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POPULATION ABUNDANCE, DISTRIBUTION, FORECASTING MODELS AND BREEDING HABITAT ECOLOGY OF DENGUE VECTORS IN PENANG ISLAND

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UNIVERSITI SAINS MALAYSIA

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by

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LIST OF ABBREVIATION AND SYMBOLS

ACF	auto correlation function
Ae.	*Aedes*
ANOVA	Analysis of variance
AR	auto regressive
ARIMA	auto regressive integrated moving average
ARMA	auto regressive moving average
BI	Breteau index
CI	Container index
cm	centimeter
DF	dengue fever
DHF	dengue haemorrhagic fever
HI	house index
KM	kilometer
L	liter
MA	moving average
N	number of samples
p	significant
PACF	partial auto correlation function
PP	pupae per person
r	correlation
R	regression
RH	relative humidity
S.E	standard error
SOM	self-organizing map
Temp	temperature
^{o}C	Celsius
%	percentage
±	plus minus
χ^2	chi-square
ϕ	autoregressive parameter
ε	error term
θ	moving average parameter

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APPENDIX N Estimated model parameters of ARIMA (0,1,2) without constant and model statistics for SG2 series.

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APPENDIX R Map of Pulau Pinang showing the four study sites in Southwest District.

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APPENDIX U Indoor breeding sites

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APPENDIX W Outdoor breeding sites (sheets)

APPENDIX X Outdoor breeding sites (canvas and water puddle)

APPENDIX Y Various *Aedes* breeding containers

APPENDIX Z Natural breeding sites

LIST OF PUBLICATIONS & SEMINARS

1 Abu Hassan A, Nur Aida H, Dieng H. 2013. Ovitrap surveillance of dengue vectors and their larval habitats in dengue hot spots in Malaysia. The American Mosquito Control Association AMCA 2013, 79th Annual Meeting. Atlantic City. February 24-28, 2013.

2 Nur Aida H, Abu Hassan A, Anita T, Farida A. 2012. *Aedes* productivity in natural and artificial containers in Penang Island. International Symposium on Insects. Mines Wellness Hotel, Kuala Lumpur, Malaysia. December 3-5, 2012.

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5 Nur Aida H and Abu Hassan A. 2011. Distribution and habitats of *Aedes aegypti* and *Aedes albopictus* larvae in Penang Island, Northern Peninsular Malaysia. Global Conference on Entomology 2011. Chiang Mai, Thailand. March 5-9, 2011.

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KELIMPAHAN POPULASI, TABURAN, MODEL-MODEL RAMALAN DAN EKOLOGI HABITAT PEMBIAKAN VEKTOR-VEKTOR DENGGI DI PULAU PINANG

ABSTRAK

Kajian ovitrap dijalankan untuk menentukan kelimpahan populasi lapangan vektor-vektor denggi, *Aedes albopictus* (Skuse) dan *Aedes aegypti* (Linnaeus) di tiga kawasan panas denggi di Pulau Pinang mulai Februari 2008 hingga Mac 2010 (108 minggu). Tiga puluh ovitrap diletakkan di setiap kawasan kajian dan dikutip setiap minggu. Kawasan setinggan bandar (Sungai Nibong Kecil) mempunyai jumlah telur dan peringkat tak matang tertinggi di sepanjang tempoh kajian, diikuti oleh kawasan bandar (Sungai Dua) dan kawasan pinggir bandar (Permatang Damar Laut). Bilangan telur yang dikutip lebih tinggi di musim lembap berbanding ketika musim kering. Populasi telur menunjukkan korelasi yang siknifikan dengan hujan dan kelembapan relatif tetapi tidak dengan suhu. Aktiviti semburan kabus lebih berkesan menurunkan populasi telur ketika musim kering berbanding ketika musim lembap. Model "autoregressive integrated moving average" (ARIMA) diaplikasikan terhadap set data ovitrap dari ketiga-tiga kawasan kajian. Model-model dianggar dan dinilai dari segi kesesuaian dan kemampuan meramal dengan tepat dan mencukupi untuk mewajarkan penggunaannya di dalam kawalan strategik *Aedes*. Model ARIMA (1,0,0), ARIMA (2,0,0) dan ARIMA (0,1,1) dinilai sebagai paling sesuai untuk set-set data kawasan pinggir Bandar, kawasan setinggan bandar dan kawasan bandar masing-masing.

Dua spesis vektor denggi iaitu *Ae. aegypti* dan *Ae. albopictus* dikenalpasti di dalam pemantauan bekas-bekas yang berkaitan dengan 1506 bekas yang mempunyai

larva daripada 2880 rumah di empat kawasan endemik denggi di Pulau Pinang. Peringkat tak matang *Aedes* dijumpai di dalam pelbagai habitat terutamanya di dalam bekas buatan manusia. Bekas yang paling produktif ialah baldi yang merangkumi 14.74% bekas yang positif dengan larva/pupa dan menghasilkan 18.18% daripada jumlah keseluruhan peringkat tak matang *Aedes* yang dikutip. Walaubagaimanapun dari segi penggunaan bekas, bekas yang diperbuat daripada plastik dan dibuang merupakan bekas yang paling kerap ditemui ketika kajian ini dijalankan. Kedua-dua spesis berkebolehan untuk mengkoloni bekas luar dan dalam rumah, dengan *Aedes aegypti* secara dominan boleh dijumpai di dalam bekas yang terletak di dalam rumah, manakala *Aedes albopictus* menggemari bekas di luar rumah. Perkongsian tempat pembiakan di antara *Ae. albopictus* dan *Ae. aegypti* sangat rendah (hanya 57 (3.8%) daripada 1506 bekas). Perkaitan hanya siknifikan di antara spesis dari segi taburan bekas tetapi tidak di antara individu. Indeks-indeks *Aedes* dan "pupae per person" digunakan untuk menilai risiko tranmisi denggi di semua kawasan kajian. Ambang transmisi untuk Pulau Pinang ialah 0.43. Indeks "pupae per person" di semua kawasan kajian melebihi nilai ambang transmisi menunjukkan risiko denggi di kawasan-kawasan tersebut.

