Determination of Breeding Habitats and Seasonal Prevalence of Larvae of *Aedes aegypti* (L) and other Possible Vector of Dengue in Kathmandu Valley

Final Project Report

Submitted by

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SUMMARY

DF/DHF occurs primarily in tropical and subtropical areas of the world, prevalent in over 100 countries and threatens the health of approximately 100 million cases of DF, 500,000 cases of DHF, and several thousand deaths occur annually worldwide. During the past decades, dengue virus has emerged in southern Asia; DF/DHF epidemics have occurred in Bhutan, India, Maldives, Bangladesh, and Pakistan. The incidence of dengue has been increasing in recent years in densely populated areas in Nepal. Dengue viruses are transmitted from viremic to susceptible human beings by various mosquitoes of the subgenus *Stegomyia*, notably day-biting mosquitoes *Aedes aegypti* and *Aedes albopictus,* vectors throughout tropical and sub-tropical areas.

In this study, house-to-house surveys of larval breeding places and larval prevalence were conducted covering all three districts of Kathmandu valley during April, May, June, July, August, September and October in 2009. The sample sites included Koteshwar, Manahara, Gongabu-Balaju, Tokha, Jorpati and Kalanki (Kathmandu district), Tikathali, Lokanthali, Kaushaltar, Thimi, Darbar square and Bode (Bhaktapur district). Satdobato, Thalchkhel, Mahalaxmisthan, Dhobighat, Sanepa, Bagalamukhi, Dholahiti, Chibahal, Thecho, Chapagaon, Badegaon, and Godawari (Lalitpur district) of Kathmandu valley. Collection sites were selected in both town and rural residential areas. Nine major Water-filled containers discarded tires, metal /plastic drums, plastic buckets, flower pots, mud pots, cement tank, metal containers, plastic pots and miscellaneous small, discarded items such as tin cans, jars and plastic food containers were searched for the presence or absence of *Aedes* larvae and/or pupae. Adult *Aedes* mosquitoes were examined with the help of aspirators and flash-lights. All live mosquito larvae and pupae collected were reared until adult emergence and identified.

Main purpose of the selecting the areas for the study is to know if there are distribution, abundance of *Aedes aegypti* and other possible vector of dengue in urban areas of Kathmandu valley. No information is available regarding seasonal abundance of Dengue vector, *Aedes aegypti* in these districts.

Primary end points of this study were the Breteau index and the house index; the secondary end point was the container index. Larval survey techniques were used to obtain the House Index, Container Index and Breteau Index. The container preferences of *Aedes* mosquitoes were assessed by calculation of breeding preference ratio (BPR). The highest HI, CI and BI recorded for *Aedes aegypti* were 4.42, 3.63 and 9.73 respectively in October, and 9.91, 12.45 and 31.40 respectively for *Aedes albopictus* in September, 2009 (Kathmandu district). Whereas, HI, CI and BI recorded for *Aedes aegypti* in Lalitpur district were 5.50, 4.06 and 12.80 respectively in October, 2009. For *Aedes albopictus,* the highest HI, CI and BI recorded for *Aedes albopictus* in Lalitpur district were 15.64 in August, 9.83 and 25.30 in September, 2009. The highest BPR recorded for *Aedes aegypti* and *Aedes albopictus* in Kathmandu district were 1.89 and 1.89 in August, 2009. Similarly, the highest BPR recorded for *Aedes aegypti* and *Aedes albopictus* in Lalitpur district were 1.69. It was observed that discarded tires lying outdoors were the preferred breeding habitats. No breeding of *Aedes aegypti* was observed in other containers during this seven months survey. However, breeding of *Aedes albopictus* was also observed in metal drum in Mangal bazar and in metal container in Gwarko of Lalitpur district . From the present entomological investigations, it can be concluded that *Aedes aegypti* and *Ae. albopictus* are established within the urban agglomeration of Basundhara, Gongabu-Balaju area of Kathmadu district and Satdobato-Gwarko, Thalchikhel, Mahalaxmithannad Sanepa area in Lalitpur district showing larval indices. Overall, this study brings out an idea regarding the current distribution of *Aedes aegypti* and *Aedes albopictus* in Kathmandu valley for implementation of vector control operation.

From a previous study on Kathmandu (Darsie and Pradhan, 1990,1994), *Aedes aegypti* had not been previously recorded in the Kathmandu valley. And, the breeding habitats occupied by immature mosquitoes had not been previously determined. In other words, information on seasonal distribution and breeding habitats of *Aedes aegypti* and *Aedes albopictus* in Kathmandu valley was lacking. Since *Aedes aegypti* has been newly emerged increasing its range throughout the country, an entomological investigation has been directed toward detecting its presence or absence in Kathmandu district by the principal investigator. The Herewith, the principal investigator report the first (1st) collection and identification of *Aedes aegypti* in Kathmandu and Lalitpur district in 2009. Indeed, this finding constitutes the first record from the districts and the collection represents the Basundhara and Gongabu-Balaju and Gwarko–Satdobato area for this species within the Kathmandu valley.

There has been a significant increase in the human population, demographic and vehicular movement of the people and accommodation-based tourism facilities in Nepal. Continued and increased urbanization and vehicular movement and use in Kathmandu valley have been resulted in increased amount of non-biodegradable objects (tires). The accumulation of all sorts of discarded or unused tires in dwellings has resulted in occurrence of *Aedes aegypti* and *Aedes albopictus.* As a result, crowded urban human populations live in intimate contact with large mosquito populations, thus creating ideal conditions for increased mosquito-borne disease transmission. Finally, increased and more rapid travel possibly lead to increased movement of dengue viruses within the area.

Alternative strategies are needed which can be implemented through participation continuous participation of people in the community and which compliment the efforts of the vector control teams. The *Aedes aegypti* control programme is needed to make people aware of the threat poses to their health, and to educate them on how they can reduce this threat by either eliminating the potential breeding habitats which harbour the mosquito larvae or by preventing mosquitoes from having access to water-holding containers for egg-laying that are used in or around houses.

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CHAPTER 1

1.0 INTRODUCTION

1.1 Background

Dengue fever and dengue haemorrhagic fever are caused by the dengue virus, which belongs to the genus *Flavivirus*, family Flaviviridae, that consists of 4 dengue virus serotypes (DEN-1, DEN-2, DEN-3, and DEN-4), all of which can cause dengue fever and dengue haemorrhagic fever (Miyagi & Toma 2000). Dengue viruses are transmitted from viremic to susceptible human beings by various mosquitoes of the subgenus *Stegomyia*, notably *Aedes aegypti* and *Aedes albopictus*.

The observations over 100 years have shown that the epidemiology of dengue varies a great deal with respect to both geography and time. This is due not only to modifications in human ecology (population increase, urbanization, more frequent travel), but also to ecological adaptations of certain mosquito species in particular with respect to their geographic distribution and abundance which in turn result in changes in their ecology. Over the last two decades, in Kathmandu, a, 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.

Several studies have reported that *Aedes albopictus* and *Aedes aegypti* may share the same habitat (Gilotra *et al.* 1967, Chan *et al.* 1971a, Sprenger & Wuithiranyagool 1986, O'Meara *et al.* 1993). Because of this association, it has been hypothesized that some parts of Southeast Asia, *Aedes aegypti* has completely replaced the indigenous *Aedes albopictus* in urban areas (Pant et al.1973, Service 1992). Conversely, observations on the spread of *Aedes albopictus* in the southern coastal states of the United States indicate that the expansion appeared to be occurring at the expense of *Aedes aegypti*. The introduction of *Aedes albopictus* has been accompanied by a drastic and rapid decline of *Aedes aegypti* populations (Nasci *et al*. 1989, O'Meara *et al.* 1993).

The above studies have brought up some laboratory studies of larval competition. Laboratory studies of larval competition conducted with different Asian strains *Aedes albopictus* and *Aedes aegypti* showed that *Aedes aegypti* is better able to compete than *Aedes albopictus* (Macdonald 1956a, Gilotra *et al.* 1967, Moore & Fisher 1969, Sucharit *et al.* 1978, Service 1992). Information obtained by these researches suggested that *Aedes albopictus* would not become established in localities inhabited by *Aedes aegypti* because of the competitive displacement of competitive exclusion. However, Hawley (1988) hypothesized that the apparent spread of *Aedes aegypti* in Southeast Asia has been caused by increased urbanization which favours breeding of this species, which is also prevalent in indoor larval habitats, whereas *Aedes albopictus* breeds more successfully in suburban and rural areas and tends not to colonize in indoor water collections.

From a previous study on Kathmandu (Darsie and Pradhan, 1990,1994), *Aedes aegypti* had not reported, and *Aedes albopictus* was found in Kathmandu. However, information on seasonal distribution and breeding habitats of *Aedes aegypti and Aedes albopictus* Kathmandu valley is lacking.

Generally, insects are exceedingly sensitive to temperature and rainfall regiments; tropical and temperate species frequently show great variations in seasonal abundance (Vezzani *et al.* 2004), and many epidemiological studies of mosquito-borne arboviruses have shown that there are relationships between environmental factors, mosquito densities and disease epidemics (Russell 1986). Therefore, by using climatological indicators, vector population increases could than be predicted earlier by routine surveillance, thus increasing the time available for planning and conducting control operations (Moore 1985). Epidemic haemorrhagic fever is a disease of the rainy season and of the period of *Aedes aegypti* abundance (Halstead 1966). In Chiang Mai, northern Thailand, *Aedes* egg population remained low in the dry season, but increased/decreased exponentially during the first/latter half of the rainy season, respectively (Mogi et al.1988). This seasonal pattern was similar to the seasonal distribution of dengue haemorrhagic fever cases in the area. Foo *et al.* (1985) analyzed a ten-year data on rainfall and dengue cases; it had provided statistical evidence of an association between increases in dengue cases and heavy rain in Selangor, Malaysia.

The density of insect populations is typically determined by counting the number of individuals in a random sample (Schaalje *et al.* 1991). Exact counts of insects are often difficult to obtain because of the small size of insect and of the high numbers per sample. Binomial or presence-absence sampling is often useful for estimating population density because the only information needed from a sampling unit is whether or not the organism is absent (Schaalje *et al.* 1991). An approach was developed for the estimation of insect population density, which eliminates the necessity of counting every individual per sample (Gerrard & Chiang 1970). Using population parameters obtained by preliminary surveys, presence-absence sampling allows density estimation simply from the proportion of positive samples. Danielson & Berry (1978) applied presence-absence sampling on redbacked cutworm by assuming that the insect fitted in the negative binomial distribution. However, Gerrard & Chiang (1970) applied presence-absence sampling on corn rootworm egg without assuming any particular distribution pattern. Many studies on *Aedes* population density estimation whether using ovitraps method or larval survey method, can be made using presence-absence sampling without assuming any distribution pattern (Mogi et al. 1990, Lee 1992a, Lee & Inder Singh 1991, Bellini et al. 1996, Lee 1996, Lee & Chang 1997).

Based on the above studies, it brings out an idea to study the current distribution of *Aedes aegypti* and *Aedes albopictus* in Kathmandu valley, and the determination of potential breeding habitats of these two mosquitoes collected from sampling sites. Furthermore, it is interesting to study the relationship between *Aedes* mosquito abundance and physical parameters and the relationship between *Aedes* mosquito abundance, physical parameters in Kathmandu valley.

1.2 Specific Objectives

General

To report observation on breeding habitats and seasonal prevalence of larvae of *Aedes aegypti* (L) and other possible vector of dengue in Kathmandu valley

The specific objectives of this study are as follows:

1. To ascertain the presence of *Aedes aegypt*i and other possible vectors of dengue virus in the Kathmandu valley,
2. To determine potential breeding habitats of *Aedes aegypti* and other possible vectors of dengue virus in Kathmandu valley.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Dengue Fever (DF) and Dengue Haemorrhagic Fever (DHF)

The dengue virus is in the genus *Flavivirus* (Miyagi & Toma 2000). There are three types of dengue fever, namely classical dengue fever (DF), dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) (Singh 2000). The term "haemorrhagic fever" was first applied to illness in South-East Asia in the Philippines in 1953 (Halstead 1966). Dengue is caused by four antigenically related virus serotypes which are dengue type 1, 2, 3 and 4 viruses (Miyagi & Toma 2000).

DF/DHF occurs primarily in tropical and subtropical areas of the world, prevalent in over 100 countries (Lee 2005) and threatens the health of approximately 100 million cases of DF, 500,000 cases of DHF, and several thousand deaths occur annually worldwide (WHO, 2002). Dengue is the cause of an estimated 500,000 hospitalizations each year with some 24,000 death each year. Dengue virus induces clinical illness, which ranges from a nonspecific viral syndrome (dengue fever [DF]) to severe and fatal hemorrhagic disease (dengue hemorrhagic fever [DHF]). Dengue fever is seen in syndromes that are age-dependent (Halstead 1980). Infants and children may have undifferentiated febrile illness or mild febrile disease with maculopapular rash. Older children and adults usually have an overt illness characterized by fever, headache, myalgia, and gastrointestinal symptoms, often terminating with a macupapular rash. Dengue haemorrhagic fever or dengue shock syndrome proceeds through two stages (Halstead 1980). The illness begins with abrupt onset of fever accompanied by dengue-like symptoms; during or shortly after the fall in temperature, the condition of the patient suddenly deteriorates, the skin becoming cold, the pulse rapid, and the patient becomes lethargic and restless. In some children the range of pulse pressure progressively narrows, the patient becomes hypotensive and if not treated, may expire in as little as 4-6 hours.

