Immature Production of Dengue Virus Vectors in Residential and Non-residential Areas of Lalitpur Municipality, Nepal

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ABSTRACT

Global incidence of dengue has grown over recent decades, with half of the population now at risk. Vector control is the main way to control dengue disease, as many dengue vaccines are still under research. *Aedes aegypti* and *Aedes albopictus* are the vector species, responsible for dengue transmission in the world.

The repeated larvae and pupae sampling of eight times in dry and wet seasons (May to September) was conducted within 100 houses including residential and non-residential. Dipping method using standard dippers were used for immature mosquito collection.

This study found that non- residential areas are preferred breeding sites for dengue mosquitoes compared to residential premises. The *Stegomyia* indices, House Index (HI), Container Index (CI) and Breteau Index (BI) were found higher in non-residential houses than that of residential houses.The statistical analysis shows strong significant differences, $p<0.05$ when compared between two seasons (dry and wet). Seven different types of containers classified by shape, use and materials contribute 72-74% of immature dengue mosquitoes. This study concludes that for dengue mosquitoes' production, dark coloured containers found in both residential and non-residential sites are highly productive. Thus, further studies covering all seasons and households are highly recommended in the study sites leading to effective vector control actions targeting all types of productive wet containers available in the study area and elsewhere.

Keywords: Dengue, Immature, Mosquitoes, Non-residential, Residential

1. Introduction

Aedes-borne diseases including dengue, chikungunya and zika are a growing problem worldwide. Dengue fever, in particular, has increased 30-fold, extending its range in new countries, from urban to the rural areas, in the past 50 years (Gubler 1998; WHO 2008; WHO 2009). It is one of the fastest-growing global infectious diseases, with 100–400 million new infections each year (Brady & Hay 2020) and an estimated 3.83 billion people living in areas suitable for dengue transmission (Messina *et al*. 2019). Additionally, 96 million people with dengue infections were recorded globally in 2010, of which 70% were from Asia. Among this 34% were recorded from India alone (Bhatt *et al*. 2013).The disease is further classified into three types, classical dengue fever (DF), dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) (Hadinegoro 2012). A small single stranded RNA virus of genus Flavivirus and family Flaviviridae causes dengue fever, which consist of four serotypes, DENV 1, DENV 2, DENV 3, and DENV 4 (WHO 2009). Dengue fever was first recorded in Nepal in 2004 from Chitwan district (Pandey *et al*. 2004). The outbreak of dengue fever started in Nepal from 2006, which was recorded from nine districts of low land region of Nepal (Pandey *et al*. 2008; Malla *et al*. 2008). More cases of dengue from Kathmandu valley were recorded from dengue outbreak in 2010 (Pandey *et al*. 2013) and all four dengue virus serotypes are expanding their range in new geographical areas of the country, Nepal, which will further increase the risk of dengue outbreaks in new areas (Pun 2011).

The virus is transmitted through the bite of *Ae. aegypti* and *Ae. albopictus* (Gubler 2002; Gratz 2004; Ponlawat & Harrington 2005; Bonizzoni *et al*. 2013). Both species coexist in Nepal. *Ae. aegypti* is considered the principal vector of dengue. On the other hand, *Ae. albopictus* alone has been confirmed as the vector in some dengue outbreak areas (Paupy *et al*. 2009). It has also driven the global emergence of chikungunya virus in as well (Weaver & Forrester 2015). Anthropogenic changes such as urbanization, alterations in land use, increased cross country trade, travel networks and vehicular movement, climate change etc. have impacted their distribution and geographical expansion (Kolimenakis *et al*. 2021).

Ae. albopictus originated in the forests of Southeast Asia (Paupy *et al*. 2009) and first documented in 1956 in Nepal including Halchowk, Kathmandu (Peters & Dewar 1956). Though, no scientific publication came through regarding the presence of *Ae. aegypti* in Nepal until 2006 when this species was recorded in the selected urban settings of different districts of Terai region near Indian border namely Morang (Biratnagar), Parsa (Birgunj), Chitwan (Bharatpur), Dang (Tulsipur) and Banke (Nepalgunj) (WHO 2006; Malla *et al*. 2008). In Kathmandu valley, *Ae. aegypti* was reported in the year 2009 for the first time (Gautam *et al*. 2009). Both species are expanding its geographical range up to an altitude of 1,350 m and sparsely in 1,700 to 2,100 m in Nepal and distributed in sub- tropical regions (Dhimal *et al*. 2015) including Lalitpur district of Nepal (Gautam *et al*. 2009).

