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EFFECT OF TEMPERATURE ON THE VECTOR EFFICIENCY OF *AEDES AEGYPTI* FOR DENGUE 2 VIRUS

DOUGLAS M. WATTS,* DONALD S. BURKE,** BRUCE A. HARRISON,† RICHARD E. WHITMIRE,‡ AND ANANDA NISALAK§

*Department of Pathogenesis and Immunology, Disease Assessment Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21701-5011,
**Department of Virus Diseases, Walter Reed Army Institute of Research, Washington, DC 20012,
†Walter Reed Biosystematics Unit, NHB Stop 165, Smithsonian Institute, Washington, DC 20506,
‡United States Army Medical Research Unit–Kenya, Box 401, APO New York 09675, and
§Department of Virology, U.S. Army Medical Component, AFRIMS, Rajvithi Road, Bangkok 4, Thailand

Abstract. The effect of temperature on the ability of *Aedes aegypti* to transmit dengue (DEN) 2 virus to rhesus monkeys was assessed as a possible explanation for the seasonal variation in the incidence of dengue hemorrhagic fever in Bangkok, Thailand. In two laboratory experiments, a Bangkok strain of *Ae. aegypti* was allowed to feed upon viremic monkeys infected with DEN-2 virus. Blood-engorged mosquitoes were separated into two groups and retained at constant temperatures. Virus infection and transmission rates were determined for *Ae. aegypti* at intervals ranging from 4 to 7 days during a 25-day incubation period. Results of the first experiment for mosquitoes infected with a low dose of DEN-2 virus and maintained at 20, 24, 26, and 30°C, indicated that the infection rate ranged from 25% to 75% depending on the incubation period. However, DEN-2 virus was transmitted to monkeys only by *Ae. aegypti* retained at 30°C for 25 days. In the second experiment, the infection rate for *Ae. aegypti* that ingested a higher viral dose, and incubated at 26, 30, 32, and 35°C ranged from 67% to 95%. DEN-2 virus was transmitted to monkeys only by mosquitoes maintained at ≥30°C. The extrinsic incubation period was 12 days for mosquitoes at 30°C, and was reduced to 7 days for mosquitoes incubated at 32°C and 35°C. These results imply that temperature-induced variations in the vector efficiency of *Ae. aegypti* may be a significant determinant in the annual cyclic pattern of dengue hemorrhagic fever epidemics in Bangkok.

Epidemics of dengue hemorrhagic fever (DHF) occur annually in Bangkok, Thailand, where *Aedes aegypti* (L) has been incriminated as the primary vector of the four dengue (DEN) virus serotypes.[1,2] While all serotypes have been associated with epidemics, DHF epidemics have been attributed most frequently to DEN-2 virus infections.[3,4] Case rates begin to increase during the latter half of the hot-dry season, March through May, and attain peak case rates during the rainy season, June to November. The incidence of DHF cases subsides markedly during the cool-dry season, November to March.[5] Initially, the association of DHF epidemics with the rainy season was attributed to an increase in the population density of *Ae. aegypti*.[2] Data generated subsequently failed to support this observation and also revealed that an increase in the longevity of this mosquito was negatively correlated with the increased incidence of DHF.[6] However, observations on the seasonal feeding pattern of *Ae. aegypti* suggested that annual DHF epidemics were more likely the result of increased frequency of feeding on humans during the hot-dry and rainy seasons.[7,8] Also, Yasuno and Tonn[7] alluded to the possibility that variation in case rates might reflect the influence of seasonal temperature fluctuations on the extrinsic incubation period of DEN viruses in *Ae. aegypti*. Definitive data demonstrating that the extrinsic incubation period of DEN viruses was temperature-dependent have not been published, but this phenomenon has been documented for other virus-mosquito vector systems.[9–16]

An analysis of the incidence of DHF cases in relation to meteorologic variables in Bangkok for the past two decades provided indirect evidence that temperature influenced the vector efficiency of *Ae. aegypti* for DEN viruses.[17] The present

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study was designed and conducted to assess the ability of *Ae. aegypti* to transmit DEN-2 virus under environmental temperatures approximating those of the different seasons in Bangkok, Thailand.

