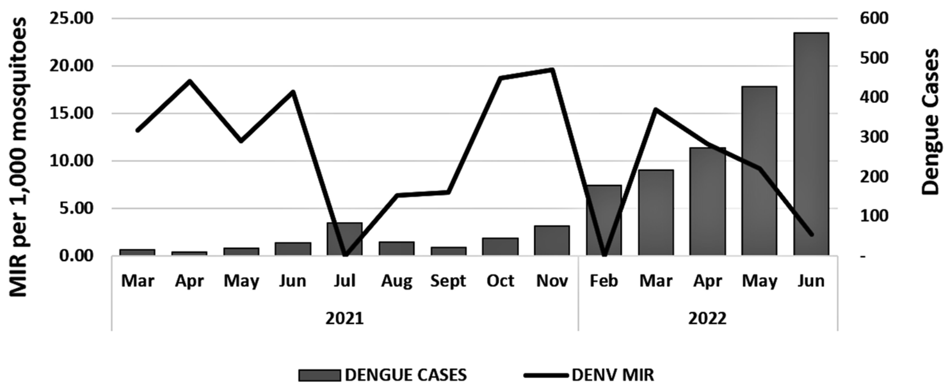

Fig. 4 MIRs of combined DENV serotypes (1–4) in *Ae. albopictus* plotted against the reported dengue cases in Cebu city during the wet (June-November 2021; February and June 2022) and dry (March-May 2021 and 2022) seasons. MIR, minimum infection rate; DENV, Dengue

Second, ZIKV's MIRs in *Ae. albopictus* differed as influenced by temperature was significant (Table 7), although with negative insignificant correlation (Spearman's correlation coefficient, *rs* = -0.44, *P*=0.117). ZIKV's MIR was higher at 27 °C, consistent with Mordecai et al. [17], who reported that ZIKV transmission occurs from 22.7 to 34.7 °C. The optimum is at 30.6 °C (range: 22.9 to 38.4 °C) for ZIKV vector competence in *Ae. aegypti* [75]. At constant 23 °C, adult *Ae. albopictus* carries higher ZIKV's RNA load [75]. ZIKV transmission rate by *Ae. albopictus* in Europe is influenced also by the viral load and temperature [76]. These studies [17, 74–76] suggest that temperature drives ZIKV transmission by *Ae. aegypti* and *Ae. albopictus*; beyond the maximum thermal range, ZIKV transmission may decline. This might be attributed to the current study's negative correlation between MIR of ZIKV in *Ae. albopictus* and temperature.

Third, the mean MIR of ZIKV in *Ae. albopictus* did not differ by season and by two-site categories (Table 4). The latter result was consistent between *Ae. albopictus* populations at highest and lowest altitudes in Albania [52]. Current results may suggest that *Ae. albopictus* transmitted ZIKV all throughout the year and in both lowland and highland sites in Cebu city. Moreover, results imply a whole-year round implementation of the DOH enhanced *4 S* strategy in this climate change as in related studies [34, 35].

Conclusion

In conclusion, the MIRs of CHIKV in *Ae. albopictus* were affected by RF, whereas those of ZIKV were affected by RH and temperature. DENVs in *Ae. albopictus* varied seasonally; DENV-2 and DENV-4 differed seasonally across selected highland and lowland study sites in Cebu city. Seasonal temperature, RF, and RH did not influence the MIRs of DENVs in *Ae. albopictus* across the two-site categories by altitude. One limitation of this study was the use of PAG-ASA weather data nearest to the study sites but were not measured on-site. However, this study aimed to explore the relationships between the MIRs of the arboviruses in the local study sites with the weather data, hence, an encompassing weather data of Cebu was assumed sufficient to demonstrate such relationships.

This study recommends for an all-year round implementation of the Philippine DOH's enhanced *4 S* strategy in this climate change because of the lack of the seasonal impact on ZIKV, DENV-1 and DENV-3 in *Ae. albopictus*; similar as in our previous studies [34, 35]. Water pipelines [34, 35] and vector control services are highly recommended in the highlands and not just in the lowlands to minimize the practice in storing water in containers that serve as potential breeding sites of dengue mosquitoes as mentioned in the above results. The MIR data of these three arboviruses in *Ae. albopictus* will be useful in future arboviral modeling at which weather conditions can potentially be used as indicators in predicting possible arboviral outbreaks. Thus, findings of this study are very relevant not just in the Philippines but also in the tropics and subtropics.

