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Research Article

SPATIAL ANALYSIS OF VECTOR BREEDING INDICES IN RELATION TO THE RISK OF DENGUE FEVER IN TIRUNEVELI, TAMIL NADU, INDIA

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ABSTRACT

Spatial analysis of entomological parameters in relation to the incidence of dengue cases was carried out by using various entomological indices such as house index, container index, breteau index and pupal index. Various entomological parameters in three pre-identified risk areas were compared. Except container and breteau indices of *St. aegypti* in relation to water storage breeding sources, all other larval indices with respect to type of habitats as well as species did not differ significantly (P>0.05). This indicates that the risk factors are highly variable and dynamic and not necessarily continue in its magnitude. The container and breteau indices of *St. aegypti* in relation to water storage breeding sources was found significantly (p<0.05) higher in high risk areas and had shown significant relationship between the areas selected as low, medium and high incidence of dengue cases. This clearly indicates that the entomological indications with respect to the vector mosquito can be used as risk factors to predict the outbreaks and preparedness to prevent the same.

Keywords: Dengue fever, Larval indices, *Aedine* msquitoes, *Stegomyia aegypti*.

INTRODUCTION

Dengue, a mosquito borne disease affects millions of people worldwide and is considered an emergent mosquito-borne viral disease in terms of mortality and morbidity (Gubler, 2002) in both developing and developed countries. It is caused by Dengue virus (DENV). Dengue fever (DF) is rapidly growing arbo-viral disease of public health concern. The transmission, incidence and severity of dengue are associated with the geographic expansion and distribution of dengue vectors and viruses (Gubler, 1998, 2004). Vector surveillance provides information on the vector distribution, population dynamics, breeding sources as well as risk factors related to transmission. Source reduction, a part of Vector Control Program is appropriate control method and the incidence of cases can be reduced/prevented with reduction of vector breeding. Information on the risk factors through vector surveillance is crucial for initiating preparedness to prevent the incidence /outbreak of dengue cases. In this context, a study was carried out to analyze the risk factors. This study was aimed at deriving recommendations appropriate to the situation so as to prevent the dengue cases and its outbreak.

MATERIALS AND METHODS

An observational analytical study, yearlong entomological survey was carried out in the selected villages in Tirunelveli district (Figure 1). It is Located at latitude of 8.7139° N, 77.7567° E. The population of low incidence, medium incidence and high incidence study sites were 19772, 97657 and 111185 respectively (Table 1). Based on the incidence of dengue in 2012, the villages in Tirunelveli were stratified in three strata (Low, moderate and high). From each stratum 2 villages were selected (Table 1) and there was a cohort of six study sites for longitudinal survey. Manur and Valliyur represent low stratum (with no confirmed cases), Palayamkottai and VK Puthur (confirmed cases above 1 and below 10) represent moderate and Vadakarrai and Kadayanalur represent high incidence stratum (confirmed cases above 10). The incidence of dengue confirmed cases was 0.31, 0.041 and 0 respectively in high, medium and low incidence areas (Table 1).

Survey was carried out to collect the immature of mosquitoes. The basic sampling unit was the

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house/premise. As many as 180 houses (30 houses per village) were selected by systematic sampling with a random start for entomological monitoring every month. The premises where breeding was found was considered as positive house. Water holding habitats were considered as breeding sources. The wet containers bearing immature of mosquitoes were regarded as the positive breeding sources. Each selected house was thoroughly searched for the presence of breeding sources and the searching was continued till all containers have been examined.

Table 1. Dengue incidences (in year 2012) in different areas.

Village strata	Population	Dengue cases (in 2012)	Dengue incidence
Low risk areas (Manur &Valliyur villages)	19, 772	0	0.00
Medium risk areas (Palayamkottai & VK-Puthurvillages)	97, 657	4	0.04
High risk areas (Vadakarrai & Kadayanalurvillages)	1, 11, 185	34	0.31

During sampling, a dipper of 20 cm wide and 10cm deep was dipped ten times at a sampling point of each of the selected systems/sites for collection of pupae and late instar larvae. The collection of *Aedes* immatures was carried out by followed the specific protocols (Balakrishnan *et al.*, 2006; Balaya *et al.*, 1969). The contents were passed through a strainer of 20 meshes/cm mosquito nylon netting to retain the immatures. The immatures were then transferred to the plastic containers (half - filled with breeding site water) by using pipette. The plastic containers containing the immatures were labeled with breeding source code, locality code, house identification code and date of collection. The samples were carried to the laboratory in Vector Control Research Centre (VCRC), Pondicherry. In laboratory the samples were transferred into plastic containers (100 ml capacity) and labeled. The containers were covered with nylon net secured with a rubber band. The immatures were reared to reach the adult stages. Larval food was added daily. Every day emerging adults were collected using oral/mechanical aspirator and were transferred into test tubes. The adults were immobilized by tapping and were transferred to petridishes for examination. The adults were examined using binocular stereo-dissection microscope. Using the taxonomic key the adults were identified up to species level and were recorded.