POPULATION ABUNDANCE, FORECASTING MODELS AND BREEDING HABITAT ECOLOGY OF DENGUE VECTORS IN PENANG ISLAND

ABSTRACT

Ovitrap study was carried out to determine the field population abundance of dengue vectors, *Aedes albopictus* (Skuse) and *Aedes aegypti* (Linnaeus) in three dengue hotspots in Penang Island from February 2008 to March 2010 (108 weeks). Thirty ovitraps were placed in each study area and collected weekly. The urban squatter area (Sungai Nibong Kecil) had the highest total number of eggs and immatures throughout the sampling period, followed by urban area (Sungai Dua) and suburban area (Permatang Damar Laut). The amount of eggs collected were higher in wet season compared to dry season. The egg population showed a significant correlation with rainfall and relative humidity but not with mean temperature. Fogging activities were effectively reduced *Aedes* population during dry season as compared to wet season. An autoregressive integrated moving average (ARIMA) model was applied to the ovitraps data set from the three study sites. The models estimated were judged to both fit and forecast with sufficient accuracy to warrant their use in strategic *Aedes* control. The ARIMA (1,0,0), ARIMA (2,0,0) and ARIMA (0,1,1) models were judged to best fit the suburban area, urban squatter area and urban area data sets respectively.

Two species of dengue vectors, *Ae. aegypti* and *Ae. albopictus* identified in the surveys of containers associated with 1506 larvae inhabited containers from 2880 households in four dengue endemic areas on the Island. *Aedes* immatures were found in a wide range of habitats but were particularly abundant in artificial containers. The most productive containers were buckets and comprised 14.74% of larvae/pupae positive containers and provided 18.18% of total *Aedes* immature collected. However, in terms

of container usage, discarded plastic-made containers were the most commonly found during this study. Both species were able to colonize indoor and outdoor containers with *Ae. aegypti* predominantly can be found in indoor containers, whereas *Ae. albopictus* prefer outdoor containers. Shared breeding between *Ae. albopictus* and *Ae. aegypti* was very low (only found in 57 (3.8%) out of 1506 containers. Association between species only significant in term of container distribution but not in individual immature. The *Aedes* and pupae per person indices were applied to assess the dengue transmission risk in all study areas. Transmission threshold for Penang Island is 0.43. Pupae per person index in all study sites exceeded the threshold value indicating risk for dengue transmission in the areas.

CHAPTER 1

GENERAL INTRODUCTION

Dengue fever (DF) and dengue haemorrhagic fever (DHF) are caused by the dengue virus which belonging to Genus Flavivirus, that consists of four serotypes (DEN 1, DEN 2, DEN 3 and DEN 4) (Gubler 1997a). The geographical spread of dengue is increasing. In 1950s only five countries documented with the disease. But to date, there are more than 100 countries around the world reporting the incidence of DF and DHF (Guha-Sapir and Schimmer 2005).

Dengue viruses are transmitted from viremic to susceptible human beings by various mosquitoes of subgenus *Stegomyia*, notably *Ae. aegypti* and *Ae. albopictus*. Both species have been known to bite hosts during daytime and breed in and around human habitation. *Aedes aegypti* remains the principal vector for dengue haemorrhagic fever with *Ae. albopictus* being regarded as a secondary vector (Halstead 1994). *Aedes albopictus* has been incriminated as the vector responsible for dengue and dengue haemorrhagic fever epidemics in several locations including Hawaii, Japan, Indonesia, southern China, Thailand, Singapore, and Malaysia (Rai 1991).

The ovitrap is the most common surveillance and sampling method to detect the presence of *Aedes* mosquito and are characterized by low operating cost (Bellini et al. 1998). Although association between weather and dengue vectors is well documented (Sulaiman and Jeffery 1994, Dieng et al. 2011, Al Thabiany 2012), the study on how the association of climatic parameters with *Aedes* egg population in dengue hotspots of Penang Island is yet to be established. Climatic factors such as temperature, precipitation and humidity could have major influence on the

distribution (Teng and Apperson 2000) and the abundance (Okogun et al. 2003) of vectors. The seasonal patterns of dengue fever had been associated vector population size, with a higher risk of dengue during warmer and wetter periods (de Melo et al. 2012). The regression analysis and Time Series analysis incorporated with ovitrap data are valuable tools in the analysis of the long term temporal effects of various climatic parameters on the affected populations. Ovitrap data also have been successfully used to monitor the impact of various types of control measures involving source reduction and insecticides.

There is evidence to suggest *Ae. albopictus* has been replaced by invasion of *Ae. aegypti* (Service 1992). Laboratory experiments with Southeast Asian populations have shown that *Ae. aegypti* outcompetes *Ae. albopictus* (Service 1992). However, Hawley (1988) hypothesised that the apparent spread of *Ae. aegypti* in Southeast Asia has been caused by increased urbanisation which favours breeding of this species, which is also prevalent in indoor larval habitats, whereas *Ae. albopictus* breeds more successfully in suburban and rural areas and tends not to colonise indoor water holding containers. The reverse situation has been reported in the USA. This is probably due to an increase in *Ae. albopictus* abundance and a decrease in *Ae. aegypti* and there is no evidence of interspecific competition between the two species (Rai 1991).

In Penang Island, *Ae. aegypti* is distributed in most urban areas whereas *Ae. albopictus* sparsely found in urban areas but found with high abundance in sub urban and rural areas (Dieng et al. 2010, Saifur et al. 2012). The mixed breeding of *Ae. aegypti* and *Ae. albopictus* in water holding containers in residential areas of Penang also have been established (Dieng et al. 2010, Saifur et al. 2012). Based on the above information, there is a need to look into ecology of *Aedes* mosquitoes in Penang

Island as well as to establish the degree of association between *Ae. aegypti* and *Ae. albopictus* in areas where both species are present.

In dengue vector surveillance study, inspection of the infested containers such as ovitraps to assess the vectors populations do not normally provide a good indicator of adult mosquito and their productivity. Focks and Chadee (1997) and Focks et al. (2000) suggested that pupal survey is more appropriate for assessing the productivity of adult *Aedes* population. They have demonstrated that pupal index was more reliable for the prediction of dengue outbreaks, as well as an excellent indicator for the monitoring of dengue vector control and surveillance programme (Focks 2003). Pupal survey will provide information on the relative importance of various types of *Ae. aegypti* and *Ae. albopictus* breeding containers.