Abbreviations

CHIKV	Chikungunya
DENV	Dengue
ZIKV	Zika
H	Highland
L	Lowland
WS	Wet season
DS	Dry season
MIR	Minimum infection rate
RF	Rainfall
RH	Relative humidity
CFR	Case fatality rate
m ASL	Meters above sea level
O/L	Ovicidal/larvicidal
USC-MRL	University of San Carlos Mosquito Research Laboratory
DW	Distilled water
RT-PCR	Reverse transcriptase polymerase chain reaction
dNTP	Deoxynucleotide triphosphate
RFW	RNAse-free water
NFW	Nuclease-free water
PGC	Philippine Genome Center

PAG-ASA Philippine Atmospheric Geophysical and Astronomical Services Administration
K-W Kruskal-Wallis

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12985-024-02310-4.

Additional file 1: Table S1. Primer sequences for arboviral detection.

Additional file 2: Dataset S1. Minimum infection rates (MIRs) for Chikungunya (CHIKV), Dengue (DENV) and its serotypes, and Zika (ZIKV) from adult *Ae. albopictus* pools collected in Cebu city highland (S1-S3) and lowland (S4-S6) sites in dry (March-May 2021 and March-May 2022) and wet seasons (June-November 2021; February and June 2022).

Additional file 3: Table S2. Dengue cases in Cebu city, Philippines in dry (March-May 2021 and March-May 2022) and wet seasons (June-November 2021; February and June 2022).

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Author contributions
F.E. conceptualized the research. F.E. and R.R.Y. supervised the research. F.E., R.R.Y. and M.M.C. established field coordinators for egg collections in highland and lowland sites. R.R.Y., A.O.N., M.M.C. and K.S. performed experiments. F.E., R.R.Y., and A.O.N. analyzed data. F.E., A.O.N., and R.R.Y. wrote the manuscript. All authors reviewed and approved the final manuscript.

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Data availability
The meteorological data and dengue cases supporting the conclusions of this article are included within the article and its additional files. They are also available from Philippine Atmospheric, Geophysical and Astronomical Services Administration (PAGASA) and Department of Health, respectively, though only accessible upon request through the Philippine Freedom of Information (foi.gov.ph).

Declarations

Ethics approval and consent to participate
The approval for all protocols in data gathering was acquired from the Institutional Animal Care and Use Committee (IACUC) at the University of San Carlos, Cebu city, Philippines.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Mosquito Research Laboratory, Department of Biology, University of San Carlos– Talamban Campus, 6000 Cebu city, Philippines
[2]Department of Science and Technology, Science Education Institute, Taguig City, Metro Manila 1631, Philippines