Dengue is now the arboviral disease that causes the greatest public health impact on man. It is likely that dengue and DHF will continue to spread to wherever the mosquito vectors are found in densities high enough to permit transmission of the causative virus (Gratz, 1991). The incidence of dengue has been increasing in recent years in densely populated areas in Nepal

During the past decades, dengue virus has emerged in southern Asia; DF/DHF epidemics have occurred in Bhutan, India, Maldives, Bangladesh, and Pakistan (WHO, 2007; Islam *et al.*, 2006; Jamil *et al*., 2007). From August through November 2006, the number of febrile patients increased in 4 major hospitals in the Terai region of Nepal: Nepalgunj Medical College, Bheri Zonal Hospital in Nepalgunj, Tribhuban Hospital in Dang, and Narayani subregional hospital in Birgunj (Pandey *et al*. 2008). DF/DHF have been considered to be a possible public health threat to Nepal because DF/DHF epidemics have occurred recently in India and Pakistan, which reported several thousand cases and >100 deaths (*Gupta et al.* 2006). DF case in Nepal was reported in 2004 (Pandey *et al.* 2004). During 2006 a total of 140 DF cases in Nepal heve been estimated. It has been identified in 5 major urban areas of Terai region bordering with India, i.e. Biratnagar (Morang), Birganj (Parsa), Bharatpur (Chitwan), Tulsipur (Dang) and Nepalganj (Banke) (Pandey *et al.* 2008). The prevalence of dengue virus antibody was reported to be 10.4% in the southwestern region of Nepal (Sherchand *et al.* 2001). These reports suggest that dengue virus has been circulating in Nepal for several years and has remained a serious health problem in this country, Thus, DF/DHF has likely been misdiagnosed and illness caused by dengue virus underestimated in Nepal.

Dengue fever and dengue haemorrhagic fever are maintained in a cycle between humans and domestic day-biting mosquitoes *Aedes aegypti* and *Ae. albopictus,* vectors throughout tropical and sub-tropical areas. *Aedes albopictus* has been repeatedly incriminated as a vector during dengue outbreaks, particularly in Southeast Asia (Shroyer 1986). Jumali *et al.* (1979) compared the efficiency in transmission of dengue-3 virus by oral route of *Aedes albopictus* and *Aedes aegypti* and found that both species were equally efficient. However, Rosen *et al*. (1985) showed that there is a significantly larger proportion of the *Aedes albopictus* that became infected by each dengue virus than *Aedes aegypti*. It is believed that *Aedes albopictus* originated in the tropical forest of Southeast Asia (Smith 1956).

Human activities associated with settlement, agriculture, climate change may increase larval habitats of *Aedes aegypti* and *Ae. albopictus.* Females of these species deposit their eggs in a variety of water-holding containers, such as jars used for domestic water storage, tires, and disposed items that have filled with rain water. Larvae of vector mosquitoes hatch promptly upon flooding, small water bodies that are often numerous, scattered, temporary, and close to human dwellings. Eggs can be transported great distances and for long times in old car tires, soft drink containers, etc. *Aedes aegypti* prefers the clean water found in many types of domestic containers inside or near human dwellings, whereas *Ae. albopictus* is more likely to be found in natural containers or outdoor man-made habitats containing a greater amount of organic debris (Rattanarithikul and Panthusiri 1994). Although difference, the preferred breeding habitats of these two species slightly overlap (Gould *et al.* 1970). Because an effective vaccine is not yet available, dengue control can be achieved by making water-holding containers unavailable for immature development (source reduction), by killing adult mosquitoes with insecticides, or by interfering with mosquito-human contact (Reiter and Gubler, 1997). In depth, study is essential to confirm the real situation of the distribution of vectors and breeding sites which are responsible for causing disease in the country and it is sure that this study may fulfill the requirement to some extent.

2.2 Classification of *Aedes aegypti* and *Aedes albopictus*

Classification of *Aedes aegypti* and *Aedes albopictus* published by Knight & Stone (1977) is as below:

Aedes aegypti

***Aedes* albopictus**

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Order: Diptera

Family: Culicidae

Subfamily: Culicinae

Genus: *Aedes*

Genus: *Aedes*

Species: a*egypti*

Species: *albopictus*

2.3 Distribution of *Aedes aegypti* and *Aedes albopictus*

2.3.1 In Nepal

The occurrence of *Aedes albopictus* previously known from Kathmandu district (Darsie and Pradhan,1990) and has been well established. The first record of *Aedes albopictus* for Nepal date back to 1956 (Peters and Dewar 1956). And, other reports from Nepal are, Joshi *et al.* (1965), Shrestha (1966) and Huang (1979). Thirteen species of the genera *Aedes*, *Culex*, *Mimomyia* and *Uranotaenia* were reported from Nepal for the first time by Pradhan (mainly) and Darsie in 1989. Darsie and Pradhan (1990) published an extensive account of the mosquitoes of Nepal, including all previous published works. They reported 130 species in 14 genera including 24 species of *Aedes.* Darsie *et al.*, reported 11 species in genus *Aedes* from different districts of Nepal in 1992. In 1994, 54 species of genera *Aedes* were reported by Darsie and Pradhan (1994). However, in both editons (1990 and 1994) no report and identification keys regarding *Aedes aegypti* were included. *Aedes aegypti* has been identified in 5 major urban areas of terai region bordering India, i.e. Biratnagar (Morang), Birganj (Parsa), Bharatpur (Chitwan), Tulsipur (Dang) and Nepalganj (Banke) during the entomological surveillance in Japanese Encephalities endemic districts after the Dengue outbreak in 2006 in Nepal (WHO, 2006). *Aedes aegypti* had not been previously recorded in the Kathmandu district . And, the breeding habitats occupied by immature mosquitoes had not been previously determined. Since this species has been newly emerged increasing its range throughout the country, an entomological investigation has been directed toward detecting its presence or absence in Kathmandu district by the principal investigator. Herewith, I report the first (1st) collection and identification of *Aedes aegypti* in Kathmandu and Lalitpur distric, during June and July, 2009. Indeed, this finding constitutes the first record from the districts and the collection represents the Basundhara and Gongabu-Balaju and Gwarko–Satdobato area for this species within the Kathmandu valley.

2.3.2 In other countries

In general, *Aedes aegypti* is an indoor breeder while *Aedes albopictus* is an outdoor breeder. It is commonly associated with dengue haemorrhagic fever in urban areas. However, *Aedes albopictus* is a local species and more prevalent in peri-urban areas, and is associated with dengue fever. This mosquito is an aggressive day-biting species and a known in Japan (Sabin 1952), South east Asia (Russell *et al.* 1969), the Seychelles (Metselaar *et al.* 1980), and southern China (Qui *et al.*1981), perhaps second in importance only to *Aedes aegypti* (Knudsen 1995) a primary vector for dengue fever viruses in tropical areas. Although it is a secondary vector to *Ae. aegypti* in urban areas (Chan *et al.* 1967), its importance in an endemic rural cycle in Asia is recognized (Gubler 1987). Its public health significance is well documented (Rosen et al.1985, Shroyer 1986, Boromisa *et al.* 1987, CDC 1987, Mitchell *et al.* 1987, Craven *et al.* 1988, Moore *et al.* 1988, Francy *et al.* 1990, Reeves 2000).

Rudnick *et al.* (1965) did a mosquito survey and reported that *Aedes aegypti* was present in the urban areas of Penang Island, Malaysia whereas *Aedes albopictus* was present in the urban, rural and forested areas. From a previous study on Penang Island, *Aedes aegypti* had not spread beyond the city limit of Georgetown to the rest of the Island (Yap 1975a), and *Aedes albopictus* females preferred to breeds in the outdoor ovitraps than indoor ones. However, in 1991, a nationwide survey was done in urban towns of Peninsular Malaysia (Lee 1991). He reported that both *Aedes aegypti* and *Aedes albopictus* were found breeding indoor and outdoor in a variety of containers. However, the dominant indoor breeder appeared to be *Aedes aegypti*, whilst both species showed equal preference for outdoor containers. In his study, he gave an idea that *Aedes aegypti* was beginning to edge out *Aedes albopictus* because of the interspecific competition (Lee 1991). He claimed that the use of household insecticide products such as mosquito coil, liquid vaporizer, mat and aerosol deter the mosquito from breeding in the houses. Since then, *Aedes aegypti* choose to breeding outdoor rather than indoor. Therefore, the competition between *Aedes aegypti* and *Aedes albopictus* began. *Aedes aegypti* and

Aedes albopictus mixed breeding was found in 18.9% of water containers surveyed and occurred mainly in outdoor containers of large size (Hwang 1994). He also found that more *Aedes aegypti* than *Aedes albopictus* was observed to breed indoors and outdoors.

In Asia, *Aedes albopictus* occurs as far north as Beijing, China, at 40º latitude (WHO 1980). *Aedes albopictus* was first documented in the United States in Texas in 1985 (Sprenger & Wuithiranyagool 1986). A year later, this mosquito was found in Florida at a tire dump site near Jacksonville (O'Meara *et al.* 1995). It has spread to 678 counties in 25 states in a 10 year period (Moore & Mitchell 1997). But the distribution was only concentrated in the southeastern United States. Establishment of this mosquito was less common northwards and westwards, presumably because of the less hospitable environment. The distribution of *Aedes aegypti* currently is limited to urban habitats in southern Texas, Florida and in New Orleans (Lounibos 2002). *Aedes albopictus* was discovered in Italy in 1990 through tire trade (Carrieri *et al.* 2003). Due to the high biological adaptability and its ability to overwinter in embryonic diapause, it spread rapidly to colonize various areas in the central and northern regions of Italy.

2.3.3 Factors Influencing to Mosquito Distribution

From the global viewpoint, environmental changes, including urbanization, improvement of highway and trunk road networks, and importation or transportation of used tires that are stacked or thrown away, are considered important socio-economic factors in the spread of *Aedes albopictus* (Dutta *et al.* 1998). Moore & Mitchell (1997) also stated that the dispersion of *Aedes albopictus* appeared to be related to the proximity of a county to interstate highways. The one all-important cause of the present extended distribution of the species is very clearly by man (Christophers 1960). Beside that, there are also several factors that prevent its establishment such as the decrease of mosquito habitats due to abnormally dry weather, abnormally cold weather, and improvements in handling discarded materials such as used tires (Kobayashi *et al.* 2002). The density of the vector mosquitoes is related to the climatic condition (Vezzani *et al.* 2004). *Aedes* prefer clean clear water with low oxygen partial pressure, like rain water to breed (Lo & Narimah 1984). Therefore, the habit of storage of rain water inside homes for domestic use will provide a suitable breeding site for them. This statement is also supported by Christophers (1960) who claimed that a dry climate and domestic habits that lead to the storage of water may also be favourable for mosquito breeding. Both human host and mosquito vectors are affected by the environment in which they thrive. With crowding, squatters and slums, poor sanitary amenities such as water supply, people tend to store water for their daily needs. Thus, it will create potential breeding places for the vector mosquitoes, which are domesticated and tend to follow human movement and development (Lo & Narimah 1984).

2.3.4 *Aedes albopictus* and *Aedes aegypti* Breeding Habitat Preference and larval indices

Except for some African strains, *Aedes aegypti* lives in close association with man, mainly breeding in artificial containers in the domestic environment. *Aedes albopictus* uses both artificial and natural breeding sites. Sumodan (2003) studied the potential of rubber plantation as breeding source for *Aedes albopictus* in Kerala, India. In the plantations where tapping had been suspended, sap-collecting containers had rainwater collection and also mosquito breeding. *Aedes albopictus* had adapted commensurably to the discardable plastic tea cups, a new man-made breeding site for this vector species (Hiriyan et al. 2003). In Malaysia, *Aedes albopictus* is usually found at forest fringes, in secondary forests, and in green areas in towns (Abu Hassan 1994, Macdonald 1956b). It is one of the most common anthropophilic mosquitoes in Malaysia, and it breeds in man-made containers, tree holes, plant axils, and bamboo stumps. *Aedes aegypti* is an artificial container breeder in the coastal towns. On the Island of Anguilla, West Indies, *Aedes aegypti* is able to breed in rock hole as well (Parker et al. 1983). *Aedes aegypti* and *Ae. albopictus* larvae were found breeding in almost all indoor and outdoor, temporary and/or permanent collections of water, either alone or in association with each other in residential areas of residential areas of Calcutta City *Aedes aegypti* showed preference for breeding in chowbachhas, indoors and *Aedes albopictus* for collections of water in flower pots and discarded containers and outdoors. The larval indices of both the species were highest during monsoons and post-monsoons (Tandon and Ray, 2000). A one year study on the breeding habitats of *Aedes aegypti* (L.) in

Jakarta, Indonesia revealed that the infestation rate of covered containers was significantly higher than that of uncovered containers, perhaps because loose-fitting lids allowed entrance of gravid females to the attractive darkened interior of the container Nelson *et al.* (1976). The breeding habitats of the dengue vector, *Aedes aegypti* and *Aedes albopictus*, were studied using larval collection method inside and outside houses in 6 villages of Barru, South Sulawesi, Indonesia from July 1994 to August 1995. The high Breteau indices of *Aedes aegypti* and *Aedes albopictus* suggested that these species may play an important role in the transmission of dengue hemorrhagic fever in Barru where epidemics of the fever occur occasionally Ishak *et al.* (1997).