MATERIALS AND METHODS

Monkeys

Adult rhesus monkeys (*Macaca mulatta*) were obtained commercially and maintained in mosquito-proof rooms according to standard laboratory procedures. Heparinized blood was obtained from monkeys prior to use in the experiments and centrifuged at 300 × *g* for 20 min. A 1:10 dilution of each plasma was tested for hemagglutination inhibition (HI) antibody by employing 8 units of sucrose acetone-extracted DEN-2 and Japanese encephalitis (JE) virus-infected, suckling mouse brain antigens.[18] A similar dilution of heat-treated (56°C × 30 min) plasma was assayed for DEN-1, 2, 3, and 4 and JE virus antibody by plaque reduction neutralization tests (PRNT),[19] with 50% endpoints, employing LLC-MK2 cells.

Mosquitoes

The *Ae. aegypti* adults were F_2 progeny from eggs oviposited by adults collected as larvae in a low socioeconomic sector of Bangkok. Eggs were hatched, and larvae were reared to adults, according to standard procedures, at 25°C and at 70% to 80% RH. All mosquitoes were 6- to 10-days-old when initially used in each experiment. After blood engorgement, a continuous supply of 10% sucrose and an oviposition substrate were provided.

Virus

DEN-2 virus was isolated during 1978 from the blood of a DHF patient hospitalized at the Children's Hospital, Bangkok. The virus had been passaged twice in LLC-MK2 cells and was identified before and after passage by PRNT employing DEN virus 1, 2, 3, and 4 monospecific antisera.[19] Each antiserum was prepared by a single intravenous injection of adult rhesus monkeys with 0.5 ml of approximately $1 \times 10^{5.0}$ plaque forming units (PFU)/ml of each DEN virus type. On day 28 post-inoculation, blood was obtained from each monkey; serum was collected and stored at −20°C. Specificity and titer of each antiserum was determined by serial 2-fold dilutions tested against 80 to 100 PFU of each DEN virus serotype in a PRNT.[19]

Infection of mosquitoes

Mosquitoes were infected by allowing them to feed on viremic monkeys that had been inoculated via the saphenous vein with 0.5 ml of $10^{5.0}$ PFU/ml of DEN-2 virus. Immediately after the mosquitoes fed, blood was obtained from monkeys and centrifuged at 300 × *g* for 20 min. The plasma component of each blood sample was stored at −70°C until assayed for virus at 1:5 or serial $\log_{10}$ dilutions by direct and delayed plaque assay in LLC-MK2 cells.[20] In addition, plasma obtained on day 28 post-inoculation was tested for DEN-2 virus HI and neutralizing antibody as described previously.[18,19] Blood-engorged mosquitoes were incubated at temperatures approximating the hot-dry, the rainy, and the cool-dry seasons for Bangkok, Thailand.

Viral transmission

At 3- to 7-day intervals during the incubation period, DEN-2 virus transmission was attempted at room temperature. A sample of mosquitoes representing each temperature was transferred to a 0.5-l cylindrical carton, one end of which was enclosed by nylon netting. Each carton was taped securely to the shaven abdomen of a monkey to allow mosquitoes to feed through the nylon netting. Immediately after feeding, mosquitoes were stored at −70°C until assayed for virus. Blood was obtained from monkeys before the mosquitoes fed and again 28 days later. Serial 2-fold dilutions of plasma of each blood specimen were tested for DEN-2 virus HI antibody,[18] and a 1:10 dilution of each plasma was assayed for virus-specific neutralizing antibody by PRNT.[19] The absence of DEN virus HI and neutralizing antibody in the first plasma specimen and the presence of antibody in the second was considered evidence of virus transmission.

Viral infectivity assays

The *Ae. aegypti* used in preliminary experiments were triturated individually in 1.0 ml of medium RPMI 1640 containing 10% heat-treat-

ed fetal bovine serum, 500 μl/ml of streptomycin, and 500 U/ml of penicillin. Mosquito suspensions were clarified by centrifugation at 1,000 × *g* for 30 min at 4°C and tested for virus by direct and delayed plaque assay.[20]

