

# Abstracts

## **Session 1** **Vector Biology and Control**

## Plenary Paper

### S1.1 Emerging threats of vector borne diseases in Malaysia

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A number of vector borne diseases were discovered decades ago but they still persist at the turn of this century. In fact some have become of greater public health importance now than before. One good example is Dengue which is increasing over the years. It has acquired a higher status since it poses a great threat to the affluent people in urban areas. Another viral disease Chikungunya, is also resurfacing in this country and the vector is *Ae. aegypti* which is also the vector for dengue. Among the parasitic vector borne diseases, filariasis is very much under control. However, of late we see an increase in the number of *Wuchereria bancrofti* cases being imported into the country. *Culex quinquefasciatus* is the main vector, it is abundant in the country and is susceptible to the parasite. Malaria which kills a child every second in Africa is well under control in Malaysia. However, our neighbouring developing countries are not so fortunate and malaria is a serious problem there. Now due to good diagnostic techniques we are seeing simian malaria in humans. Perhaps this could lead in the future to simian malaria being transmitted from humans to humans. Human resources are needed to develop and implement sustainable prevention programmes. This is lacking in most developing countries. Global travel has become very affordable and thus infectious agents can be easily introduced into areas where they have been absent. Thus the emergence/resurgence of vector borne disease is always there.



### S1.2 Seasonal abundance and biting cycle of *Anopheles maculatus* in Penang Island

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Study on the bionomics of adult *Anopheles maculatus* was carried out from February 2006 until January 2007 in Gambir Hill, Penang Island, Malaysia. Adult collections were made over a 12 month period using the cow baited trap. A total of 3,476 adult females were collected. *Anopheles maculatus* was found to be abundant in April/May and October/November. The biting activity of *An. maculatus* was observed throughout the night over a period of 12 hours. The biting pattern fluctuated throughout the night peaking at 2200 – 2300 and 0100 – 0200 hour.

### S1.3 Incrimination of *Anopheles latens* as vector of *Plasmodium knowlesi* and other simian malaria parasites in Sarawak, Malaysian Borneo

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A large focus of naturally acquired *Plasmodium knowlesi* infections in humans was reported in the Kapit Division of Sarawak, Malaysian Borneo. In order to identify the vector(s) of *P. knowlesi*, all-night human bait collections and monkey-baited traps were used to obtain mosquitoes attracted to humans and monkeys, respectively. A total of 2,706 anophelines representing 12 species were collected over a 12 month period. *Anopheles latens* was the predominant species collected (44% of anophelines caught) and the only species where sporozoites were found (n=9). DNA from the salivary glands of these 9 infective mosquitoes was extracted for analysis by nested-PCR with primers specific for human and simian malaria parasites. Results showed that 4 of these *An. latens* were positive for *P. knowlesi* while the remaining 5 infective mosquitoes were found to carry other simian malaria parasites such as *P. coatneyi* and *P. inui*. *Plasmodium* circumsporozoite (*csp*) genes of PCR positive samples were amplified, cloned and sequenced. Analyses of the DNA sequences showed that the *P. knowlesi* isolates from mosquitoes were phylogenetically indistinguishable from those isolated from humans and monkeys. Some *csp* genotypes were also shared between isolates from humans, monkeys and mosquitoes. The results confirm *An. latens* as the natural vector of *P. knowlesi* and other simian malaria parasites and, together with epidemiological data, strongly suggest that human knowlesi malaria is a zoonoses in Kapit, Sarawak, Malaysian Borneo.



### S1.4 Insecticide susceptibility of *Aedes aegypti* in North-East of Thailand

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Larvae and adults of *Aedes aegypti* collected from Nakornratchasima province (which is a high risk area for Dengue haemorrhagic fever) and a nearby province, Buriram were tested using the standard WHO guideline to determining their susceptibility to insecticides that have been used for dengue control: temephos, permethrin, deltamethrin, cyfluthrin, cypermethrin and fenitrothion. The results showed that the Nakornratchasima strain of larvae was susceptible to temephos but the Buriram strain was tolerant. Resistance to permethrin and cypermethrin was detected in both strains. There was also resistance to fenitrothion in the Buriram strain. Both strains were tolerant to other insecticides. Biochemical assay of enzymes in mosquitoes was conducted to detect the emergence of insecticide resistance and to define the mechanisms involved in pyrethroid and organophosphate resistance. The results revealed significant increase of monooxygenase levels in both strains but was higher in Nakornratchasima strain which showed higher resistance to permethrin when compared with laboratory susceptible strain. The esterase activity was found to have increased levels only in Buriram strain which is related to organophosphorus resistance.