Ae. aegypti have become widely distributed in tropical regions of the Asian, South American, and African continents and *Ae. albopictus* is commonly found in most of the countries of Asia, Africa, America, and Europe (WHO 2009; Braks *et al*. 2003). Common breeding habitat for *Aedes aegypti*is in artificial containers with clear water, where as *Aedes albopictus* prefer to breed in natural water holding containers (Christophers 1960; Bonizzoni *et al*. 2013). The eggs of these species can survive in adverse climatic conditions like long winter and droughts (Sota & Mogi 1992). The larvae of both species feed on microorganisms, organic detritus and other food particles found in the water holding containers (Braks *et al*. 2004). Adult stages of these species are aggressive day biting mosquitoes with bimodal biting behaviour. *Ae. aegypti* has peak biting period at dawn and dusk and *Ae. albopictus* biting peaks from 06:00-09:00 to 15:00-20:00hr GMT (Ho *et al*. 1973; Chen *et al*. 2014). The adult female feed on human blood and disperse for food, oviposition and searching for mate. Dispersal for oviposition of this mosquito is pertinent for the disease propagation (Lambrechts *et al*. 2010; Muir & Kay 1998; Honorio *et al*. 2009). Female *Ae. aegypti* is highly anthropophilic in nature and well adapted in urban areas (Ponlawat & Harrington 2005). While *Ae. albopictus* has adapted to anthropogenic changes in the environment, feeding more frequently on humans and domestic animals, although it remains more abundant in vegetated rural and suburban areas (Hawley 1988). Density is high when there is greater population of human settlements with low socioeconomic status (Tauil 2001). The size and the biological status are the determinant to transmission dynamics of the disease. Rainfall, high temperature, high humidity, and moisture are the important drivers of vector reproduction and also help to enhance the vectorial capacity. Additionally, temperature also affects the gonotrophic cycle and survival of the primary vector of dengue (Yang *et al*. 2009).

The classical *Stegomyia* indices show the absence or presence of the vector. Pupal productivity surveys are a much better representative indicator for adult vector abundance in dry and/or inwet season because the total number of *Aedes* pupae is used as a proxy indicator for adult dengue vector density, as roughly 80% of pupae develop to adult mosquitoes (Focks & Alexander 2006). Additionally, it explicitly depicts the most productive *Aedes* water container types in the dry and the wet seasons coupled with variation of the pattern among different residential or non-residential settings leading to targeted management of the most mosquito-productive containers for eliminating all potential breeding habitats in various socio-ecological settings. Abundance of immature dengue mosquitoes were found higher in non-residential areas compared to residential areas (Baak-Baak *et al*. 2014). Dos *et al*. (2010) also argued that the study on dengue vectors in Brazil shows that non-residential sites were key sites for vector surveillance than that of residential areas. The pupal demographic survey of *Ae. aegypti* in non-residential areas of Peruvian city of Iquitos shows that such areas are highly productive compared to residential areas (Morrison *et al*. 2006).

Vector control is the main way to control dengue, as many dengue vaccines are still under research (Jacobs 2000; Koenraadt *et al*. 2007; Deng *et al*. 2020). Some other methods are spraying larvicides, introducing predatory fish in water holding containers etc. (Baak-Baak *et al*. 2014; Dos *et al*. 2010; Kroeger *et al*. 2006). Although, it is necessary, at this juncture, to conduct larval and pupal-demographic surveys which pave a path toward effective methods for vector control (Ponlawat *et al*. 2005; Nathan *et al*. 2006) through eliminating mosquitoes breeding containers from residential and non-residential areas.

Different studies on dengue virus and vector surveillance have been conducted previously from lower tropical and sub-tropical regions including container preference of *Ae. albopitus* in Kathmandu and Lalitpur district (Gautam *et al*. 2012). However, most of the dengue vector surveillance was only focused on residential sites often neglecting non-residential sites, which might be potential breeding sites in large volume for dengue mosquitoes. Furthermore, there is a lack of studies on breeding site characteristics and immature dengue mosquitoes' production in residential and non-residential areas.Keeping this in mind, this study aims to compare immature production of *Ae. aegypti* and *Ae. Albopictus* as well as to find out the most productive containers in residential and non-residential areas of Lalitpur district of Nepal.