The distribution of DEN-2 virus in a sample of individual *Ae. aegypti* employed in actual experiments was determined as follows. Mosquito heads were removed and assayed individually for virus by the direct fluorescent antibody technique (DFAT).[21, 22] Heads of uninfected and parenterally DEN-2 virus-infected *Ae. aegypti* were included as controls for these assays. DEN virus antisera for the DFAT were obtained from convalescing DHF patients and conjugated with fluorescein isothiocyanate. The thorax-abdomen of each mosquito was placed in a drop of medium RPMI 1640 and the salivary glands were extracted with sterile insect minuten pins. Glands were disrupted by sonic energy, either as individual specimens in 0.2 ml or as pools of 5 pairs of glands in 0.5 ml of medium RPMI 1640, supplemented as described above for testing individual *Ae. aegypti.* Suspensions prepared from individual glands were tested for virus in *Toxorhynchites splendens* (Weidmann)[22, 23] and suspensions derived from pooled glands were tested by the direct and delayed plaque assay.[20] Assay of suspensions in *Tx. splendens* was performed by inoculating each of 8 mosquitoes intrathoracically with aliquots of 0.85 μl per mosquito. These mosquitoes were then incubated for 14 days at 32°C and then stored at −70°C until virus assay.

After extracting salivary glands from an individual *Ae. aegypti,* the corresponding thorax-abdomen components were triturated and suspensions were assayed for DEN-2 virus in LLC-MK2 cells, as described above. Undiluted suspensions of each thorax-abdomen were tested in the first and second experiments. Viral infectivity titers were also determined for additional whole mosquitoes from the second experiment by assaying serial 10-fold dilutions of each mosquito suspension. The body component of individual *Ae. aegypti* was labeled such that the viral assay results could be analyzed for individual mosquitoes.

The relation of DEN virus titer in mosquitoes to temperature was analyzed by Tukey's Studentized Range (HSD) test.[23] Recovery of DEN-2 virus from plasma of monkey blood specimens and from suspensions derived from whole mosquitoes, thorax-abdomens, and salivary glands was confirmed by PRNT employing DEN-2 virus, monospecific antiserum.[19]

Environmental temperature

Environmental temperature data recorded for Bangkok from 1958 through 1978 were received from the Ministry of Communications, Bangkok, Thailand. These records were used to prepare an annual temperature profile.[17] Data were collected with a maximum-minimum thermometer and a hygrothermograph located at a meteorological station in Bangkok. Also, during 1978 and 1979 temperature was monitored continuously with a mechanical hygrothermograph in an *Ae. aegypti*-infested house located approximately 1 km from where this species was collected for viral transmission trials.

RESULTS

Environmental temperature

The annual temperature profile for Bangkok, based on an analysis of 20 years of daily temperature recordings, is presented in Figure 1. The mean temperature during the cool-dry season, November through February, ranged from 25.5 to 28.0°C, with a mean minimum of 21.0 to 25.0°C and a mean maximum of 31.0 to 33.5°C. The hot-dry and rainy season (March through October) mean temperature ranged from 28.0 to 30.0°C, and the mean minimum and mean maximum ranged from 25.0 to 26.0°C, and 32.0°C to 34.5°C, respectively. Year-to-year temperature variations were slightly greater during the cool-dry seasons than during the hot-dry and rainy seasons. A comparable cyclical pattern was recorded inside an *Ae. aegypti*-infested house where temperatures were from 1 to 2°C lower than the above.

Rhesus monkey model for infecting Aedes aegypti *with DEN-2 virus*

A preliminary experiment was conducted to determine the duration of DEN-2 viremia in rhesus monkeys and to ascertain the day post-infection that the viremia would produce maximum infection rates in *Ae. aegypti.* Mosquitoes fed upon each of these monkeys on days 2 through 10 post-inoculation and were retained for 14 days

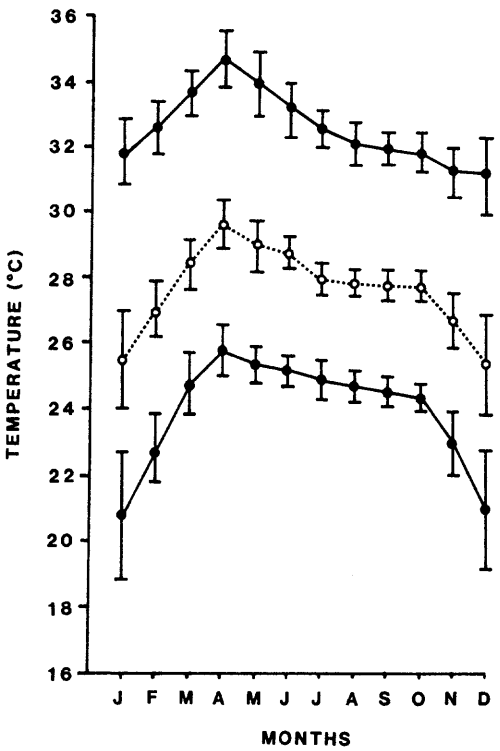

FIGURE 1. Average monthly temperature for Bangkok, 1958–1978. Top line = maximum mean temperature with range, middle line = mean with range, and bottom line = minimum mean with range.