The objectives of this study are as follows:

1. To determine the distribution and abundance of both *Ae. aegypti* and *Ae. albopictus* eggs in three dengue endemic (hot spot) areas of Penang Island.
2. To elucidate the climatological factors that may influence population abundance of dengue vectors, *Ae. aegypti* and *Ae. albopictus* in selected dengue endemic areas by ovitraps.
3. To assess the effect of fogging events on *Aedes* egg population by ovitraps
4. To predict *Aedes* population abundance using Time Series analysis.
5. To study the relative abundance of *Aedes* infested containers and their immature forms through entomological surveys.
6. To determine the association between *Ae. aegypti* and *Ae. albopictus* in their sharing habitats and their significance in larval productivity.
7. To use pupae per person index as an indicator to estimate productivity of *Aedes* population.

CHAPTER 2

LITERATURE REVIEW

2.0 Dengue General Consideration

Dengue and dengue hemorrhagic fevers (DF and DHF) are caused by four virus serotypes (DEN-1, DEN-2, DEN-3 and DEN-4) belonging to the genus *Flavivirus*, family Flaviviridae (Gubler and Clark 1996). DF and DHF is the most important emerging viral disease affecting nearly half of the world's population. In recent years, dengue has become a major international public health concern. The global prevalence of dengue has increased drastically over the past few years (Kyle and Harris 2008). The disease is now present in more than one hundred countries (Jahan 2011) of Africa (Ananda et al. 2011), the Americas (José et al. 2011), Southeast Asia and the Western Pacific (Atul et al. 2011) as well as the Middle East (Ravanini et al. 2011). Many countries and areas in Asia and in Latin America have been experiencing unusually high levels of DF and DHF incidence. This has been shown by increased frequency of epidemics alongside a geographic spread of both the vectors and the virus. They are the most important mosquito-borne viral diseases in Southeast Asia. In 1995, DF/DHF was the most important arboviral disease affecting humans. About 2.5 billion people throughout the world are estimated to live in areas at risk from epidemic transmission (Miyagi and Toma 2000). Each year an estimated 40-50 million cases of dengue occur, and depending on the year, tens to hundreds of thousands of cases of DHF (Gubler and Clark 1996).

According to Gubler and Clark (1996), the reasons for this worldwide increase in dengue activity and the emergence of DF/DHF outside of Asia are not fully understood, but several important factors have influenced the epidemiology of

dengue during this period. First, the world has experienced unprecedented population growth during the past 50 years, especially in developing tropical countries. This has resulted in unplanned and uncontrolled urbanization, which in turn, has resulted in the deterioration of the housing system, inadequate water supply, sewer and waste management systems. There have been associated with increased populations of rodents, mosquitoes, and other animals living in intimate association with crowded human populations. Second, the dispersal and packaging of consumer goods in nonbiodegradable plastic have contributed to the geographic expansion of the distribution of *Aedes* by providing breeding sites. Third, increased commercial air travel has provided the ideal mechanism for the rapid movement of infected travellers, and therefore, dengue viruses between population centres of the tropics. Finally, mosquito control measures used since 1970 have been ineffective in reducing mosquito populations to levels that interrupt transmission. The result of this has been repeated introductions and movement of new virus serotypes and strains into, and between, tropical urban centres that are highly permissive for dengue transmission. Collectively, these factors have been responsible for the expanding distribution, increased frequency of epidemic activity, and the emergence of DHF in new geographic areas (Gubler and Clark 1996).

2.1 Dengue in Malaysia

The earliest report of a dengue fever in Malaysia was from Penang Island in 1902 (George 1987). However, the first report of the sinister dengue fever with haemorrhagic manifestations was made in the same island only in 1962 where 41 cases and 5 deaths were recorded (Rudnick et al. 1965). Since then, the disease has become endemic throughout the country (Singh 2000a). Major outbreaks were reported in 1974, 1978, 1982, 1990, and 1995 (Lam 1993, Poovaneswari 1993, Hairi

et al. 2003). In the last decade, cases of dengue have become more severe (Hairi et al. 2003). The infection is predominant in urban areas where 61.8% of the total population lives and the rapid industrial and economic development created many man-made opportunities for *Aedes* mosquito breeding (Teng and Singh 2001).

All the states in the country are affected. The majority of the cases are confined to the more developed and highly populated states but there is a changing trend where more and more cases are being reported from less problematic dengue states like Perlis, Kedah, Pahang, Terengganu, Kelantan and Sabah (Singh 2000b).

After a major outbreak DHF in 1973, a plan of action for the prevention and control of DF and DHF was put into immediate effect and the disease was made notifiable in 1974. The Destruction of Disease Bearing Insect Act (DDBIA 1975) was introduced in 1975.

There was a dramatic increase in the number of dengue cases in the past 20 years. In 1995 a total of 6543 cases were reported and this figure increased to 14225 cases in 1996. In 1997 a total of 19429 cases were reported, an increase of 36.3% over the 1996 figure. In 1998 a total of 27381 cases were reported, an increase of 40.9% over the 1997 figure. In 1999 a total of 9947 cases were reported, a decrease of 63.7% over the 1998 figure. The dramatic reduction in dengue cases in 1999 was mainly due to the National Anti-mosquito and Cleanliness Campaign and the greater awareness of the public during the Japanese Encephalitis outbreak (KKM 2002).

Sentinel virological surveillance within Klang Valley shows DEN 3 to be predominant in 1993, 1994 with increase in DEN 2 virus in 1995. In 1996 the predominant virus was both DEN1 and DEN 2, while in 1997 it was DEN 1. In 1998 the predominant virus was DEN 2 followed by DEN1 and in 1999 it was DEN 2. In 2002, from 83 virology test results received from the Institute of Medical Research,

28.9% was DEN1 virus, 24.1% was DEN 2, 33.7% was DEN 3 and 13.3% was DEN4 virus.

The number of reported cases of DHF, which accounts for only a small proportion of total infections, provides an index of total infections on a regional basis. Although reported cases fluctuated widely on yearly basis, the trend over the last decade has been upward; 27.5 cases/100,000 population in 1990 and the cases increased to 123.4 cases/population in 1998 during the global pandemic (Ang and Satwant 2001). In the year 2000, based on notification of clinically-diagnosed cases of 16.3 cases/ 100,000 population was reported. But unfortunately, dengue continues to be a public health problem in Malaysia when the incidence of dengue increases again from 36.4 cases/ 100,000 population in 2001 to 63.6 cases/100,000 population in 2002 (KKM 2002). The pattern of DF incidence is predominant in urban areas where 61.8% of the country's population lives, as compared to only 34% in 1980 (MOH 2005).