2. Methods:

2.1 Study area:

Two wards of Patan city of Lalitpur district, Nepal were chosen for the study. Lalitpur sub-metropolitan city is located between N 27 °39" and E 27° 41", with the elevation of 457 m to 2831m above sea level. The city lies near to capital city of Nepal (Kathmandu). It is one of the oldest cities of Nepal which consist of old houses and historical places (Fig. 1).

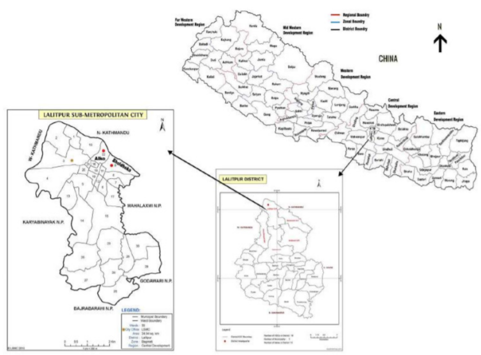

Fig. 1: Map of Study Area

2.2 Study design and sampling:

A series of cross-sectional entomological survey with repeated sampling in May-June (dry season) and August-September (wet season), 8 times for 4 months period, and 2 surveys in each month was performed in residential and non-residential areas of the city in altitudes ranged from 1200 to 1300m. Hundred houses were randomly selected covering both areas from two wards (Aliko and Bholdhoka) of the city. A team of 5 persons were employed to conduct larval and pupae surveillance from 7 to 11 am. The non-residential areas include cement block factories, mud statue factories, metal workshops, tire repair shops, temple, furniture factories, government and private offices, rice mills, electronic shops, restaurants, garbage recycling centres, kindergarten and schools and grocery shops.

Oral informed consent was taken from the head of the each household before starting the collection of larval mosquitoes. In case where the household head disagreed, the house was dropped from the collection plan and the immediate next one was selected for survey.

2.3 Entomological Survey:

All the water holding containers from the residential and non-residential areas were screened for the presence and absence of immature *Aedes* mosquitoes and were collected by using standard larvae collection procedure (Su *et al*. 2016). The containers were inspected using flashlight. Immature mosquitoes were collected using dippers of different size and pipettes (Vikram *et al*. 2016). All larvae and pupae were transferred to plastic bags and labelled with house code, container code, date and locality. According to Koenraadt *et al*. (2007), all the wet containers were recorded based on the shape, use and materials (SUM) method. Other associated variables include, presence of cover (yes or no), location (indoor or outdoor), size (length, height and opening), water depth, shade (yes, no or partially), under roof (yes, no or partially), water source (rain fed or manually), insecticide used (yes or no) and container washed (yes or no). There was no active vector control method applied in the area during surveillance. Weekly or monthly temperature was not included in the analysis.

2.4 Laboratory work:

All the collected larvae and pupae were brought to the laboratory at the Natural History Museum, Kathmandu, Nepal for rearing and identification and transferred to plastic cup and covered with thin muslin cloth and rubber bands. Plastic cups were kept in the laboratory under normal temperature conditions for rearing. The labelled plastic cups were checked once a day for adult emergence. Adult were then transferred to test tubes by using aspirator and killed with chloroform. Larval mosquitoes that did not emerge to adult were preserved in 70% alcohol in vials and prepared slide. Adult mosquitoes were identified to species level by using taxonomic keys, dissecting microscope, hand lenses (10X triplet hand lens) and pointed forceps. The slides containing larvae were observed under compound microscope and identified using the standard keys (Darsie & Pradhan 1990; Rueda 2004; Fenemore 2006).

2.5 Data analysis:

Traditional *Stegomyia* indices were used to evaluate the population densities of the dengue mosquitoes in residential and non-residential areas, such as house index (HI), container index (CI) and Breteau index (BI). These techniques were commonly used as standard parameters in most of the developing countries (Petric *et al*. 2014).