at 32°C. DEN-2 virus was recovered from the blood of each of 3 monkeys on days 2 through 5 or 6 following inoculation (Table 1). However, infection rates were highest for mosquitoes that fed upon monkeys on day 3 and/or 4 post-inoculation. In all subsequent experiments, mosquitoes were fed upon monkeys on day 4 post-infection. DEN virus HI or neutralizing antibody was not detected in plasma obtained from monkeys before inoculation with virus. On day 28 post-infection, HI antibody titers were ≥1:40, and virus-specific neutralizing antibody titers were ≥1:10 for all monkeys.

Dengue virus infection and transmission experiments

In the first experiment, 300 *Ae. aegypti* ingested blood from a DEN-2 virus-infected monkey. Five separate groups of 60 fully blood-engorged mosquitoes were maintained in environmental chambers at 20, 24, 26, 28, and

TABLE 1

Dengue 2 virus viremia in rhesus monkeys

Monkey number	Days post-inoculation*	Viremia**	*Aedes aegypti* infection rate†
228	2	+	00 (0/5)
	3	+	20 (1/5)
	4	+	60 (3/5)
	5	+	00 (0/5)
	6	+	00 (0/5)
258	2	+	00 (0/5)
	3	+	00 (0/5)
	4	+	80 (4/5)
	5	+	00 (0/5)
	6	0	00 (0/5)
294	2	+	20 (1/5)
	3	+	60 (3/5)
	4	+	20 (1/5)
	5	+	00 (0/5)
	6	+	00 (0/5)

* Viremia not detected on day 1 or after days 5 or 6 through day 10 post-inoculation.
** + = virus positive, 0 = virus negative.
† Percent infected (No. pos./No. tested).

30°C. At intervals ranging from 3–7 days during the 25-day incubation period, virus transmission trials were conducted by allowing the surviving mosquitoes (5–13/group) an opportunity to refeed as a group on an individual recipient monkey. Overall feeding rates for mosquitoes at all temperatures on incubation day 3 ranged from 42%–83%, and from 70%–100% during subsequent virus transmission trials. All mosquitoes maintained at 28°C died before incubation day 12 due to a malfunction of the incubator.

An infective dose of DEN-2 virus was not transmitted to monkeys by mosquitoes maintained at 20, 24, and 26°C during the 25-day incubation period (Table 2). However, mosquitoes retained for 25 days at 30°C transmitted virus, as evidenced by an HI antibody titer of 1:160 and a neutralizing antibody titer of ≥1:10 in plasma of the recipient monkey.

Although a viremia was not demonstrable for the monkey used to provide an infectious bloodmeal to the mosquitoes, it was infected, as indicated by its development of an HI antibody titer of ≥1:40 and DEN-2 virus neutralizing antibody titer of ≥1:10. Data presented in Table 2 show that mosquitoes became infected, and that the frequency and pattern of virus recovery varied in relation to the incubation period and temperature. Virus recovery rates for thorax-abdomens increased from 25% (5/20) on day 3 to 74% (14/19) on day 25, and rates increased from 25%

TABLE 2

Transmission of dengue 2 virus to rhesus monkeys by Aedes aegypti *and distribution of virus in mosquitoes at temperatures ranging from 20 to 30°C*

Incubation period (days)	Virus transmission and distribution	Temperature (°C) 20	24	26	30	% (No. pos./ No. tested)
3	Transmission	−/7*	−/8	−/10	−/5	
	Heads	0/5**	0/5	0/5	0/5	
	Salivary glands	0†	0	0	0	
	Thorax-abdomen	0/5‡	1/5	0/5	4/5	25 (5/20)
7	Transmission	−/9	−/7	−/10	−/11	
	Heads 0/4	0/4	0/4	0/4		
	Salivary glands	0	0	0	0	
	Thorax-abdomen	0/4	3/4	1/4	2/4	38 (6/16)
12	Transmission	−/10	−/9	−/11	−/12	
	Heads 0/5	0/5	0/5	0/5		
	Salivary glands	0	0	0	0	
	Thorax-abdomen	1/5	1/5	3/5	2/5	35 (7/20)
18	Transmission	−/5	−/5	−/5	−/7	
	Heads 0/5	0/5	0/5	0/5		
	Salivary glands	0	+	+	+	
	Thorax-abdomen	2/5	1/5	2/5	2/5	35 (7/20)
25	Transmission	−/6	−/3	−/5	+/8	
	Heads 0/5	0/5	0/5	0/5		
	Salivary glands	0	+	+	+	
	Thorax-abdomen	3/5	3/4	4/5	4/5	74 (14/19)
% (No. thorax-abdomens pos./No. tested)		25 (6/24)	39 (9/23)	42 (10/24)	58 (14/24)	

