A SHORT SURVEY ON THE DISTRIBUTION AND ABUNDANCE OF *AEDES* SPP IN UNIVERSITI SAINS MALAYSIA KELANTAN IN RELATION TO DENGUE VIRUS

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ABSTRACT: *Aedes* has become the most significant public health concern in Malaysia due to dengue fever disease. As *Aedes* vectors are expanding their geographical distribution, the population dynamics of the adult mosquitoes are highly significant in areas where dengue is prevalent. The objective of this study was to conduct a short survey on the abundance of *Aedes* mosquito in Universiti Sains Malaysia (USM), Health campus and its relation to dengue virus. Two areas were selected in the campus as sampling location: hostel, and Hospital USM area. The mosquitoes were reared from field collected eggs and the number of adult, species, and sex of mosquitos produced were recorded. RT-PCR analysis was performed on mosquito population from Hospital USM sampling area for dengue virus detection. Results showed that *Ae. albopictus* was the predominant species in both sampling areas. A spatial and ecological coexistence of *Ae. aegypti* and *Ae. albopictus* were observed in Hospital USM area. However, there was no population of *Ae. aegypti* recorded in the hostel area. Analysis of RT-PCR on samples showed that one pool from *Ae. albopictus* samples was positive for dengue virus. Although *Ae. albopictus* has long been considered as a secondary vector in dengue virus transmission in Malaysia and Southeast Asia, the increasing abundance of *Ae. albopictus* should be of particular concern in the absence of *Ae. aegypti* as the former vector could potentially evolve to become the primary vector for transmitting dengue virus in a peri-domestic area.

Keywords: *Aedes* spp, biology and distribution, dengue virus

Introduction

Mosquitoes have a nearly worldwide distribution, being found throughout the tropics and temperate regions. They thrive in a variety of habitats including fresh water and brackish water (clear, turbid and polluted) but not in marine habitats with high-salt concentrations. Apart from being found in many parts of the world, mosquitoes are among the most important arthropod vectors for various diseases that are caused by viruses or parasites. At present, its disease transmissions are very difficult and challenging to control and eradicate completely. The most important pest and vector species belong to the genera *Anopheles*, *Culex*, *Aedes*, *Psorophora*, *Haemagogus*, and *Sabethes*. In Malaysia, common mosquitoes that medically important in disease transmissions are from the genera *Aedes, Anopheles,* and *Culex* which transmit serious diseases such as malaria, dengue, Chikungunya, encephalitis and filariasis (Becker *et al.*, 2010). In addition, the recent rapid emergence of Zika disease has become global attention among public healthcare services because of its highly suspected association with related newborn microcephaly (Dyer 2015; Hajra *et al.*, 2016). Among the genera, *Aedes* is an important public health concern in Malaysia due to dengue. Depending on the geographical factor, *Aedes aegypti*, *Aedes albopictus*, *Aedes polynesiensis* and *Aedes scutellaris* are known to transmit the dengue virus (Gubler, 1998). However, in Malaysia only *Aedes aegypti* and *Aedes albopictus* are commonly associated with dengue virus transmission (Smith, 1956; Gubler, 1998; Norzawati *et al.*, 2015; Ong, 2016).

Pediatric Dengue Vaccine Initiative (PDVI) estimated more than half of the world populations (55%) are at risk of being infected with dengue. The endemic outbreak of this disease has expanded to areas which were unaffected before. Malaysia, Cambodia, Philippines, and Vietnam are among World Health Organization (WHO) Western Pacific region countries that are severely affected by dengue. In Malaysia, a total of 32,435 dengue cases with 53 deaths were reported from Jan 30 till June 30, 2018, compared to 49,726 cases with 110 deaths during the same period in 2017 (CPRC, 2018). Once infected with dengue virus, patients could exhibit a wide range of illnesses from mild fever to fatal hemorrhagic fever and dengue shock syndrome (Zhang *et al.*, 2014). Virus serotype immunity status, and genetic variation are among the determining factors of disease severity (Gubler, 1998). Dengue virus belongs to *Flaviviridae* family and there are four dengue virus serotypes which are antigenically related namely dengue-1, dengue-2, dengue-3 and dengue-4. Strong resemblance of epitopes of these

four dengue virus serotypes makes diagnosis difficult. Treatment is also a challenging matter, since there is no cross-protective immunity among these four serotypes (Gubler, 1998). This means that a person can still be infected with the other serotypes after being affected with one of them and had development immunity towards it.