Findings of the survey were analysed using Microsoft Excel 2013 spread sheet and SPSS version 21. Descriptive analysis was conducted to carry out the container characteristics, immature mosquitoes' infestation, and percentages in residential and non-residential areas. Container productivity of *Aedes aegypti* and *Aedes albopictus* were classified by shape, use and material and ranked from highest to lowest. Negative binomial regression model to test the significance difference between two areas at 95% confidence level for both species was carried out using SPSS.

3. Results

3.1 Container characteristics:

Of the 1779 wet containers, 1259 from residential areas and 520 from non-residential areas were screened covering 694 outdoor and 565 indoor locations and 332 outdoor and 188 indoor locations of residential and non-residential areas respectively (Table 1). The size of the container and water depth in both areas are shown in Table 2.

Table 1. Container characteristics in residential and non-residential areas of Lalitpur district, Nepal.

	Residential areas (n - 68)	%	Non-residential areas (n - 32)	%	Total (n)	Total (%)
Number of wet containers	1259	71	520	29	1779	100
Location						
Outdoor	694	68	332	32	1026	58
Indoor	565	75	188	25	753	42
Cover lid						
Yes	615	76	190	24	805	45
No	644	66	330	34	974	55

Filling method						
Rain	439	65	232	35	671	38
Manual	820	74	288	26	1108	62
In shade						
Yes	385	73	142	27	527	29
Partially	97	63	57	37	154	9
No	777	71	321	29	1098	62
Under roof						
Yes	735	74	260	26	995	56
Partially	22	56	17	44	39	2
No	502	67	243	33	745	42
Wash before refill						
Yes	715	76	229	24	944	53
No	544	65	291	35	835	47

Table 2. Size of the container and water depth.

	Residential	Non-residential
Maximum		
Length (cm)	200	200
Width (cm)	100	100
Height (cm)	250	250
Opening (cm)	200	200
Minimum		
Length (cm)	5	5
Width (cm)	2.5	2.5
Height (cm)	5	8
Opening (cm)	4	5
Average (cm)		
Length (cm)	41.9	46
Width (cm)	20.5	22.37
Height (cm)	56.9	57.3
Opening (cm)	40	44.7
Water depth (cm)		
Maximum	197	206
Minimum	1	1
Average	38.3	37.7

3.2 Shape, use and material:

The main container types were drums (793), buckets (504), pots (270), tanks (94), gallons (55), tires (42) and jars (21). Among these, 72% of the drums were in residential houses and 28% in non-residential houses. Of the buckets, 76% were in residential houses and 24% in non-residential houses. The corresponding residential and non-residential Fig.s for pots were 68% and 32%, for tanks 56% and 44%, for gallons 76% and 24%, and for jars 67% and 33% respectively. Pots were made up of either metal and plastic or clay, and drums were either plastic or metal. Most of the plastic drums were black, yellow and blue in colour, whereas metal drums were blue or brown. Buckets were made from plastic or metal, tires from rubber, and tanks from cement. Most of the plastic pots were used for washing such as hand and face washing, brushing, and cleaning. No use of metal pots in non-residential areas was observed, whereas metal pots were used for irrigation in gardens and drinking water for pets in residential areas. Clay pots in residential areas were used for ornamental flowers. Drums, buckets and cement tanks were found to be used for daily washing propose (dishwashing, bathing, cooking and clothes washing). Large cement tanks were used for all types of washing and drinking and cement tanks in non-residential areas were used for making statue, cement blocks and rings in non-residential houses. Jars and gallons were used to store drinking water. Discarded tires were found lying outdoor near non-residential houses such as workshops, repairing shop, recycling centre etc.

3.3 Mosquito immature infestation:

A total of 136 containers (136/1779 = 7.6%) were infested with *Ae. aegypti* larvae and pupae. These were pots (n = 58), drums (n = 42), buckets (n = 24), tires (n = 10), and tanks (n = 2). For *Ae. albopictus* all together 152 containers (152/1779=8.5%) were found positive for larvae and pupae. These were pots (n = 62), drums (n = 43), buckets (n = 37), tires (n = 9) and a tank (n = 1). A total of 122 containers (6.9%) were infested with *Culex* spp; these were drums (n = 44), pots (n = 32), buckets (n = 23), tanks (n = 18), and tires (n = 5). Thirty-four containers (2%) were positive for other *Aedes* mosquito pots (n = 14), drums (n = 10), buckets (n = 7), tires (n = 2) and tank (n = 1).