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References

1. Benedict MQ, Levine RS, Hawley WA, Lounibos LP. Spread of the tiger: global risk of invasion by the mosquito *aedes albopictus*. Vector Borne Zoonotic Dis. 2007;7:76–85.
2. Paupy C, Ollomo B, Kamgang B, Moutailler S, Rousset D, Demanou M, et al. Comparative role of *Aedes albopictus* and *Aedes aegypti* in the emergence of dengue and chikungunya in Central Africa. Vector Borne Zoonotic Dis. 2010;10(3):259–66.
3. Calvez E, Mousson L, Vazeille M, O'Connor O, Cao-Lormeau VM, Mathieu-Daudé F, et al. Zika virus outbreak in the Pacific: vector competence of regional vectors. PLoS Negl Trop Dis. 2018;12(7):e0006637.
4. Vazeille M, Madec Y, Mousson L, Bellone R, Barré-Cardi H, Sousa CA, et al. Zika virus threshold determines transmission by European *Aedes albopictus* mosquitoes. Emerg Microbes Infect. 2019;8(1):1668–78.
5. Eiras AE, Pires SF, Staunton KM, Paixão KS, Resende MC, Silva HA, et al. A high-risk Zika and dengue transmission hub: virus detections in mosquitoes at a Brazilian university campus. Parasit Vectors. 2018;11(1):359.
6. Dos Reis IC, Gibson G, Ayllón T, de Medeiros Tavares A, de Araújo JMG, da Silva Monteiro E, et al. 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 Trop. 2019;197:105061.
7. Suwanmanee S, Surasombatpattana P, Soonthornworasiri N, Hamel R, Maneekan P, Missé D, et al. Monitoring arbovirus in Thailand: surveillance of dengue, Chikungunya and Zika virus, with a focus on coinfections. Acta Trop. 2018;188:244–50.
8. Maia LMS, Bezerra MCF, Costa MCS, Souza EM, Oliveira MEB, Ribeiro ALM, et al. Natural vertical infection by dengue virus serotype 4, Zika virus and Mayaro virus in *Aedes (Stegomyia) aegypti* and *Aedes (Stegomyia) albopictus*. Med Vet Entomol. 2019;33(3):437–42.
9. CDCP (Center for Disease Control and Prevention). Dengue Clinical Case Management. 2018; https://www.cdc.gov/dengue/training/cme/ccm/index.html. Accessed 2023 September 4.
10. Silva JVJ Jr, Ludwig-Begall LF, Oliveira-Filho EF, Oliveira RAS, Durães-Carvalho R, Lopes TRR, et al. A scoping review of Chikungunya virus infection: epidemiology, clinical characteristics, viral co-circulation complications, and control. Acta Trop. 2018;188:213–24. https://doi.org/10.1016/j.actatropica.2018.09.003.
11. GBD (Global Burden of Disease) Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990–2016: a systematic analysis for the GBD study 2016. Lancet. 2017;390(10100):1211–59. https://doi.org/10.1016/S0140-6736(17)32154-2.
12. Department of Health. Epidemic-Prone disease case surveillance: morbidity week no. 52. c2022. https://doh.gov.ph/sites/default/files/statistics/2022-EDCS-Weekly-Surveillance-Report-No-52.pdf. Accessed 9 Jan 2023.
13. World Health Organization. Dengue situation update in the Western Pacific Region. c2021. https://apps.who.int/iris/bitstream/handle/10665/341149/Dengue-20211229.pdf?sequence=606&isAllowed=y. Accessed 9 Jan 2023.
14. World Health Organization. Dengue situation update in the Western Pacific Region. c2022. https://apps.who.int/iris/bitstream/handle/10665/352792/Dengue-20221215.pdf?sequence=576&isAllowed=y. Accessed 9 Jan 2023.
15. Research Institute of Tropical Medicine. National summit on Zika Virus disease. c2019. https://ritm.gov.ph/zikasummit/. Accessed 29 Sept 2019.
16. Huber JH, Childs ML, Caldwell JM, Mordecai EA. Seasonal temperature variation influences climate suitability for dengue, Chikungunya, and Zika transmission. PLoS Negl Trop Dis. 2018;12(5):e0006451.
17. Mordecai EA, Cohen JM, Evans MV, Gudapati P, Johnson LR, Lippi CA, et al. Detecting the impact of temperature on transmission of Zika, dengue, and chikungunya using mechanistic models. PLoS Negl Trop Dis. 2022;16(6):e0010514. https://doi.org/10.1371/journal.pntd.0010514.
18. Bellone R, Anna-Bella F. The role of temperature in shaping mosquito-borne viruses transmission. Front Microbiol. 2020;11:584846. https://doi.org/10.3389/fmicb.2020.584846.
19. Harris M, Caldwell JM, Mordecai EA. Climate drives spatial variation in Zika epidemics in Latin America. Proc Biol Sci. 2019;286(1909):20191578. https://doi.org/10.1098/rspb.2019.1578.
20. Fuller TL, Calvet G, Estevam CG, Angelo JR, Abiodun GJ, Halai U-A, et al. Behavioral, climatic, and environmental risk factors for Zika and Chikungunya virus infections in Rio De Janeiro, Brazil, 2015-16. PLoS ONE. 2017;12(11):e0188002. https://doi.org/10.1371/journal.pone.0188002.
21. Carreto C, Gutiérrez-Romero R, Rodriguez T. Climate-driven mosquito-borne viral suitability index: measuring risk transmission of dengue, Chikungunya