* − = virus not transmitted, + = virus transmitted, / number of mosquitoes that refed on an individual monkey.
** No. of heads exhibiting virus-specific fluorescence/No. examined.
† 0 = suspension of ≤5 salivary glands virus negative, + = virus positive.
‡ No. of thorax-abdomens yielding virus/No. tested.

(6/24) to 58% (14/24) as the incubation temperature increased from 20 to 30°C. Regardless of the incubation period, virus was obtained from the thorax-abdomens but not from salivary glands of mosquitoes maintained at 20°C. In contrast, virus was recovered 5–9 days earlier from the thorax-abdomens of mosquitoes incubated at the higher temperatures, and on days 18 and 25 from salivary glands. Virus-specific fluorescence was not observed in head tissue of any of these mosquitoes.

The results of the first experiment suggested that a higher incubation temperature and an increase in the mosquito-infectious DEN-2 virus dose would be required to demonstrate an increase in the frequency of virus transmission by *Ae. aegypti*. Therefore, in a second experiment, 450 mosquitoes ingested blood from a monkey with a DEN-2 viremia of $10^{3.3}$ PFU per 1.0 ml of plasma. Groups of 60 mosquitoes were incubated at 26, 30, 32, and 35°C, and virus transmission trials were conducted as described in the first experiment. The refeeding rate for mosquitoes ranged from 46%–75% at day 3, to ≥90% on subsequent days. Mosquitoes retained at 32–35°C were not available for virus transmission trials on day 25 due to mortality.

DEN-2 virus was transmitted to monkeys by *Ae. aegypti* incubated at 30, 32, and 35°C, but not by mosquitoes at 26°C (Table 3). The extrinsic incubation period was 12 days at 30°C and 7 days at 32–35°C. DEN-2 HI antibody titers for these and additional monkeys that were infected by mosquitoes during subsequent virus transmission trials ranged from 1:80 to 1:160; and neutralizing antibody titers were ≥1:10.

The frequency and pattern of DEN-2 virus recovery from mosquitoes in relation to temperature and the incubation period are also presented in Table 3. Overall, virus was recovered from thorax-abdomens of 67% of the mosquitoes on day 3 and from ≥80% at subsequent incubation periods. Similarly, rates increased from 72% (18/25) for mosquitoes maintained at 26°C to 93% (14/15) for mosquitoes at 35°C. Virus recovery from salivary glands of mosquitoes

TABLE 3

Transmission of dengue 2 virus to rhesus monkeys by Aedes aegypti *and distribution of virus in mosquitoes at temperatures ranging from 26 to 35°C*

Incubation period (days)	Virus transmission and distribution	Temperature (°C) 26	30	32	35	% (No. pos./No. tested)
3	Transmission	−/6*	−/8	−/9	−/6	
	Heads	0/5**	0/5	0/5	0/5	
	Salivary glands	ND†	ND	ND	ND	
	Thorax-abdomen	3/5	3/5	4/5	ND	67 (10/15)
7	Transmission	−/12	−/10	+/12	+/10	
	Heads	0/5	0/5	0/5	1/5	
	Salivary glands	0/5‡	0/5	1/5	3/5	
	Thorax-abdomen	4/5§	3/5	5/5	4/5	80 (16/20)
12	Transmission	−/11	+/12	+/9	+/12	
	Heads	0/5	3/5	3/4	3/5	
	Salivary glands	1/5	5/5	4/5	5/5	
	Thorax-abdomen	4/5	5/5	5/5	5/5	95 (19/20)
18	Transmission	−/10	+/10	+/10	+/10	
	Heads	0/5	3/5	3/5	5/5	
	Salivary glands	1/5	5/5	4/5	5/5	
	Thorax-abdomen	3/5	5/5	4/5	5/5	85 (17/20)
25	Transmission	−/10	+/9	ND	ND	
	Heads	2/5	4/5	ND	ND	
	Salivary glands	4/5	4/5	ND	ND	
	Thorax-abdomen	4/5	4/5	ND	ND	80 (8/10)
% (No. thorax-abdomens pos./No. tested)		72 (18/25)	80 (20/25)	90 (18/20)	93 (14/15)	