2.3.5 Replacement of *Aedes albopictus* by *Aedes aegypti*

There is evidence to suggest that in Southeast Asia, indigenous *Aedes albopictus* has been replaced in many areas by the invasion of *Aedes aegypti* (Service 1992, Rudnick *et al.* 1967). Laboratory experiments with Southeast Asian populations have shown that *Aedes aegypti* out-competes *Aedes albopictus* (Service 1992). It is quite possible that this phenomenon can be brought about by progressive urbanization which tends to cause reduction in the amount of vegetation, outdoor shade and naturally occurring containers as habitats for mosquito breeding and an increase in artificial containers (Hawley 1988).

2.3.6 Replacement of *Aedes aegypti* by *Aedes albopictus*

Some countries, other than Southeast Asia, have shown a widespread of *Aedes albopictus*. In October 1999, Fontenille & Toto (2001) captured biting *Aedes albopictus* females in Southern Cameroon. By 2000, *Aedes albopictus* was already widespread in Southern Cameroon. In Mobile, Alabama, *Aedes albopictus* was first detected during a CDC sponsored ovitrap survey in the Hótoric District. A comparison of ovitrap and larval survey done in 1957, 1984, 1987 and 1990, indicated that *Aedes albopictus* had replaced *Aedes aegypti* in urban Mobile (Hobbs *et al.* 1991). In 1985, *Aedes albopictus* was introduced into Houston, Texas (Sprenger & Wuithiranyagool 1986). Then, a well establishment of *Aedes albopictus* in the continental USA. By 1988, large breeding populations were common in Texas, Louisiana and the southern states east of the

Mississippi River (Nasci *et al.* 1989). Live larvae of *Aedes albopictus* had been found entering South Africa in used tire casings imported from Japan on 3 separate occasions. It is suggested that undetected populations of *Aedes albopictus* may have already become established in Africa because large areas of Africa experience climatic conditions similar to those where *Aedes albopictus* is already established (Cornel & Hunt 1991). In South Florida, the displacement of *Aedes aegypti* by *Aedes albopictus* could be because of larval resource competition based on the result conducted using tires (Juliano 1998). Some reasons have been suggested to explain the replacement of *Aedes aegypti* by *Aedes albopictus*, (a) sterility of offspring from interspecific matings; (b) reduced fitness of *Aedes aegypti* form parasites brought in with *Aedes albopictus* and; (c) superiority of *Aedes albopictus* in larval resource competition (Lounibos 2002).

2.3.7 The Effect of Mosquito Invasion in Public Health

Invaded mosquitoes may affect human health by (i) simultaneous introduction of a novel vector and novel pathogen, (ii) acquisition by an introduced vector of a native pathogen, or (iii) independent introductions of a novel vector and a novel pathogen (Juliano & Lounibos 2005). Novel vector and novel pathogen are defined as a newly invasive vector and pathogen in an area. The introduced vector also defined as a newly invasive vector but there is difference between them. When novel vector invaded to an area, it is the only one vector in that area. While, when introduced vector invaded to an area, it is not the only one vector because there are one or more than one vector which can transmit the same pathogen in a same area.

Simultaneous introduction of a novel vector and a novel pathogen may be sufficient to create a new public health threat, with potential to cause large outbreaks of disease, particularly because the host population will likely have limited immunity. For example, yellow fever originated in Africa and appears to have been spread initially by the slave trade of the 16-17th centuries (Lounibos 2002). Outbreaks of yellow fever occurred in many North American cities, as far as New York City, and it is virtually certain that these outbreaks occurred when both vector and virus arrived via ship (Tabachnick 1991). If an invasive vector takes on a role in an existing disease transmission cycle, it changes the nature of an existing public health threat, presumably for worse. For example, *Aedes aegypti* is the major vector of urban dengue in tropical Asia, where dengue is likely native. Thus, since its invasion of Asia in the late 19th century, *Aedes aegypti* became the primary vector of a resident pathogen. If an invasive vector acquires, sometime after establishment, an association with an independently introduced pathogen, susceptible hosts are exposed to a novel pathogen with a new vector. This situation may be particularly prone to unpredictable disease dynamics. In Hawaii, *Aedes albopictus* was established before the introduction of dengue virus in 2001, which probably brought by infected humans (Juliano & Lounibos 2005).

2.3.8 Species Competitions

The presence of other organisms may limit the distribution of some species through competition. Factors that may contribute to species displacement include changes in physical conditions, environmental modification by one of the competing species, cannibalism, predation, active interference (e.g., inhibition of mating, feeding, or ovipostition), disease, parasitism, and generic drift (Moore & Fisher 1969). In the context of invasions, effect of competition and predation can be important because they can be impacts of invasive species on native species, and because these processes may act as barriers to invasion (Juliano & Lounibos 2005). In general, the likelihood that exotic species will colonize new regions depends on their capacity to adapt to the new environmental conditions and to compete with the pre-existing species (Carrieri et al. 2003).

There are many researchers reporting on the interspecific competition between *Aedes aegypti* and *Aedes albopictus*, and yet the results are varied. Mixed rearing of *Aedes albopictus* with *Aedes aegypti* delayed the development of *Aedes albopictus* larvae. Development of *Aedes albopictus* larvae was slower than that of *Aedes aegypti* larvae under various experimental conditions (Lee 1994, Moore & Fisher 1969). Laboratory experiments on competition between *Aedes aegypti* and *Aedes albopictus* using different detritus resources (e.g. liver powder, yeast, dead insects) have tended to yield approximate competitive equality, or even an advantage for *Aedes aegypti* (Barrera

1996, Daugherty *et al*. 2000). In Illinois State, *Aedes albopictus* showed significantly greater survivorship compared with *Aedes aegypti* because *Aedes albopictus* is more superior in resource-harvesting ability (Yee *et al*. 2004). Another study in Florida showed that *Aedes albopictus* had significantly higher metamorphic success and eggs hatched more rapidly than *Aedes triseriatus* (Lounibos *et al*. 2001).

Also, it was more successful than *Aedes triseriatus* in survival to emergence in the presence of the predatory larvae of the native mosquito *Toxorhynchites rutilus.* Under laboratory conditions at 25°C, Carrieri *et al.* (2003) observed that *Aedes albopictus* is more successful than *Culex pipiens* in exploiting artificial microhabitats when food is scarce, because it has greater efficiency in converting food into biomass and rapidity in larval development. However, food-biomass conversion coefficient dropped significantly when the temperature falls below 25°C. Alto & Juliano (2001) also proved that temperature could influence the larval trophic activity.

2.3.9 Seasonal Abundance of *Aedes* Mosquitoes

In Malaysia, the variation in *Aedes albopictus* larval populations and high densities of biting mosquitoes in various months was related to the rainfall (Abu Hassan *et al.* 1996). As an example, the high larval population and high densities of biting mosquitoes observed in November 1990 was possibly due to the increase in rainfall in September and October 1990. Deposition of *Aedes aegypti* eggs decreased during periods of no rainfall but sharply increased 2 weeks after significant rainfall in Texas (Hoffman & Killingsworth 1967). In Victoria peak abundance of adult *Aedes sagax* (Skuse) typically followed floods or heavy rains in winter, spring and autumn (Russell 1986). In Buenos Aires, Argentina, the presence of *Ochlerotatus albifasciatus* was positively related to the amount of rain ($p<0.001$), whereas negatively correlated to air temperature ($p<0.05$) within a 5.2 to 29.7°C range because high temperature is unfavourable to larval development (Fischer *et al.* 2002). The year-round presence of immature stages of *Ochlerotatus albifasciatus* indicates that the climatic conditions of the city of Buenos Aires are not limiting to the development of this species. And thus, differences in habitat availability may be partially responsible for the detected seasonal fluctuations of mosquito. Rainfall always provide breeding habitat for mosquitoes. Zyzak *et al.* (2002) stated that as rainfall-produced surface pools accumulated, an accompanying expansion of oviposition sites occurred resulting in a sustained, elevated *Culex igripalpus* population in Florida. The extension of dry season and increment of temperature results the reduction of adult mosquito.

2.3.10 Relationship between Incidence of Dengue Cases and Dengue Vectors

Pattern of dengue transmission are influenced by the abundance, survival, and behaviour of the principal mosquito vector, *Aedes aegypti*; the level of immunity to the circulating virus serotype in the local human population; density, distribution and movement of humans; and time required for development of virus in *Aedes aegypti* (Halstead 1990). Abundant vector populations are often a prerequisite for epidemic and epizootic transmission of arboviruses. The *Aedes aegypti* densities were correspondence to dengue fever incidence (Moore *et al.* 1978). In Puerto Rico, from 1973-1975, dengue transmission increased when average Breteau indices rose above 20 (Moore *et al.* 1978). The result was the same as Foo *et al.* (1985), in Selangor. In year 1966, in Singapore, there was a general correspondence between the epidemic curve and the population fluctuations of *Aedes aegypti* and *Aedes albopictus* (Chan *et al.* 1971b). The simultaneous rise in the populations of these mosquitoes in February-March was followed by an increase in the incidence of cases, which continued to rise sharply in the subsequent months (Chan *et al.* 1971b). The slightly fall in the incidence of the cases from July-October was associated with the fall in the populations of the mosquitoes. Another study also suggests that the presence of *Aedes albopictus* in or around household premises was associated with an increased risk of dengue transmission in Dhaka, India (Ali *et al.* 2003).

2.3.11 Relationship between Incidence of Dengue Cases and Physical Parameters

Some studies showed that there is an association between rainfall and dengue outbreak. In Selangor, Malaysia, a monthly rainfall of 300mm or more would cause a major dengue outbreak in the state after a lag period of about two to three months (Foo *et al.* 1985). Abundance of *Aedes albopictus* was significantly correlated with rainfall four weeks before collection dates using ovitraps (Lourenco-de-Oliveira *et al.* 2004). In Malaysia, the epidemics of dengue from 1973-1982 were related to the two monsoons: SW Monsoon in the first half, and NE Monsoon in the second half of the year (Lo and Narimah 1984). Endemicity is low during the period from January-April, when it begins to rise, reaching a peak in July/August, and then declines. This variation is related to the storage of water during the drought season from January-April, and the drizzling rainfall before the heavy monsoons, which all create a suitable breeding places for the vector (Lo and Narimah 1984). There are some other studies which reported the use of rainfall data to predict malaria epidemics. Mouchet *et al.* (1998) pointed out that temperature and rainfall influence malaria transmission; the former determines the length of the larval mosquito cycle and the sporogonic cycle of the parasite in the mosquito; the latter determines the number and productivity of breeding sites and consequently the vector density. Rainfall in highland areas has been associated with entomologic transmission parameters (Lindblade *et al.* 1999) and malaria incidence (Fontaine *et al.* 1961, Mouchet *et al.* 1998), and it has been suggested that monitoring rainfall levels could provide sufficiently early warning of highland malaria epidemics. However, elevated temperatures may interact with rainfall to create epidemic conditions (Lindblade *et al.* 1999), and this interaction must be investigated further before rainfall alone can be used as a suitable indicator of epidemic risk.

2.4 Justification

Dengue fever/dengue haemorrhagic fever is the most important arbovirus disease of human in terms of morbidity and mortality. Occurrence of Dengue was confirmed in

Kathmandu valley. *Aedes aegypti* and *Ae. albopictus* are the most important vectors. Rapid urbanization often results in the proliferation of the small peridomestic breeding sites such as discarded tires, tins etc. as well as an inadequate provision of water supply and disposal facilities. The mosquitos breed in relatively clean water in domestic water containers or water vessels. Female mosquitos feed on man during the day time, are infected with dengue virus and transmit viruses to man after an 8 to 10 day extrinsic incubation period. Mechanical transmission of the virus may also occur if the infected mosquito immediately bites on another person..This study would be useful for the possible inclusion of the Kathmandu valley in the national Dengue prevention and control program.

Some entomological studies were carried out to know the distribution of *Aedes aegypti* in Nepal and reported the species first time in different districts of terai areas of Nepal. Not any study has been carried out in Kathmandu valley, regarding the occurrence of *Aedes aegypti*, and its breeding habitats and transmission by the vector. Hence this study aimed to target to survey in Kathmandu valley. Keeping this in mind, in depth study was carried out to confirm the real situation of the distribution of vectors and breeding habitats and it is sure that this study may fulfill the requirement to some extent.

Larval control has not been often used in Nepal in the past because larval source reduction initiatives have always been seen as expensive and largely associated with the government. An appropriate community intervention is clearly needed. The major challenges to community involvement in larval source reduction activities are in educating people about the sources of the mosquitoes and motivating people to assume responsibility for controlling mosquitoes in and around their homes, responsibilities often assumed to be that of government. Some governments, for example Singapore, strictly enforce legislation making it unlawful for citizens to allow larval habitats for *Stegomyia aegypti* mosquitoes on private property. Study of breeding habitats of mosquitoes may therefore be feasible. For integrated development of community-based vector control programs, not only should consideration be given to the larval ecology of *Aedes aegypti*, but also to the sociological significance of the various container habitats and the selection of control strategies most appropriate for their management.

Overall, the study shows the nature of potential breeding habitats, the number of them, and their proximity to houses taken together ought to enhance feasibility of controlling the larval stages of *Aedes agypti*. Because vector control activities are beyond the budgetary capacities dengue effected areas, community initiative or support is a likely prerequisite for any successful intervention. The concept of community participation has gained popularity in many malaria-control strategies . Larval control has not been often used in Nepal in the past because larval source-reduction initiatives have always been seen as expensive and largely associated with the government. The challenge is to change this perception and stimulate community involvement in larval control activities. Control of mosquito production may therefore be feasible.