3.4 Mosquitoes in residential and non-residential areas:

All together 2107 larvae and pupae were recorded from the whole survey, of which 484 were *Ae. aegypti*, 304 from the residential land 180 from the non- residential sites.Whereas776 *Ae. albopictus*, 479 from residential and 297 from non-residential (Table 3). Most abundant species was *Ae. albopictus* (n = 776), and then *Culex* spp. (n = 713), followed by *Ae. aegypti* (n = 484), other *Aedes* species (n = 96), *Anopheles* species (n = 24) and other unidentified mosquitoes were 14.

Table 3: Number and proportion of immature mosquitoes collected in residential and non-residential areas in May, June, August, and September 2016.

Species	Residential		Non-residential		Total	
	Number	%	Number	%	Number	%
Aedes aegypti	304	63	180	37	484	100
Larvae	209	63	121	37	330	100
Pupae	95	62	59	38	154	100
Aedes albopictus	479	62	297	38	776	100
Larvae	264	61	169	39	433	100
Pupae	215	63	128	37	343	100
Anopheles spp.	12	50	12	50	24	100
Culex spp.	448	63	265	37	713	100
Aedes spp.	64	67	32	33	96	100
Unidentified	6	43	8	57	14	100
Total	1313		794		2107	

3.5 The *Stegomyia* indices by areas:

The House Index, Container Index and Breteau Index for immature dengue mosquitoes was higher in non-residential houses than in residential houses (Table 4).

Table 4. The *Stegomyia* indices of *Aedes aegypti* and *Aedes albopictus* in residential and non-residential areas.

	Area		
	Residential	Non-residential	Total
Total no. of wet containers encountered	1259	520	1779
Average no. of wet containers per house	2.3	2.1	2.2
Number of positive houses	102	63	165
Number of positive containers	110	65	175
Container Index (CI)	8.7	12.5	9.8
House Index (HI)	18.8	24.6	20.6
Breteau Index (BI)	20.2	25.4	22
Number of pupae positive containers	71	39	110
Total number of pupae	310	187	497
Pupae per house index (PHI)	57	73	62

CI = Percentage of water holding containers infested with immature dengue mosquitoes.

HI = Percentage of houses infested with immature dengue mosquitoes.

BI = Number of dengue mosquito positive containers per 100 houses.

PHI = Number of pupae per house.

3.6 Seasonal distribution of immature mosquitoes:

In the dry season the highest number of mosquitoes recorded was of *Culex* spp., the second highest was *Ae. albopictus*, followed by *Aedes aegypti* and then other *Aedes* spp. (Fig. 2). For the post-monsoon season the most abundant mosquitoes was *Ae. albopictus*, and *Ae. aegypti* followed by *Culex* spp. (Fig. 2).

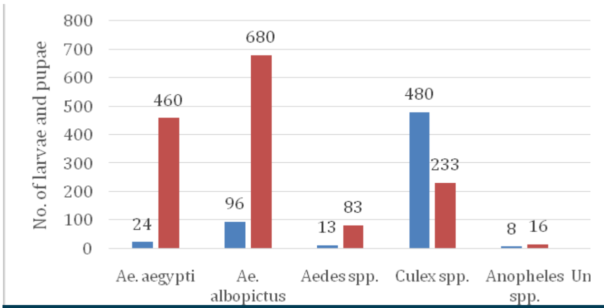

Fig. 2: Total larvae and pupae collected in dry (May-June) and wet (August-September) seasons in 2016.

3.7 Container productivity:

Containers were ranked from most to least productive are tabulated in table 5. The most productive containers for *Ae. aegypti* classified by shape, use and material were plastic drums used for water storage and washing .However, those did not produce more than 16% of all immature collected (Table 5). As many as seven different classes of containers (various shape, use and material combinations) produced 72% of *Ae. aegypti*. The various categories consisted of cement and plastic tanks used for washing; mud and metal pots, buckets and mud drums used for washing; plastic and metal pots used for irrigation; glass and metal pots without use; metal and plastic pots used for pets; mud pots used for flowers; plastic drums used for irrigation; plastic drums used for drinking, plastic buckets used for irrigation, wood and plastic buckets without use, and plastic buckets used for drinking purpose.