* − = virus not transmitted, + = virus transmitted, / number of mosquitoes that refed on an individual monkey.
** No. DFAT pos./No. examined.
† ND = Not done.
‡ No. salivary glands yielding virus/No. tested.
§ No. thorax-abdomens yielding virus/No. tested.

maintained at 26°C was delayed in comparison to mosquitoes incubated at 30–35°C, but by day 25, virus recovery rates for salivary glands were approximately the same at each temperature. Evidence of virus-specific fluorescence was not observed in heads of mosquitoes at 26°C until day 25, whereas viral antigen was observed in heads as early as day 7 for mosquitoes maintained at 35°C.

The effect of temperature on the replication of DEN-2 virus in *Ae. aegypti* that ingested the highest viral dose is depicted in Figure 2. On incubation day 3, the mean viral titer for 5 individual mosquitoes at 26°C was significantly lower ($P = 0.004$) than the titer for mosquitoes incubated at 30 and 32°C. An overall increase in viral titers occurred concurrent with an increase in the incubation period for mosquitoes at each temperature. Virus titers for mosquitoes maintained at 26°C remained lower through incubation day 12, but the difference was not significant (day 7, $P = 0.6570$; day 12, $P = 0.4823$). Subsequent titers on days 18 and 25 were comparable, regardless of the incubation temperature.

Data summarized in Figure 3 for both experiments indicate that the incubation period and temperature thresholds required for the transmission of DEN-2 virus by *Ae. aegypti* ranged from 7 to 25 days at ≥30°C.

DISCUSSION

Experimental studies on the vector competence of mosquitoes for several arboviruses have demonstrated conclusively that the duration of the extrinsic incubation period was influenced markedly by environmental temperatures.[9–16] Similarly, data reported herein revealed that the extrinsic incubation period of DEN-2 virus in *Ae. aegypti* varied in relation to temperature. The extrinsic incubation period was 7 days for mosquitoes maintained at temperatures ranging from 32–35°C, whereas the extrinsic incubation period for mosquitoes incubated at ≤30°C was 12 days

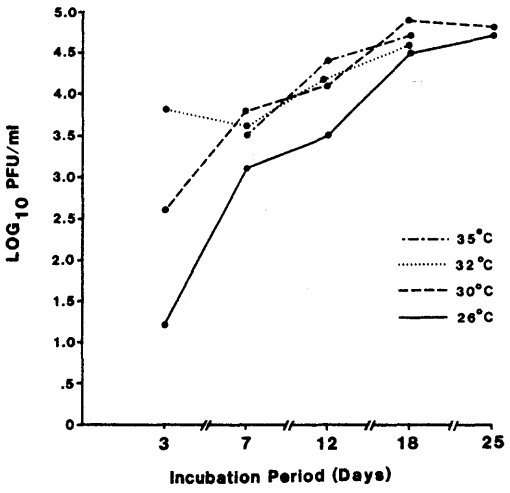

FIGURE 2. Dengue 2 viral replication pattern in *Aedes aegypti* in relation to temperature and incubation period. (Data not available for day 3 at 35°C, and day 25 at 32 and 35°C.)

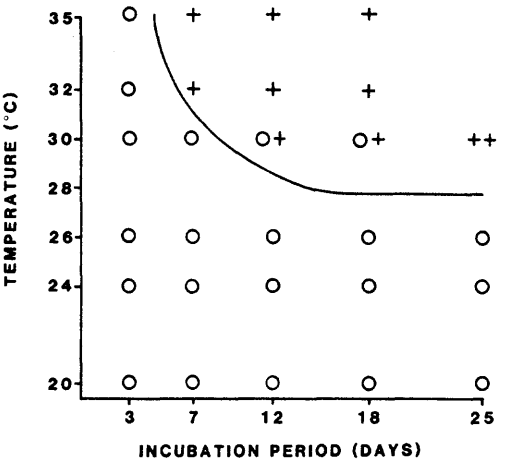

FIGURE 3. Summary of the influence of temperature on the extrinsic incubation period of dengue 2 virus in *Aedes aegypti.* O = no virus transmission; + = virus transmission; solid line is threshold of temperature and incubation period required for virus transmission.