### S1.5 Potential of *Litsea elliptica* Blumae extract against the dengue vector *Aedes aegypti* Linnaeus

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*Litsea elliptica* Linn. leaf extract viz: hexane fraction, chloroform fraction, ethyl acetate fraction and methanol fraction and essential oil were investigated for anti-mosquito potential including larvicidal, adulticidal, ovicidal and repellent activities against *Aedes aegypti*, the vector of dengue haemorrhagic fever. These fractions and essential oil were screened for larvicidal activity on early fourth instar larvae of *Ae. aegypti* following WHO standard protocol. The hexane fraction was found to show the highest larvicidal effect with the median lethal concentration (LC<sub>50</sub>) value of 1.88 ppm and the LC<sub>90</sub> value of 10.76 ppm, respectively. Ovicidal activity was also demonstrated using the hexane fraction against the eggs of *Ae. aegypti* with LC<sub>50</sub> value at 39.46 ppm and the LC<sub>90</sub> value at 269 ppm. The mortality of the newly hatched larvae, reared from eggs in this media, reached 100% at concentrations as low as 100 ppm, 2 days post hatching. However, when tested for the adulticidal activity, the essential oil showed promising adulticidal effect with the LC<sub>50</sub> and LC<sub>90</sub> values of 0.04 and 0.90 mgcm<sup>-2</sup> respectively. It was then further tested for biting deterrence in human volunteers. In laboratory, *L. elliptica* essential oil possessed repellent effect against *Ae. aegypti* with ED<sub>50</sub> value of 0.03 mgcm<sup>-2</sup>. Topical application of the essential oil did not induced dermal irritation. *L. elliptica*, therefore, can be considered as a probable source of some biologically active compounds used in the development of mosquito control agents.



### S1.6 Weekly variation on susceptibility status of *Aedes* mosquitoes against temephos in Selangor, Malaysia

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Larvae of *Aedes aegypti* and *Aedes albopictus* obtained from 6 consecutive ovitrap surveillance (OS) in Taman Samudera and Kg. Banjar were evaluated for their susceptibility to temephos. Larval bioassays were carried out in accordance with WHO standard methods, with diagnostic dosage (0.012 mg/L) and operational dosage (1 mg/L) of temephos, respectively. *Aedes aegypti* and *Ae. albopictus* obtained from six OS in Taman Samudera showed resistance to diagnostic dosage of temephos with percentage mortality between 5.3 to 72.0 and 9.3 to 56.0, respectively, while *Ae. aegypti* and *Ae. albopictus* obtained from Kg. Banjar showed resistance to temephos with percentage mortality between 16.0 to 72.0 and 0 to 50.6, respectively. Only two strains of *Ae. aegypti* from Kg. Banjar were susceptible to temephos with 93.3% (OS 2) and 100% (OS 3) mortality. The 50% mortality of lethal time (LT<sub>50</sub>) for all strains of *Ae. aegypti* and *Ae. albopictus* tested against operational dosage of temephos showed range between 36.07 to 75.69 minutes and 58.65 to 112.50 minutes, respectively, and complete mortality was achieved after 24 hours. Our results indicated that there is weekly variation of the resistance status for *Ae. aegypti* and *Ae. albopictus*. *Aedes* susceptibility to temephos is changing from time to time in these two study sites. It is essential to continue monitoring the resistance of this vector to insecticides in order to ensure the efficiency of program aimed at vector control and protection of human health.

## S1.7 Outdoor surveillance of dengue vectors breeding in a selected area in Kuala Lumpur

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An outdoor larva survey was conducted in Kampung Baru, Kuala Lumpur. The results confirmed that *Aedes albopictus* is more abundant as an outdoor container breeder compared to *Aedes aegypti*. Plastic containers and pot flowers were the most abundant outdoor breeding containers, with 43.30% and 19.10% breeding, respectively. On the other hand, bottles and paint pail were less abundant with 4.46% and 3.82% breeding, respectively. We also observed that the main adult mosquito hiding places were bedroom, kitchen and closet. Our study indicated that in indoor collection, *Culex* species were most abundant, representing 92.7% of total captured.