Table 5: Most productive *Aedes aegypti* containers as classified by shape, use and material.

	Container class						
Rank	**Shape**	**Use**	**Material**	**No. positive container**	***Ae. aegypti* larvae + pupae**	**Container productivity (%)**	**Cumulative productivity (%)**
1	Drum	Washing	Plastic	21	78	16.1	16.1
2	Pot	Garbage	Plastic	25	74	15.3	31.4
3	Bucket	Washing	Plastic	14	48	9.9	41.3
4	Tire	Garbage	Rubber	10	50	10.3	51.6
5	Pot	Washing	Plastic	11	38	7.9	59.5
6	Drum	Washing	Metal	6	31	6.4	65.9
7	Drum	Dishwashing	Plastic	11	30	6.2	72.1
8	Various	Various	Various	37	135	27.9	100
Total				136	484	100	

Container productivity: Percentage of total pupae produced by each container class.

For *Ae. albopictus* the containers were ranked in the same way as for *Ae. aegypti*. Discarded plastic pots were found to be the most productive container for *Ae. Albopictus* which produced 18.4% of all immature collected. Seven different different classes of containers produced 73.4% of *Ae. albopictus*. The various category consisted of plastic tanks, metal pots metal and mud drums, metal and mud buckets used for washing;

plastic and metal pots used for irrigation; metal and glass pots without use; plastic pots used for pets; mud and plastic pots used for flowers; plastic drums, plastic buckets used for storage and drinking; cement drums used for dish washing; plastic and mud buckets use for irrigation, wood and plastic buckets without use and plastic buckets used for dish washing purpose.

Table 6: Most productive Aedes albopictus containers as classified by shape, use and material.

Rank	Shape	Use	Material	No. of positive containers	Ae. albopictuslarvae + pupae	Container productivity	Cumulative productivity
1	Pot	Garbage	Plastic	26	143	18.4	18.4
2	Bucket	Washing	Plastic	18	103	13.3	31.7
3	Drum	Washing	Plastic	22	95	12.2	43.9
4	Pot	Washing	Plastic	13	67	8.6	52.5
5	Drum	Dishwashing	Plastic	13	64	8.2	60.7
6	Pot	Garbage	Mud	8	53	6.8	67.5
7	Tire	Garbage	Rubber	9	46	5.9	73.4
8	Various	Various	Various	43	205	26.4	100
Total				152	776	100	

Container productivity: Percentage of total pupae produced by each container class.

3.8 Comparisons of mosquito density between areas and seasons:

The negative binomial regression model analysis on comparing *Ae. aegypti* (larvae + pupae), *Ae. albopictus* (larvae + pupae) and *Culex* spp. between areas in each season and between seasons are shown in table 7. Not significantly differed between residential and non-residential but significantly differed between the dry and wet seasons.

Table 7: Incidence rate ratios (IRR) (95% confidence intervals) of immature mosquitoes per container in relation to areas (for each season) and season (across areas) in 2016.

Variable	Level	*Ae. Aegypti*		*Ae. Albopictus*		*Culex* spp.
		Larvae	Pupae 1	Larvae	Pupae	
Dry season(n = 945)						
House type	Non-residential	1	-	1	1	1
	Residential	0.81 [0.16-4.13] P=0.805	-	1.12 [0.26-4.72] P=0.882	1.29 [0.10-16.73] P=0.845	0.90 [0.38-2.14] P=0.807
Wet season (n = 833)						
House type	Non-residential	1	1	1	1	1
	Residential	0.71 [0.39-1.30] P=0.274	0.67 [0.35-1.30] P=0.242	0.59 [0.30-1.18] P=0.134	0.67 [0.36-1.27] P=0.220	0.43 [0.16-1.13] P=0.087
Across house types (n=1779)						
Season	Wet season	1	1	1	1	1
	Wet season	14.48 [8.27-25.34] P<0.0001	1.16×10 into 8 [--] P=0.98	5.79 [3.34-10.05] P<0.0001	14.45 [8.03-25.98] P<0.0001	0.55 [0.30-1.00] P=0.051

[1)]No *Aedes aegypti* pupae collected in the dry season.