and longer. This pattern of temperature-induced variation in the vector efficiency of *Ae. aegypti* for DEN-2 virus paralleled the seasonal cyclic pattern of the incidence of DHF cases in Bangkok, Thailand. Epidemics of DHF have been documented annually during the hot-dry and rainy seasons,[5] with mean daily temperatures of 28–30°C. However, case rates invariably decreased markedly during the cool seasons, with mean daily temperatures of 25–28°C. The variation in the extrinsic incubation period, together with previous observations,[7,8] that *Ae. aegypti* feed more frequently on humans during the hot-dry and rainy seasons, may be significant determinants of the seasonal variation in the incidence of DHF cases in Bangkok.

The effect of temperature on the transmission of DEN viruses by *Ae. aegypti* has been studied, but convincing evidence of a temperature-dependent extrinsic incubation period for this virus-mosquito system was lacking prior to this study. A previous report indicated that DEN virus was transmitted by per os-infected *Ae. aegypti* maintained at 22°C, but incubation at 16.4°C rendered the mosquitoes noninfectious.[24] The absence of appropriate virological techniques precluded the identification of a specific virus serotype. Virus transmission trials were conducted with an unspecified generation of *Ae. aegypti* infected per os with low passaged strains of DEN-2 virus.[25] Virus transmission trials with mosquitoes (n = 4) maintained at 32°C were conducted on incubation days 6 and 10, whereas trials involving sibling mosquitoes (n = 9) held at 27°C were conducted after incubation for 13 and 21 days. DEN-2 virus was transmitted by these mosquitoes, but trials were not conducted with mosquitoes maintained at 27°C before incubation day 13. It was not possible to exclude virus transmission capability for the latter mosquitoes on incubation day 6, as was observed for mosquitoes maintained at 32°C. A subsequent study conducted with a low passaged strain of DEN-2 virus and *Ae. aegypti* (n = 8) at 13 and 21°C failed to demonstrate that the extrinsic incubation period was temperature-dependent.[26]

The permissive temperature range for DEN-2 virus transmission by *Ae. aegypti* was influenced by the titer of the mosquito-infecting virus dose. An extrinsic incubation period of 25 days at 30°C for mosquitoes infected with the low virus dose was reduced to 12 days for mosquitoes infected with the high virus dose. These results were consistent with the concept that the duration of the extrinsic incubation period and hence, vector efficiency, varied directly in relation to the titer of the mosquito-infecting virus dose.[27] An increase in the virus dose might therefore be expected to decrease the extrinsic incubation period of DEN-2 virus in *Ae. aegypti,* thus extending transmission capability to mosquitoes maintained at cool-sea-

son temperatures. Viremia levels associated with human DEN-2 virus infections in Bangkok have not been reported, but titers in the blood of DEN-2-infected humans[28] in Indonesia were higher than the maximum virus dose ingested by *Ae. aegypti* during this study. Although a higher infecting virus dose may affect virus transmission capability to mosquitoes at cool-season temperatures, the extrinsic incubation period would also be expected to decrease likewise for infected mosquitoes maintained at the temperatures of the hot-dry and rainy seasons. Thus, the overall inferred temperature-induced vector efficiency pattern for DEN-2 virus-infected *Ae. aegypti* would not be expected to change appreciably.

Studies conducted with Japanese encephalitis (JE) virus-infected *Culex quinquefasciatus* Say[29] indicated that the viral infection was confined to the midgut of this mosquito at 10°C. While this precluded virus transmission, a sample of these mosquitoes attained virus transmission status following incubation for 4 days at 26.5°C. Since DEN-2 virus infection was readily demonstrable in salivary glands of *Ae. aegypti* maintained at 24 and 26°C, an even shorter incubation period may have affected virus transmission capability at slightly elevated temperature. That the temperature exceeds 26°C during the cool seasons in Bangkok was evidenced by the reported mean maximum of 28°C. This suggested that virus transmission at cool-season temperatures was precluded by the failure of DEN-2 virus to attain titers of sufficient magnitude in the salivary glands to infect the monkeys. Viral titers associated with the salivary glands were not determined in this study, but DEN-2 virus transmission by *Ae. aegypti* has been reported to vary directly in relation to the amount of infection in the salivary gland tissue.[30] Also, viral titers increased in salivary glands concurrent with an increase in the incubation temperature for St. Louis encephalitis (SLE) virus-infected *Culex pipiens* (L).[31]