In 2009, 41486 cases and 88 deaths were reported. This was equivalent to approximately 147 cases per 100,000 populations. DF contributed 94% (38749 cases) of the total cases and 6% (2,737 cases) was Dengue Hemorrhagic Fever. States showing the highest Incidence Rate (IR) of all Dengue cases (per 100,000 populations) were Selangor (365), Federal Territory of Kuala Lumpur (217), Sarawak (179), Penang (155), Perak (114), and Negeri Sembilan (104). The other states have IR less than 100 cases per 100,000 populations. The case fatality rate in 2009 was 0.31%, a slight decrease compared to the previous year (MOH 2009).

Overall there was an increase of 11% of dengue cases occurrence in year 2010 which were 46171 cases compared to 2009. The total of dengue related death recorded in 134 cases in 2010. The number of dengue cases decreased in 2011 which

were 19884 with 36 deaths (MOH 2009). Figure 2.1 shows the number reported dengue cases in Malaysia for the last 11 years (2001-2012). Up until 28th July 2012, a total of 13162 cases with total mortalities of 26 cases were reported, this is an increase of 1278 cases or 11% compared to 11884 cases for the same period in 2011 (MOH 2012).

2.2 Dengue in Penang Island

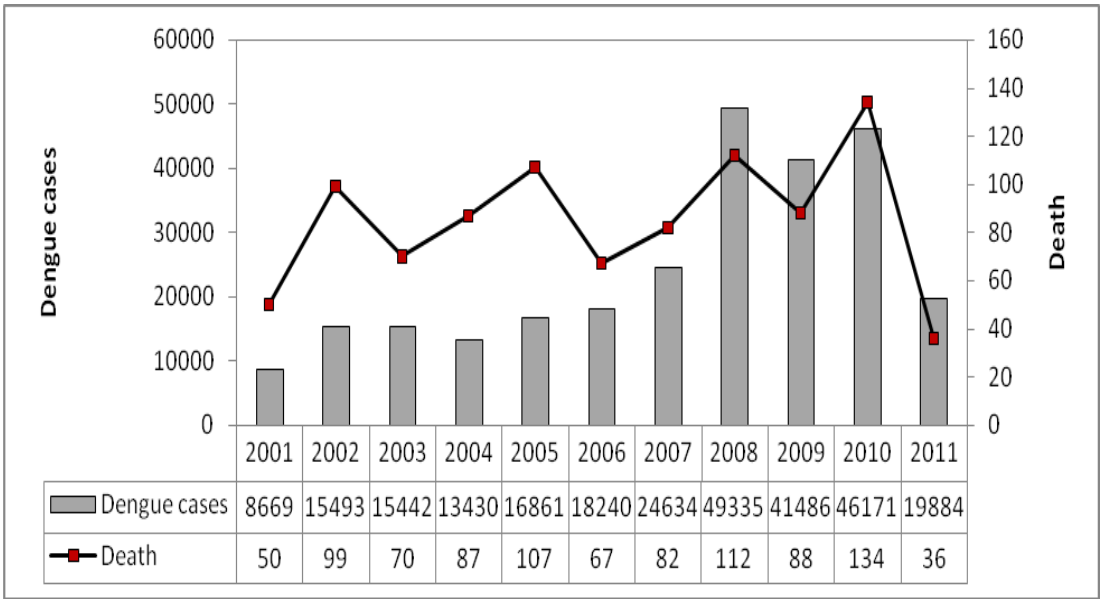
In 2003 the predominant dengue virus in Penang Island was DEN 2, while in 2004 it was DEN 1 and DEN 3. The predominant virus in 2005 was DEN 1 followed by DEN 2 and and DEN4 (KKM 2005). Figure 2.2 shows the number of reported cases of dengue in Penang (2001 - 2011). The reported dengue cases have generally increased in the past 11 years. In the year 2012, starting from January 1 to July 16, a total of 501 cases with 1 death were reported (MOH 2012).

2.3 Dengue Control in Malaysia with focus on the statistics of Penang Island

Dengue Control Programme was established in the year 1973 under the Epidemiology Unit, Public Health Services Division, Ministry of Health. In 1981 the programme was integrated with the Vector-borne Disease Control Programme. In 1993, the Dengue Control Programme together with other vector-borne disease control programmes was integrated with the Disease Control Programme, Public Health Services Division (Singh 2000a).

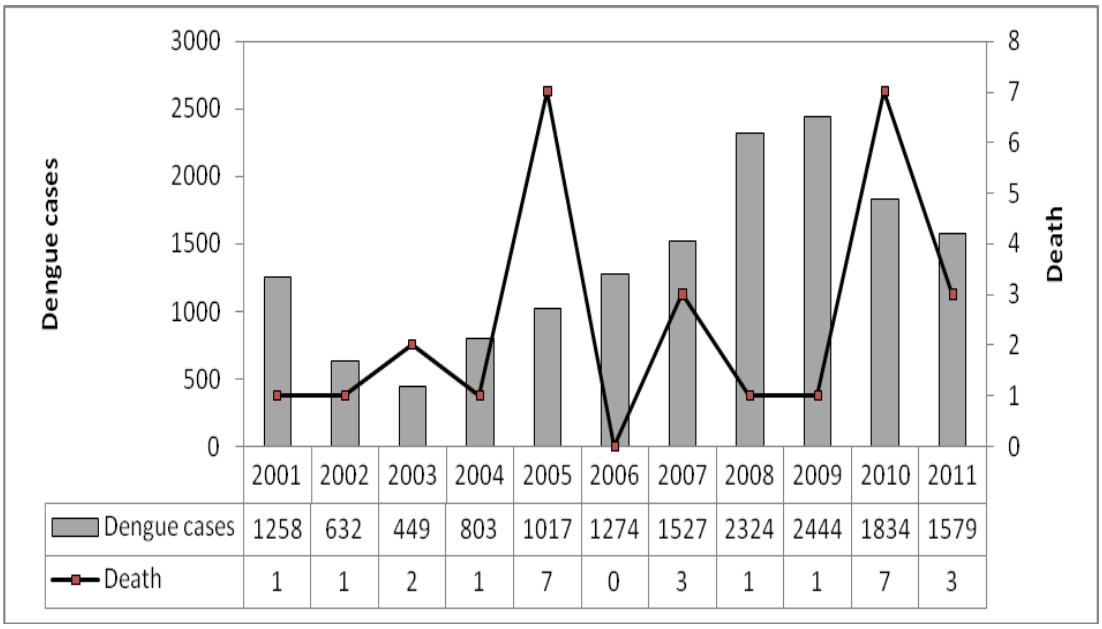
The objectives of Dengue Control Programme are:

1) To reduce the morbidity and mortality of DF/DHF so that it will no longer pose a public health problem.