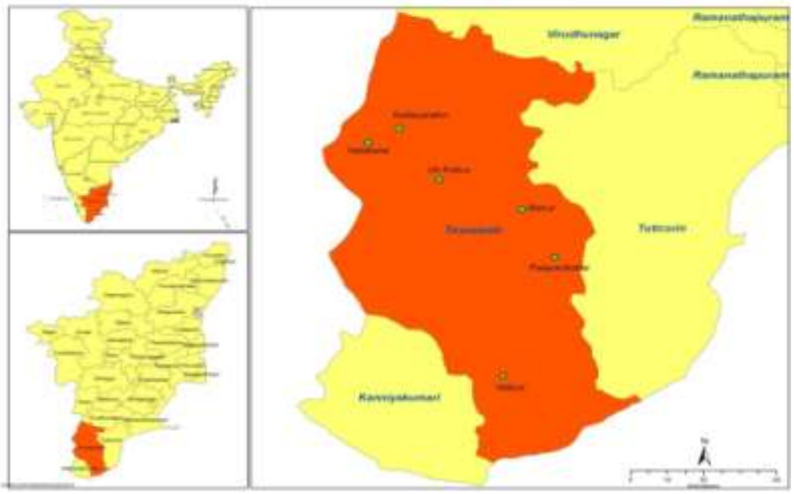

Figure 1. Physical map showing study sites selected in Tirunelveli district.

The larval indices were used for monitoring infestation. The indices are useful for determining general distribution, seasonal changes and principal larval habitats, as well as for evaluating environmental sanitation programs. They have direct relevance to the dynamics of disease transmission. However, the threshold levels of vector infestation that constitute a trigger for dengue transmission are influenced by many factors, including mosquito longevity and immunological status of the human population. These larval indices were calculated as given below.

House Index (HI): Percentage of houses infested with *Aedine* larvae or pupae.

$$\mathrm{HI} = \frac{\text{Number of houses infested}}{\text{Number of houses inspected}} \text{X } 100$$

Container Index (CI): Percentage of water-holding containers infested with *Aedine* larvae or pupae.

$$\mathrm{CI} = \frac{\text{Number of containers with aedine larvae or pupae}}{\text{Number of containers examined}} \text{X } 100$$

Breteau Index (BI): Number of positive containers per 100 houses inspected.

$$BI = \frac{\text{Number of containers with aedine larvae or pupae}}{\text{Number of houses inspected}} X\ 100$$

Pupal Index (PI): is the number of pupae per 100 houses.

$$PI = \frac{\text{Number of pupae}}{\text{Number of houses inspected}} X\ 100$$

The vector breeding indices were compared with incidence of cases in each of the selected sites. The analysis was done with the help of Excel spread sheet 2013 and SPSS. Regression analysis was carried out using monthly data and the incidence in different sites to examine the correlation. Confidence interval was also calculated for its significance. Dengue cases recorded in Tirunelveli district during 2013-14 for different months were obtained from the "Directorate of Public Health and Preventive Medicine, Chennai, Govt. of Tamil Nadu".

RESULTS AND DISCUSSION

Various kinds of breeding sources were examined in this study and all these were categorized into two types. One kind is regarded as "water storage breeding sources" and other as "rain dependent discarded breeding sources". "Water storage breeding sources" are those breeding sources which are containers used for prolonged water storage throughout the year while as rain dependent discarded breeding sources" included discarded breeding sources which were found filled with rain water and thus are positive during the rainy seasons. Thus the vector breeding indices has been obtained on the basis of types of habitat. Three species (*Stegomyia aegypti*, *Stegomyia albopicta* and *Fredwardsius vittatus*) of *Aedine* mosquitoes were encountered in this study and the term "*Aedine/Aedes* mosquitoes/species" has been used to include all the three species of mosquitoes encountered. Analysis of *Aedine* mosquitoes as well as that of vector mosquitoes was performed separately.

A total of 720 houses were examined from the two (low risk) villages for one year. As many as 196 (HI=27.2%) houses were found to have breeding sources supporting *Aedine* mosquitoes (irrespective of breeding type) (Figure 2). The mean number of positive houses for the *Aedine* mosquitoes (irrespective type of breeding sources) per 10 houses examined was found 2.7. Out of total 720 houses examined 168 houses (HI=23.3%) contained positive water storage breeding sources (Figure 2) and the mean number of positive houses supporting the water storage breeding sources (irrespective of species) per 10 houses examined was found 2.3. Only 28 (HI=3.9%) houses had rain dependent habitats supporting *Aedine* breeding (Figure 2).