CHAPTER 3

3.0 Materials and Methods

3.1 Study sites

The sample site are selected on the basis of topography, Dengue receptive areas. Probability of transmission of Dengue by the target vector species *Aedes aegypti* and other possible vector species is high. In addition, most of the areas may be receptive areas.

Kathmandu District is located in Kathmandu Valley, Bagmati Zone, Nepal. It covers an area of 395 km² in the hills of Bagmati zone, from 27'27"E to 27'49"E and 85'10"N 85'32"N. The district is surrounded by Bhaktapur district and Kabhrepalanchok district in the East, Dhading and Nuwakot districts in the West, Nuwakot and Sindhupalchok districts in the North and Lalitpur and Makwanpur districts in the South. The altitude of the district ranges between 1262 m. to 2732 m. above the sea level. Bhaktapur district, a part of Bagmati zone, covers an area of 119 km². Lalitpur District, a part of Bagmati Zone, covers an area of 385 km² . Collection sites included Koteshwar, Manahara, Gongabu-Balaju, Tokha, Jorpati and Kalanki (Kathmandu district), Tikathali, Lokanthali, Kaushaltar, Thimi, Darbar square and Bode (Bhaktapur district). Satdobato, Thalchkhel, Mahalaxmisthan, Dhobighat, Sanepa, Bagalamukhi, Dholahiti, Chibahal, Thecho, Chapagaon, Badegaon, and Godawari (Lalitpur district) of Kathmandu valley.

In this study, house-to-house surveys of larval breeding places and larval prevalence were conducted covering all three districts of Kathmandu valley during April, May, June, July, August, September and October in 2009. For each household, the number and categorization of potential larval habitats with and without water, were noted. Water-filled containers were examined for the presence of mosquito larvae and/or pupae. Adult *Aedes* mosquitoes were examined with the help of aspirators and flash-lights during morning hours (0700–0900 hrs) from those breeding habitats. Approximately 70 houses in Kathmandu district, 51 houses in Bhaktapur district and 61 houses in Lalitpur district were randomly sampled and larval breeding habitats were identified. Collection sites were selected in both town and rural residential areas of three districts. All water containers in those houses were randomly checked for the presence or absence of *Aedes* larvae.

3.2 Ethical clearance

Since this study does not involve any human and animal ethical clearance did not felt necessary to obtain.

3.3 Entomological studies

Main purpose of the selecting the areas for the study is to know if there are distribution, abundance of *Aedes aegypti* and other possible vector of dengue in urban areas of Kathmandu valley. No information is available regarding seasonal abundance of Dengue vector, *Aedes aegypti* in these districts. Entomological studies on relative distribution of immatures of *Aedes sp.* were conducted in Koteshwar, Manahara, Gongabu-Balaju, Tokha, Jorpati and Kalanki (Kathmandu district), Tikathali, Lokanthali, Kaushaltar, Thimi, Darbar square and Bode (Bhaktapur district). Satdobato, Thalchkhel, Mahalaxmisthan, Dhobighat, Sanepa, Bagalamukhi, Dholahiti, Chibahal, Thecho, Chapagaon, Badegaon, and Godawari (Lalitpur district) of Kathmandu valley from April to October, 2009.

Entomologic data collected through routine systems. A total of 140 houses in April, 164 houses in May, 246 houses in June and 335 houses in July, 430 houses in August, 389 houses in September and 320 houses in October were sampled and all possible larval breeding habitats were surveyed by two entomological teams (two persons per team) per month. Surveys were conducted by searching for containers containing water and recording their types and site locations. Larval habitats were examined for all accessible water containers in and around the house. Larval habitats were also surveyed in all natural and artificial containers including all trash near and around dwellings (less than 10 m from the selected house). Adult *Aedes* mosquitoes were examined with the help of aspirators and flash-lights during morning hours (0700–0900 hrs) from those breeding habitats. All live mosquito larvae and pupae were collected by dipper and brought to the insectary at the JCRC college and Central Department of Zoology , Tribhuvan University, Kirtipur laboratory, Kalimati, Kathmandu. All live mosquito larvae and pupae collected were reared (Collins, 1970) until adult emergence. Adult mosquitoes emerged from reared larvae were identified by using the conventional key for *Aedes* species (Darsie and Pradhan 1990; Rueda 2004).

3.4 Data analysis

The raw data was compiled in the table and taken different photographs. All necessary data relevant to the species of the different study areas of the country was analyzed.

Primary end points of this study were the Breteau index and the house index; the secondary end point was the container index. Larval survey techniques were used to obtain the House Index (HI, percentage of houses positive for *Aedes* larvae), Container Index (CI, percentage of containers positive for *Aedes* larvae) and Breteau Index (BI, number of containers positive for *Aedes* larvae per 100 houses) (Service, 1976) as per guidelines (WHO, 2003). The container preferences of *Aedes* mosquitoes were assessed by calculation of breeding preference ratio (BPR) (Sharma, 2002; Singh *et al* 2008).

3.5 Limitation of the study

Only *Aedes aegypti* and *Aedes albopictus* was be focused during study period. This study describes the vector's larval habitats in a Nepali context and as a result of visits to limited numbers of localities and inadequate period (only seven months), in each district, the result do not reflect a complete figure regarding the seasonal abundance of *Aedes aegypti* and *Aedes albopictus* . Therefore, one year complete study is recommended. Furthermore, District wise detail survey will be needed to more accurately identify the priority larval habitats within each community or group of communities before deciding on the most appropriate means of control. It must be noted that continuity of this study is necessary.

CHAPTER 4

4.0 Results

To identify the potential breeding habitats of *Aedes* mosquitos, all accessible water containers, seven months entomological investigation was carried out in 3 districts of Kathmandu valley from April to October, 2009 in the houses with a view to study the prevalence and distribution of areas for *Aedes* species.

All kinds of potential breeding habitats in the study areas like discarded tires, metal/plastic drums, containers, plastic buckets, flower pots, mud/plastic pots, tanks, etc. screened for the presence of immature stages of *Aedes* mosquitoes are shown in table 1-14. The data on larval survey were also analyzed and calculated in terms of Container Index (CI), House Index (HI) and Breteau Index (BI) as per guidelines (WHO 2003). Breeding Preference Ratio (BPR) of *Aedes aegypti* and *Ae. albopictus* in different breeding habitats were calculated. Prevalence indices and breeding preference ratio of *Aedes aegypti* and *Aedes albopictus* in June-October is summarized and tabulated in table 1-10. Breeding habitat type and % of containers with water examined in Kathmandu valley in June-october, 2009 are figured in figure 2-6. Monthly distribution of *Aedes aegypti* and *Aedes albopictus* larvae in Kathmandu valley are figured in figure 7 and 8.

A total of 114 wet containers in 53 households were searched in Koteshwar, Manahara, Gongabu-Balaju, Tokha, Kalanki and Jorpati (Kathmandu district), 63 wet containers in 42 households were searched in Tikathali, Lokanthali, Kaushaltar, Thimi, Darbar square and Bode (Bhaktapur district) and 76 wet containers in 45 households were searched in Satdobato-Gwarko, Bagalamukhi, Dholahiti, Chibahal, Thecho, Chapagaon, Badegaon and Godawari (Lalitpur district) for *Aede*s larvae in April, 2009. Not even one container found to be positive for Adult and larva in April.

Similarly total 200 wet containers in 69 households were searched in Koteshwar, Manahara, Gongabu-Balaju, Tokha, Kalanki and Jorpati (Kathmandu district), 150 wet containers in 44 households were searched in Tikathali, Lokanthali, Kaushaltar, Thimi, Darbar square and Bode (Bhaktapur district) and 121 wet containers in 51 households were searched in Satdobato-Gwarko, Bagalamukhi, Dholahiti, Chibahal, Thecho, Chapagaon, Badegaon and Godawari (Lalitpur district) for *Aede*s larvae in May, 2009.

Not even one container found to be positive for Adult and larva in May also. A total of 268 wet containers in 89 households were searched in Koteshwar, Manahara, Gongabu-Balaju, Tokha, Kalanki and Jorpati (Kathmandu district), 202 wet containers in 68 households were searched in Tikathali, Lokanthali, Kaushaltar, Thimi, Darbar square and Bode (Bhaktapur district) and 325 wet containers in 89 households were searched in Satdobato-Gwarko, Bagalamukhi, Dholahiti, Chibahal, Thecho, Chapagaon, Badegaon and Godawari (Lalitpur district) for *Aede*s larvae in June, 2009, out of which 2 houses in Kathmandu district for *Aedes aegypti* and *Ae. albopictus* and 2 in Lalitpur district were found positive for *Aedes albopictus* breeding. Average Rainfall (mm) in Kathmandu valley is shown in figure 1. Breeding habitat type and percentage containers with water examined in Kathmandu valley in June, 2009 are figured in figure 2 and 3.

Figure 2. Average Rainfall (mm) in Kathmandu Valley

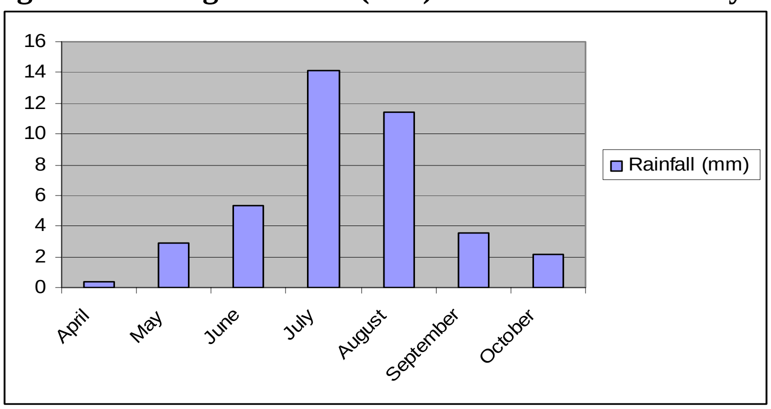
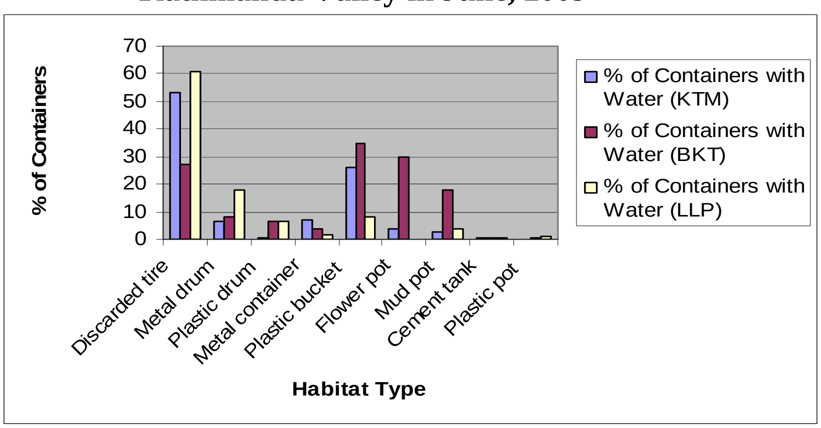


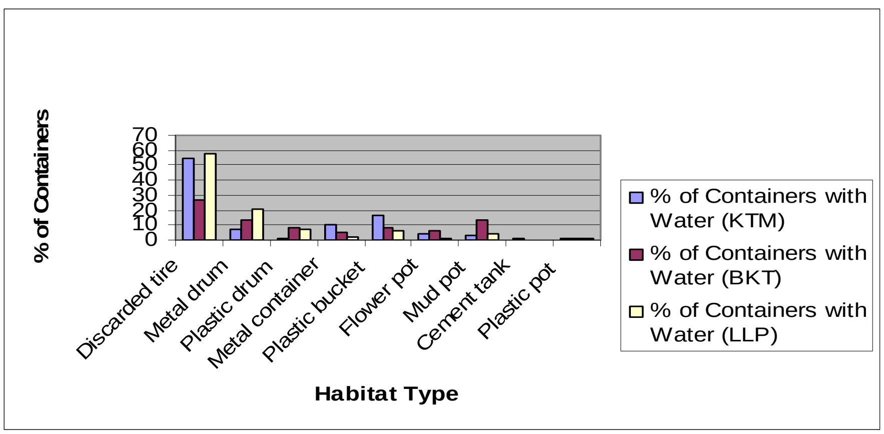
Figure 3. Breeding Habitat Type and % of Containers with Water Examined in Kathmandu Valley in June, 2009



In the present study, larvae of *Aedes aegypti* were recorded in Kathmandu district (Basundhara and Gongabu-Balaju area) from two localities within the urban agglomeration of Kathmandu for the first time in June, 2009.

A total of 313 wet containers in 117 households were searched in Koteshwar, Manahara, Gongabu-Balaju, Tokha, Kalanki and Jorpati (Kathmandu district), 232 wet containers in 89 households were searched in Tikathali, Lokanthali, Kaushaltar, Thimi, Darbar square and Bode (Bhaktapur district) and 387 wet containers in 129 households were searched in Satdobato-Gwarko, Bagalamukhi, Dholahiti, Chibahal, Thecho, Chapagaon, Badegaon and Godawari (Lalitpur district) for *Aede*s larvae in July, 2009, out of which 2 houses in Kathmandu district for *Aedes aegypti* and *Ae. albopictus* and 2 in Lalitpur district were found positive for *Aedes aegypti* and 2 houses for *Aedes albopictus* breeding in Kathmandu and Lalitpur district.