As *Aedes* vectors are expanding their geographical distribution, this can infer the risk of dengue transmissions especially in receptive and prone areas (Huang *et al.*, 2014; Boonklong and Bhumiratana 2016). Several studies had demonstrated that competent vectors are present in various geographic locations (Gubler and Rosen 1976). This subsequently helps to introduce viruses into new susceptible human populations. It was reported that the distribution and abundance of the *Aedes* mosquitoes are related to the human population's behavior and activities towards the environment. This includes modification of spaces and presence of vegetation in urban and rural areas. Apart from that, meteorological factors such as temperature, relative humidity and rainfall also contribute to the population density of *Aedes* (Serpa *et al.*, 2013). In addition, the epidemiology of diseases transmission is also determined by vector behavior in the environment. The objective of this study was to survey the population and abundance of *Aedes* mosquito spp in the USM Health campus. We hypothesized that most areas would be dominated with only one species at a time.

Materials and Methods

Ovitrap settings of Aedes mosquito eggs

Ovitrap or oviposition trap is a popular method of obtaining eggs from *Aedes* mosquito species. Ovitrap, as described by Lee (1992) was used in this study. It consists of a simple cylindrical black plastic container and a hard board (paddle). The ovitrap were filled with tap water and paddles were placed vertically in it, serving as a substrate for female mosquitoes to lay eggs. Two sampling areas in Universiti Sains Malaysia Kubang Kerian, Kelantan were selected in this study i.e Desasiswa Murni (6.09801,102.2834069) and Hospital Universiti Sains Malaysia (Hospital USM) (6.0990448,102.2786647) (Figure 1). The locations were chosen based on the presence of different habitat types. Desasiswa Murni is the students' hostel areas, well shaded, often with many shrubs, or bushes, and well-tended lawns. On the other hand, Hospital USM sampling areas were consisted primarily of concrete lots, covered garages, building coverages with little vegetation or bushes and trees for shade. Some of the

areas had stagnant water and piles of discarded materials. Both locations are situated in USM campus area with the distance from each point as 417.62 m apart. 20 ovitraps were placed randomly at each area covering 50 meter radius from sampling point location (Figure 2 and 3). Ovitraps were placed on the ground level in a shady and hidden area to attract female *Aedes* mosquitoes to lay eggs. Each ovitrap was labeled with basic information of the research study and they were left for seven (7) days in the field.

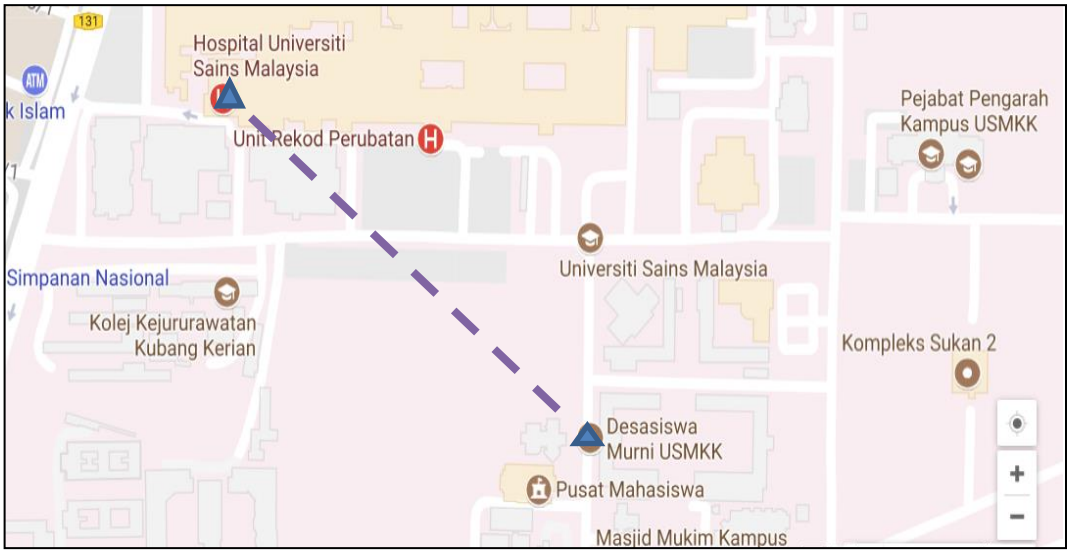

Figure 1: Two ovitrap setting locations in Universiti Sains Malaysia Health Campus

