THE EFFECT OF TEMPERATURE AND HUMIDITY ON DENGUE VIRUS PROPAGATION IN *AEDES AEGYPTI* MOSQUITOS

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Abstract. The effect of temperature and relative humidity on dengue virus propagation in the mosquito as one of the possible contributing factors to dengue hemorrhagic fever (DHF) outbreaks was studied. *Ae. aegypti* mosquitos were reared under standard conditions and inoculated intrathoracically with dengue virus. Virus propagation in the mosquitos was determined at the temperature and relative humidity of all 3 seasons of Yangon and for the simulated temperature and relative humidity of Singapore. The virus propagation was detected by direct fluorescent antibody technique (DFAT) with mosquito head squash and the virus titer was determined by plaque forming unit test (PFUT) in baby hamster kidney-21 cells. The results show that the infected mosquitos kept under the conditions of the rainy season and under the simulated conditions of Singapore had a significantly higher virus titer ($p=<0.05$) when compared with the other 2 seasons of Yangon. So it is thought that the temperature and relative humidity of the rainy season of Yangon and that of Singapore favors dengue virus propagation in the mosquito and is one of the contributing factors to the occurence of DHF outbreaks.

INTRODUCTION

Dengue hemorrhagic fever (DHF) is a major cause of pediatric morbidity and mortality and an emerging public health problem occurring in both epidemic and endemic forms. As the magnitude of the dengue problem is fast increasing, scientists have strived to find out the causes and factors which might contribute to the transmission of DHF.

DHF outbreaks have been reported in Myanmar mostly in the rainy season when the mosquito house index or premise index *(ie* the percentage of houses positive for *Aedes aegypti* larvae) is over 60%. Only a few cases were reported in the hot and cold seasons when the house index was below 60% (Soe Thein, 1991). Therefore DHF outbreaks have been mainly attributed to vector abundance because mosquito indices increase in the rainy season. But in countries like Singapore, prevention and control strategies of vector mosquitos are very successful and the mosquito premise index has been brought down to a record level of 1. In spite of this, nationwide outbreaks have occurred with even greater frequency and intensity in subsequent years (WHO, 1992). Therefore DHF outbreaks could not be due to high vector density alone. It is thought among other factors, virus propagation in the mosquito due to the temperature and relative humidity of the environment could be one of them. In this study, the virus propagation in the mosquito was determined for the temperature and relative humidity of the 3 seasons of Yangon and for the simulated temperature and relative humidity of Singapore.

MATERIALS AND METHODS

Aedes aegypti mosquitos used in this study were from the laboratory colony of Medical Entomology Research Division, Department of Medical Research. Mosquitos were reared from eggs to adults under standard conditions (Gerberg, 1970).

Only male mosquitos wer utilized in the study for safety reasons since males cannot transmit the infection should they escape in the laboratory and also because male mosquitos are as susceptible to infection as females and virus titers as determined in the male mosquitos did not appear significantly different from those determined in female insects (Rosen and Gubler, 1974).

The virus strain used in this study was dengue 2. This seed virus was prepared by cell culture in C6/36 mosquito cell line. The cells used for determination of virus titer in the mosquitos were baby hamster kidney-21 (BHK-21) cells.

The mosquitos were reared and male mosquitos sorted out first. The mosquitos were grouped into two groups. One group was inoculated intrathoracically with dengue 2 virus according to the method of Rosen and Gubler (1974) and the other group were left uninoculated and kept as a control to study natural mortality. Infection experiments of the mosquitos were done four times. Each time was done separately for the rainy season, cold season and hot seasons of Yangon and another for the simulated conditions of Singapore. The infected mosquitos were kept in holding cages and 5 mosquitos each were removed from the cages on day 5, day 8, day 11, day 14 and day 17 after inoculation respectively and then kept at -80°C for further study.

Dengue virus development in all groups of mosquitos was studied by 2 methods. Mosquito head squash with direct fluorescent antibody technic (DFAT) was performed according to the technology of Kuberski and Rosen (1977). The virus titer in all groups of mosquitos were determined by the plaque forming unit test in BHK-21 cells according to the technic by Morens *et al* (1985).

