

CONTAINER PREFERENCE OF THE ASIAN TIGER MOSQUITO (*Aedes albopictus*) IN KATHMANDU AND LALITPUR DISTRICTS OF NEPAL

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ABSTRACT

In various countries the Asian Tiger Mosquito (*Aedes albopictus*) is a known vector of dengue and chikungunya viruses and other pathogens, but its ecology and role in disease transmission in Nepal has not been studied yet. Here, we report on an investigation of the seasonal distribution of potential artificial breeding habitats of *A. albopictus* in urban areas of the Kathmandu and Lalitpur districts of Nepal. Larval collections were performed from April 2009 to March 2010 simultaneously in all the wet containers present in and around the houses of the study areas. Altogether 1873 water containers in Kathmandu district and 1807 in Lalitpur district were searched in 12 months, out of which 107 (5.7%) and 129 (7.1%), respectively, were found positive for *A. albopictus* breeding. Within the urban agglomeration of both districts, the breeding was found to vary between localities. Immature stages of *A. albopictus* were recorded in pre-monsoon, monsoon and post-monsoon seasons as 1.9%, 6.7% and 5.6%, respectively, in wet containers in Kathmandu district. In Lalitpur district, *A. albopictus* larvae were detected in 4.6%, 7.6% and 7.1% of the wet containers in the pre-monsoon, monsoon and post-monsoon, respectively. The breeding preference ratio during all seasons was highest for discarded tires lying outdoors in both Kathmandu and Lalitpur districts. Among nine container types searched and examined, 95% of discarded tires were found positive for *A. albopictus* larvae and pupae, followed by metal drums (2%) and plastic drums (1.25%). Continued and increased urbanization and vehicle movement in Kathmandu and Lalitpur districts have resulted in increased amounts of non-biodegradable containers such as tires around human dwellings, thereby creating ideal breeding habitats for *A. albopictus*.

Key Words: *Aedes albopictus*, breeding preference ratio, Nepal, season

INTRODUCTION

In Nepal, mosquitoes have great public health importance as vectors for the transmission of various diseases including dengue fever (Pandey *et al.* 2004, 2008). Dengue fever (DF) and its severe manifestations such as Dengue Haemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) have emerged as an international public health problem that is now endemic in more than 100 countries. Of the 2.5 billion people who live in dengue endemic countries and are at risk of contracting DF/DHF, 1.3 billion live in 10 countries of the WHO South-East Asia Region that also includes Nepal (WHO 2011). During the past decades, dengue virus which emerged from Southeast Asia gradually became prevalent in Bhutan, India, Maldives, Bangladesh, Pakistan, Indonesia,

Myanmar, Nepal, Sri Lanka, Thailand and Timor-Leste (WHO 2007). In Nepal, DF cases were for the first time reported from Chitwan in 2004 (Pandey *et al.* 2004), followed by a report of a suspected dengue fever outbreak in Banke district (Pandey *et al.* 2008). A first DF case was reported in Kathmandu on 11 September 2006 (WHO 2006) while Sherchand *et al.* (2001) had earlier reported on the prevalence of dengue virus antibody in southwestern Nepal. All these reports suggest that dengue virus has been circulating in Nepal for several years and is a serious public health threat to the country.

At a global scale, dengue viruses are primarily transmitted by *Aedes aegypti* and *Aedes albopictus* mosquitoes. While *A. aegypti* is considered to be the primary vector of dengue viruses and has repeatedly been incriminated as a driving force in the worldwide emergence of DF, the importance of *A. albopictus*, e.g., in an endemic rural cycle in Asia, is well recognized (Gubler 1987) and its public health significance well documented (e.g., Russell *et al.* 1969, Qui *et al.* 1981, Rosen *et al.* 1985, Shroyer 1986, Boromisa *et al.* 1987, Mitchell *et al.* 1987, Craven *et al.* 1988, Moore *et al.* 1988, Francy *et al.* 1990, Reeves 2000).