Figure 4. Breeing Habitat Type and % of Containers with Water Examined in Kathmandu Valley in July, 2009



In the present study, larvae of *Aedes aegypti* were recorded in Kathmandu district (Basundhara and Gongabu-Balaju area) and Lalitpur district (Satdobato-Gwarko area) from 2 localities and Lalitpur district within the urban agglomeration of Kathmandu valley for the first time. During this study (in June), the average temperatures in the field ranged from 19.08 °C to 30.64 °C. The highest rainfall was 32.7 mm and the lowest was 1mm including few traces. Similarly in July, the average temperatures in the field ranged between 20.67 °C to 29.92 °C. The highest rainfall was 99 mm and the lowest was 0.2 mm including few traces (table 11-17).

Houses infested with *Aedes* larvae can be expressed as House Index (HI), water containers that are infested by *Aedes* larvae can be expressed by the Container Index (CI) and larval prevalence is determined by the Breteau Index (BI). The over all HI, CI and BI for *Aedes aegypti* were 2.24, 1.11 and 3.37 in Kathmandu district (Basundhara and Gongabu-Balaju) and for *Aedes albopictus* 2.24, 0.74 and 2.24 respectively (table 3 & 4) in June, 2009. The over all House Index (HI), Container Index (CI) and Breteau Index (BI) for *Aedes albopictus* were 2.24. 0.61 and 2.24 respectively in Satdobato-Gwarko area (Lalitpur district) (table 1 & 2) in June, 2009.

Table 1. Prevalence indices and Breeding Preference Ratio (BPR) of *Aedes aegypti* in different localities of Kathmandu Valley in June, 2009

District	**HI (%)**	**CI (%)**	**BI (%)**	**Breeding Preference Ratio (BPR)**	**Remarks**
Kathmandu	2.24	1.11	3.37	1.88	Discarded tire
Bhaktapur	0	0	0	0	
Lalitpur	0	0	0	0	

Table 2. Prevalence indices and Breeding Preference Ratio (BPR) of *Aedes albopictus* in different localities of Kathmandu Valley in June, 2009.

District	**HI (%)**	**CI (%)**	**BI (%)**	**Breeding Preference Ratio (BPR)**	**Remarks**
Kathmandu	2.24	0.74	2.24	1.88	Discarded tire
Bhaktapur	0	0	0	0	
Lalitpur	2.24	0.61	2.24	1.64	Discarded tire

Similarly, the over all HI, CI and BI for *Aedes aegypti* were 1.70, 1.27 and 3.41 (Basundhara and Gongabu-Balaju) and 1.55, 0.77 and 2.32 (Satdobato-Gwarko). HI, CI and BI for *Aedes albopictus* were 1.70, 1.27 and 3.41 and 12.5 respectively in Kathmandu district in July, 2009. The over all House Index (HI), Container Index (CI) and Breteau Index (BI) for *Aedes albopictus* were 2.32, 1.03 and 3.1 in Lalitpur district respectively in Satdobato-Gwarko area (table 3 & 4) in July, 2009.

Table 3. Prevalence indices and Breeding Preference Ratio (BPR) of *Aedes aegypti* in different localities of Kathmandu Valley in July, 2009

District	HI (%)	CI (%)	BI (%)	Breeding Preference Ratio (BPR)	Remarks
Kathmandu	1.70	1.27	3.41	1.81	Discarded tire
Bhaktapur	0	0	0	0	
Lalitpur	1.55	0.77	2.32	1.74	Discarded tire

Table 4. Prevalence indices and Breeding Preference Ratio (BPR) of *Aedes albopictus* in different localities of Kathmandu Valley in July, 2009

District	HI (%)	CI (%)	BI (%)	Breeding Preference Ratio (BPR)	Remarks
Kathmandu	1.70	1.27	3.41	1.81	Discarded tire
Bhaktapur	0	0	0	0	
Lalitpur	2.32	1.03	3.1	1.54	Discarded tire and Metal drum

In August, September and October the highest and lowest temperatures were 18.8°C to 31.6°C, 16.4°C to 31.2°C and 11.1°C to 29.8°C respectively. Similarly the lowest and the highest rainfall in August, September and October were 0.4 (mm) to 59.6 (mm), 0.4 (mm) to 27.4 (mm) and 0.3 (mm) to 45.1(mm) respectively (table17-19). A total of 334 wet containers in 141 households in Kathmandu district, 257 wet containers in 112 households in Bhaktapur district and 423 wet containers in 177 households in Lalitpur district in August; 305 wet containers in 121 households in Kathmandu district, 248 wet containers in 106 households in Bhaktapur district and 417 wet containers in 162 households in Lalitpur district in September; 303 wet containers in 113 households were searched in Kathmandu district, 241 wet containers in 98 households in Bhaktapur district and 344 wet containers in 109 households in Lalitpur district in October, 2009 were searched. Out of which 5 houses and 11containers in Kathmandu district and 6 houses and 14 containers in Lalitpur district for *Aedes aegypti* breeding were found positive in August, September and October.

Figure 5. Breeding Habitat Type and % of Containers with Water Examined in Kathmandu Valley in August, 2009

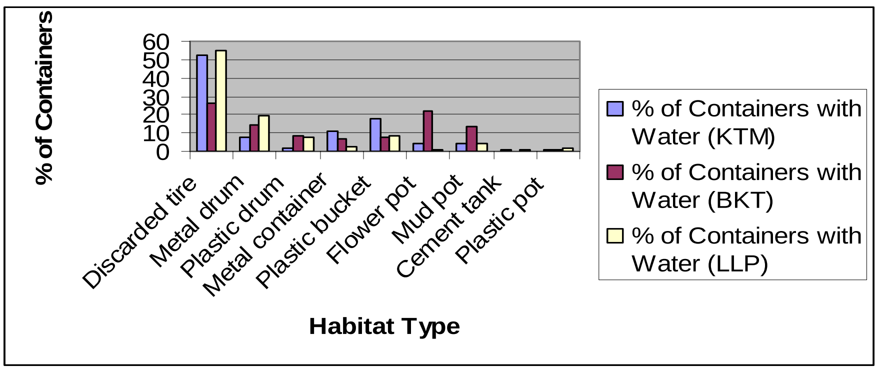


Figure 6. Breeing Habitat Type and % of Containers with Water Examined in Kathmandu Valley in September, 2009

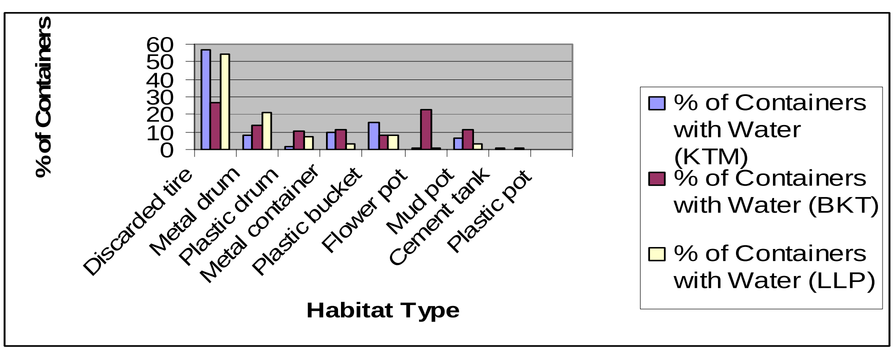
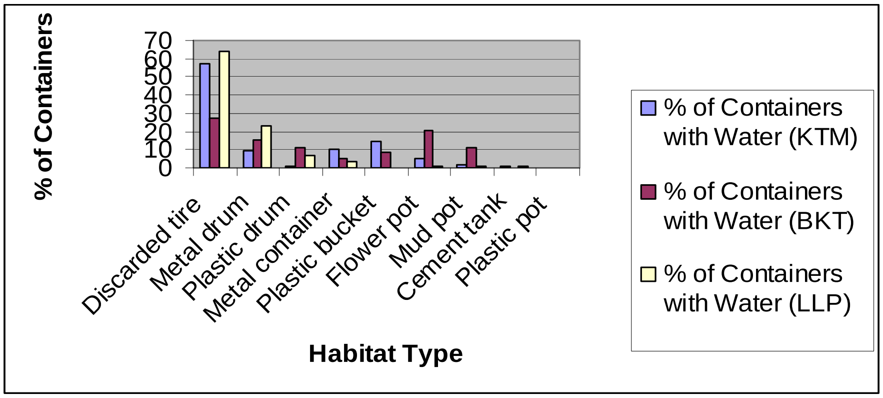


Figure 7. Breeing Habitat Type and % of Containers with Water Examined in Kathmandu Valley in October, 2009



The over all HI, CI and BI for *Aedes aegypti* were 3.54, 3.29 and 7.80 in Kathmandu district and 3.38, 3.30 and 7.90 in Lalitpur district respectively (table 7). HI, CI and BI for *Aedes albopictus* were 8.51, 11.37 and 26.95 in Kathmandu district and 5.64, 9.69 and 23.16 in Lalitpur district respectively (table 5 & 6) in August, 2009.

Table 5. Prevalence indices and Breeding Preference Ratio (BPR) of *Aedes aegypti* in different localities of Kathmandu Valley in August, 2009

District	HI (%)	CI (%)	BI (%)	Breeding Preference Ratio (BPR)	Remarks
Kathmandu	3.54	3.29	7.80	1.89	Discarded tire
Bhaktapur	0	0	0	0	
Lalitpur	3.38	3.30	7.90	1.28	Discarded tire

Table 6. Prevalence indices and Breeding Preference Ratio (BPR) of *Aedes albopictus* in different localities of Kathmandu Valley in August, 2009

District	HI (%)	CI (%)	BI (%)	Breeding Preference Ratio (BPR)	Remarks
Kathmandu	8.51	11.37	26.95	1.89	Discarded tire

Bhaktapur	0	0	0	0	
Lalitpur	5.64	9.69	23.16	2.79	Discarded tire, metal drum and metal container

In September, HI, CI and BI for *Aedes aegypti* in Kathmandu and Lalitpur district were 4.13, 3.60 and 9.09 and 3.70, 3.35, 8.64 respectively (table 7). HI, CI and BI for *Aedes albopictus* in Kathmandu and Lalitpur district were 9.91.12.45 and 31.40 and 6.17, 9.83 and 25.30 respectively in September, 2009 (table 8).

Table 7. Prevalence indices and Breeding Preference Ratio (BPR) of *Aedes aegypti* in different localities of Kathmandu Valley in September, 2009

District	HI (%)	CI (%)	BI (%)	Breeding Preference Ratio (BPR)	Remarks
Kathmandu	4.13	3.60	9.09	1.77	Discarded tire
Bhaktapur	0	0	0	0	
Lalitpur	3.70	3.35	8.64	1.82	Discarded tire

Table 8. Prevalence indices and Breeding Preference Ratio (BPR) of *Aedes albopictus* in different localities of Kathmandu Valley in September, 2009

District	HI (%)	CI (%)	BI (%)	Breeding Preference Ratio (BPR)	Remarks
Kathmandu	9.91	12.45	31.40	1.77	Discarded tire
Bhaktapur	0	0	0	0	
Lalitpur	6.17	9.83	25.30	2.59	Discarded tire, metal drum and metal

					container

Similarly, HI, CI and BI for *Aedes aegypti* were 4.42, 3.63 and 9.73 in Kathmandu district and 5.50, 4.06 and 12.8 in Lalitpur district respectively in October, 2009 (table9). HI, CI and BI for *Aedes albopictus* in Kathmandu and Lalitpur district were 7.07, 5.61 and 15.04 and 8.25, 6.39 and 20.1 respectively in October, 2009 (table 10).

Table 9. Prevalence indices and Breeding Preference Ratio (BPR) of *Aedes aegypti* in different localities of Kathmandu Valley in October, 2009

District	HI (%)	CI (%)	BI (%)	Breeding Preference Ratio (BPR)	Remarks
Kathmandu	4.42	3.63	9.73	1.74	Discarded tire
Bhaktapur	0	0	0	0	
Lalitpur	5.50	4.06	12.8	1.55	Discarded tire

Table 10. Prevalence indices and Breeding Preference Ratio (BPR) of *Aedes albopictus* in different localities of Kathmandu Valley in October, 2009

District	HI (%)	CI (%)	BI (%)	Breeding Preference Ratio (BPR)	Remarks
Kathmandu	7.07	5.61	15.04	1.74	Discarded tire
Bhaktapur	0	0	0	0	
Lalitpur	8.25	6.39	20.1	1.68	Discarded tire and metal drum

The highest HI, CI and BI recorded for *Aedes aegypti* were 4.42, 3.63 and 9.73 respectively in October, and 9.91, 12.45 and 31.40 respectively for *Aedes albopictus* in

September, 2009 (Kathmandu district). Whereas, HI, CI and BI recorded for *Aedes aegypti* in Lalitpur district were 5.50, 4.06 and 12.80 respectively in October, 2009. For *Aedes albopictus,* the highest HI, CI and BI recorded for *Aedes albopictus* in Lalitpur district were 15.64 in August, 9.83 and 25.30 in September, 2009. It was observed that discarded tires lying outdoors were the preferred breeding habitats. No breeding of *Aedes aegypti* was observed in other containers during this seven months survey. However, breeding of *Aedes albopictus* was also observed in metal drum in Mangal bazar and in metal container in Gwarko of Lalitpur district .