For statistical analysis, the Anova method was used to analyse if the difference in virus titer means were statistically significant. Then, Scheffe's multiple range test was used to detect which means were different from each other.

The developmental stages in the life cycle of the mosquitos were also studied. The time taken from eggs through 4 stages of instar larvae, pupa completion, adult emergence and completion was noted and compared. The number of adults emerging at the end of each cycle for all 4 weather conditions was also noted down and compared.

RESULTS

Mosquito head squash with the direct fluorescent antibody technic was done on 5 groups of mosquitos each collected on their respective days for all 3 seasons of Yangon and also for the simulated conditions of Singapore. Positive results were detected in those mosquitos collected on the 14^{th} and 17^{th} days of the rainy season and also on those same days for the mosquitos kept under the conditions of Singapore (Table 1).

The virus infectivity titer determined by the PFUT tests were also done for the same groups of mosquitos. The infectivity titer was as shown in Table 2. The results show that the viral infectivity titer in the rainy season was significantly higher than the other 2 seasons of Yangon. Similarly, the viral infectivity titer under the conditions of Singapore was generally higher than all the 3 seasons of Yangon.

The natural mortality of the mosquitos in all groups was noted down and compared and is shown in Table 3. In the cold season, the mortality by the 20^{th} day was 55%. In the hot season, mortality began early and was the highest where by on the 17^{th} day there were no surviving mosquitos left at all.

In the rainy season and under the simulated conditions of Singapore, the mosquito survival rates were 93% and 90%, both of which were much higher than that of the hot and the cold seasons of Yangon.

The developmental stages in the life cycle of the mosquito were also studied. As shown in Table 4, the stages of mosquito development were all quicker in the rainy season and under Singapore conditions when compared to the other 2 seasons of Yangon. The number of adults which emerged at the end of the developmental cycle for all 3 seasons as well as Singapore was noted down and compared.

In the hot season, the highest mortality of larvae and pupae were seen and at the end of the cycle approximately about 70% emerged as adults. In the cold season, adult emergence was about 78%. For the rainy season and Singapore conditions, the adult emergence was approximately about the same which was about 82%.

DISCUSSION

Dengue hemorrhagic fever and dengue shock syndrome are among the leading causes of pediatric morbidity and mortality and a serious health problem in many countries throughout the world. DHF has been described as a multifactorial disease (Wong, 1986). The aim of this study was to gain further knowledge of these contributing factors.

DHF outbreaks occur in Myanmar mostly in the rainy season which has been mainly attributed to the abundance of the vector mosquito. But in countries like Singapore, nationwide outbreaks occur despite very effective and successful vector control. It is seen in contrast to Myanmar, Singapore has DHF outbreaks with a very low mosquito

Table 1

Results of mosquito head squash for viral detection by DFAT for 3 seasons of Yangon and Singapore.

Condition	Positivity on days				
	5	8	11	14	17
Rainy season * (T = 23-30°C) ** (RH = 90%)	–	–	–	+	+
Cold season * (T = 16-33°C) ** (RH = 65%)	–	–	–	–	–
Hot season * (T = 25-37°C) ** (RH=66%)	–	–	–	–	–
Singapore * (T = 24-31°C) ** (RH = 87%)	–	–	–	+	+

* T = Temperature
**RH = Relative humidity

Table 2

Results of viral infecitvity titer.

Condition	Viral infectivity titer on days (PFU/ml)				
	5	8	11	14	17
Yangon					
Rainy	16,000	17,600	35,200	52,000	102,000
Cold	5,760	8,000	14,000	17,900	19,200
Hot	1,600	1,520	920	880	–
Singapore	20,000	25,600	48,000	67,200	108,000

premise index. So, factors other than vector density could be affecting outbreaks. Among them, the virus propagation in the mosquito influenced by the temperature and relative humidity of the environment could be one.