	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
Dengue cases	8669	15493	15442	13430	16861	18240	24634	49335	41486	46171	19884
Death	50	99	70	87	107	67	82	112	88	134	36

Figure 2.1: Reported dengue cases in Malaysia (2001 - 2011). Source: Disease Control Division, MOH, Kuala Lumpur.



	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
Dengue cases	1258	632	449	803	1017	1274	1527	2324	2444	1834	1579
Death	1	1	2	1	7	0	3	1	1	7	3

Figure 2.2: Reported dengue cases in Penang Island (2001 - 2011). Source: Disease Control Division, MOH, Kuala Lumpur.

2) To reduce the breeding of *Aedes* mosquitoes to a level of below 1% *Aedes* Premise Index.
3) To increase public support and community participation in the prevention and control of dengue.

The targets set for controlling DF/DHF are as follows:

1) Not more than 50 cases of DF/100,000 population.
2) Not more than 2 cases of DHF/100,000 population.
3) Case fatality rate of DF/DHF not more than 0.2%.
4) Case fatality rate of DHF not more than 1.0%.

The strategies used in the control of DF/DHF are as follows:

1) Epidemiological surveillance through prompt case notification through telephone followed by written notification.
2) Laboratory diagnosis through the use of rapid screening tests and confirmation by standard laboratory technique.
3) Improved clinical management through case detection and institution of supportive management of care in hospital.
4) Disease control through case investigation and follow up
5) Vector control through source reduction, done by search and destroy activities, anti-adult operation through chemical fogging, and legislation.
6) Entomological surveillance through larval survey and adult survey.
7) Interagency collaboration and co-operation for control of dengue in specific population sub-group and high risk areas, e.g. school and construction sites.
8) Health education activities including community participation through community involvement in activities related to dengue control.

Dengue fever and dengue haemorrhagic fever is a notifiable disease in Malaysia since 1974 (MOH 2002). It is compulsory for all medical officers to notify the disease under the Prevention and Control of Infectious Diseases Act, 1988. All cases diagnosed clinically are to be notified by the medical officer to the nearest District Health Office within 24 hours. One should not wait for laboratory confirmation of the case before notification. Early notification is very essential for control measures to be instituted immediately. Delay in notification will lead to delay in control measures taken by health personnel which will further lead to occurrence of outbreaks. All cases notified are investigated to pin-point the source of infection (MOH 2002).

2.4 Vector Control and Fogging

The estimated mean economic cost of dengue to Malaysia is US$ 133 million (range US$ 88 million to US$ 215 million) per annum (Lee et al. 2010). The immediate cost is substantial and amounts to US$ 3.5 to US$ 8.5 per capita and is equivalent to 3% to 7% of government spending on healthcare (WHO 2006). The most important parameters accounting for the variation of the total cost are the reporting rate, the annual variation in reported cases, the hospitalization rate, vector control and cost per ambulatory case (Lee et al. 2010).

According to Lam (1993), the strategies used in the prevention and control of dengue are contained in the Vector-borne Diseases Control Programme Sixth Malaysia Plan (1991-1995). These strategies are directed both at the larval and adult stages of the *Aedes* mosquitoes. For larval control, the activities carried out are source reduction measures, use of Abate larvicide, regular house inspection and enforcement of the Destruction of Disease-bearing Insects Act (DDBIA, 1975). Control measures include fogging activities when a case is notified and conducting

case investigations and contact tracing. Health education activities are carried out routinely as an integrated approach for the prevention and control of dengue (Lam 1993).

The practice of conventional chemical fogging to control dengue outbreak in Malaysia was introduced in 1986 and has not been changed since then (Omar et al. 2011). In the conventional strategy of chemical fogging to control dengue outbreak, thermal fogging was conducted within 24 hours upon receiving notification of a clinical case of acute dengue (Omar et al. 2011). For a single case of dengue, perifocal fogging 200 m around the patient's house using the portable thermal fogging is carried out. While for outbreak situation, ULV fogging is used to cover the whole locality (Lam 1993). During dengue outbreak, the first adulticidal treatment is normally followed by a second application 7-10 days later. The two-treatment cycle is based on the life cycle of the *Aedes* mosquitoes and the incubation period of the virus in the mosquito. The outbreak is declared as over once a 20 day transmission-free period is achieved (Lam 1993).

Space-spraying formulations have traditionally been oil-based (WHO 2003). The oil carrier inhibits evaporation of small fog droplets. Only insecticide products with high flash points should be used for thermal fogging. Diesel is used as a carrier for thermal fogging, but creates a thick smoke and oily deposits, which may lead to public rejection. For environmental reasons, water-based formulations have been made available in recent years. These formulations may also contain substances that prevent rapid evaporation. Insecticides used to control mosquito vectors in Penang were supplied by Malaysian MOH. Table 1 shows the types of insecticides received by MOH, Penang Office. Table 2 lists selected insecticides and dosage suitable for space spraying against mosquitoes as recommended by WHO (2003).

Table 2.1: Insecticides for vector control received by MOH, Penang Office in 2005 (KKM 2005).