4. Discussion and Conclusion

The present study highlights the importance of key productive container types for dengue vectors which play pivotal role for the development to their adult stage, as these were different from the *Stegomyia* indices. This difference has been determined elsewhere previously also (Focks *et al*. 2000; Focks & Alexander 2006; Lenhart *et al*. 2006; Romero-Vivas *et al*. 2006; Troyo *et al*. 2007).

As the specific findings are detailed, the *Stegomyia* indices, Container index, House index, and Breteau index were higher in non-residential compared to residential area. The number of mosquitoes per containers was found higher in non-residential (0.60) than in the residential area (0.40). However, when testing, there were no significant differences found between areas for all species (*Ae. aegypti*, *Ae. albopictus, Culex* spp.). It means that, P value is greater than 0.05 in both dry and wet seasons for *Ae. aegypti*, *Ae. albopictus* and *Culex* spp. Non-residential areas include garbage recycling centres (1), metal workshops (2), tire repair shop (1), cement block factories (2), Offices (6), School (2), grocery shops (3), temple (2), restaurants (6), electronic shop (1), furniture factories (2), rice mill (1), and mud statue factories (3).

The reason for higher production of mosquitoes in non-residential areasin the present study may be due to more bushes in outdoor premises and locations and presence of most favourable breeding containers such as discarded plastic pots, and rubber tires fill up with fresh rainwater in repairing shop and recycling centres. The mosquito infestation was higher in residential houses compared to non-residential premises but some of the non-residential houses (recycling centres) were highly infested with *Ae. aegypti* mosquito than residential houses specially those houses which were nearby to highly infested residential houses in Rio de Janeiro, Brazil (Dos *et al*. 2010). It shows the presence of high productivity of breeding containers may be influenced by highly infested houses nearby in non-residential premises. In the present study, the density (mosquitoes/containers) was lower in the residential areas in comparison to non-residential areas. Though, overall, there were no significant differences in mosquito productivity betweenthe areas.The percentage of pupal *Ae. aegypti* production in non-residential sites in the Amazonian city of Iquitos, Peru (Morrison *et al*. 2006) and in Merida city, Mexico (Baak-Baak *et al*. 2014) when comparative studies were carried out between residential and non-residential sites,the greater number of productions of *Ae. aegypti* immature were recorded in vacant lots where there were abundant vegetation and often being located near residential premises and contained large or small size discarded water filled containers which became favourable place to breed adult mosquitoes and suitable place for the immature development compared to residential houses. Further, non-residential premises such as tire repair shops, metal workshops were infested highly with *Ae. aegypti* than residential premises (Lagrotta *et al*. 2008).

In the present study, people found to be use plastic drums, the most productive containers for *Ae. aegypti,* for washing purpose. Other containers observed were discarded plastic pots. Those were responsible for 31.3% of larvae/pupae production. Likewise, discarded plastic pots and plastic buckets used for washing furthering higher container productivity (31.7%) for *Ae. albopictus*. As many as seven different containers class (various shape, use and material combinations) only found to be produce 72-74% of all immature *Ae. aegypti* and *Ae. albopictus*. Most of the black, blue, and yellow coloured middle size plastic drums and buckets used for washing in residential houses were kept outside with lid remained open favouring oviposition for *Aedes* mosquitoes. The small size discarded plastic pots lying outdoors in non-residential areas can accumulate rainwater and favourable breeding place for dengue mosquitoes. No immature *Aedes* mosquitoes were recorded from those containers with covered lid, light and transparent coloured plastic gallons and jars, but very few numbers were collected from large sized plastic and cement tanks.

Findings of Koenraadt *et al*. (2007) showed that the most productive containers classified by shape, use, and material for pupal *Ae. aegypti* were earthen jars and cement tank used for washing purpose, which were responsible for 59% pupae production. The large sized containers with dark coloured and organic materials harbour more immature dengue mosquitoes than that of light-coloured containers (Baak-Baak *et al*. 2014).

Discarded tires, metal drums, plastic drums, and mud pots were found as the most productive container for *Ae. aegypti* and *Ae. albopictus* from Lalitpur and Kathmandu district of Nepal (Gautam *et al*. 2012). On the other hand, the findings from this study shows that the most productive containers in Lalitpur district for *Ae. aegypti* and *Ae. albopictus* were plastic pots, drums and buckets which is due to water storage practice by the communities. The variation of productive container types reflects the environmental and social settings (Jahansson *et al*. 2009).