The container preferences of *Aedes* mosquitoes were assessed by calculation of breeding preference ratio (BPR) (Sharma, 2002; Singh *et al.* 2008). Breeding preference ratio (BPR) for *Aedes aegypti* and *Aedes albopictus* in June in Kathmandu district were 1.88 and 1.88 (table 3 & 4). In July BPR for *Aedes aegypti* and *Aedes albopictus* were 1.81 and 1.81 in Kathmandu district and 1.74 and 1.30 in Lalitpur district respectively (table 5 & 6). BPR were 1.89 in Kathmandu district and 1.28 in Lalitpur district respectively for *Aedes aegypti* in August, 2009 (table 7). For *Aedes albopictus* in Kathmandu district BPR were 1.89 and 1.69 (discarded tire), 0.25 (metal drum), 0.85 (metal container) in Lalitpur district in August, 2009 (table 8). In September, 2009 BPR for *Aedes aegypti* and *Aedes albopictus* were 1.77 and 1.77 in Kathmandu district and 1.82 and 1.69 (discarded tire), 0.23 (metal drum), and 0.67 (metal container) in Lalitpur district respectively (table 9 & 10). BPR for *Aedes aegypti* in Kathmandu district was 1.74, for *Aedes albopictus* was 1.74 and in Lalitpur district 1.55 for *Aedes aegypti* and 1.48 (discarded tire) and 0.20 (metal drum) in October, 2009 (table 11 & 12). The highest BPR recorded for *Aedes aegypti* and *Aedes albopictus* in Kathmandu district were 1.89 and 1.89 in August, 2009. Similarly, the highest BPR recorded for *Aedes aegypti* and *Aedes albopictus* in Lalitpur district were 1.69. Monthly distribution of *Aedes aegypti* larvae in Kathmandu valley are shown in figure 7 and 8.

Figure 7. Monthly distribution of *Aedes aegypti* larvae in Kathmandu valley

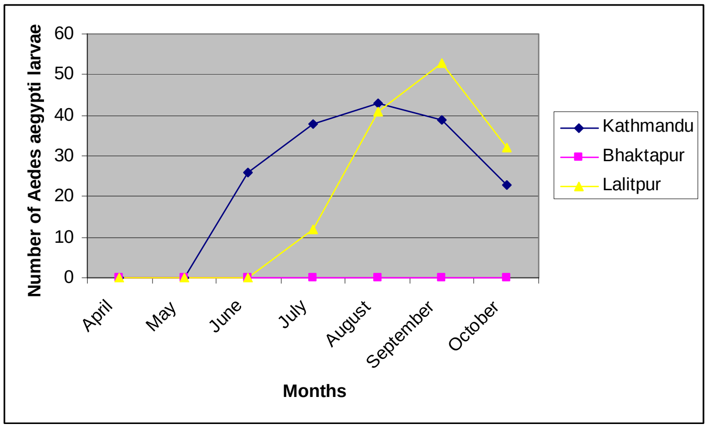
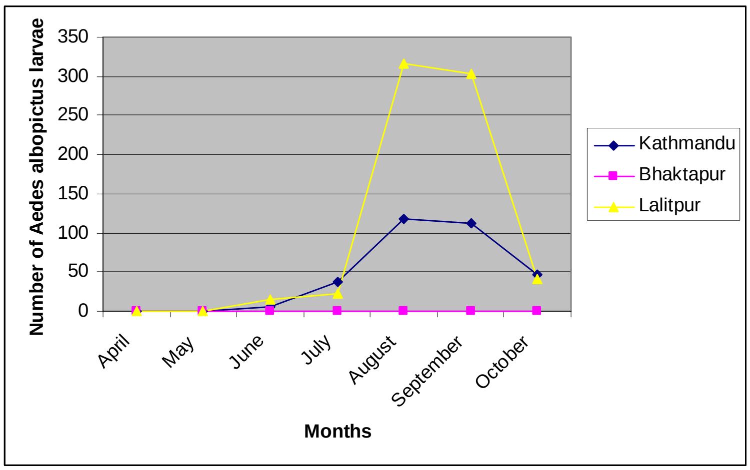


Figure 8. Monthly distribution of *Aedes albopictus* larvae in Kathmandu valley



Among all the habitats, the maximum positivity of *Aedes* larvae was recorded in discarded tires. It was also observed that discarded tires lying outdoors were the preferred breeding habitats. Although, *Aedes albopictus* larvae were found in Mangal bazar in metal drum and metal container in of Lalitpur district. No breeding of *Aedes aegypti* was observed in other containers during this seven months survey. Among all the habitats, the maximum positivity of *Aedes* larvae was recorded in discarded tires.

There has been a significant increase in the human population, demographic and vehicular movement of the people and accommodation-based tourism facilities in Nepal. These changes can have a great impact on the pvevalence of *Aedes* mosquitoes, by creating larval breeding habitats for dengue mosquitoes. From the present entomological investigations, it can be concluded that *Aedes aegypti* and *Ae. albopictus* are established within the urban agglomeration of Basundhara, Gongabu-Balaju area of Kathmadu district and Satdobato-Gwarko, Thalchikhel, Mahalaxmithannad Sanepa area in Lalitpur district showing larval indices.

CHAPTER 5

5.0 Discussion

Main larval habitats:

It is well known that *Aedes aegypti* and *Aedes albopictus* serve as dengue vectors. Larvae of these two species were found in clear and clean water in all types of artificial and natural containers (Rattanarithikul and Panthusiri 1994). Nine major container types searched in this study were 1) discarded tires-- unused tires; 2) metal /plastic drums--used for the domestic storage of water/rainwater ; 3) plastic buckets--used for domestic water usage; 4) flower pots; 5) mud pots--for domestic water usage; 6) cement tank-- used for domestic water usage; 7) metal containers; 8) and plastic pots and miscellaneous small, discarded items such as tin cans, jars and plastic food containers.

Some studies showed that there is an association between rainfall and dengue outbreak. In Selangor, Malaysia, a monthly rainfall of 300 mm or more would cause a major dengue outbreak in the state after a lag period of about two to three months (Foo *et al.* 1985). Abundance of *Aedes albopictus* was significantly correlated with rainfall four weeks before collection dates using ovitraps (Lourenco-de-Oliveira *et al.* 2004). In Malaysia, the epidemics of dengue from 1973-1982 were related to the two monsoons: SW Monsoon in the first half, and NE Monsoon in the second half of the year (Lo and Narimah 1984). Endemicity was low during the period from January-April, when it began to rise, reaching a peak in July/August, and then declined. This variation was related to the storage of water during the drought season from January-April, and the drizzling rainfall before the heavy monsoons, which all created a suitable breeding places for the vector (Lo and Narimah 1984). There are some other studies which reported the use of rainfall data to predict malaria epidemics. Mouchet *et al.* (1998) pointed out that temperature and rainfall influence malaria transmission; the former determined the length of the larval mosquito cycle and the sporogonic cycle of the parasite in the mosquito; the latter determined the number and productivity of breeding sites and consequently the vector density. Rainfall in highland areas has been associated with entomologic transmission parameters (Lindblade *et al.* 1999) and malaria incidence (Fontaine *et al.* 1961, Mouchet *et al.* 1998), and it has been suggested that monitoring rainfall levels could provide sufficiently early warning of highland malaria epidemics. However, elevated temperatures may interact with rainfall to create epidemic conditions (Lindblade *et al.* 1999), and this interaction must be investigated further before rainfall alone can be used as a suitable indicator of epidemic risk.

In this study, populations of *Aedes aegypti* and *Aedes albopictus* in some parts of the valley during the survey from in June to October was found, indicating that drought could interrupt *Aedes* abundance. April and May were the dry seasons and monsoon was confined in northeastern states of India till 22nd June and entered east Nepal only on 23rd June, delayed by 13 days. The monsoon onset was weak and advanced gradually towards western Nepal and covered the entire country by 27th June and became strong only during the end of the month. The monsoon arrived in the southern tip of India in Kerala on 23rd May, 9 days earlier than the normal onset date of 1 June. The Bay of Bengal part of the monsoon progressed very quickly and covered the northeastern parts of India by 25th May. However, the monsoon then weakened and stalled there till 22nd June and entered Nepal as a weak surge only on 23rd June. Some scattered rainfall was recorded during the first and the third week of the month due to the westerlies and the local convection. With the onset of the monsoon in the 4th week the country received some amount of rainfall. Due to the drier condition, large parts of the country remained warmer than normal in June (table 37). After a little rain in a few days, the *Aedes* mosquito population began to expand. This brief emergence of perhaps a week can be from old eggs rather than from new matings. Shortage of water supply during the dry season can increase the number of storage containers in the community but could not created larval breeding habitats. This study indicated that *Aedes aegypti* and *Aedes albopictus* preferred to breed primarily in discarded tires than different water storage containers, as well as other man-made artificial containers. In other words, it has been noted that, for survival, *Aedes* mosquitos tend to breed in discarded tires, in the wet season.

Aedes aegypti is a tropical and subtropical species found usually within the limits of 35° North and 35° South latitudes; the range traditionally declared as the borderline for the *Aedes aegypti* was 1200 meters above sea level. This range has been surmounted by the mosquito and one outbreak at 1,760 meters was detected in Mexico (Watts *et al.* 1987). Its establishment at higher levels than 1,200 meters is not a rare finding. The habitat conditions for the vector vary at sea level and at higher altitudes since weather conditions are more stable on the lowlands, the mean annual temperature is higher and and more stable and rainfall may be heavier than at the higher altitudes. Mean annual temperature has a direct influence over the duration of the life cycle and especially on the duration of the extrinsic incubation period, a vital factor in the vectorial capacity of *Aedes aegypti* (Watts *et al.* 1987). The rainfall is another parameter that is deeply involved in the bionomics of the vector. When heavy it provides abundant sites for oviposition, besides it also guarantees that the water surfaces will stay longer on the environment if not on a steady basis. In those months with higher rainfall we would expect larger vector population that are kept for longer periods and therefore transmission may happen any time during the year and when it does it would tend to stay on an endemic fashion. It is expected then that at higher rainfall and higher mean annual temperature, the vector will be in better conditions for survival, reproduction and viral replication . But transmission is also tightly related to the host population. Its distribution, number, level of organization, contacts with other populations (migratory movements) and their behaviour have also direct influences in the chances of contact with the vector and the persistence of viral transmission. The increasing tendency of urbanization would be one factor that enhances the importance of dengue as a public health problem. These urban settlements are now important centers where industrial, commercial, medical services and employment are concentrated, therefore people come and go from this areas acting as dispersal or attraction areas for dengue transmission. This situation creates very favourable conditions for dengue because migration towards these places brings in large groups of susceptibles and favours the introduction of viremic host looking for medical care. As part of the process of integration to an urban environment vehicles are increasing in Kathmandu valley where non recycling products such as discarded/unused tires are intensive, favouring the number and disperson of potential breeding sites. Therefore, uniquely, discarded tires were the principal source of breeding of both *Aedes* species found in Kathmandu and Lalitpur. Affinity to discarded tires as breeding habitat of *Aedes albopictus* is well known (Hawley 1988). Noteworthy is the low larval numbers of *Aedes albopictus* in metal drums. Only 1 metal drum and metal container was found to be positive for *Aedes albopictus* in this study, but this low infestation rate is most likely because these containers were in almost daily domestic use. As *Aedes aegypti* in all areas of the world breeds mainly in man-made larval habitats (Gratz 1991), the ideal method of control would be reduce the numbers of larval habitats to a point where the density of emerging adult populations would be too low for transmission of disease to occur. *Aedes* larval densities have been monitored since time immemorial using the House, Container and Breteau Indices. Therefore, the identification of high risk areas using these indices within every urban centre is of paramount importance. The source reduction activities should be at their lowest during June to prevent indices from going high. A concentrated effort at the time of the year when indices are increasing or lowest may more impact than a concentrated effort at the time of year when they are highest. If people keep discarded tires, it would seem reasonable to aim at organizing clean-up campaigns during April, May or June immediately preceding the rains. Input from community members is needed here. It is undisputed fact that it is necessary to know which larval habitats predominate in the area to select control strategies. *Aedes aegypti* and *Aedes albopictus* found to be prefer to breed primarily in discarded tires lying outdoor in Kathmandu and Lalitpur district but not even single container found to be positive in Bhaktapur district during this seven months survey. Since the importance of any particular larval habitat is country or perhaps even region specific, a source reduction campaign for example will have a different impact from one country or area to another.

Dengue is a disease linked to the environment and, above all, to domestic habits of the population. Continued and increased urbanization and vehicular movement and use in Kathmandu valley have been resulted in increased amount of non-biodegradable objects (tires). The accumulation of all sorts of discarded or unused tires in dwellings has resulted in occurrence of *Aedes aegypti* and *Aedes albopictus.* As a result, crowded urban human populations live in intimate contact with large mosquito populations, thus creating ideal conditions for increased mosquito-borne disease transmission. Finally, increased and more rapid travel possibly lead to increased movement of dengue viruses within the area.

Implications for integrated community-based strategies:

Previous studies have reported results regarding the effects of community knowledge on dengue prevention practices. Several studies have shown that a higher socio-economic status correlates with better knowledge scores (Kubik *et al.* 2004; McArthur *et al.* 2001; Potvin *et al.* 2000). Some studies have shown that dengue knowledge was associated with an increased use of preventive measures against the disease (Swaddiwudhipong *et al.* 1992; Van Benthem *et al.* 2002) and a reduced number of development sites for vector larvae (Chiaravalloti *et al.* 2003). Other studies found a significant reduction in the *Ae. aegypti* infestation index after community-based prevention campaigns (Sanchez *et al*. 2005; Lloyd *et al.* 1992; Clark *et al.* 1992; Fernandez *et al.* 1993).