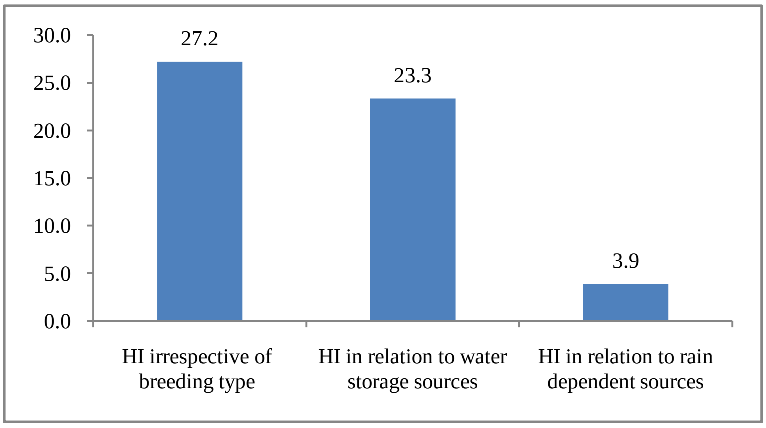

Figure 2. House index (HI) of *Aedine* mosquitoes in low risk areas (n =720).

Out of 5643 containers screened in low risk areas, 288 (CI=5.1) containers were found positive for *Aedine* species (irrespective of type of breeding sources) (Figure 3) and the mean of containers (irrespective of type) examined per house was found 7.8. The mean number of positive containers examined (irrespective of species and breeding type) per 10 houses was found 4.0.

The container index with respect to water storage containers was 5.0 per cent out of 4974 containers examined and the mean of water storage containers examined per house was found 6.9. *Aedine* breeding was observed in 38 out of 669 rain dependent containers (CI=5.7) (Figure 3) and the mean number of positive rain dependent breeding sources examined (irrespective of species) per 100 houses was 5.3. The number of water storage containers supporting *Aedine* breeding was exceptionally higher when compared to rain dependent containers though the latter supports in equal proportion. The mean number of water storage containers examined per house was almost 7 times higher than the rain dependent breeding sources. Analysis has shown that the container index of *St. aegypti* in water storage breeding sources was 1.53% while it was less than 1% in case of *St. albopicta* (Figure 4).

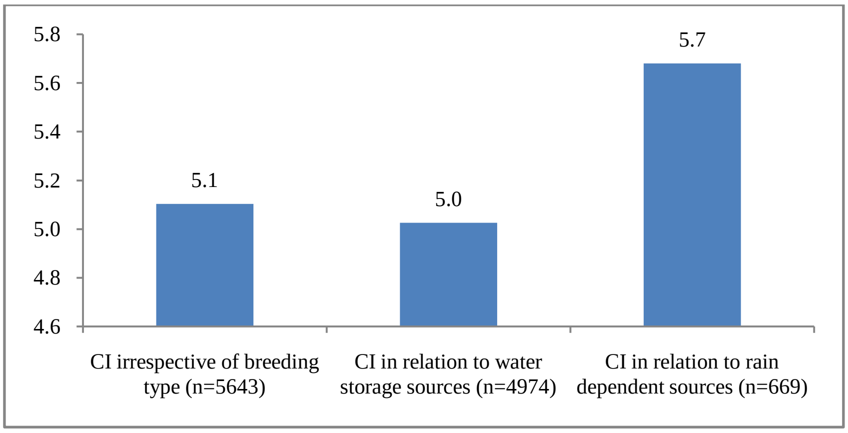

Figure 3. Container index (CI) of *Aedine* mosquitoes in low risk areas.

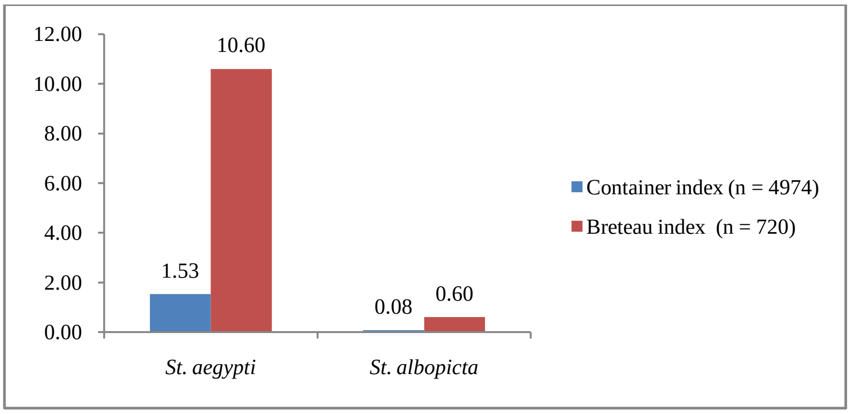

Figure 4. Container and breteau indices of different species in water storage sources in low risk areas.

The breteau index of *Aedine* mosquitoes irrespective of breeding types in low risk areas was found 40% to which water storage containers contributed as high as 34.7% (Figure 5). Only one sixth of the BI was contributed by rain dependent breeding sources (5.3%) (Figure 5). Analysis has shown that the breteau index of *St. aegypti* in water storage breeding sources was 10.60% while in case of *St. albopicta* it was very low (0.60%) (Figure 4).

A total of 810 pupae were collected out of which 792 (97.8%) were contributed by water storage breeding sources whereas less number (18; 2.2%) were collected from rain dependent breeding sources. The pupal index of *Aedine* mosquitoes irrespective of breeding type was found high (112.5), water storage containers contributing 110.0 per 100 houses while as the rain dependent containers contributing 2.5 per 100 houses (Figure 6).