Insecticides	**Total received**
Resigen	2625 L
Aqua resigen	1550 L
Deltamethrin	330Kg
Malathion	2820L
Permethrin	23L
Bacillus thuringiensis israeliensis 12AS	100L
Bacillus thuringiensis israeliensis WG	25Kg
Abate 500E	360L
Abate 1% SG	750kg

Table 2.2: Selected insecticides suitable for cold or thermal fogging for mosquito control (WHO 2003)

Compounds	**Dosage of active ingredient (g/ha)**
Organophosphates	
fenitrothion	250–300
malathion	112–600
pirimiphos-methyl	250
Pyrethroids	
cyfluthrin	1–6
deltamethrin	0.5–1.0
lambda-cyhalothrin	1.0
permethrin	5–10
resmethrin	2–4

2.5 Vectors of dengue

The two main mosquito vector species, incriminated in the transmission of dengue fever in Malaysia *are Ae. aegypti* and *Ae. albopictus* (Lo and Narimah 1984, Yap 1984, Lam 1993). A study on virus isolation from the field in Singapore indicated that both *Aedes* species showed a similar infection rate of about 5% of the collection (Chow et al. 1998). Monitoring of circulating of dengue virus serotypes have been conducted in several regions of the world since the 1990s (Chungue et al. 1993, Chow et al. 1998, Harris et al. 1998, Romero-Vivas et al. 2000, Kow et al. 2001, Chung and Pang 2002, Urdaneta et al. 2005). Most of these studies were conducted in Asia, where the four serotypes occur and the incidence of dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS) is very high. The possibility of transovarian transmission of dengue virus in *Ae. aegypti* and *Ae. albopictus* has been reported by Lee et al. (1997). Rohani et al. (1997) has isolated and detected dengue virus from *Aedes* larvae collected from dengue high risk areas. The dengue virus type 2 (DEN 2) can be transmitted transovarially in *Aedes aegypti* mosquitoes until the fifth generation under laboratory conditions (Rohani et al. 2008). Thus confirmed the maintenance of the virus in the larval stage through transovarian transmission in Malaysia.

In most Southeast Asian countries, there is a tendency of dengue fever being spread to the semi-urban and rural areas where *Ae. albopictus* is more abundant (Khatijah 2002). *Aedes albopictus* also play an important role in the epidemiology of other viral diseases such as Chikungunya fever (Mangiafico 1971, Yamanish et al. 1983). Therefore both species play an important role as vectors in the transmission of dengue virus.

Mosquito surveys in Penang revealed the presence of *Ae. aegypti* mosquitoes in relatively high abundance in the urban areas (Saifur et al. 2012). *Aedes albopictus* were presently high abundance throughout the island in urban, suburban and rural area (Dieng et al. 2010). The ability of *Ae. albopictus* to transmit dengue virus was first shown in studies involving human volunteers as early as 1926 (Rudnick et al. 1965). However dengue (all four serotypes) more commonly transmitted by *Ae. aegypti* (Gratz 2004). Among public health authorities in the newly infested countries and those threatened with the introduction, there has been much concern that *Ae. albopictus* would lead to serious outbreaks of arbovirus diseases (*Ae. albopictus* is a competent vector for at least 22 arboviruses) (Gratz 2004). *Aedes albopictus* was shown to be able to transmit all four DEN serotypes (Mitchell et al. 1987). However because *Ae. albopictus* often overlaps in distribution with *Ae. aegypti*, it is often difficult to determine the relative contribution of the two species to disease transmission. Thus, *Ae. albopictus* may serve as important maintenance vector of dengue viruses in endemic areas, and new endemic areas may be initiated by importation of vertically infected eggs (Gubler 2002). Dengue haemorrhagic fever (DHF) was a disease of the urban human population with concentration of cases having a high density of human population and these also associated with areas where both *Ae. aegypti* and *Ae. albopictus* have been widely distributed and abundant (Chan et al. 1971a)

There is no vaccine available for dengue fever. Thus, the only option left is to control the vector. The best method of eliminating *Aedes* mosquitoes are considered to be, larval source reduction by an integrated approach of environmental sanitation, insecticide spraying and biological control (Miyagi and Toma 2000, Lee 2000). Over the last two decades the rapid changes in the urban environment and demographic

structure in the country has undoubtedly influenced changes in the vector ecology and consequently the epidemiology of dengue. In addition, the increased development of transportation has caused an increase in mass migrations of workers and consequently is expected to increase in concerns regarding their future impacts on the incidence of dengue (Weinhold 2010, CDC 2011). The above reasons suggested that more studies should be done on *Ae. albopictus* as well as *Ae. aegypti* biology and ecology to understand more about their relation to dengue transmission. Silver (2008) had emphasized the importance of ecology in the control of vector borne-diseases.

2.6 Species competition and displacement

After its arrival in Asia toward the end of the 19th century, the increase in abundance of *Ae. aegypti* in many cities was accompanied by a decrease in the abundance of the native *Ae. albopictus* (Rudnick and Hammon 1960, Gilotra et al. 1967, Chan et al. 1971a, Ho et al. 1972, Hawley 1988, Tabachnick 1991). In contrast, the recent establishment and spread of *Ae. albopictus* (Sprenger and Wuithiranyagool 1986, Moore 1999) in the United States has been accompanied by a decrease in the range and abundance of *Ae. aegypti,* a resident of the Americas for centuries. *Aedes albopictus* has largely displaced *Ae. aegypti* and became the most abundant mosquito in artificial containers in most of the southeastern United States (O'Meara et al. 1995, Moore 1999). The displacement of *Ae. albopictus* by *Ae. aegypti* in certain Asian cities was suggested to be caused by destruction of *Ae. albopictus* habitats with a concomitant increase in urban habitat more suitable for *Ae. aegypti* (Chan et al. 1971d, Hawley 1988).

In Asia, *Ae. aegypti* had an overall competitive advantage over *Ae. albopictus* in urban areas and therefore displaced the latter (Rudnick 1965, Gilotra et al. 1967).

The same process, but with a reversal of competitive advantage, might explain the replacement of *Ae. aegypti* by *Ae. albopictus* in North America.