The differences between the dry and wet seasons were noticeable while the number of larvae/pupae of *Ae. aegypti* and *Ae. albopictus* were found higher in the wet season (August and September) compared to the dry season (May and June) due to the increased temperature, humidity and rainfall favoring vector breeding in the wet season. In spite of water storage for domestic use was enhanced in the dry season, pupal productivity was found higher during the wet season. This was possibly due to the vectors' preference of those containers filled with rainwater, lying in shady places, and that were remained undisturbed.

The *Stegomyia* indices, despite being poor proxies for adult abundance, indicate the absence or presence of dengue vectors. The Container index, House index, and Breteau index were also higher in wet season compared to dry season. The statistical analysis negative binomial regression model at 95% confidence interval showed highly significant differences ($P<0.05$) betweendry season and wet seasons, indicating that population of both species were higher in wet season than in dry season. In case of *Culex* spp., mosquito population were higher in dry season than wet season ($P = 0.51$).

It means in dry season, most of the containers were dry, but after monsoon most of the containerswere filled up with fresh water which became favourable breeding place for mosquitoes. According to (Gautam *et al*. 2012; Dhimal *et al*. 2015), abundance of dengue mosquitoes follows seasonal patterns in Nepal. The larva/pupae abundance in Lalitpur and Kathmandu district were significantly higher in wet season (monsoon and post- monsoon) compared to pre-monsoon and winter season when the containers were fill up with fresh water. In this study also immature mosquito abundance was significantly higher in the wet season compared to the dry season. *Ae. Albopictus* was the most abundance species recorded from this study whichmay be due to the presence of vegetation, since *Ae. albopictus* prefer vegetation. Study conducted in Mexico shows that, the most abundance species found was *Ae. albopictus* followed by *Cx. quinquefasciatus* because of abundant vegetation (Baak-Baak *et al*. 2014). In the previous study conducted by Dhimal *et al*. in 2015 in Lalitpur district, also concluded *Ae. albopictus* as the most abundant species followed by *Ae. aegypti*. Furthermore, in this study a greater number of immature *Ae. aegypti* as well as *Ae. albopictus* had recorded from the containers lying outdoor locations rather than indoor containers which coincides to the study conducted in central Nepal (Dhimal *et al*. 2015) and India (Vijayakumar *et al*. 2014).

Overall, 2107 immature mosquitoes were collected during field survey, which includes *Ae. albopictus*, *Ae. aegypti*, *Culex* spp., *Aedes* spp., *Anopheles* spp. and other unidentified species. Among them abundance of *Ae. albopictus* from residential areas in post-monsoon season was highest followed by *Culex* spp. Abundance was high in first week of August (5^{th} field) for both *Ae. aegypti* and *Ae. albopictus*. After monsoon, most of the containers contain fresh water which become favourable place for oviposition for adult mosquitoes.

This finding concludes that the most potential breeding containers were found in non-residential areas than that of residential areas. However, mosquito abundance was low and there was not any significance difference between areas. This may be due to fewer containers found in non-residential sites. Seven different container classes (various shape, use and material combinations) only produced 72–74% of immature dengue mosquitoes, thus almost all containers searched were found productive. Containers in non-residential areas near to residential sites found positive with larvae and pupae. In non-residential houses and surrounding outdoors of the study sites contain more unused disposable plastic, metal and mud pots and discarded tires with vegetation. In such containers rainwater stored during monsoon and become favourable places to breed for mosquitoes.

Dengue fever is an emerging disease for Nepal, expanding from the lowlands to higher altitudes. It means that there could be a higher chance of risk of dengue transmission in future. Vector surveillance with larval/pupal control methods in Nepal were only focused on residential areas ignoring non-residential sites. The findings of this study suggest determination of pupal productivity would be best during the wet season that provides a vector surveillance tool for the specific container types whereby the most productive wet containers can be targeted including non-residential areas for vector management. In addition, this approach can be more cost-effective than managing or treating all containers without targeting any specific container type. However, further studies should be carried out in future to quantify the immature dengue mosquito production in residential verses non-residential areas.

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