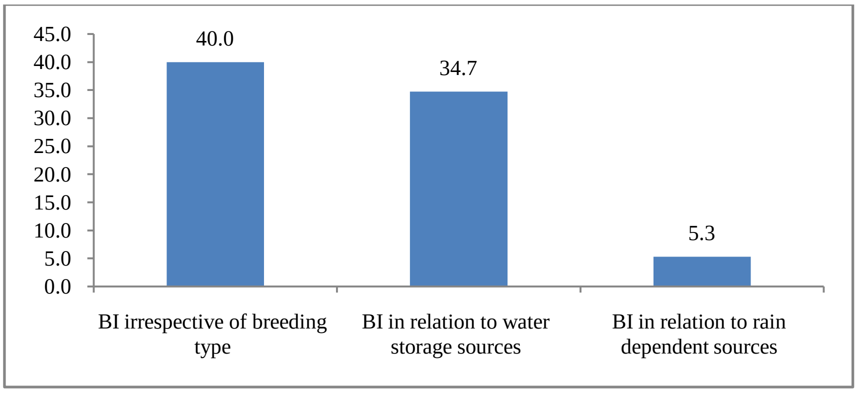

Figure 5. Breteau index (BI) of *Aedine* mosquitoes in low risk areas (n= 720).

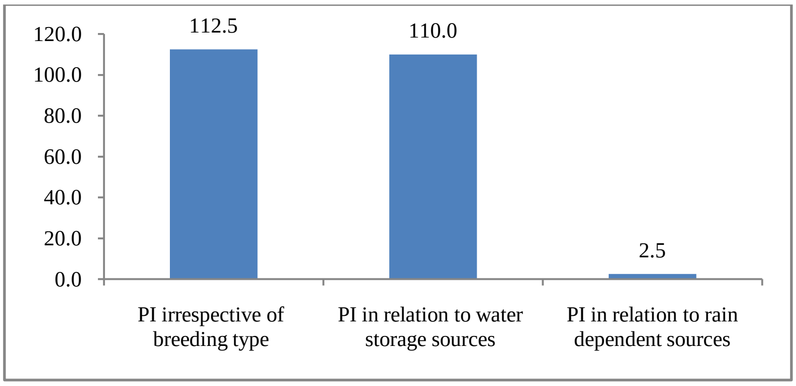

Figure 6. Pupal index (PI) of *Aedine* mosquitoes in low risk areas (n= 720).

The overall house index in both sites with medium risk of dengue was 23.5% (Figure 7) and 169 out of 720 houses were found positive for the breeding of *Aedine* mosquitoes (irrespective of breeding type). The water storage containers contributed 18.2 houses with breeding sources per 100 houses and the rain dependent breeding sources were found from only 38 (HI=5.3%) houses (Figure 7).

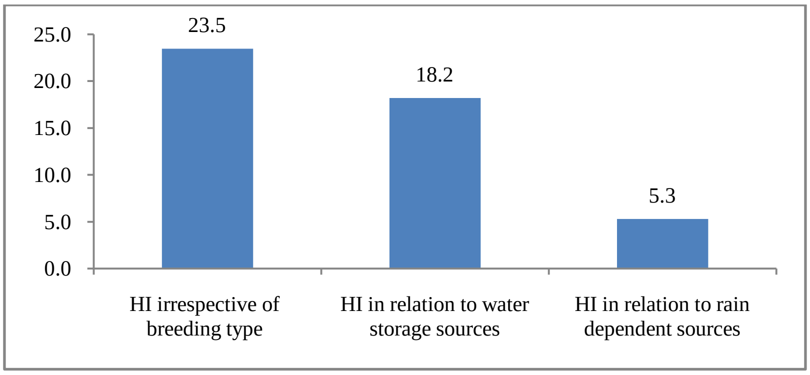

Figure 7. House index (HI) of *Aedine* mosquitoes in Medium risk areas (n = 720).

Total of 4396 containers were screened and 252 (CI=5.7) containers were found positive for the breeding of *Aedines* pecies (irrespective of type of breeding sources) (Figure 8). Total of 3907 water storage breeding sources were examined and 206 (CI=5.3) were found positive for the breeding of *Aedine* species while the CI for rain dependent breeding sources was 9.4% (Figure 8). The breteau index of *Aedine* mosquitoes irrespective of breeding types in medium risk areas was found 35 per 100 houses, water storage breeding sources contributing 28.6 and rain dependent breeding sources 6.4 % (Figure 9).