and Zika in Mexico. Int J Health Geogr. 2022;21(1):15. https://doi.org/10.1186/s12942-022-00317-0.
22. Shil P, Kothawale DR, Sudeep AB. Rainfall and chikungunya incidences in India during 2010–2014. Virus Disease. 2018;29(1):46–53. https://doi.org/10.1007/s13337-018-0428-6.
23. Furuya H. Estimating vector-borne viral infections in the urban setting of the 2020 Tokyo olympics, Japan, using mathematical modeling. Tokai J Exp Clin Med. 2017;42(4):160–4.
24. Bogoch II, Brady OJ, Kraemer MUG, German M, Creatore MI, Brent S, et al. Potential for Zika virus introduction and transmission in resource-limited countries in Africa and the Asia-Pacific region: a modelling study. Lancet Infect Dis. 2016;16(11):1237–45.
25. Dhimal M, Dahal S, Dhimal ML, Mishra SR, Karki KB, Aryal KK, et al. Threats of Zika virus transmission for Asia and its Hindu-Kush Himalayan region. Infect Dis Poverty. 2018;7(1):40.
26. Wong PS, Li MZ, Chong CS, Ng LC, Tan CH. *Aedes* (*Stegomyia*) *Albopictus* (Skuse): a potential vector of Zika virus in Singapore. PLoS Negl Trop Dis. 2013;7(8):e2348.
27. Kamaraj US, Tan JH, Xin Mei O, Pan L, Chawla T, Uehara A, et al. Application of a targeted-enrichment methodology for full-genome sequencing of dengue 1–4, Chikungunya and Zika viruses directly from patient samples. PLoS Negl Trop Dis. 2019;13(4):e0007184.
28. Balingit JC, Carvajal TM, Saito-Obata M, Gamboa M, Nicolasora AD, Sy AK, et al. Surveillance of dengue virus in individual *Aedes aegypti* mosquitoes collected concurrently with suspected human cases in Tarlac City, Philippines. Parasit Vectors. 2020;13(1):594.
29. Edillo FE, Sarcos JR, Sayson SL. Natural vertical transmission of dengue viruses in *Aedes aegypti* in selected sites in Cebu City, Philippines. J Vector Ecol. 2015;40(2):282–91.
30. Dominguez N, Nerissa MA, Current, DF /DHF Prevention and Control Programme in the Philippines. WHO Regional Office for South-East Asia. 1997. https://apps.who.int/iris/handle/10665/148536. Accessed 29 Sept 2019.
31. Reinhold JM, Lazzari CR, Lahondère C. Effects of the environmental temperature on *Aedes aegypti* and *Aedes albopictus* mosquitoes: a review. Insects. 2018;9(4):158.
32. Schrauf S, Tschismarov R, Tauber E, Ramsauer K. Current efforts in the development of vaccines for the prevention of Zika and Chikungunya virus infections. Front Immunol. 2020;11:592.
33. Dhimal M, Gautam I, Joshi HD, O'Hara RB, Ahrens B, Kuch U. Risk factors for the presence of chikungunya and dengue vectors (*Aedes aegypti* and *Aedes albopictus*), their altitudinal distribution and climatic determinants of their abundance in Central Nepal. PLoS Negl Trop Dis. 2015;9(3):e0003545.
34. Edillo F, Ymbong RR, Bolneo AA, Hernandez RJ, Fuentes BL, Cortes G, et al. Temperature, season, and latitude influence development-related phenotypes of Philippine *Aedes aegypti* (Linnaeus): implications for dengue control amidst global warming. Parasit Vectors. 2022;15(1):74.
35. Edillo F, Ymbong RR, Cabahug MM, Labiros D, Suycano MW, Lambrechts L, et al. Yearly variations of the genetic structure of *Aedes aegypti* (Linnaeus) (Diptera: Culicidae) in the Philippines (2017–2019). Infect Genet Evol. 2022;102:105296.
36. Medeiros AS, Costa DMP, Branco MSD, Sousa DMC, Monteiro JD, Galvão SPM, et al. Dengue virus in *Aedes aegypti* and *Aedes albopictus* in urban areas in the state of Rio Grande do Norte, Brazil: importance of virological and entomological surveillance. PLoS ONE. 2018;13(3):e0194108.
37. Garcia-Rejon JE, Navarro JC, Cigarroa-Toledo N, Baak-Baak CM. An updated review of the invasive *Aedes albopictus* in the Americas; geographical distribution, host feeding patterns, arbovirus infection, and the potential for vertical transmission of dengue virus. Insects. 2021;12(11):967.
38. Lacour G, Chanaud L, L'Ambert G, Hance T. Seasonal synchronization of diapause phases in *Aedes albopictus* (Diptera: Culicidae). PLoS ONE. 2015;10(12):e0145311.
39. Belkin JN. The mosquitoes of the South Pacific (Diptera, Culicidae). 2nd ed. Berkeley, CA: University of California Press; 1962.
40. Casagrande JT, Pike MC. An improved approximate formula for calculating sample sizes for comparing two binomial distributions. Biometrics. 1978;34(3):483–6.
41. Guedes DRD, Cordeiro MT, Melo-Santos MAV, Magallanes T, Marques E, Regis L, et al. Patient-based dengue virus surveillance in *Aedes aegypti* from Recife, Brazil. J Vector Borne Dis. 2010;47:67–75.