The Asian Tiger Mosquito *A. albopictus* is an aggressive day-biting and globally invasive species whose origins are thought to be in Southeast Asia (Russell *et al.* 1969). Originally considered a secondary vector of viruses such as dengue virus, *A. albopictus* was recently demonstrated to be involved in the transmission of chikungunya and dengue viruses in several countries bordering the Indian Ocean, Central Africa and Europe (e.g., see Benedict *et al.* 2007 and references therein). This species was reportedly also responsible for dengue epidemics in Japan and Taipei during World War II (Hotta 1998).

Females of *A. albopictus* deposit their eggs in a variety of artificial as well as natural water filled containers (Rattanakul and Panthusiri 1994). Human activities associated with settlement, agriculture, urbanization and also climate change have a huge potential for increasing the number, diversity and suitability of larval habitats for this species. Along with the globalization of trade and travel, this has resulted in a dramatic and still ongoing global expansion of the geographic distribution of this species (Benedict *et al.* 2007), increasing public health concerns about its role as a disease vector. Although there is a rapidly growing body of literature on the ecology of *A. albopictus* and its breeding site preferences in various countries and different regions (e.g., Chan *et al.* 1971, Sprenger and Wuithiranyagool 1986, Nasci *et al.* 1989, Cornel and Hunt 1991, Hobbs *et al.* 1991, Abdul Kader *et al.* 1997, Ishak *et al.* 1997, Moore and Mitchell 1997, Fontenille and Toto 2001, Tsuda and Takagi 2001, Tsuda *et al.* 2002, Lounibos 2002, Hiriyan *et al.* 2003, Hiriyan and Tyagi 2004, Regu *et al.* 2008, Singh *et al.* 2008, Sumodan 2008, Rao 2010), no such study had been conducted in Nepal where this species is known to have been present in the area of the capital Kathmandu since at least 1969 (Darsie and Pradhan 1990). Here, we report on an entomological survey of *A. albopictus* larvae and pupae carried out to study the artificial container preference of this species in the urban areas of Kathmandu and Lalitpur districts of Nepal from April 2009 to March 2010.

MATERIALS AND METHODS

Study area

Kathmandu valley is a bowl-shaped valley in central Nepal and lies at a mean elevation of about 1350 m above sea level. It comprises the three administrative districts with the highest population density in the country, i.e., Kathmandu, Lalitpur and Bhaktapur districts. The valley is surrounded by Bhaktapur district and Kabhrepalanchowk district in the east, Dhading and Nuwakot districts in the west, Sindhupalanchowk and Nuwakot districts in the north, and Makwanpur district in the south.

Kathmandu district is located at 27°42'N 85°20'E and covers an area of 395 km². Similarly, Lalitpur district is situated in the southeastern part of the Kathmandu valley between 27°32'13"N and 27°49'10"N and 85°11'31"E and 85°31'38"E and covers an area of 119 km². As per the latest census of 2011, Kathmandu metropolitan city has the highest population density (20,966 per km²) followed by Lalitpur sub-metropolitan city (14,966 per km²) among the urban areas in Nepal. The Kathmandu valley is in the warm temperate zone with a range of monthly maximum temperatures of 19.1-29.1° C, minimum temperatures of 2.4-20.2° C and 8.3-263.4 mm precipitation (DHM 2012).

A total of 13 localities within Kathmandu municipal limits (Basundhara, Samakhushi, Gongabu, Balaju, Banasthali, Sitapaila, Kalanki of Kathmandu district and Gwarko, Satdobato, Mahalaxmasthan, Ekantkuna, Nakkhu, Sanepa of Lalitpur district) were surveyed in the pre-monsoon (March-May), monsoon (June-September), and post-monsoon (October-November) seasons. Larval collections were performed simultaneously in each locality following the single larval technique of WHO (2003) to determine the *Aedes* breeding status of all the wet containers present in and around the houses and their premises. The number of houses visited in each locality varied depending on the expanse of the area and the types of houses.

