The Discriminative Infection of Dengue Virus in *Aedes aegypti* at Subspecific Level

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Abstract: After years of studies in *Aedes aegypti* in Thailand it was revealed that there is a species complex at subspecific level comprising of the dark form or the type species and the pale form. The two forms were studies for susceptibility to dengue 2 virus by oral feeding. The dark form is more susceptible than the pale form.

Key words: Dengue virus, *Aedes aegypti*

INTRODUCTION

The dengue fever is still public health problem and causes to death by dengue hemorrhagic fever and dengue shock syndrome. It has become a serious health problem because of 1) increase number of cases 2) expansion of epidemic areas and 3) appearance of severe clinical manifestations dengue hemorrhagic fever (DHF) / dengue shock syndrome (DSS) and expanded dengue syndrome (encephalopathy and hepatic involvement). At present, there is no effective control measures to prevent dengue; dengue vaccine is still under development and vector control could not provide expected effect. Up to now no antidengue drugs are available.

One of the remarkable achievements in dengue is the reduced case fatality as result of improved cases management, particularly intravenous infusion to correct hemoconcentration which can lead to fatal outcome by hypovolemic shock.

Three factors in maintaining dengue virus transmission are-
1. host susceptibility to virus; young ages from 1/2 year to 15 years are susceptible, the infants and older ages are not. Infection in the older age group for 15 to 24 indicated waning herd immunity and the continuous introduction of dengue virus into the dengue receptive area e.g. Singapore
2. viremic status-usually viremia is short in man and monkey therefore the
susceptible vectors and number must be enough in nature to carry on transmission. These factors included the interrupted feeding behavior of *Aedes aegypti*.

3. Vectors and environment - The vector of dengue viruses are *Aedes aegypti*, *Aedes albopictus* and other. *Aedes aegypti* is the main vector in urban areas, extensive researches in the vector of dengue revealed two subspecies 1) *queenslandensis* and the pale form, outdoor breeder 2) *formosus* the jungle breeder in Africa. The other form *Aedes mascarensis* was found in Madagascar. The multiple factors in *Aedes aegypti* make control difficult.

Recently we discovered *Aedes aegypti* pale form and maintained it in laboratory and we have opportunity in studying dengue infection between *Aedes aegypti* (type form) and pale form and we found the differences in the infection rates between the two. *Aedes aegypti* type form is more susceptible than the other. Our preliminary report on susceptibility to dengue virus in both form of *Aedes aegypti* aiming at to study the susceptibility to dengue virus by oral feeding in order to determine 1) the sensitivity to virus infection by oral route 2) the ability to disseminate virus to brain and salivary gland 3) the capability of virus replication in mosquitoes.

**MATERIALS AND METHODS**

I. Preparation of dengue virus antigen

Den-2 16681 infected *Toxorhynchites splendens* were triturated and virus suspension was made in PBS pH7.5+30% fetal calf serum (FCS)

II. Oral feeding

Mosquito: *Aedes aegypti* - dark form is the colony from Phrae Province maintained in laboratory for 20 years. The pale form is the colony from Chanthaburi Province and has been selected for pure pale form for 4-5 generations. *Aedes queenslandensis*, the fully pale form are included (Sucharit and Surathin, 1994).

Infectious blood meal was prepared from virus suspension, mixed with equal volume of 10% sugar solution and washed guinea pig red cells. These blood meal was then placed as drops on the nylon mesh to feed the mosquitoes. Only fully engorged mosquitoes were transferred to the clean carton and incubated at 30°C for 7, 14, 21 or 28 days.

III. Detection of dengue antigen

After incubation for 28 days, squashed head and body of *Aedes aegypti* were checked by direct-immunofluoresent (DFAT) using fluoro-isothiocyanate (FITC) conjugate to determine the infection and dissemination rate.

IV. Virus assay by mosquito infectious dose 50 (MID50) titration
1. Titrations of infectious blood meal were done by intrathoracic mosquito inoculation using *Toxorhynchites splendens*.

2. Titrations of individual mosquito suspension from each harvesting day were done by the same route in order to determine the capacity of virus replication.

Inoculated *Toxorhynchites* were kept in 30°C incubator for 14 days and checked for dengue antigen by DFAT.

**RESULTS**

The study of susceptibilities to virus infection by oral feeding has shown that-

1. Pale form *Aedes* can be infected with DEN2 virus by oral route which indicates its susceptibility to dengue virus (Table 1).

2. However, the overall infection rate of pale form by $\chi^2$ was significantly lower ($P < 0.05$) than those of dark form (27% and 46% respectively).

3. On the other hand dissemination rate of pale form *Aedes aegypti* seems to be lower but showed no significant difference ($p > 0.05$) from dark form (22.8% and 39.7% respectively).

4. The DEN-2 16681 virus could replicate in both pale and dark form, but the capacity of virus replication in pale form seem to be lower than dark form. However statistical analysis of the results showed that there is no significant difference of virus replication at 95% confidence limit.

**Table 1**  Infection and dissemination rates of *Aedes aegypti* (pale form and dark form) after oral infection with DEN-2 16681.

<table>
<thead>
<tr>
<th><em>Aedes aegypti</em></th>
<th>Titer of blood meal log 10 MID50/ml</th>
<th>Infection* No. of infected/no. of tested (%)</th>
<th>Dissemination** No. of infected/no. of tested (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pale form</td>
<td>8.0 8.0</td>
<td>17/63 (27.0)</td>
<td>15/63 (23.8)</td>
</tr>
<tr>
<td>dark form</td>
<td>8.5 8.5</td>
<td>29/63 (46.0)</td>
<td>25/63 (39.7)</td>
</tr>
</tbody>
</table>

* Determine by DFAT on body squashes  
** Determine by DFAT on head squashes

**DISCUSSION**

High infection rates of dengue virus in type form or dark form *Aedes aegypti* which is indoor breeder and anthropophilic favours cycle of transmission. On the other hand, lower infection rates in pale form which is outdoor breded and anthropophilic is less favourable in cycle (Table 1).
Table 2  Geometric mean titers of DEN-2 16681 in Aedes aegypti (pale form and dark form) after oral infection.