Figure 2: Location of Desasiswa Murni in USM campus area

Figure 3: Location Hospital Universiti Sains Malaysia (Hospital USM)

Rearing and culturing of Aedes mosquito

All ovitraps were collected after seven days of setting up in the field. Ovitraps were brought back to the laboratory/insectarium for processing. The ovitrap contents and paddles were poured into individual plastic trays and added with water to allow eggs hatching. A powder form of larval food mixed with dried cow liver, cat food, yeast, vitamin B complex, and cereals were added to the tray as food supply. Larvae that pupated were separated from the colony by transferring them into a small bowl filled with water. The bowl was placed into a cage to contain emerged adult. Date of adult emergence, species, sex and sampling location were all noted down. Emerged mosquitoes were collected using mouth aspirator and pooled in a plastic tube. The tubes were labeled and kept in a -80 °C freezer for PCR analysis. In this study, PCR was performed only on samples collected in Hospital USM area where reported dengue cases had been notified within a 200m radius of the case residence. The sampling activity of the *Aedes* sp population was carried out for three consecutive months from June to August 2015.

Observations of Aedes life cycles

20 *Aedes* 1st instar larvae used in this study were collected from Desasiswa Murni and Hospital USM field sampling area respectively. Larvae were transferred individually into 40

paper cups using a disposable pipette and labeled accordingly. Sufficient larval food was provided throughout the observation period to promote larval growth and development. Observations and recording of the life cycle duration were carried out every four hours. Species and gender of emerged adult mosquito were recorded. The cup opening was covered with a net cloth to contain adult mosquito from escaping the individual cup.

Reverse transcriptase polymerase chain reaction (RT-PCR) analysis

RNA extraction

Viral RNA from mosquitoes samples was extracted using Analytik Jena innuPREP Virus RNA kit according to the manufacturer's instruction. In brief, pools of head-thorax (from 10-20 adult mosquitoes) were ground in 500 µl of 1% DMEM and centrifuged at 20 000 g for 15 minutes. 450 µl lysis solution was added to 150 µl of head-thoraxes suspension together with 20 µl proteinase K before being mixed vigorously and incubated for 10 minutes. Then, 600 µl binding solution was added and mixed before being transferred to a spin column and centrifuged at 10 000 g for 1 minute. Subsequently, the bound RNA was washed twice using washing solution HS and LS. The spin filter was centrifuged at a maximum speed twice to remove traces of ethanol. The RNA was eluted from the column by adding 60 µl heated RNase free water and incubated for 2 minutes before centrifugation at 8000 g for 1 minute. The extracted RNA was stored at -80˚C until further use.

Detection of dengue virus by conventional RT-PCR

A conventional RT-PCR was performed using MyTaq™ One-Step RT-PCR kit to detect the presence of dengue virus according to the manufacturer's instruction. 5 µl RNA extract from 10 samples were used as template in a 25 µl reaction. The primer sequences are as follows: DV1, 5′-GGRACKTCAGGWTCTCC-3′; DV3, 5′-AARTGIGCYTCRTCCAT-3′ (Diaz-Badillo *et al.,* 2014; Seah *et al.,* 1995). The expected size of the amplicon product is 470 bp (DV1 and DV3). The RT-PCR profiles were: One cycle of reverse transcription at 50°C for 30 min, one cycle of polymerase activation at 95°C for 5 min, followed by 39 cycles of denaturation step at 95°C for 15s, annealing step at 60°C for 30s and extension step at 70°C for 1 min and finally, one cycle for final extension at 70°C for 10 min. The PCR products were electrophoresed on 1.5% agarose gel in 0.5 X TBE buffer.

Data Analysis

Data were analyzed using SPSS v.22. Distribution and abundance of mosquito species were recorded accordingly in percentages for species and sex. For development duration, mean of time duration in days was calculated for each stage from 1st instar larvae until adult emergence. Paired samples T-test were assessed statistically for group differential.