Site selection

Surveys were conducted in urban localities of the ring road area of both districts representing Kathmandu valley. The localities were selected so as to cover a broad representation of various residential areas, automobile workshops, commercial and small industry plots, private junkyards, jetties etc.

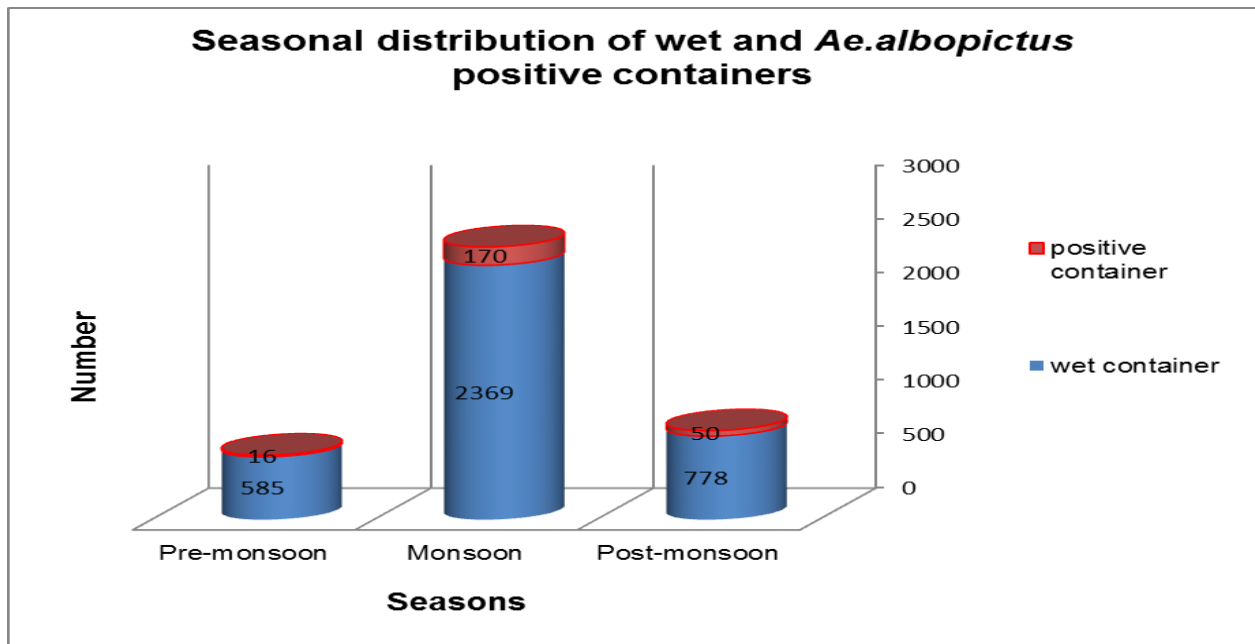
Entomological surveys

To identify the potential breeding habitats of *A. albopictus*, households in the urban areas of Kathmandu and Lalitpur districts were visited once in each season by an entomological team of two persons between April 2009 and March 2010. Altogether 256 households were visited in the pre-monsoon, 1023 in the monsoon and 545 in the post-monsoon period. All accessible artificial larval breeding habitats like discarded tires, metal drums, plastic drums, other metal containers, plastic buckets, flower pots, mud pots, cement tanks, and other plastic containers in indoor and outdoor

locations were inspected using dippers. We did not examine natural habitats such as tree holes, rocks and puddles of water. All trash near and around dwellings (less than 10 m from the selected house) was also examined. All live larvae and pupae were collected, brought back to the laboratory of the Central Department of Zoology, Tribhuvan University, Kirtipur, Kathmandu, where they were reared until adult emergence. Adult mosquitoes emerged from the reared larvae were identified using the taxonomic keys by Darsie and Pradhan (1990). The container preference of *A. albopictus* was assessed by calculating the breeding preference ratio (BPR) (Sharma 2002) as presented in the figures and tables.