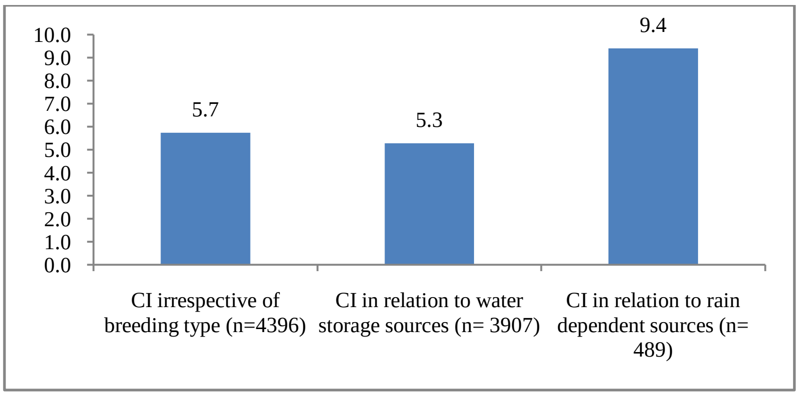

Figure 8. Container index (CI) of *Aedine* mosquitoes in Medium risk areas.

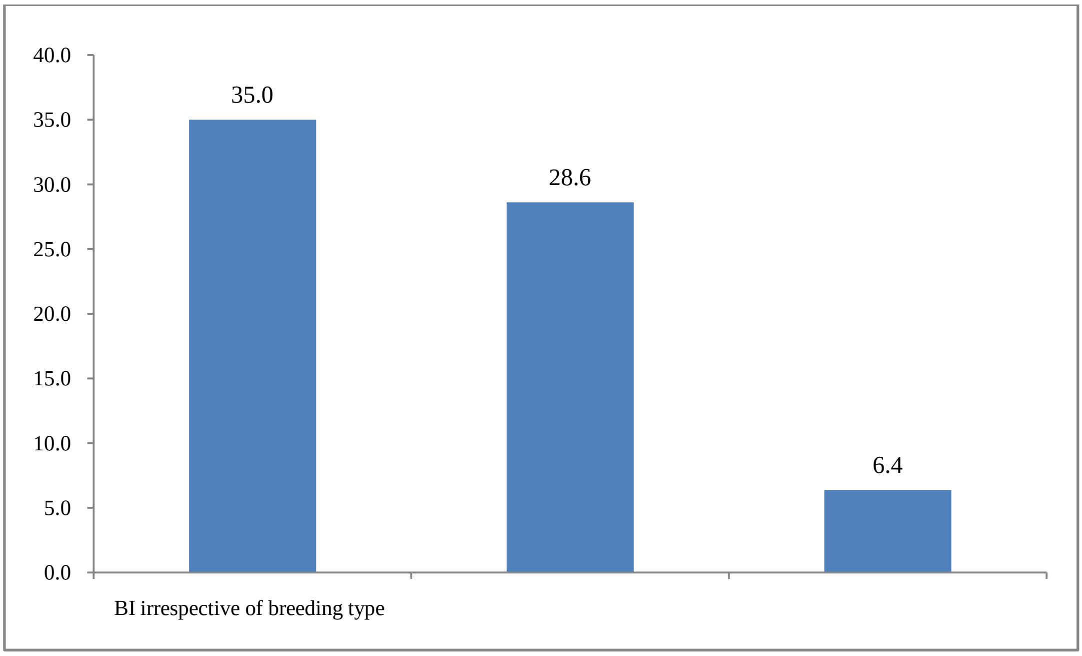

Figure 9. Breteau index (BI) of *Aedine* mosquitoes in Medium risk areas (n= 720).

A total of 645 pupae were collected from medium risk areas, out of which 623 (96.6%) were collected from water storage breeding sources and only 22 (3.4%) were contributed by rain dependent sources. Mean number of *Aedine* pupae was 9.0 per 10 houses inspected irrespective of habitat type while it was 8.7 and 0.3 for water storage and rain dependent breeding sources respectively. The pupal index of *Aedine* mosquitoes irrespective of breeding types was 89.6 while it was 86.5 in water storage sources and very low (3.1) in rain dependent breeding sources (Figure 10).

Container and breteau indices of *St. aegypti* in relation to water storage breeding was 1.38% and 7.50% respectively (Figure 11). Between low and medium risk areas these two parameters were comparable. In case of *St. albopicta* very low container index and breteau index was observed (Figure 11).