Apparently the temperature required to attain effective vector efficiency varies depending on the particular arbovirus-mosquito vector system.[32] Variation based on laboratory studies, however, must be interpreted with caution because of different environmental conditions, experimental designs, model systems, and procedures. Nevertheless, observations reported for western equine encephalitis virus (WEE), and strains of *Culex tarsalis* Coquillett implied that maximum vector efficiency was confined to a temperature ≤25°C.[16] Virus transmission rates for mosquitoes maintained at 32°C decreased markedly and virus replication and dissemination to salivary glands were interrupted as the incubation period increased. Evidence obtained in field studies also indicated that the extent of WEE virus transmission to humans and sentinel chickens paralleled these laboratory observations in that transmission rates were reduced at very high ambient temperatures.[32] In contrast, maximum transmission efficiency was attained for DEN-2 virus by *Ae. aegypti* at 32 and 35°C, with no apparent evidence that elevated temperature interfered with virus replication and dissemination. However, the latter observation for DEN-2 virus was consistent with laboratory results described for other flavivirus-mosquito systems.[9, 12, 13, 15] Data reported for JE[33] and for SLE[13, 32] viruses implied that virus transmission rates under field conditions were highest at maximum summer temperatures. More rigorously designed studies are required to substantiate these findings, but our results lend further support to the contention that the temperature required for maximum virus transmission differs depending on the particular arbovirus-mosquito system.

The frequency and pattern of DEN-2 virus recovery from the thorax-abdomens of *Ae. aegypti* infected with the lower virus dose varied in relation to the incubation temperature through incubation day 18. Apparently, this reflected variation of viral titers among individual mosquitoes as a result of temperature-induced differential in the rate of virus replication. This is supported by the overall marked increase and comparable virus recovery rates observed on incubation day 25 for the mosquitoes that ingested the low dose of DEN-2 virus. Similarly, that temperature influenced the rate of virus replication was evidenced by the reduced viral titers for mosquitoes that ingested the high viral dose, and maintained at 26°C on and before incubation day 12. These results confirm previous observations that temperature influenced the rate of virus replication and exerted no apparent effect on the establishment of infection in the mosquitoes.[11]

Since the recovery of DEN-2 virus from thorax-abdomens of *Ae. aegypti* varied in relation to the incubation period and temperature, true infection rates were more accurately reflected by results obtained for mosquitoes incubated for the maximum periods. Based on previous observations,[15, 28, 34] these rates would have been ex-

pected to vary directly in relation to the amount of virus ingested by the mosquitoes. However, the difference for DEN-2 virus in *Ae. aegypti* was not remarkable, as indicated by rates that ranged from 60%–80% and from 80%–100% for mosquitoes that ingested the low and the high virus doses, respectively. These results suggested that both virus doses exceeded the threshold level required to infect the midgut of most mosquitoes.

The observations reported here must be interpreted with caution in regard to the possible influence of temperature on the transmission of DEN-2 virus under field conditions. Nevertheless, the data clearly imply that temperature-induced variation in the vector efficiency of this mosquito is among the critical determinants of the seasonal variation in the incidence of DHF cases in Bangkok. Evidence reported for yellow fever virus and *Ae. aegypti*[27] suggested that a fluctuating temperature regime may have been more representative of field conditions. However, observations for the latter virus-vector system and for eastern equine encephalitis (EEE) virus-infected *Ae. triseriatus* showed fluctuating temperatures to have an intermediate effect on the extrinsic incubation period,[11] thus suggesting that the results for DEN-2 virus and *Ae. aegypti* were indicative of those expected under fluctuating temperatures. In addition, the relevance of these laboratory results to field conditions was evident by the experimental design and model system. The use of F_1 generation *Ae. aegypti* and a low-passage DEN-2 virus reduced the possibility of laboratory-induced changes in their biological properties. Both the mosquitoes and virus originated from Bangkok. Whether or not these strains were representative of the total natural mosquito and virus populations in Bangkok is problematic. However, the susceptibility of another Bangkok strain of *Ae. aegypti* to infection with a different strain of DEN-2 virus was comparable to our results.[36] Variation in the susceptibility of geographically isolated strains of *Ae. aegypti* to infection with DEN-2 virus has been reported, but this phenomenon has not been reported for *Ae. aegypti* within a specific geographic location such as Bangkok.

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