RESULTS AND DISCUSSION

In the present study, a total of 3732 wet containers were examined for *A. albopictus* larvae and pupae and overall 236 (6.3%) of the containers were found positive for these. The highest percentage of positive containers was recorded in the monsoon season (7.2%) followed by the post-monsoon (6.4%). We observed a statistically significant association between the number of *A. albopictus* positive containers and the total number of wet containers found (Chi-square value = 425.8256, df = 360, P-value = 0.009582). This shows that rainfall is an important factor for the breeding of *A. albopictus* in this area of Nepal.



The total number of wet containers examined and the number of containers that were positive for *A. albopictus* larvae and pupae are presented in Figure 1. Among the nine container types searched and examined, 95% (n=225) of those positive for *A. albopictus* larvae and pupae were discarded tires, followed by metal drums (2%; n=5) and plastic drums (1.25%).

In the present study, overall 1873 water containers in Kathmandu district and 1807 in Lalitpur district were searched in three seasons. Out of these, 107 (5.7%) and 129 (7.1%) were found positive for *A. albopictus* breeding in the respective districts. Details of breeding varied between the localities surveyed within the urban agglomeration of both districts. Immature stages of *A. albopictus* were recorded in 1.9%, 6.7% and 5.6% of the wet containers in the pre-monsoon, monsoon and post-monsoon seasons, respectively, in Kathmandu district.

In Lalitpur district, *A. albopictus* larvae were detected in 4.6%, 7.6% and 7.1% of the wet containers in the pre-monsoon, monsoon and post-monsoon, respectively. There was no statistically significant association between study districts and *A. albopictus* positive containers.

Details of the breeding preferences of *A. albopictus* larvae in Kathmandu and Lalitpur district are provided in Tables 1-6.

Pre-monsoon survey

In this investigation 316 containers with water were searched in Kathmandu district during the pre-monsoon period. Of these, 39.9% were discarded tires followed by metal drums (32.3%) and other metal containers (12.7%). Similarly, in Lalitpur district, of the 217 containers with water searched 31.3% were metal drums, 28.6% were discarded tires and 20.3% were plastic buckets.

Table 1. Breeding preference ratio (BPR) of *Aedes albopictus* in different artificial breeding habitats in Kathmandu district, Nepal, during the pre-monsoon season.

Type of breeding habitat	No. of containers with water	X%	No. of containers with larvae	Y%	BPR (Y/X)
Discarded Tire	126	39.87	6	100	2.51
Metal Drum	102	32.28	0	0	0
Plastic Drum	5	1.58	0	0	0
Metal Container	40	12.66	0	0	0
Plastic Bucket	23	7.28	0	0	0
Flower Pot	10	3.16	0	0	0
Mud Pot	8	2.53	0	0	0
Cement Tank	2	0.63	0	0	0
Plastic Pot	0	0	0	0	0
Total	316		6		

Table 2. Breeding preference ratio (BPR) of *Aedes albopictus* in different artificial breeding habitats in Lalitpur district, Nepal, during the pre-monsoon season.

Type of breeding habitat	No. of containers with water	X%	No. of containers with larvae	Y%	BPR (Y/X)
Discarded Tire	62	28.57	6	60	2.10
Metal Drum	68	31.34	3	30	0.96
Plastic Drum	20	9.22	0	0	0
Metal Container	0	0	0	0	0
Plastic Bucket	44	20.28	1	10	0.49
Flower Pot	0	0	0	0	0
Mud Pot	13	5.99	0	0	0
Cement Tank	0	0	0	0	0
Plastic Pot	10	4.61	0	0	0
Total	217		10		

In Kathmandu and Lalitpur districts in the pre-monsoon season, *A. albopictus* breeding was detected in six water containers (discarded tires) in Kathmandu and ten water containers (six discarded tires, three metal drums and one plastic bucket) in Lalitpur district (Tab. 1+2). We found a statistically significant association between type of container and *A. albopictus* presence (Chi-square value = 94.186, df = 72 and P value = 0.0408).