## S1.8 Biological efficacy of commercial household insecticides aerosol spray against *Aedes aegypti* (Linn.) under simulated field conditions

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Simulated field study on biological efficacy of commercial household insecticide aerosol was conducted. The bioefficacy of five pyrethroid aerosols named as A01 to A05, were tested in cabins measuring 10' width x 20' length x 8' height. The cabins were furnished to simulate bedroom conditions. Each aerosol product was tested against lab-bred *Aedes aegypti* based on the manufacturer's recommended dosage. Ten cages of mosquitoes were placed in the following locations: one in the middle of the room; two cages on and underneath the bed; three cages were placed inside, behind and on top of the wardrobe; and one cage was placed on the desk and three were placed inside the drawers (tightly closed, partially closed and open). Prior to the experiments, the discharge rate of each aerosol can was determined. Lab-bred 2-5 days old, sugar-fed *Ae. aegypti* adults were placed inside the test cages. The aerosol was then discharged in the cabin at the recommended dosage. After 30 minutes, the mosquitoes were collected into clean paper cups & their mortality recorded after 24 h. The results indicated that all aerosols induced complete mortality in caged *Ae. aegypti* females, except in cages that were hidden completely inside drawers and wardrobes. Insecticide droplet analysis was also observed and the uniformity value (ratio of VMD:NMD) for the tested aerosols varied. It was concluded that generally insecticidal aerosols in cans were effective for mosquito control provided they are properly used.

### **S1.9 The effects of vaporized acetone against adult *Stegomyia aegypti* (Linn.)**

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Acetone is formed endogenously in human and it is removed through urine and exhaled air. However, acetone of occupational source has been classified as hazardous under the Malaysian Occupational & Health Regulations 2000 (Use and Standard of Exposure of Chemicals Hazardous to Health) due to its high flammability and harmful effects to human. Inhalation of acetone at concentrations above the permissible exposure level may cause acute effects on human central nervous system, characterized by nausea, headache, dizziness and unconsciousness. Acetyl coenzyme-A is involved in the production of endogenous acetone in human and formation of acetylcholine in mosquito nervous system. Therefore, it was suspected that the presence of acetone at mosquito nervous synapse may affect the normal process of impulse transmission in it. This study investigated the possibility of acetone to affect mosquito nervous system. Incapability of mosquito to coordinate movement (knockdown) was used as an indicator for the affects of acetone on mosquito nervous system. *Stegomyia aegypti* adults were released into enclosed glass chambers containing airborne acetone at several doses ranging from 0 to 153 mL/m<sup>3</sup>. The numbers of mosquito- knockdown were recorded at an interval of one minute for a period of 20 minutes. Acetone vapour at the dose of 76.5 and 153 mL/m<sup>3</sup> was found to knockdown 33 and 64 % of the mosquitoes, respectively. The results provide a basis for detailed study to elucidate the effects of acetone vapour on the mosquitoes.



### **S1.10 Larvicidal activity of crude ground betel nuts against *Stegomyia aegypti* (Linn.)**

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*Areca catechu* nuts also known as betel nuts is traditionally used as one of the ingredient in betel quid, an alternative medicine to strengthen gum, as appetite stimulants etc. It is also chewed for the mild psychoactive effects. Frequent chewing of betel nut leads to epilepsy, symptoms associated with oral submucous fibrosis and pre cancerous oral lesions. These harmful health effects are due to excessive oral consumption of arecoline contained in the betel nut. Arecoline mimics acetylcholine, which is an important mosquito neurotransmitter. As such it is seen as a potential candidate to be screened for its larvicidal activity. *Stegomyia aegypti* larvae were released into glass containers containing solutions of distilled water mixed with crude ground mature betel nuts at the dose of 10, 20, 30, 40 and 50 g/L. The numbers of larvae that died at the 24<sup>th</sup> & 48<sup>th</sup> hours of post treatment were recorded. The solutions at the dose of above 30 g/L resulted in the death of more than 50 % (24<sup>th</sup> hour) and 85 % (48<sup>th</sup> hour) of the larvae. The results provide the basis for further study to detect and quantify the lethal doses of the active ingredients in the betel nut.