42. Thermo Fisher Scientific. Is your RNA intact? Method to check RNA integrity. c2021. https://www.thermofisher.com/ph/en/home/references/ambion-tech-support/rna-isolation/tech-notes/is-your-rna-intact.html. Accessed 19 Jul 2021.
43. Laras K, Sukri NC, Larasati RP, Bangs MJ, Kosim R, Djauzi, et al. Tracking the re-emergence of epidemic Chikungunya virus in Indonesia. Trans R Soc Trop Med Hyg. 2005;99(2):128–41.
44. Lanciotti RS, Calisher CH, Gubler DJ, Chang GJ, Vorndam AV. Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. J Clin Microbiol. 1992;30(3):545–51.
45. Balm MN, Lee CK, Lee HK, Chiu L, Koay ES, Tang JW. A diagnostic polymerase chain reaction assay for Zika virus. J Med Virol. 2012;84(9):1501–5.
46. Hall TA, BioEdit. A user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucleic Acids Symposium Series. 1999;41:95–98.
47. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990;215(3):403–10.
48. Walter SD, Hildreth SW, Beaty BJ. Estimation of infection rates in population of organisms using pools of variable size. Am J Epidemiol. 1980;112(1):124–8.
49. Wiwanitkit S, Wiwanitkit V. Ckikungunya virus infection and relationship to rainfall, the relationship study from Southern Thailand. J Arthropod Borne Dis. 2013;7(2):185–7.
50. Heath CJ, Grossi-Soyster EN, Ndenga BA, Mutuku FM, Sahoo MK, Ngugi HN, et al. Evidence of transovarial transmission of chikungunya and dengue viruses in field-caught mosquitoes in Kenya. PLoS Negl Trop Dis. 2020;14(6):e0008362.
51. Chadsuthi S, Iamsirithaworn S, Triampo W, Cummings DA. The impact of rainfall and temperature on the spatial progression of cases during the chikungunya re-emergence in Thailand in 2008–2009. Trans R Soc Trop Med Hyg. 2016;110(2):125–33.
52. Mercier A, Obadia T, Carraretto D, Velo E, Gabiane G, Bino S, et al. Impact of temperature on dengue and chikungunya transmission by the mosquito *Aedes albopictus*. Sci Rep. 2022;12(1):6973.
53. Waldock J, Chandra NL, Lelieveld J, Proestos Y, Michael E, Christophides G, et al. The role of environmental variables on *Aedes albopictus* biology and chikungunya epidemiology. Pathog Glob Health. 2013;107(5):224–41.
54. Mubbashir H, Munir S, Kashif R, Nawaz HB, Abdul B, Baharullah K. Characterization of dengue virus in *Aedes aegypti* and *Aedes albopictus* spp. of mosquitoes: a study in Khyber Pakhtunkhwa, Pakistan. Mol Biol Res Commun. 2018;7(2):77–82.
55. Johari NA, Voon K, Toh SY, Sulaiman LH, Yap IKS, Lim PKC. Sylvatic dengue virus type 4 in *Aedes aegypti* and *Aedes albopictus* mosquitoes in an urban setting in Peninsular Malaysia. PLoS Negl Trop Dis. 2019;13(11):e0007889.
56. Wijesinghe C, Gunatilake J, Kusumawathie PHD, Sirisena PDNN, Daulagala SWPL, Iqbal BN, et al. Circulating dengue virus serotypes and vertical transmission in *Aedes* larvae during outbreak and inter-outbreak seasons in a high dengue risk area of Sri Lanka. Parasit Vectors. 2021;14(1):614.
57. Isa I, Ndams IS, Aminu M, Chechet G, Dotzauer A, Simon AY. Genetic diversity of Dengue virus serotypes circulating among *Aedes* mosquitoes in selected regions of Northeastern Nigeria. One Health. 2021;13:100348.
58. Whitehorn J, Kien DT, Nguyen NM, Nguyen HL, Kyrylos PP, Carrington LB, et al. Comparative susceptibility of *Aedes albopictus* and *Aedes aegypti* to Dengue virus infection after feeding on blood of viremic humans: implications for public health. J Infect Dis. 2015;212(8):1182–90.
59. Romiti F, Casini R, Magliano A, Ermenegildi A, De Liberato C. *Aedes albopictus* abundance and phenology along an altitudinal gradient in Lazio region (Central Italy). Parasit Vectors. 2022;15(1):92.
60. Christofferson RC. A reevaluation of the role of *Aedes albopictus* in dengue transmission. J Infect Dis. 2015;212(8):1177–9.
61. Sedda L, Vilela APP, Aguiar ERGR, Gaspar CHP, Gonçalves ANA, Olmo RP, et al. The spatial and temporal scales of local dengue virus transmission in natural settings: a retrospective analysis. Parasit Vectors. 2018;11(1):79.
62. Chung YK, Pang FY. Dengue virus infection rate in field populations of female *Aedes aegypti* and *Aedes albopictus* in Singapore. Trop Med Int Health. 2002;7(4):322–30.
63. Ferreira-de-Lima VH, Andrade PDS, Thomazelli LM, Marrelli MT, Urbinatti PR, Almeida RMMS, et al. Silent circulation of dengue virus in *Aedes albopictus* (Diptera: Culicidae) resulting from natural vertical transmission. Sci Rep. 2020;10(1):3855.
64. Abdulsalam FI, Antunez P, Yimthiang S, Jawjit W. Influence of climate variables on dengue fever occurrence in the southern region of Thailand. PLOS Glob Public Health. 2022;2(4):e0000188.