<table>
<thead>
<tr>
<th>Aedes aegypti</th>
<th>Geometric mean titers log 10 MID50/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days</td>
</tr>
<tr>
<td></td>
<td>7</td>
</tr>
<tr>
<td>pale form</td>
<td>4.1</td>
</tr>
<tr>
<td>dark form</td>
<td>4.1</td>
</tr>
</tbody>
</table>

In laboratory infection several species of common man-biting Aedes were much more susceptible to oral infection with each of the 4 dengue serotypes than Aedes aegypti e. g. Ae. pseudoscotellaris 84.0% (21/25), Ae. malayensis 84.0% (21/25), Ae. albopicuts 60% (15/25), Ae. polynesinensis 52% (13/25), Ae. aegypti 16.7% (3/18), Ar. subalbatus 0% (0/10), Cx. molestus 0% (0/14) (Rosen et al., 1985).

Geographic strains of Aedes aegypti varied in susceptibility to oral infection with dengue viruses susceptibility is recessive and the factors controlling susceptibility were the same for 4 types. No difference was observed between resistant and susceptible mosquito strains in the rate or the amount of viral replication after infection by parenteral route, or in their ability to transmit dengue 2 virus after infection by the oral route (Gubler et al., 1979).

Variation in susceptibility to oral infection with arboviruses among strains of mosquitoes from different geographic localities has also been reported in Aedes albopictus / dengue viruses (Gubler and Rosen, 1976), Aedes albopictus /chikungunya virus (Tesh et al., 1976), Culex tarsalis / western equine encephalomyelitis virus (Hardy et al., 1978). Strains of Aedes triseriatus from La crosse virus-endemic areas had lower susceptibility than these from nonendemic areas, suggesting that selection was toward higher resistance to the mosquito population (Grimstad et al., 1977). This was not the case for dengue virus, since the African strains of Aedes aegypti were among the most resistant. Distribution of dengue viruses have very little known in West Africa.

Transovarial transmission of all four dengue serotypes was demonstrated in Aedes albopictus. The highest rate were observed in DEN1 and the lowest in DEN2 but only DEN1 was demonstrated in Aedes aegypti at a relatively low rate (Rosen et al., 1983).

The vaccine virus was markedly less efficient in its ability to infect Aedes aegypti orally (66/397 against 220/396, 3.7 to 8.3 log 10 MID50/ml of viruses). None of 16 infected mosquitoes transmitted the vaccine virus while 3/22 of the mosquitoes transmitted parent virus (Miller et al., 1982). Vaccine viral antigens of dengue, bunyaviruses and alphaviruses were found in large amounts in the mesenteral tissue only fore and hind guts as well as ovaries, ventral nerve chord, salivary gland and fat body. It would appear that although virus
was replicating in the midgut it was unable to mature and escape into the hemocoel or unable to attach and replicate in the secondary organ systems.

The dissemination of dengue type 3 (DEN3) virus in parenterally infected female *Aedes aegypti* mosquitoes was studied by detecting viral antigen using immunocytochemically. Antigen was first detected in fat body cells near the site of inoculation, intersuscepted foregut, salivary glands and nervous tissue. Nervous tissue appeared to be the primary site of amplification. Muscle, tracheae, malpighian tubules and posterior midgut did not become infected. After 7 day 100% of salivary gland were infected (Linthicum et al., 1996).

Wallis et al. (1984) studied genetic heterogeneity among Caribbean population of *Aedes aegypti* and revealed some relationship between geographic proximity and genetic distance, the overall picture among islands is one of gene frequency patchiness with some collections clearly not conforming to any geographic pattern attributable to high rates of gene flow among islands and with mainland American continent and the activities of various vector control agencies in the region.

Man mosquito association favours dengue virus transmission which depends on various factors (a) mosquito factors: 1) breeding habitat; type form is more productive in its indoor nature 2) biting habit; both are anthropophilic and diurnal periodic in nature but the dark form is endophilic while pale form is exophilic. These conditions favour the dark form in transmission of dengue virus. 3) longevity: indoor habit favour the dark form has longer life 4) gonotrophic cycle: seem to be the same in both forms 5) movement: although *Aedes aegypti* is endophilic it can fly about a mile away from the point of release 6) susceptibility: the dark form is more susceptible to dengue 7) frequency of feeding: interrupted feeding favour more transmission 8) number: the dark form is endophilic therefore more number is expected from being protected from preys and physical hazard 9) physiological and behavioral plasticity: the dark form is found both endo and exophilic while the pale form is mostly exophilic. Moreover the dark form is more resist to insecticide (b) man factor 1) viremic state 2) behavior indoor 3) age 4) sex 5) race 6) environment & socioeconomic.

Factors operating in the mosquito *Aedes aegypti* type form and pale form for dengue virus infection may be different in 1) genetic difference at subspecific level (Sucharit and Surathin, 1994), 2) isozyme difference in esterase 3) insecticide resistance and 4) faster rate of development indicated by early split of chromosome in the type form (to be published)

The presence of (1) a vasodilatory peptide of the tachykinin family in the salivary glands of *Aedes aegypti* (Ribeiro, 1992) (2) Apyrase (Apy) gene that encodes and ATP-diphosphohydrolase preventing platelet aggregation in the host (Smartt et al., 1995) (3) other coagulation factors may be related to dengue virus transmission.
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