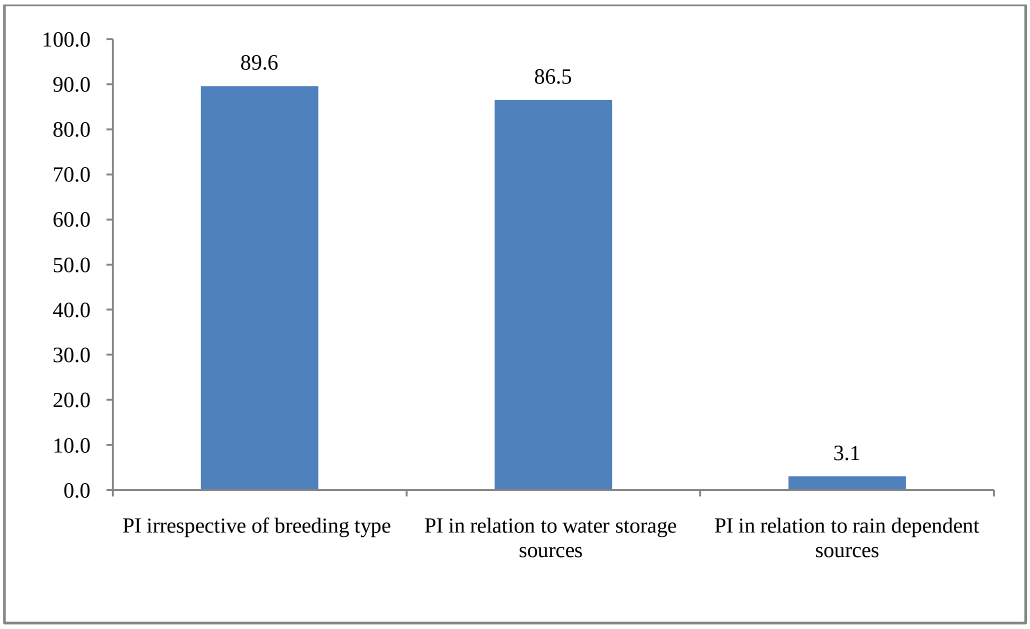

Figure 10. Pupal index (PI) of *Aedine* mosquitoes in Medium risk areas (n= 720).

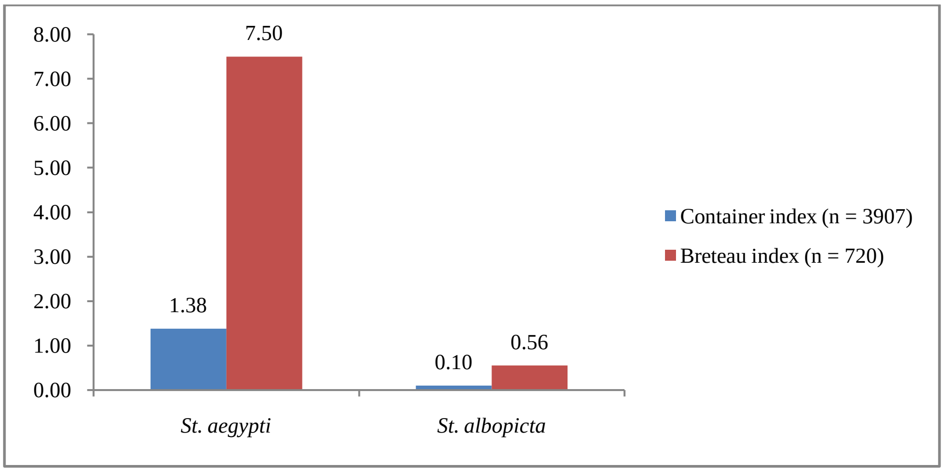

Figure 11. Container and breteau index of different species in water storage sources in medium risk areas.

A total of 720 houses from two sites identified as high risk areas were examined out of which 161 houses (HI=22.4%) (Figure 12) were found with *Aedine* breeding sources (irrespective of breeding type). At least two out of 10 houses inspected were found with positive breeding sources. A total of 128 houses were found to have *Aedine* mosquito infestation in water storage containers (HI=17.8) while 33 houses had rain dependent breeding sources with HI = 4.6% (Figure 12). About 4 out of 20 houses had water storage sources with *Aedine* breeding while only 1 out of 20 houses had rain dependent container with *Aedine* breeding.

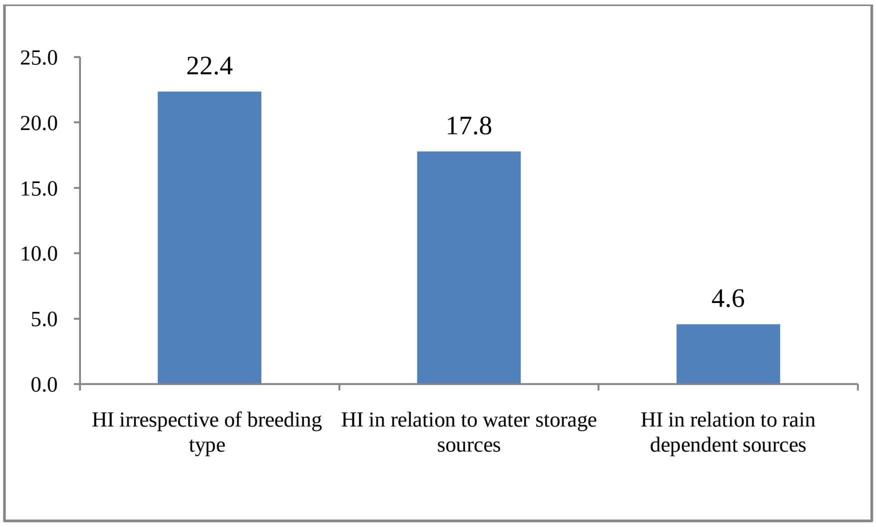

Figure 12. House index (HI) of *Aedine* mosquitoes in High risk areas (n = 720).