65. Akter R, Hu W, Gatton M, Bambrick H, Naish S, Tong S. Different responses of dengue to weather variability across climate zones in Queensland, Australia. Environ Res. 2020;184:109222.
66. Shabbir W, Pilz J, Naeem A. A spatial-temporal study for the spread of dengue depending on climate factors in Pakistan (2006–2017). BMC Public Health. 2020;20(1):995.
67. Lai YH. The climatic factors affecting dengue fever outbreaks in southern Taiwan: an application of symbolic data analysis. Biomed Eng Online. 2018;17:148.
68. Campbell KM, Lin CD, Iamsirithaworn S, Scott TW. The complex relationship between weather and dengue virus transmission in Thailand. Am J Trop Med Hyg. 2013;89(6):1066–80.
69. Chan M, Johansson MA. The incubation periods of dengue viruses. PLoS ONE. 2012;7(11):e50972.
70. Xiao FZ, Zhang Y, Deng YQ, He S, Xie HG, Zhou XN, et al. The effect of temperature on the extrinsic incubation period and infection rate of dengue virus serotype 2 infection in *Aedes albopictus*. Arch Virol. 2014;159(11):3053–7.
71. Sayono S, Nurullita U, Sumanto D, Handoyo W. Altitudinal distribution of *Aedes* indices during dry season in the dengue endemic area of Central Java, Indonesia. Ann Parasitol. 2017;63(3):213–21.
72. Sultana A, Tuno N. Effects of temperature and humidity on the fecundity and longevity of *Aedes albopictus* and *Aedes flavopictus* (Diptera: Culicidae). J Expt Biosci. 2021;12:31–8.
73. Reiskind MH, Lounibos LP. Effects of intraspecific larval competition on adult longevity in the mosquitoes *Aedes aegypti* and *Aedes albopictus*. Med Vet Entomol. 2009;23(1):62–8.
74. Tesla B, Demakovsky LR, Mordecai EA, Ryan SJ, Bonds MH, Ngonghala CN, et al. Temperature drives Zika virus transmission: evidence from empirical and mathematical models. Proc Biol Sci. 2018;285(1884):20180795.
75. Jian XY, Jiang YT, Wang M, Jia N, Cai T, Xing D, et al. Effects of constant temperature and daily fluctuating temperature on the transovarial transmission and life cycle of *Aedes albopictus* infected with Zika virus. Front Microbiol. 2023;13:1075362.
76. Gutiérrez-López R, Figuerola J, Martínez-de la Puente J. Methodological procedures explain observed differences in the competence of European populations of *Aedes albopictus* for the transmission of Zika virus. Acta Trop. 2023;237:106724.

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