There are several alternative explanations for the Asian and North American shifts in mosquito distributions after invasions (Barrera 1996). Many workers have assumed that because one species has been replaced by the other in some habitats or whole areas, competitive displacement has occurred (Hawley 1988). Field experiments in both the United States (Juliano 1998) and Brazil (Braks et al. 2003) have shown a strong competitive advantage for *Ae. albopictus* larvae that seems to be independent of population origin or environmental conditions (Braks et al. 2003). Although resource competition among larvae seems to account for the displacement of *Ae. aegypti* in some regions of the United States, it does not explain the persistence of *Ae. aegypti* in southern cities, often coexisting with *Ae. albopictus.* Juliano et al. (2002) proposed that local coexistence of the species is possible because warm dry climates favor *Ae. aegypti* by alleviating the effects of competition from *Ae. albopictus* through differential mortality of *Ae. albopictus* eggs. Coexistence of the species observed in tropical Asia might be also caused by this phenomenon. In these regions of sympatry, *Ae. aegypti* and *Ae. albopictus* rarely share identical habitats in southeast Asia (Hawley 1988).

2.7 Feeding behaviour and Oviposition

The feeding behaviour of *Ae. aegypti* is unique among mosquito species because compared with male, females seldom feed on sugar (Edman et al. 1992, Van Handel et al. 1994). Most female mosquitoes including *Ae. aegypti* and *Ae. albopictus* are anautogenous and depended on blood meal to produce eggs although they can survive by feeding on sugar meal alone (Clements 1992a). In each

gonotrophic cycle, *Ae. aegypti* imbibe human blood frequently (Scott et al. 1993a and 1993b).

Scott et al. (1997) found that females fed on human blood and needed to feed every day to survive. A reproductive advantage was observed over female *Ae. aegypti* that fed on human blood (Naksathit and Scott 1998). This may explain why *Ae. aegypti* fed so often on human (Scott et al. 1993b). The most important food for the female is blood (Clements 1963), however, sugar obtained from flowers and some plants are used extensively as energy source by both males and females (Clements 1992b).

Aedes albopictus oviposits in both natural and artificial containers while *Ae. aegypti* lay eggs in practically all types of man-made containers and in some natural containers (WHO 1995). Females *Ae. aegypti* (Strauss et al. 1965) and *Ae. albopictus* (Strauss et al. 1965, Rozenboom et al. 1973), were found to deposit their eggs in more than one container. Female *Ae. aegypti* preferred black colour for oviposition (Gubler 1971a, WHO 1995), with secondary preference for darker shaded colours such as blue, green and red (Foo and Yap 1983). Snow (1971) surmised that *Ae. aegypti* were most sensitive to green–orange light (470–610 nm) and thus avoided those colors while seeking more cryptic oviposition sites.

Yap (1975b) found that gravid *Ae. albopictus* in rural Malaysia oviposited more in red and black ovitraps than in blue, yellow, green, white, and plain (unpainted) ovitraps. Later, in laboratory studies gravid female *Ae. albopictus* laid significantly more eggs in black, red, and blue ovitraps than in green, yellow, white, or clear (unpainted glass) ovitraps (Yap et al. 1995).

Oviposition is rhythmical induced by a light period followed by dark. Without this stimulus, oviposition is highly irregular and non periodic (Haddow et al.

1961). Chadee and Corbet (1989), in a study using *Ae. albopictus* observed that oviposition in the laboratory occurred during photophase and 56% of all its eggs were laid 2 hours before the end of photo phase. He and his co-worker also reported oviposition in the evening 'twilight' and near the end of scotophase. In Japan, Tsuda et al. (1989) conducted a field study using *Ae. albopictus* where the recorded 79% of oviposition occur in two to three hours period each day. They also found that environmental condition of the day influence the peak oviposition. The number of eggs laid by *Ae. aegypti* depend on the source of the blood (Woke 1937a and 1937b) and also the physiological age of the female (the number of eggs decreases as the age increases) (Gubler and Bhattacharya 1971, Hien 1976b).

Dieng et al. (2010) found a major effect in the level of adaptation to indoor/domestic environment on the number of *Ae. albopictus* gonotrophic cycles in the laboratory. The fifth generation of females derived from wild mosquitoes showed a much higher number of gonotrophic cycles than their original female's generation counterparts. They observed the original wild female mosquitoes and the fifth generation of females achieved 7 and 14 gonotrophic cycles, respectively. The fifth generation survival rate was higher than that of original wild mosquitoes. Nur Aida et al. (2011) found that the mean number of eggs laid per female *Ae. albopictus* in its first gonotrophic cycle was 77.4. Gubler (1970) observed lower mean eggs for *Ae. albopictus* in their first gonotrophic cycle. He found that the mean number of eggs laid per *Ae. albopictus* female in the first gonothrophic cycle ranged from 51.8 to 71.8 depending on the blood source. *Aedes albopictus* female laid an average of 221 eggs throughout its life span and the mean number of female offsprings produced by a single female from a cohort during the course of its lifespan was calculated to be 69

females (Nur Aida et al. 2008a). Longer development time in immature *Ae. albopictus* was associated with higher total mortality (Nur Aida et al. 2008b).

Saifur et al. (2012) found that the numbers of gonotrophic cycles ranged from 1 to 8 in wild indoor and outdoor derived *Ae. aegypti*, while gonotrophic cycle ranged from 1 to 10 in the fifth generation. They also observed that the survival rates of all three groups of test mosquitoes decreased from first to last gonotrophic cycle. The mean number of gonotrophic cycle indicated equivalent fitness of both indoor and outdoor females to reproduce their generation. Egg production tended to decrease as the rank of gonotrophic cycle progressed in all generations of females (Saifur et al. 2012).

2.8 Environmental factors associated with *Aedes* mosquitoes

Temperature, rainfall and relative humidity are physical factors that influence the abundance of the mosquitoes. According to Lee (1990), with no changing seasons in our country's weather, therefore there is no significant difference in larval numbers throughout the year. Mosquitoes are remarkably selective in their choice of breeding habitats. The female mosquitoes will respond differently to visual, chemical and tactile stimuli in selecting the suitable habitats for larval development (Gubler 1971). In general, insects are exceedingly sensitive to temperature and rainfall regiments and tropical and temperate species frequently show great variations in seasonal abundance (Samways 1995).