The realistic objectives of vector control programs in the foreseeable future must be to reduce infestation levels to an extent that the risk of dengue transmission is eliminated or at least minimized. Because of limited resources, Nepal is experiencing difficulty in providing an adequate and continuous"protective insecticidal shield" for their populace. Seasonal variation in community-based *Aedes* control programmes, a more central concern may be seasonal variation in household container habitats. Alternative strategies are needed which can be implemented through community participation and which compliment the efforts of the vector control teams.

Financing disease control depends on political will. Partially, this will depends on better educated and awared populations that are attaining a higher culture that allows them to demand a better standard of living such as clean environment. The better integrated clean communities do not have the large mosquito populations that container communities do. The matter of container productivity of adult mosquitoes has not been addressed in this study. Nevertheless, the above determination of potential larval habitats of *Aedes* mosquitoes in Kathmandu valley can provide some orientation for focusing control efforts on the management or elimination of the vector's most common habitats. The selection of the most appropriate and cost-efficient strategies for this management can be further guided by consideration of the main container categories in terms of their infestation. For those categories considered by the community or householder, removal of discarded tires may be an acceptable option. Possible breeding sources such as drums, buckets etc. provide a means of storing potable water in communities where there is an inadequate pipe-borne supply or no supply at all. In dealing with rainwater drums the options include provision of house connections from a central water supply. The physical exclusion of mosquitoes could be through the use of protective covers; the introduction of larvivorous fish or other biological control agents such as predatory copepods; or the use of appropriate insecticides. Although miscellanous small containers, the by product of an increasingly materialistic, consumer-and import-oriented urban Nepali society, may be the potential larval habitats. There are numerous other potential larval habitats which are the discarded products of contemporary life-styles; they no longer fulfil a useful purpose in the peridomestic environment. These can be targeted for removal or destruction. However, in managing limited resources for vector control, including the vital educational component that will provide the basis for any effective and suatainable community action, consideration should be given to the efforts and rewards of a particular action. In the case of old or discarded tires, because they are preferred oviposition sites, the relative ease with which they can be removed from the domestic environment and their relative rate of accumulation suggests that a clean-up campaign strategy would make a significant impact on vector abundance. Although the considerable differences do not exist among localities, depending on wealth, economy, basic sanitation services and various other factors, within each district a thorough understanding of the infestation characteristics is needed. In this instance, the considerable investment in time, effort and money required to conduct clean-up campaigns, and the continuing rapid accumulation of these containers, would yield short-term and minimal gains. An indirect, long-term option in this case would be to strengthen or establish solid waste disposal services.

Since acceptable dengue vaccine for mass use is unavailable, efforts to prevent dengue hemorrhagic fever rely mainly on anti-vector programs that require the continuous participation of people in the community. It is known that *Aedes aegypti* and *Aedes albopictus* serve as dengue vectors. Larvae of these two species were found in clear and clean water in all types of artificial and natural containers (Rattanarithikul and Panthusiri, 1994). Although these two species have great epidemiological importance, there have been no published studies in Kathmandu valley focusing on larval breeding habitats and larval distribution and the distribution as well as *Aedes* larval indices during the wet and dry season. This study describes the vector's larval habitats in a Nepali context and as a result of visits to limited numbers of localities and inadequate period (only seven months), in each district and do not reflect a complete figure regarding the seasonal abundance of *Aedes aegypti* and *Aedes albopictus*. To facilitate the vector control program in the country, further studies on seasonal distribution and larval breeding habitats of these mosquitoes are urgently needed.

In the study areas larval indices did not develop until June. In such areas the occurrence and prevalence of *Aedes aegypti* and *Ae. albopictus* may vary from month to month depending on wheather a districtwise subsequent study will be needed to more accurately identify the priority larval habitats within each community or group of communities before deciding on the most appropriate means of control. It must be noted that continuity of this study is necessary. For detail study of breeding habitats and seasonal abundance in the larval density of *Aedes aegypti* and *Ae. albopictus* in Kathmandu valley, seven months program is not adequate. One year complete study is therefore recommended. As vector control is the only way of interrupting by reducing transmission of the disease for the time being, the methods of attacking the larval or adult stages to ensure a rapid, sustained, high level of reduction of adult, female populations prior to or at the time of an out break or epidemic must be reviewed, taking into account experience under different ecological and socioeconomic conditions. If a significant fraction of all potential *Ae. aegypti* breeding sites can be destroyed, removed or effectively controlled, whether by episodic community-wide programmes or quite, sustained personal efforts, such activities should reduce the cost of public sector programmes. Messages which inform people about the biology of *Aedes aegypti* and the role that this mosquito plays in transmitting dengue virus may motivate people to take actions to reduce or control main types of mosquito breeding sites.

CHAPTER 6

6.0 Conclusion and Recommendation

6.1 Conclusion

All over the world, the *Aedes* mosquito population is probably higher today than ever before, because nearly all consumer goods are now packed in non-biodegradable plastics or tin containers and refuse collection services and waste disposal practices are grossly deficient. On the other hand, the proliferation of drums and tyres increases the population of *Aedes aegypti* and hence the risk of dengue fever and dengue shock syndrome in towns and cities. Environmental, socioeconomic, political and social factors have a strong impact on dengue, and are associated with the re-emergence of dengue as a serious issue. Climate change and ecosystem alterations have provided ideal conditions for expanding the geographical distribution of pathogens and vectors, and increases in migration and international traffic favour the spread of the vector and the disease. The world's population has tripled in the last 70 years is also contributing to increasing the number of vector breeding sites. Also, the presence of dengue in large urban centres, and especially in ‘megacities’, associated with urbanization that is neither planned nor controlled and poverty, with the absence of basic services (electricity, running water, sewer systems, refuse collection), poses new challenges and requirements for prevention activities and control. Such activities are expensive and require great coordination and synchronization, and the incorporation of extrasectorial actors, such as the tire industry.

In Kathmandu valley, squatter settlements are growing in peri-urban areas and housing problems are becoming acute. Water-supply and waste-management systems are poor in the valley; this facilitates vector proliferation and persistence. The high presence of containers that can contain water and that are not biodegradable also facilitates vector persistence, because these containers remain for long periods in the environment and must be eliminated properly by man. In other words, higher urban population densities, urban water storage practices and poor or non-existent mechanisms for waste water and solid waste disposal create numerous breeding sites for vectors. In the study unused tires remain a significant factor for oviposition, as there are no specific or regulated methods employed to eliminate them. The population normally discards them outside the houses. The population does not cover drums with plastic or nylon sheeting to prevent oviposition. Therefore, regular visits of health inspectors and health awareness would create considerable awareness among populations to be more cautious about avoiding oviposition by mosquitoes. The intervention by the health officials in association with community participation would reduce the wet and dry containers.

The *Aedes aegypti* control programme is needed to make people aware of the threat that dengue haemorrhagic fever poses to their health, and to educate them on how they can reduce this threat by either eliminating the discarded tires/domestic containers which harbour the mosquito larvae or by preventing mosquitoes from having access to water-holding containers for egg-laying that are used in or around houses. Automobile tires discarded, stored or used around people’s homes provide perfect larval habitats for the dengue mosquito. It should be noted that water storage often occurs in the presence of piped water systems because of intermittent water supply and collected rainwater. Houseowners are legally required to prevent mosquito breeding in Nepal. For instance, in

Fiji, the numbers of drums breeding *Aedes aegypti* were reduced after community consultation and intervention messages. Therefore, to mobilize communities, campaigns must address community concerns. In addition, health inspectors must demonstrate the need to eliminate discarded tires properly and cover drums to prevent mosquito breeding, and they must also help communities to manage and destroy discarded tires. Targeted source reduction is one way of selectively attacking the most important types of containers. Dengue control programmes must include detail surveillance of mosquito populations to assess the effectiveness of source reduction campaigns, and targeted mosquito control measures. The success of any prevention programme depends on either convincing individuals to change their behaviour or changing the environment to remove factors that place individuals at risk of disease.

For dengue control activities, community education programmes may not be sufficient to generate sustainable behavioural change, unless other factors are taken into consideration as part of the overall strategy. In Kathmandu and Bhaktapur districts, management of discarded tires/water containers would be a key requirement for the control of larval production sites. Because of water shortage problems, there is a need to design appropriate covers or biological control methods for water storage containers as an important component of a sustainable control programme. If community participation is viewed as a means to shift responsibility and costs from the government to residents without the provision of adequate services to support residents in their ability to carry out the recommended control measures, the prospects for sustainability may not be realistic.

6.2 Recommendations

Dengue poses as much of an epidemiological challenge as it does a challenge to good governance and public administration. An epidemic is largely a failure in public health administration. No country has been able to eliminate this dangerous disease. However it is possible to keep it under control.

(1) *Aedes* populations can be controlled either by disposing of the containers which are their larval habitats - ‘source reduction’ - by applying larvicides to the containers or by space sprays to control adult mosquitoes. The valley needs to destroy all the spots where *Aedes* mosquitoes breed, primarily discarded tires that hold water.

(2) The enormous number of containers in urban areas in which *Ae. aegypti* may develop, makes control by larviciding costly and difficult. Even if done very carefully, larviciding will have only a delayed effect on the densities of the adult vector populations and would thus not be a satisfactory means of controlling an epidemic outbreak of DHF. Furthermore, there is a declining acceptance by inhabitants of the application of chemical larvicides to household water containers. So, items that collect rainwater or that are used to store water which may be conducive for breeding of the mosquitoes (containers, drums, buckets, or used automobile tires) should be covered. This will eliminate the mosquito eggs and larvae and reduce the number of mosquitoes present in these areas.

(3) For travelers to areas with dengue, as well as people living in areas with dengue, the risk of being bitten by mosquitoes indoors is reduced by utilization of windows and doors that are screened.

(4) Anti-*Aedes* control measures be integrated into a general mosquito control program with cooperation at regional and national levels. This approach best suits the expectations and needs of the population for an improvement in their quality of life. It also corresponds to the expectations of political decision-makers concerned about ensuring sustainable development for the populations they represent.

(5) The training of staff involved in mosquito control be stressed. Given the limited range of active insecticides, the increase in resistance and the importance of preserving zones where mosquitos have been controlled or will be controlled, all mosquito control requires competent and motivated staff, capable of designing, implementing, and evaluating the integrated control that best responds to the circumstances.

(6) Environmental management that promotes the elimination of vector breeding sites should be a priority in control programmes. Programmes that involve the creation of

strategic partnerships should include intersectoral participation of public and private corporations with a strong component of community participation, as well as participation of different ministries and institutions with a greater direct relationship to the various components that lead to continued dengue transmission (e.g. ministry of health, of protection of the environment, of finance, of construction, of transportation, of sports), universities, nongovernmental organizations, importers of tires, tire repair shops, municipal government, among others. There could also be partnerships between the ministries of health and education, promoting dengue prevention during the teaching process among elementary-school students. These partnerships can be promoted by the state, through the promulgation and implementation of laws that serve as a framework. For example, Puerto Rico, the United States of America, Spain, Costa Rica, Israel and Brazil have established decrees or laws for the adequate control and management of used tires-the habitual breeding site of the vector in many countries and for which few or no adequate mechanisms exist for final disposal. The tire-recycling industry can be established which can employ the people directly, and can involve few companies and factories. Municipalities can promote tire recycling. Some models of use of discarded tires can be applied in the construction of athletic fields, as roofing materials, vibration insulation and carpets etc.

(7) Local clubs can have active role. The networking of such clubs can link actions to various institutions of the community, visualizing an improvement of the quality of life. Such clubs can promote actions in the health-environment axis, such as strategies for the rational use of water, dengue prevention, and waste management, among other topics. Social mobilization is a broad scale movement to engage people's participation in achieving a specific developmental goal through self-reliant efforts. It involves all relevant segments of the society. With sensitization campaigns coordinated with other institutions and communities, clubs can involve neighbours via the use of participatory strategies and actions in the implementation of programmes. Many groups with diverse interests interact: the private sector, civil society, municipal authorities, different ethnic groups, castes, and social classes, men and women. All can play a role in the management of the urban ecosystem. Large budgets are not necessarily needed to implement community programmes for the prevention and control of dengue; it is this

philosophy, including different social actors for a common cause that clubs can promote. But management guidance is needed and this is a role that should be played by health workers.

(8) Community participation, often indispensable, is real and based on systematic feedback, the results of which are automatically taken into account in the design of education campaigns and actions in the field. Preventing epidemic disease requires a coordinated community effort to increase awareness about dengue, how to recognize it, and how to control the mosquito that transmits it. A campaign for creating awareness, no doubt, is helpful to eradicate *Aedes* mosquitoes from the valley and elsewhere in the country. For example a public awareness raising campaign should start over radio and television (also through the electronic, print and other media). Residents are responsible for keeping their yards and patios free of sites where mosquitoes can be produced.

(9) The new generation of programmes should be designed taking into account the local sanitation structure (water distribution and waste disposal) as well as information on community organizations and the roles of different family members. Well-targeted detail study is urgently needed to make progress in dengue prevention and control.

(10) Last, but not the least, we all need to be united to wrestle against this deadly disease.