Monsoon survey

Of the 1217 containers with water surveyed in Kathmandu district in the monsoon season, 54.4% were discarded tires, 18.7% were plastic buckets and 9.45% were metal containers. Altogether 82 water containers (all of them discarded tires) were found positive for *A. albopictus* in Kathmandu district (Tab. 3).

In Lalitpur district, of the 1152 containers with water surveyed during the monsoon season, 58.85% were discarded tires followed by plastic buckets (10.5%) and plastic drums (9.6%). Eighty-eight water holding containers were positive for *A. albopictus* in Lalitpur district during the monsoon season (Tab. 4).

Container preference reflected by the breeding preference ratio (BPR) was highest for discarded tires during the monsoon season in Kathmandu district (1.84) and Lalitpur district (1.56) followed by metal containers (0.64) and metal drums (0.62).

Table 3. Breeding preference ratio (BPR) of *Aedes albopictus* in different artificial breeding habitats in Kathmandu district, Nepal, during the monsoon season.

Type of breeding habitat	No. of containers with water	X%	No. of containers with larvae	Y%	BPR (Y/X)
Discarded Tire	662	54.41	82	100	1.84
Metal Drum	91	7.48	0	0	0
Plastic Drum	14	1.50	0	0	0
Metal Container	115	9.45	0	0	0
Plastic Bucket	228	18.73	0	0	0
Flower Pot	43	3.53	0	0	0
Mud Pot	50	4.11	0	0	0
Cement Tank	8	0.66	0	0	0
Plastic Pot	6	0.49	0	0	0
Total	1217		82		

Table 4. Breeding preference ratio (BPR) of *Aedes albopictus* in different artificial breeding habitats in Lalitpur district, Nepal, during the monsoon season.

Type of breeding habitat	No. of containers with water	X%	No. of containers with larvae	Y%	BPR (Y/X)
Discarded Tire	678	58.85	81	92.04	1.56
Metal Drum	106	9.20	5	5.68	0.62
Plastic Drum	111	9.64	0	0	0
Metal Container	41	3.56	2	2.27	0.64
Plastic Bucket	121	10.50	0	0	0
Flower Pot	11	0.95	0	0	0
Mud Pot	62	5.38	0	0	0
Cement Tank	7	0.61	0	0	0
Plastic Pot	15	1.30	0	0	0
Total	1152		88		

Post-monsoon survey

As shown in Table 5, a total of 340 and 438 containers with water were searched in the post-monsoon season in Kathmandu and Lalitpur districts, respectively.

In Kathmandu district, 57.9% of these were discarded tires, 13.2% plastic buckets and 9.4% metal containers. A total of 19 containers (all of them discarded tires) were found positive for *A. albopictus* larvae and pupae.

Similarly, in Lalitpur district during the post-monsoon 67.1% of the surveyed containers were discarded tires, 18.0% metal drums, and 6.4% plastic drums. Altogether 31 containers (again all of them discarded tires) were positive for *A. albopictus* larvae and pupae (Tab. 6).

Table 5. Breeding preference ratio (BPR) of *Aedes albopictus* in different artificial breeding habitats in Kathmandu district, Nepal, during the post-monsoon season.