### **S1.11 Determination by age grading of *Culex quinquefasciatus* suspected as primary vector of bancroftian filariasis in Bekasi, West Java**

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Survey study had been conducted in 7 villages of the municipality of Bekasi, known to be endemic with filariasis in West Java from March to May 2005, to determine the primary mosquito vector in the area. The study is part of a large, ongoing study to map the areas of Bekasi municipality known to be endemic with filariasis, to measure the prevalence of the disease and to study the knowledge and attitude of the population on filariasis. Result of the study showed that out of 9 species of mosquito collected at night (*Culex quinquefasciatus*, *Cx. tritaeniorhynchus*, *Cx. sitiens*, *An. vagus*, *An. tessellatus*, *An. barbirostris*, *Ar. subalbatus*, *Ae. aegypti*, and *Ae. albopictus*), *Cx. quinquefasciatus* is the primary vector for bancroftian filariasis in the area, followed by *Cx. tritaeniorhynchus*. Abdominal dissection of female *Cx. quinquefasciatus* showed remarkable result where 5.7% of the total female population has an ovarial dilatation of more than 5, which is not common in the other areas of Indonesia.



### **S1.12 Influence of several intrinsic and extrinsic factors on the longevity of the Tropical bed bug, *Cimex hemipterus* (Fab.) (Hemiptera: Cimicidae)**

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Bed bug infestation world-wide has become a major concern to travellers and pest management industries. To design an effective pest management strategy against bed bugs, it is essential to understand the biology of bed bugs. Unfortunately, this subject matter is not well-studied and little is known, especially with regards to the tropical bed bug, *Cimex hemipterus*. This study was initiated to address the need to understand more about the environmental physiology of the tropical bed bug. Throughout this study, the longevity of *C. hemipterus* was found to be influenced by a number of intrinsic and extrinsic factors including sexes of the bugs, whether they are mated or unmated, deprivation of blood meals and temperature changes. Six strains of bed bugs were studied and both sexes of unmated bugs showed longer longevity period when compared to that of the mated bugs. A significant difference ( $P < 0.05$ ) was observed for the longevity of males and females of the unmated bugs, and *vice versa* for the mated ones. Two out of six strains of bed bugs were randomly chosen to conduct study of blood deprivation and temperature. Blood-deprived bed bugs showed a significant shorter longevity period ( $P < 0.05$ ), ranging from 60 % to 65% when compared to blood-fed bugs. High temperature of 35°C was found to greatly reduce the bug's longevity and temperature within 25-30°C also caused a slight reduction in their longevity compared to those bugs that were kept in lower temperature (20°C) and relative humidity of  $70 \pm 5\%$ .

### S1.13 Determination of housefly as a mechanical vector of flu viruses

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A study on houseflies was carried out to establish whether houseflies can transmit flu A virus mechanically at different time frame interval. The initial findings indicated that the diluents of virus in tissue culture fluid with bovine serum albumen indicated positive RT-PCR, and the persistency of flu A virus on fly legs could be detected as long as 24 hr. RT-PCR is a sensitive technique and reliable that can be used to detect influenza A virus in endemic areas of poultry farming and assured of rapid detection in surveillance system to encounter epidemic of the disease. Detection of flu A virus by PCR does not quantify the presence of virus, since the virus requires to be grown into tissue culture and this will confirm the viability of the virus. However, further epidemiological studies are needed before a final conclusion can be drawn regarding the significance of houseflies as transmitter of influenza virus and impact among poultry farming where abundance of flies are available.



### S1.14 Toxicity of five gel baits against the German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae)

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This study evaluates the efficacy and attractiveness of five commercially available gel baits (Advion® [containing indoxacarb], Avert® [containing abamectin], Infiniti® [containing hydramethylnon], Maxforce FC® and Maxforce Select® [containing fipronil]) against *Blattella germanica* using consumption assay. The feeding bioassay enables the duration of cockroach mortality after consuming an adequate amount of baits to be assessed, while simultaneously evaluating the amount of time taken by the cockroaches to approach the baits. Four different starvation levels (non-starved, 24h, 48h and 72h starved) of adult males, non-gravid females and intermediate nymphs were carried out in this experiment. Bait droplets, each weighing  $0.1960 \pm 0.0053$  mg, were introduced according to the experimental design. The time when baits consumption began and time of mortality were recorded once the cockroaches begin to consume the gel baits up to the point where the cockroaches were observed dead. The efficacy of gel baits are as follows: Maxforce FC® > Advion® > Maxforce Select® > Avert® > Infiniti®. Males were most susceptible, while intermediate nymphs showed the highest tolerance. However, the number of droplets and the starvation level of German cockroaches did not affect the efficacy of the gel baits. In terms of attractiveness, they were as follows: Maxforce Select® = Maxforce FC® = Avert® > Advion® > Infiniti®. Meanwhile, males were the fastest stage to approach the baits, and nymphs were the slowest. As the level of starvation increases, the speed of the cockroaches approaching the bait increases, except those that were starved for 48h and 72h.



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