CHAPTER 7

7.0 References

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Annex

Table 11. Daily Temperature, Rainfall and Relative Humidity During the Study in April, 2009

Date	Max. temp. (ºC)	Min. temp. (ºC)	24 hrs Rainfall (mm)	Max. Relative Humidity (%)	Min. Relative Humidity (%)
2009-04-01	26.8	10.5	5.8	98	48
2009-04-02	27.8	13.0	trace	95	50
2009-04-03	29.2	13.3	0	97	46
2009-04-04	30	7.9	0	96	42
2009-04-05	30	13.7	0	96	47
2009-04-06	29.2	13.6	0	94	50
2009-04-07	29.9	13.4	0	95	27
2009-04-08	30.4	12	0	84	30
2009-04-09	30.6	14.2	0	80	35
2009-04-10	29.3	10.3	0	83	33
2009-04-11	30.3	10.2	0	94	32
2009-04-12	30.9	11.9	0	80	35
2009-04-13	30.8	13.6	0	80	37
2009-04-14	31.8	16.5	0	75	47
2009-04-15	32.4	16	0	86	38
2009-04-16	33.7	16.2	0	95	38
2009-04-17	33.6	14.3	trace	97	26
2009-04-18	32.5	11.7	5.5	89	42
2009-04-19	31.4	13.2	0	80	36
2009-04-20	31.6	16	0	84	40
2009-04-21	32.8	15.8	0	98	38
2009-04-22	33.4	14.2	0	90	24
2009-04-23	33.2	34.4	0	78	26
2009-04-24	35.2	12.4	0	69	20
2009-04-25	33.2	11.5	0	64	23
2009-04-26	32.9	13.3	0	78	34
2009-04-27	33.2	12.5	0	75	28
2009-04-28	32	13.2	0	70	27
2009-04-29	32	13.8	0	74	31
2009-04-30	31.4	16.4	0	89	47
Average	31.38	13.96	0.40	84.64	34.96

Table 12. Daily Temperature, Rainfall and Relative Humidity During the Study in May, 2009

Date	Max. temp. (ºC)	Min. temp. (ºC)	24 hrs Rainfall (mm)	Max. Relative Humidity (%)	Min. Relative Humidity (%)
2009-05-01	31.1	16	trace	86	64
2009-05-02	33	19.3	0	90	49
2009-05-03	29.8	14.3	13	90	44
2009-05-04	30	15	11.5	95	52
2009-05-05	31.4	15	0	96	35
2009-05-06	31.4	13	1.4	93	28
2009-05-07	33	14.2	0	87	30
2009-05-08	33.5	15	0	74	28
2009-05-09	33.1	14	0	85	27
2009-05-10	32.1	15.3	trace	80	40
2009-05-11	26.2	14.7	17.1	96	42
2009-04-12	27.5	14	7.1	98	46
2009-05-13	25.7	12.6	10.2	98	83
2009-05-14	29	12.2	0	98	52
2009-05-15	27.4	14.6	1.6	96	52
2009-05-16	28.2	15.3	0	95	45
2009-04-17	28.6	17.4	trace	95	53
2009-05-18	29.9	17.4	0	89	56
2009-05-19	32.8	18.4	0	90	52
2009-05-20	29	19.9	trace	88	50
2009-05-21	30.4	18	6.5	89	84
2009-05-22	27.5	19	0.5	90	62
2009-05-23	28	18	0	90	48
2009-05-24	29.9	16.6	1	98	65
2009-05-25	23.5	18.6	5.7	95	88
2009-05-26	23.4	17	0	98	79
2009-05-27	27.7	17	0	98	61
2009-05-28	28.2	16.6	1	95	61
2009-04-29	27.6	15.5	0	96	56
2009-05-30	28.8	17.6	1	90	62
2009-05-31	23.6	16.5	1	95	65
Average	29.0	16.06	2.91	92.23	53.16

Table 11. Daily Temperature, Rainfall and Relative Humidity During the Study in June, 2009

Date	Max. temp. (ºC)	Min. temp. (ºC)	24 hrs Rainfall (mm)	Max. Relative Humidity (%)	Min. Relative Humidity (%)
2009-06-01	28.2	16.3	1	98	61
2009-06-02	30.2	16.2	trace	96	59
2009-06-03	28.7	17	4	96	86
2009-06-04	28.8	18.4	trace	96	65
2009-06-05	29	19.9	25.5	93	70
2009-06-06	28.5	18.6	2.7	90	68
2009-06-07	31.5	17	0	97	52
2009-06-08	32.2	17.7	0	92	47
2009-06-09	31.8	16.6	0	90	34
2009-06-10	31.9	15.8	0	90	40
2009-06-11	31.7	18	0	88	47
2009-06-12	31.2	19	5.2	96	62
2009-06-13	30.7	19.0	0	94	53
2009-06-14	31.5	18.5	0	94	48
2009-06-15	32	18.5	0	90	46
2009-06-16	32.6	19.5	0	90	45
2009-06-17	33.6	18.8	0	95	49
2009-04-18	33.1	18	0	94	46
2009-06-19	32.1	19	trace	90	66
2009-06-20	34.2	19.3	0	90	66
2009-06-21	33.5	21.4	0	84	60
2009-04-22	31.7	20.1	20	_	_
2009-06-23	28	20.3	trace	94	80
2009-06-24	29.8	20.3	0	92	62
2009-06-25	31.5	20.2	32.7	94	70
2009-06-26	29.8	21.2	14.6	96	63
2009-06-27	26.4	20.6	11.5	96	76
2009-06-28	25.1	21	9.5	96	77
2009-06-29	30.2	20.4	0	95	71
2009-06-30	27.4	20.4	13	98	88
Average	30.64	19.08	5.37	93.07	60.57

Table 14. Daily Temperature, Rainfall and Relative Humidity During the Study in July, 2009

Date	Max. temp. (ºC)	Min. temp. (ºC)	24 hrs Rainfall (mm)	Max. Relative Humidity (%)	Min. Relative Humidity (%)
2009-07-01	26	20.2	43.3	96	85
2009-07-02	29.5	20	3.9	94	57
2009-07-03	30	20.2	0	94	75
2009-07-04	30.3	20.3	20.3	59	44
2009-07-05	30.3	19.5	7.2	95	79
2009-07-06	30.3	20.5	9.1	96	70
2009-04-07	30.5	21	76.1	93	63
2009-07-08	31.4	21.4	2.8	97	77
2009-07-09	31.6	21	0	96	67
2009-07-10	31	20.6	0	93	52
2009-04-11	29.2	22	4.5	95	90
2009-07-12	25.3	20	5.2	96	83
2009-07-13	30.4	20	18.8	96	64
2009-07-14	31.4	20.6	0	94	78
2009-07-15	31.4	21.3	7.6	98	65
2009-07-16	30.4	21.4	0.2	96	74
2009-07-17	31.2	20.5	0	95	68
2009-07-18	31.4	21.8	trace	91	67
2009-07-19	31.4	20.5	14.4	97	63
2009-07-20	31.8	20.2	8.1	94	74
2009-07-21	31	21	0.6	96	66
2009-07-22	30.2	20.6	2.1	96	66
2009-07-23	30.4	20	1.2	98	66
2009-07-24	29.6	22	trace	98	68
2009-07-25	31.5	20.6	0	96	62
2009-07-26	28.3	21.4	14.9	96	85
2009-07-27	25.1	20.8	99	96	85
2009-07-28	28	19.6	33.8	98	76
2009-07-29	25.6	20.5	3.1	98	86
2009-07-30	29.6	20.6	7.9	96	64
2009-07-31	29.5	20.4	26.5	96	70
Average	29.92	20.67	14.15	94.43	70.13

Table 15. Daily Temperature, Rainfall and Relative Humidity During the Study in August, 2009

Date	Max. temp. (ºC)	Min. temp. (ºC)	24 hrs Rainfall (mm)	Max. Relative Humidity (%)	Min. Relative Humidity (%)
2009-08-01	29.5	20.3	7.7	96	68
2009-08-02	31.1	21.4	0	96	64
2009-08-03	31.6	21.0	0	96	75
2009-08-04	30.2	21.0	0	93	70
2009-08-05	29.6	22.0	3.2	96	75
2009-08-06	27.6	21.5	8.3	89	88
2009-08-07	27.6	20.2	21.8	98	74
2009-08-08	30.5	21.6	0	96	77
2009-08-09	30.9	20.6	0.2	96	68
2009-08-10	28.3	21.5	1.7	96	80
2009-08-11	30.8	21.0	21.3	98	64
2009-08-12	29.6	21.5	14.4	95	67
2009-08-13	29.6	21.7	58.7	94	73
2009-08-14	26.0	21.0	6.9	93	84
2009-08-15	29.3	20.0	5.2	98	76
2009-08-16	27.2	20.5	3.2	98	78
2009-08-17	26.5	20.0	59.6	-	-
2009-08-18	27.8	20.5	16.9	96	90
2009-08-19	30.1	20.0	0.4	96	69
2009-08-20	27.3	20.5	19.1	92	75
2009-08-21	30.2	19.2	6.6	95	65
2009-08-22	31.0	18.8	0	97	58
2009-08-23	27.5	20.2	8.0	95	74
2009-08-24	25.0	20.5	28.8	96	79
2009-08-25	28.5	19.4	0	96	69
2009-08-26	28.6	19.0	0	96	69
2009-08-27	29.8	20.0	28.3	98	68
2009-05-28	29.8	19.7	2.5	97	66
2009-08-29	31.3	20.7	0	95	61
2009-08-30	29.8	20.2	15.5	98	65
2009-08-31	30.3	20.5	0.3	84	64
Average	29.12	20.51	11.43	92.25	69.54

Table 16. Daily Temperature, Rainfall and Relative Humidity During the Study in September, 2009

Date	Max. temp. (ºC)	Min. temp. (ºC)	24 hrs Rainfall (mm)	Max. Relative Humidity (%)	Min. Relative Humidity (%)
2009-09-01	30.4	21.8	15.8	96	63
2009-09-02	29.6	18.5	11.8	96	74
2009-09-03	28.4	19.5	0	98	75
2009-09-04	29.5	19.5	3.0	94	72
2009-09-05	29.0	18.9	2.6	96	62
2009-09-06	26.4	18.4	15.7	96	70
2009-09-07	27.3	19.3	7.0	96	71
2009-09-08	27.7	19.7	0.4	98	73
2009-09-09	26	18.9	1.9	98	73
2009-09-10	26.5	18.6	1.3	95	72
2009-09-11	29.5	17.2	8.4	98	61
2009-09-12	30.2	17.2	0	96	55
2009-09-13	30.6	18.5	0	96	60
2009-09-14	30.0	20.0	traces	96	68
2009-09-15	29.5	20.2	0	94	68
2009-09-16	30.0	18.8	0	96	63
2009-09-17	30.3	18.4	0	96	55
2009-09-18	29.8	20.0	0	96	72
2009-09-19	29.1	17.8	0	94	65
2009-09-20	30.5	17.8	traces	94	65
2009-05-21	28.4	19.0	0	96	68
2009-09-22	30.0	17.9	0	95	53
2009-09-23	29.5	18.1	0	96	58
2009-09-24	29.4	18.3	27.4	96	64
2009-09-25	28.8	18.9	9.8	95	72
2009-09-26	30.5	17.6	0.5	95	60
2009-09-27	31.0	18.5	0	95	63
2009-09-28	31.0	17.6	0	94	63
2009-09-29	30.2	18.0	1.2	98	68
2009-09-30	31.2	16.4	0.7	96	60
Average	29.34	18.64	3.58	95.83	65.16

Table 17. Daily Temperature, Rainfall and Relative Humidity During the Study in October, 2009

Date	Max. temp. (ºC)	Min. temp. (ºC)	24 hrs Rainfall (mm)	Max. Relative Humidity (%)	Min. Relative Humidity (%)
2009-10-01	29.8	17.6	8.0	96	66
2009-10-02	29.6	16.6	0	98	61
2009-10-03	25.7	19.2	0.3	96	88
2009-10-04	28.4	17.4	1.7	99	79
2009-10-05	27.0	18.4	2.5	96	70
2009-10-06	26.0	19.4	3.2	97	84
2009-10-07	24.2	19.3	5.3	98	74
2009-10-08	24.6	17.7	45.1	98	59
2009-10-09	29.0	17.7	0	98	59
2009-10-10	28.5	15.2	0	98	50
2009-10-11	27.5	15.0	0	98	56
2009-10-12	26.0	15.0	traces	92	54
2009-10-13	28.1	13.0	0	98	57
2009-10-14	28.7	14.0	0	98	50
2009-10-15	29.0	13.8	0	95	50
2009-10-16	28.3	13.8	0	95	48
2009-10-17	29.0	14.8	0	98	47
2009-10-18	29.0	13.4	0	98	48
2009-10-19	27.7	13.0	0	96	50
2009-10-20	27.8	12.0	0	96	50
2009-10-21	27.4	12.2	0	98	56
2009-10-22	27.6	13.0	0	99	49
2009-10-23	26.1	11.1	0	98	54
2009-10-24	26.6	10.5	0	96	50
2009-10-25	26.9	11.5	0	98	51
2009-10-26	26.4	12.0	0	98	48
2009-10-27	25.9	11.6	0	98	60
2009-10-28	26.8	13.1	0	94	48
2009-10-29	26.7	12.5	0	98	56
2009-10-30	26.8	11.5	0	97	48
2009-10-31	27.3	11.5	0	98	47
Average	27.36	14.41	2.13	97.06	37.48

Breeding Habitats of *Aedes aegypti* and *Aedes albopictus*

Breeding Habitats of *Aedes aegypti* and *Aedes albopictus*

Breeding Habitats of *Aedes aegypti* and *Aedes albopictus*