Type of breeding habitat	No. of containers with water	X%	No. of containers with larvae and pupae	Y%	BPR (Y/X)
Discarded Tire	197	57.94	19	100	1.73
Metal Drum	28	8.24	0	0	0
Plastic Drum	9	2.65	0	0	0
Metal Container	32	9.41	0	0	0
Plastic Bucket	45	13.24	0	0	0
Flower Pot	16	4.71	0	0	0
Mud Pot	9	2.65	0	0	0
Cement Tank	4	1.18	0	0	0
Plastic Pot	0	0	0	0	0
Total	340		19		

Aedes albopictus is known to be able to breed in a wide variety of natural and artificial container habitats. In most published studies this species was commonly found in indoor and outdoor habitats like earthen ware jars, tin cans, ant traps, rubber tires, bowls, drums and tin cans (e.g., Chan et al. 1971), containers used for collecting rubber sap in rubber plantations during the rainy season (Sumodan 2008), shed leaf sheaths of areca nut palms and cocoa pods (Regu et al. 2008, Hiriyan and Tyagi 2004), plastic cups (Hiriyan et al. 1997), coconut shells and plastics dumped around houses (Rao 2010), and drum cans in the hills and mountain areas of Indonesia (Ishak et al. 1997).

Table 6. Breeding preference ratio (BPR) of *Aedes albopictus* in different artificial breeding habitats in Lalitpur district, Nepal, during the post-monsoon season.

Type of breeding habitat	No. of containers with water	X%	No. of containers with larvae and pupae	Y%	BPR (Y/X)
Discarded Tire	294	67.12	31	100	1.49
Metal Drum	79	18.03	0	0	0
Plastic Drum	28	6.39	0	0	0
Metal Container	15	3.42	0	0	0
Plastic Bucket	8	1.83	0	0	0
Flower Pot	5	1.14	0	0	0
Mud Pot	6	1.37	0	0	0
Cement Tank	3	0.68	0	0	0
Plastic Pot	0	0	0	0	0
Total	438		31		

Broken cans and plastic containers were primary breeding sites for *A. albopictus* during the dry season in Thailand (Chareonviriyaphap et al. 2003). In the present survey, discarded tires lying outdoors were clearly the preferred breeding habitat of *A. albopictus*. However, larvae were also observed in metal drums and plastic buckets. The unused tires lying outdoors were almost exclusively seen in the urban areas of Kathmandu and Lalitpur districts during this survey. This study highlights that old vehicle tires lying around the households and shops are the major source of *A. albopictus* breeding in the urban agglomeration of Kathmandu and Lalitpur districts.

From the present entomological survey, it can be concluded that *A. albopictus* is well-established within the urban areas of Kathmandu and Lalitpur district. Most of the areas had a high frequency of larva-positive discarded tires which are kept outdoors, are rarely eliminated and remain undisturbed most of the time, thus favouring the breeding of *A. albopictus* mosquitoes.

The availability of such tires in urban areas of these districts is a by-product of increasing vehicle use and traffic. Kathmandu valley has a high density of vehicles, and usually old tires are not disposed of properly due to lack of dumping sites and lack of awareness. Thus, local people have developed a habit of storing and stacking up old tires, sometimes with the hope to re-use or recycle them later. This accumulation of ideal *A. albopictus* breeding sites that will quickly and effectively hold rain water and rapidly warm up in sunlight even during winter, puts the urban areas of this valley at highest risk.

In addition, the water supply in the Kathmandu valley is irregular and insufficient due to electric power shortages and water source limitations. As a consequence, almost every household in the valley practices water storage usually with the capacity of the water quantity needed for one to two days. As there is great uncertainty as to when tap water will be available again, water containers such as metal drums and plastic buckets are never emptied. This situation favours the breeding of *A. albopictus* in metal drums and plastic buckets in the pre-monsoon season, when fewer rain water filled containers are available.

The best conditions for *A. albopictus* mosquito breeding and the highest numbers of positive containers and mosquito larvae were found during the monsoon followed by the post-monsoon season in both districts. At these times most of the potential habitats/containers of the area were filled with fresh water creating good breeding sites for *A. albopictus*.

The results of this study highlight the need for an intensification of vector surveillance activities in Kathmandu along with source reduction and public awareness programmes that should specifically be directed at the proper disposal of breeding containers with special attention to discarded tires.

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