Total of 5519 containers were screened and 224 containers (CI=4.1%) (Irrespective of type of breeding sources) were found positive for the breeding of *Aedine* mosquitoes (Figure 13) and the mean of containers examined (irrespective of type) per house was found 7.7 while the mean number of positive containers (irrespective of species and breeding type) per 10 houses examined was found 3.1. There were as many as 4970 water storage breeding sources in these two study sites and 182 (CI = 3.7%) supporting the breeding of *Aedine* species (Figure 13) and the mean of water storage containers examined per house was found 6.9. Out of 549 rain dependent breeding sources examined, 42 (CI=7.7%) were found supporting the breeding of *Aedine* mosquitoes (Figure 13).

The breteau index of *Aedine* mosquitoes irrespective of breeding types in high risk areas was found 31.1 while in water storage breeding sources it was found 25.3 and was low (5.8) in case rain dependent breeding sources (Figure 14).

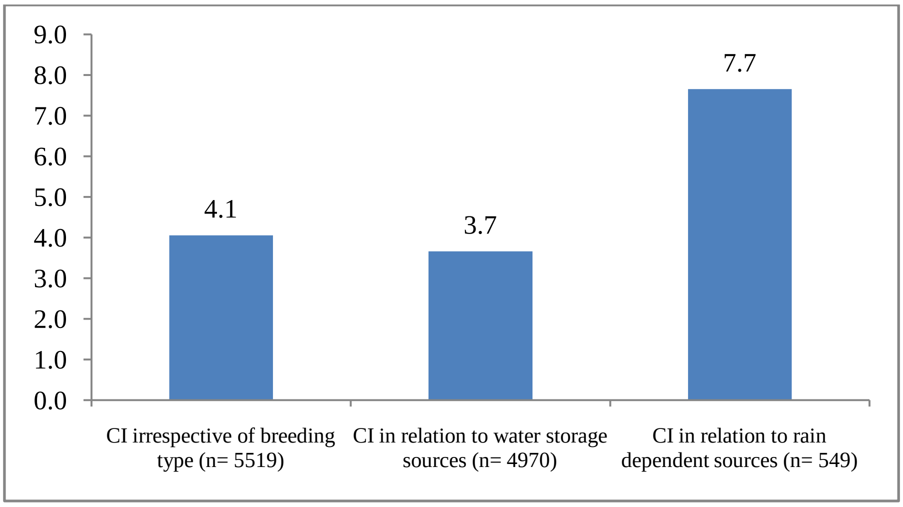

Figure 13. Container index (CI) of *Aedine* mosquitoes in High risk areas.

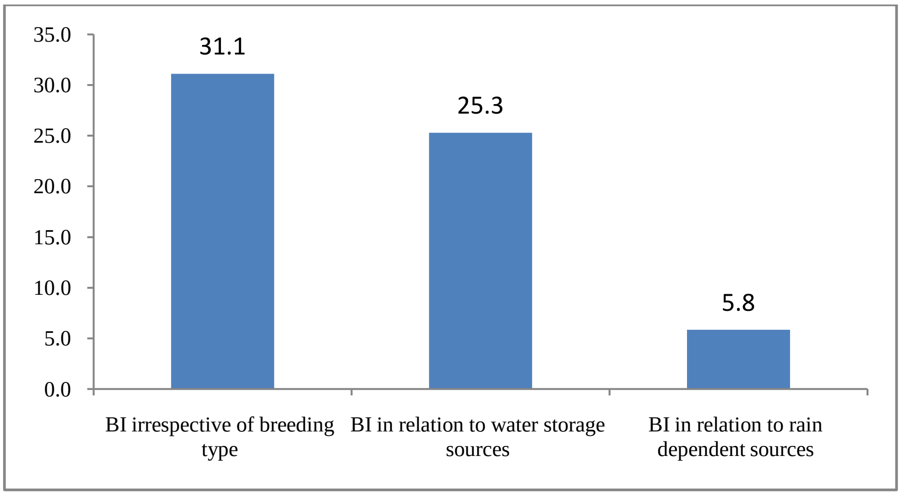

Figure 14. Breteau index (BI) of *Aedine* mosquitoes in High risk areas (n= 720).

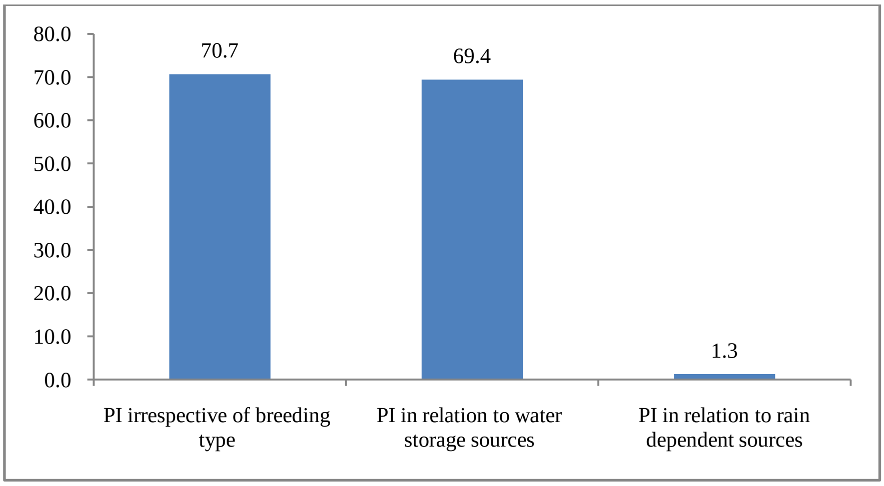

Figure 15. Pupal index (PI) of *Aedine* mosquitoes in High risk areas (n= 720).