Results

In total 40 ovitraps were set up in Desasiswa Murni and Hospital USM, *Ae. albopictus* was the predominant species in both sampling areas and it was also the only species collected from Desasiswa Murni. However, in Hospital USM study area *Ae. aegypti* and *Ae. albopictus* coexisted in the same habitat radius. Percentile of mosquito species and sex is presented in Figure 4.

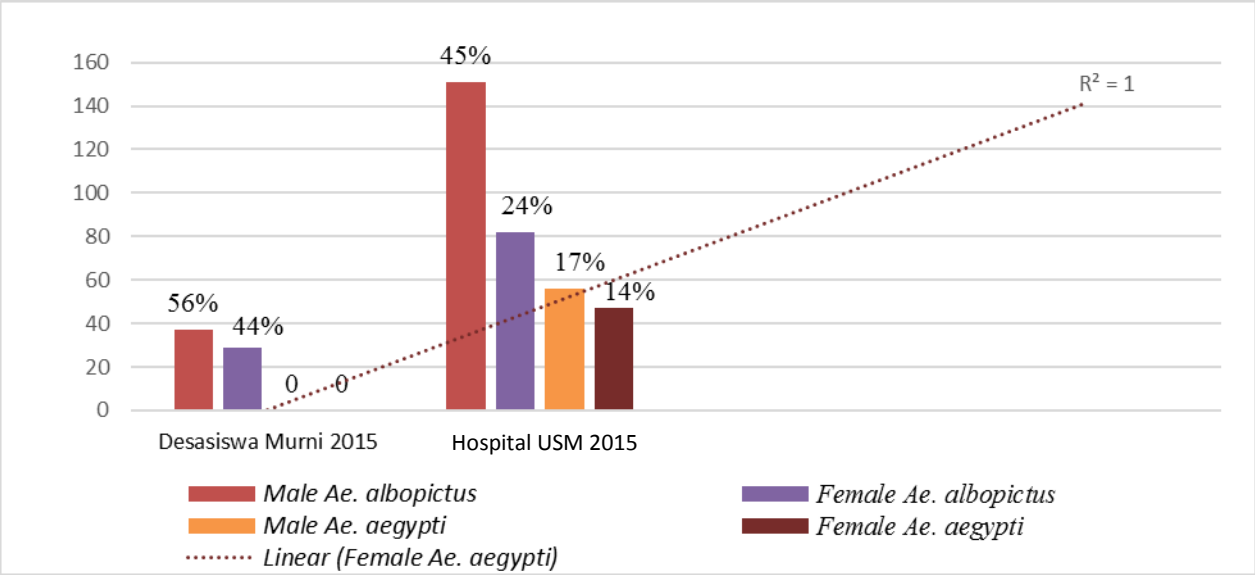

Figure 4: Population of *Aedes* sp in Desasiswa Murni and Hospital USM, Kubang Kerian, Kelantan

Following the trend, the numbers of male mosquitoes produced from both *Aedes* species were shown to be higher than female mosquitoes from both studied areas. In Desasiswa Murni the total number of adult mosquitoes was lower than Hospital USM study area. The percentage of *Ae. albopictus* male mosquitoes that emerged from the eggs collected was 56% and of female mosquitoes it was 44%. Male *Ae. albopictus* and *Ae. aegypti* were the dominant Aedes

populations in Hospital USM area, contributed 45% and 17% of total population, respectively. Percentages of female mosquitoes were 24% for *Ae. albopictus* and 14% for *Ae. aegypti*. Straight line (R^2) in Figure 4 indicates the general pattern or direction of *Aedes* sp population over time. The value of R^2 (1.0) exhibited a significant increase in female *Ae. aegypti* populations by years in both sampling areas.

Table 1 describes the mean duration development in days of *Aedes* sp from both sampling areas under laboratory condition. *Ae. albopictus* population from Desasiswa Murni area was shown to have shorter growth period when compared to *Ae. aegypti* and *Ae. albopictus* populations from Hospital USM with a total mean time from egg hatching to adult emergence of 7.9 ± 2.3 days and 9.0 ± 1.9 days, respectively. The first (L1), second (L2) and third-instar (L3) larvae presented a significant difference in the growth periods between both sampling areas with a *p*-values less than 0.05. The pupation duration from both sampling areas was longer than any larvae stages with similar duration of 3.0 days. Adult development time was moderately similar between both areas with a mean duration of 1.4 ± 0.5 days and 1.7 ± 0.5 days for Desasiswa Murni and Hospital USM areas, respectively.