In tropical and subtropical climates, *Aedes* mosquitoes is abundant all year round; however, in temperate climates such as the Midwestern United States and Japan, the active season for larval stages is limited to late spring through early fall, with larval abundance greatest in July-August (Mori and Wada 1978, Toma et al. 1982). According to Parker (1986), temperature may affect egg viability. Increased

temperatures are also likely to result in greater desiccation, perhaps causing greater mortality of egg or adult *Ae. albopictus* (Mogi et al. 1996). The development time of *Ae. albopictus* ranged from 6 to 10 days depending on the mean environmental temperature (Nur Aida et al. 2008b). Reisen et al. (1992) findings indicated that the rate of ovarian maturation after blood ingestion in mosquito, increased as a function of temperature, however, the rate of oviposition decreased at 30 ^{o}C. According to Reeves et al. (1994), females could quiescent as gravids at high temperatures and possibly delay oviposition. Laboratory experiments have demonstrated that the incubation period of dengue 2 virus could be reduced from 12 days at 30^{o}C to 7 days at 32-35 ^{o}C in *Ae. aegypti* (Watts et al. 1987), changes in weather patterns, may be the major contributing factor to the high incidence of the disease.

Bar-Zeeve (1957a and b) reported that low temperature and humidity in the cool, dry season of Northern Thailand as sub-optimal but still allowed *Aedes* to reproduce. Changes in both temperature and precipitation affect the population of *Ae. albopictus* by disturbing the reproductive and mortality rates (Akram and Lee 2004). Higher temperatures decrease larval development times (Rueda et al. 1990) and consequently there is greater capacity to produce more offspring during the transmission period. Adult female mosquitoes digest blood faster and feed more frequently in warmer climates, thus increasing virus transmission intensity (Gillies 1953). However, temperature above 34^{o}C generally will reduce the survival of vectors and parasites (Rueda et al. 1990).

Mogi et al. (1988) observed a low population of eggs during the dry season, which then increased at the beginning and later on decreased at the end of the rainy season in Chiang Mai, Thailand. Similar results on *Ae. aegypti* have also been obtained by Micieli and Campos (2003) in subtropical Argentina.

The seasonal changes in oviposition are a consequence of seasonal changes in weather conditions, and the availability of sites for laying eggs. According to Micieli and Campos (2003), the increase of *Ae. aegypti* mosquito oviposition in Aguaray and Tartagar, Argentina during the dry season might be the result of greater female activity, due to an increase in temperature and relative humidity. The decline in relative humidity induced the gonoactive female to rest, thus leading to a decrease in mosquito oviposition. High relative humidity can resulted in high hatching rates. With 100% humidity the eggs can hatch on filter papers. The low relative humidity in the other hand will cause negative impact on embryo development (Horsfall 1956). Saifur et al. (2010) observed that high-moisture substrates of 66% and 72% provided better environments for *Ae. albopictus* egg laying. They also found that the numbers of eggs laid were much lower in the drier environments. At low moisture level, gravid females retained increasing numbers of mature eggs until death, and egg retention decreased gradually with increasing moisture level.

Toma et al. (2003) reported the infestation by *Ae. albopictus* in Rome, Italy. They found that adults of *Ae. albopictus* were active from February to December, peaking in August to September. Whereas egg abundance during March to December peaked in August and September and then decreased till the end of December. Swanson et al. (2000) in their study during the summer in Illinois indicated that oviposition activity of *Ae. albopictus,* as measured by the mean number eggs per ovitrap, increased as the season progressed, peaking in early September. A study by Barker (2001) revealed the ability of *Ae. albopictus* to overwinter in Southwestern Virginia and their egg abundance during summer was greatest during late July and early August.

The studies on seasonal distribution of *Aedes* larvae in Eastern Thailand reported that even when the larvae are less abundant during the dry season, every part of the studied villages have some of them (Strickman and Kittayapong 2002). According to Usavadee et al. (2001), the population of the abundance of *Ae. albopictus* increased substantially during the rainy season (May-December) and then declined drastically in the dry season (January-April). *Aedes* larvae increased significantly in rainy season, with 60% of ovitraps become positive for *Ae. albopictus* eggs.

Previous studies have documented the influence of precipitation on container breeder mosquitoes such as *Ae. albopictus* (Sulaiman and Jeffery 1986, Nor Adzliyana 2003) and *Ae. aegypti* (Micieli and Campos 2003). Al Thabiany et al. (2012) found that *Ae. aegypti* larvae more abundant during wet season in Makkah City, Saudi Arabia. High frequency of rainfall events would ensure that small artificial containers used as larval mosquito habitats, would remain flooded thereby expanding adult mosquito populations (Patz and Reisen 2001).

The effects of rainfall on insects can be direct or indirect. Lack of rain can cause desiccation and death. Rainfall also affects humidity, which combined with temperature and wind to dictate microclimatic conditions. Rainfall will affect the water levels and current strength of these habitats. Rainfall patterns can influence a long term abundance of insect's populations. Rain may not even have an influence at a time, but instead may promote insect performance some months later (Speight et al. 1999). Furthermore, urbanization process is key factor of *Aedes* prevalence in some dengue outbreak areas.

2.9 Urbanization level and human demographic importance for dengue vectors

Human population size is dependent on urbanization level. In 2009, more than 50% of the world's population were living in cities (UN 2010). Although most developed nations already show high rates of urbanisation (about 80%), tropical countries are experiencing a remarkable expansion of their urban agglomerations.

This worldwide increase in urban population results from a combination of factors including natural population growth, migration, government policies, infrastructure development, and other major political and economical forces, including globalisation (Alirol et al. 2010).

The human population has increased from approximately 1 billion at the turn of the 20th century to 6 billion by the end of the century, and it is projected to grow to around 10 billion by 2050 (Tilman et al. 2001). The urban population of the world increased from 1.7 billion (39%) in 1980 to 2.7 billion (46%) in 1997 (Anonymous 2000) and are expected to reach 5 billion (60%) by 2030 (Anonymous 2001). Over the next 25 years, urban populations in Africa are expected to more than double, those in Asia will almost double, and those in Latin America and the Caribbean are expected to increase by almost 50% (WHO 2001).

The urban environment offers favourable grounds for the spread of epidemics, mainly because of high population densities (Alirol et al. 2010). Some diseases, such as dengue, have become permanently established in urban areas and cause regular epidemics. Epidemic forms of certain diseases, such as chikungunya, might become restricted to the urban environment (Pialoux et al. 2007). Poverty associated with rapid population growth leads to concentrations of people without the necessary infrastructure for the safe storage and distribution of water and drainage of wastewater. Drainage and water supplies are critical factors that determine the extent