The climate of Singapore is uniform the whole year round (Ho *et al*, 1971). The temperature range is nearer to that of the rainy season of Yangon when compared to that of the other 2 seasons. It also has a high humidity like the rainy season of Yangon which has a much higher humidity than the other 2 seasons.

From the results of this study, it is seen that the virus propagation in the mosquito at the temperature and relative humidity of the rainy season is significantly higher when compared to the other 2 seasons. Therefore, it is thought the temperature and relative humidity of the rainy season favors virus propagation in the mosquito.

The results also show that the virus propagation under the temperature and relative humidity of Singapore was significantly higher than in all the 3

Table 3

Natural mortality of mosquitos in the ambient temperature of the 3 seasons and the controlled conditions of Singapore

Season condition	Survival No. (%) on days									
	1	3	5	7	9	11	13	15	17	20
Cold * (T=16-33°C)** (RH=65%)	100	100	100	100	94	66	64	49	49	45
Rainy * (T=23-30°C)** (RH=90%)	100	100	100	100	98	98	98	96	93	93
Hot * (T=25-37°C)** (RH=66%)	100	98	94	92	76	64	42	8	–	–
* Singapore * (T=24-31°C)** (RH=87%)	100	100	100	100	100	100	98	96	93	90

*T= Temperature
**RH= Relative humidity

Table 4

Development of mosquito stages at different seasons.

Season	Days of development						
	1st–4th stage larva		Pupation		Adultemergence		% of adult
	start	complete	start	complete	start	complete	survival
Cold	1	13	11	15	12	17	78
Rainy	1	10	8	12	9	14	82
Hot	1	12	9	14	11	16	70
Singapore	1	10	7	12	9	14	82

seasons of Yangon. This high virus titer is thought to be one of the factors influencing DHF outbreaks. When the results of natural mortality were compared, the mortality was the highest in the hot season. For the cold season, on the 20th day there were nearly half of the mosquitos left. This high natural mortality of the hot and cold seasons may be one of the factors why there may be less DHF transmission in these two seasons as the vector density is low. In the rainy season and that of Singapore the mosquito survival rates were high with little mortality. This compares favorably with the study of Schoof, (1967) where he mentioned that high humidity increases the longevity of mosquitos. The high humidity of the rainy season and Singapore favors mosquito survival which contributes to high rate of transmission. When the results of the developmental stages in the life cycle of the mosquito were compared for the 3 seasons of Yangon, in the cold season all stages of the developmental cycle took longer than the other 2 seasons. This together with the low virus propagation in the mosquito and high natural mortality were disfavorable for DHF outbreaks.

Although in the hot season, the developmental cycle was quicker than that of the cold season, the high natural mortality (which is the highest among the 3 seasons) and the low virus propagation in the mosquito both were against virus transmission and DHF outbreaks.

The developmental cycle in the rainy season was the quickest and that under Singapore conditions nearly the same. Together this, and the high virus propagation as well as increased longevity of the mosquito, were all favorable and the best conditions pointing to an outbreak.

Apart from the time taken for the developmental cycle, the number of adults emerging at the end of it was lower in the hot season than the cold season. For the rainy season, the adult emergence was higher than the other 2 seasons. The high adult emergence together with a short developmental cycle would mean a very high mosquito density and in turn a high rate of virus transmission.

In the case of Singapore, it is thought that vector abundance is not a contributing factor in outbreaks and that the temperature and relative humidity of Singapore which are favorable for virus propagation in the mosquito could be contributing factors. Another factor which must also be given consideration is the population density of Singapore, as virus transmission is enhanced by denser human population. The population density of Singapore is much higher when compared to that of Yangon (11,606 persons per square mile *vs* 1,282 persons per square mile), (Ministry of Information and Arts, Singapore, 1994; Immigration and Manpower Department, Yangon, 1994). The dense human population favors DHF outbreaks because in a crowded area, many people living within the short flight range of the vector from its breeding source could be exposed to transmission. This together with the high virus propagation in the mosquito could be the contributing factor to DHF outbreaks.

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