Table 1: Life cycle duration of *Aedes* spp. in Desasiswa Murni and HUSM

Larvae stages	Mean of development time (Day ± SD) (n = 20)		Paired samples test
	Ae. albopictus population in Desasiswa Murni	*Aedes* sp population in Hospital USM	*p*-value
*L1	0.9 ± 0.1	1.3 ± 0.0	0.00
*L2	1.1 ± 0.4	1.8 ± 0.9	0.01
*L3	0.9 ± 0.2	0.7 ± 0.1	0.00
L4	0.6 ± 0.2	0.5 ± 0.1	0.19
Pupae	3.0 ± 2.1	3.0 ± 1.4	1.00
Adult	1.4 ± 0.5	1.7 ± 0.5	0.17
Total development	7.9 ± 2.3	9.0 ± 1.9	0.15

L = larva
**There were significant differences among Aedes sp between both populations (p< 0.05)*

Table 2 shows the results of RT-PCR assay analysis for dengue virus detection in Desasiswa Murni and Hospital USM study areas. RT-PCR was run on 10 pooled samples of *Aedes* sp, each pool consisted of head and thorax from 10-20 mosquitoes. Dengue virus was detected positive from Hospital USM studies area in 1 of the 10 pools from *Ae. albopictus* samples (Lane 2, sample: USM 2) as shown in Figure 5.

Table 2: Data for dengue virus detection in *Aedes* populations

Study Area	*Aedes* sp	No of pools	RNA RT-PCR
Desasiswa Murni	*Ae. albopictus*	10	negative
Hospital USM	*Ae. albopictus*	10	1 positive
	Ae. aegypti	10	negative

Figure 5: Agarose gel electrophoresis of PCR results from pooled *Ae. albopictus* samples. Ladder (marker) used was 100bp. Positive control (last lane on the right) was obtained from cultured dengue virus 2. Presence of dengue virus was detected in sample USM 2.

Discussions

Two *Aedes* species that are commonly associated with dengue are *Ae. aegypti* (Linnaeus) and *Ae. albopictus* (Skuse) (Christopher, 1960; WHO, 2011; Kamgang *et al.,* 2013). *Ae. aegypti* can be found worldwide, especially in a domestic environment in close proximity to humans that are abundant with artificial containers containing clean water which are often their

preferable spot to lay eggs. Observations on the larval development of *Aedes* sp from Desasiswa Murni and Hospital USM recorded that the developmental time durations were from 7.9 days to 9.0 days, which were considered as normal time duration. Similar duration had also been observed under controlled conditions when food was supplied in optimum amounts at 25°C by Hawley (1988) where their data showed that larval developmental period of *Ae. albopictus* was varied from 5 to 10 days. On the other hand, under field conditions, other factors like sex, humidity, food supply and temperatures may govern larval developmental time (Dye, 2008; Couret *et al.*, 2014). Along with the aforementioned observation we also recorded a higher number of male mosquito production in both *Aedes* species. However, Lounibos and Escher (2008) had suggested that the bigger population of male mosquitoes could be balanced by the increased fitness of female mosquito, believed to be related to delay hatching.

Ae. albopictus usually dwells in suburban areas and areas like forest fringes and rubber plantations (Hawley 1988; Service 1992). Interestingly, due to rapid urbanization, this species has evolved and adapted its habitat to co-exist with *Ae. aegypti* (Estrada-Franco and Craig 1995; Liu *et al.*, 2016; Lounibos *et al.*, 2016). In addition, *Ae. albopictus* distribution and abundance are also influenced by female mosquito behavior of oviposition, environment and the local climate (Serpa *et al.*, 2013). In this study, it was observed that only *Ae. albopictus* dwelled in Desasiswa Murni with males notably dominated the population with a 12% margin while in Hospital USM area, both *Ae. albopictus* and *Ae. aegypti* co-existed. *Ae. albopictus* had a much higher distribution rate than *Ae. aegypti*. Similar observations have also been reported by other studies conducted in the field and in laboratory settings. Field investigations by Paupy *et al.* (2010) have shown that *Ae. albopictus* was abundant and outnumbered *Ae. aegypti* to a large extent in Gabon, Nigeria particularly in suburban environments. However, the co-existence of *Ae. albopictus* and *Ae. aegypti* in Hospital USM areas could have been contributed by the variants ecology and environments in Hospital USM where some areas are close to vegetations, jungle fringes and some areas have become dumping sites for discarded items and broken furniture. In contrast, the predominance of *Ae. albopictus* population in the hostel sampling area could be influenced by the secluded area where patches of vegetation are readily available. Our findings were supported by Braks *et al.* (2003) who described that habitat variants could have some effects on the distribution and abundance of *Ae. aegypti* and *Ae. albopictus* in the Southeastern Brazil and Florida. They observed a predominance of *Ae. albopictus* in rural areas but similar abundance of both species in the suburban areas. Studies