A total of 509 Pupae of *Aedine* mosquitoes were collected out of which 500 (98.2%) were collected from the water storage breeding sources while less number (9; 1.8%) were collected from rain dependent breeding sources. The pupal index of *Aedine* mosquitoes irrespective of breeding type was found high (70.7) while in water storage breeding sources it was found 69.4 and in rain dependent breeding sources it was 1.3 (Figure 15). The container index and breteau index of *St. aegypti* in water storage breeding sources was in high risk areas were 2.07% and 14.31% respectively while in case of *St. albopicta* very low container index and breteau index were found (Figure 16).

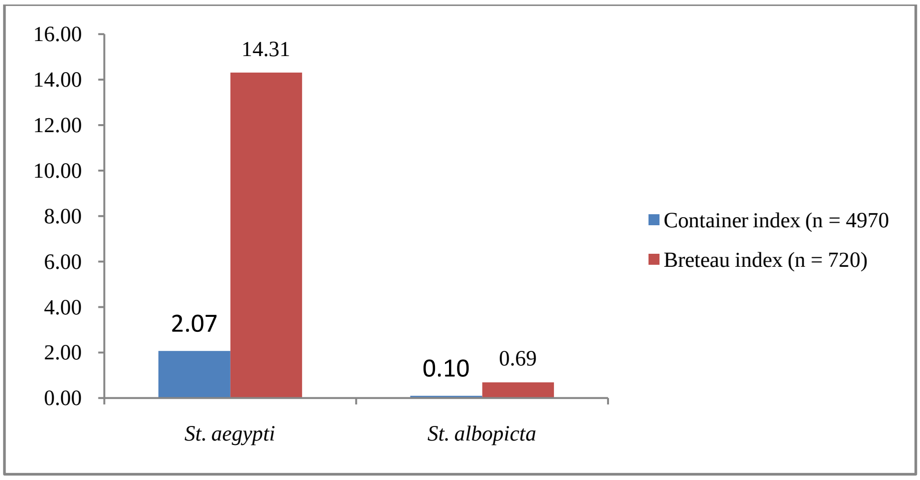

Figure 16. Container and breteau indices of species in water storage sources in High risk areas.

The comparison of entomological indices showed that the mean number of breeding sources did not show any significant difference in different levels of risk areas. Though water storage related breeding habitats are the major breeding source for the vectors, rain dependent habitats also contribute to vector breeding in certain situation as has been observed in medium incidence areas**.** Spatial analysis indicates the importance of inclusion of both rain dependent and water storage related habitats for surveillance. The container and breteau indices of *St. aegypti* in relation to water storage breeding sources was found significantly ($p<0.05$) higher in high risk areas and had shown significant relationship among the areas selected as low, medium and high incidence of dengue cases whereas all other larval indices such as house index, container index, breteau index and pupal index with respect to type of habitats as well as species did not differ significantly ($P>0.05$). This clearly indicates that the entomological indications with respect to the vector mosquito can be used as risk factors to predict the outbreaks. Community action and response to the disease outbreaks could also contribute to the risk in the villages. These larval indices were also predicted as early warning tool for dengue epidemic in previous studies (Chang *et al*., 2015).

Spatial risk identification by using local spatial-autocorrelation is concluded to help in identifying other possible causes of disease risk and further strategic planning for prevention and control measures (Hafeez *et al*., 2017). The present study also showed that there are possibilities of identifying the risks before the outbreak. In earlier studies it has been reported that the imported cases resulted in an outbreak and can be considered as risk factor (Taber *et al*., 2017) and other stuides reported that the low income areas are at more risk (Vicente *et al*., 2017). In the present study area, neither the details of epidemiology of dengue cases including the details of indigenous or imported nor distribution of cases in relation to income are available. Control measures during inter epidemic seasons based on the risk factors by using local spatial statistics and a regression modeling approach to *Ae. aegypti*, but not *Ae. Albopictus* is recommended (Vincenti Gonzalez *et al*., 2017) and human population density in southern Taiwan are closely associated with an increased risk of autochthonous dengue incidence (Tsai & Teng, 2016).

CONCLUSION

The limitation of this analysis is the assumption that the risk in a given area is static and is not supported by the results indicating that the risk factors are highly variable due to dynamics of vector breeding. Vector species *St. aegypti* has been recorded in all the surveyed districts. Association between entomological risk factors indicated that there has been a strong and significant relationship between various breeding indices with respect to *St. aegypti.* Source reduction targeting water storage breeding sources through community participation is required to prevent the outbreak.

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