by Saleeza *et al.* (2013) also concur with our findings as their studied showed that *Ae. albopictus* was the predominant larval species found outdoors. The higher survival rate of adult *Ae. albopictus* than *Ae. aegypti* could be associated to the predominance of *Ae. albopictus* as reported by Brady *et al.* (2013).

Assuming that both species are equally competent to transmit dengue virus, we performed RT-PCR analysis from the samples collected in both sampling areas. However, only one sample tested were positive for dengue virus from a pool of *Ae. albopictus* (USM 2). There were no dengue virus detected in pool samples of *Ae. aegypti* and *Ae. albopictus* from Hospital USM and Desasiswa Murni sampling areas respectively. Since this study was conducted in a short period, further investigation should be carried out to determine the specific role of both *Aedes* species in dengue virus transmissions. Epidemiological evidence clearly shows that *Ae. aegypti* and *Ae. albopictus* are responsible for the majority of dengue transmission in Southeast Asia and many other parts of the world (Smith, 1956; Failloux *et al.*, 2002; Gubler and Kuno 1997; Lee *et al.*, 2015). *Ae. aegypti* is the primary vector and *Ae. albopictus*, as the secondary vector. Other mosquito-borne diseases transmitted by these two vectors are West Nile fever, yellow fever, and chikungunya (Primavesi, 2013; Restrepo *et al.*, 2014). *Ae. albopictus*, also known as the "Asian tiger mosquito", survives around human dwellings in the daytime and is very common in Malaysia and other Asian countries (Huang, 1972; Coffey *et al.*, 2014). Whereas, *Ae. aegypti* is mainly abundant in the indoors and urban areas in tropical countries (Service, 1993).

The distribution and abundance of dengue vectors, *Ae. aegypti* and *Ae. albopictus* are commonly affected by climatic factors (Higa, 2011; Brady *et al*., 2013) and environmental changes (e.g vegetation, building density) (Kamgang *et al.*, 2010). As their life cycles are highly adaptable to the human environment, any changes from human activity such as urbanization result in a great impact on their distribution (Higa, 2011). However, there were studies reported that the spread of *Ae. albopictus* and the declining *Ae. aegypti* populations might be linked to inter-species competition (O'Meara *et al.*, 1995; Juliano *et al.*, 2007). As cited by Kamgang *et al.* (2010), previous studies have suggested *Ae. albopictus* preferentially colonizes environments with vegetation and mainly breeds in natural containers such as tree holes and leaf axils, whereas *Ae. aegypti* prefers to breed in artificial containers located in environments with higher building density (Chan *et al.*, 1971; Cox *et al.*, 2007). According to Higa (2011), as dengue virus circulates only between humans and vector mosquitoes, thus the

spatial distribution of the vectors highly affects the epidemiology of the disease and the vector presence is a limiting factor of transmission. Mendenhall *et al.* (2017) suggests that detection of virus in field-caught vectors is an important evidence of potential vector capacity in natural settings. Therefore, surveillance activity by the vector control team should include a peri-domestic area in the vector management program.

Conclusions

This study has shown that both *Ae. aegypti* and *Ae. albopictus* were present in the USM Health Campus area. *Ae. albopictus* were predominant particularly in the peri-domestic area, and the increasing abundance of *Ae. albopictus* should be of particular concern due to its potential trait to evolve and become a primary vector in transmitting dengue virus in a certain favorable area.

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