Seroprevalence of dengue in a rural and an urbanized village: A pilot study from rural western India

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ABSTRACT

Background & objectives: Dengue is highly prevalent in tropical and subtropical regions. The prevalence of dengue is influenced by number of factors, i.e. host, vector, virus and environmental conditions including urbanization and population density. A cross sectional study was undertaken to determine the seroprevalence of dengue in two selected villages that differed in the level of their urbanization and population density.

Methods: Two villages with demographically well-defined populations close to Pune, a metropolitan city of western India, were selected for the study. Age stratified serosurvey was carried out during February to May 2011 in the two villages—a rural village A, located 6 km from the national highway with a population density of 159/km²; and an urbanized village B, located along the highway with a population density of 779/km². Assuming a low seropositivity of 10%, 702 serum samples were collected from village A. Sample size for village B was calculated on the basis of seropositivity obtained in village A, and 153 samples were collected. Serum samples were tested for the presence of dengue virus (DENV)-specific IgG. Simple proportional analyses were used to calculate and compare the seroprevalence.

Results: Of the 702 samples collected from village A, 42.8% were found positive for anti-DENV IgG. A significantly higher seropositivity for DENV (58.8%) was found in village B. In village A, there was an age dependent increase in seroprevalence; whereas, in village B, there was a steep increase from 17% positivity in 0–10 yr age group to 72% in the 11–20 yr age group. The seroprevalence was almost similar in the older age groups.

Interpretation & conclusion: The observations suggested that prevalence of dengue is probably associated with urbanization and host population density. Areas that are in the process of urbanization needs to be monitored for prevalence of dengue and its vector, and appropriate vector control measures may be implemented.

Key words Antidengue IgG; dengue; seroprevalence; urbanization

INTRODUCTION

Dengue has become one of the most important vector-borne diseases over the last few decades, with a steady rise in the global incidence. It is estimated that over two-fifths (2.5 billion) of the world population live in dengue endemic areas, of which 50 million (2%) are infected annually. In India, according to the yearly reports of the National Vector Borne Disease Control Programme (NVBDCP), Delhi there is constant increase in the number of reported cases with 50,222 cases reported during 2012, which increased to 129,166 cases in 20161,2. Dengue, once considered an urban disease, has invaded rural areas due to unplanned urbanization, increase in population and improved transportation, all of which favour the breeding of the vector Aedes aegypti3.

Similar to other developing countries, the accelerated growth of the cities in India has resulted in the urbanization of nearby rural areas, which are mostly unplanned and unsupported by civic amenities. Unplanned urbanization and densely populated foci are considered to be strong risk factors for spread of dengue3,5. Dengue has become endemic in rural areas of India as well, increasing the scale of the dengue challenge in the country6.

Pune is a metropolitan city in the western Indian state of Maharashtra, which is endemic to dengue and Vadu is a rural area in close proximity. The Vadu rural health program (VRHP) run by the King Edward Memorial Hospital Research Centre (KEMHRC), Vadu has 22 villages (and a population close to 100,000) under close demographic surveillance, since 2003. To determine the effect of urbanization and population density on dengue seroprevalence, two villages from Vadu were selected.
for the age stratified serosurvey. One village was rural and located in the interior part with a population of 2621, whilst the other was urbanized and had a population of 17,666.

MATERIAL & METHODS

Characterization of villages

The two villages (Pimple Jagtap and Koregaon Bhima) were characterized as rural or urban according to the Indian Government Census (2011) criteria, which states that a place is defined as urban if it satisfies the following three criteria: (i) a minimum population of 5000; (ii) at least 75% of the male working population engaged in non-agricultural pursuits; and (iii) a population density of at least 400/km² (1000/mile²).

Sample size calculation

As there was no data on the prevalence of dengue for the study area, a conservative assumption of 10% prevalence was made. Considering 2% error in estimation, the sample size was calculated with the help of Epi Info software version 3. After adjustment of 10% in sample quality, and handling and testing error, the total sample size was 700 for village A, and 702 samples were collected and tested for presence of DENV specific IgG. The seropositivity calculated for village A was used as reference value to calculate sample size for village B. The sampling was age-stratified, with five age groups, AG1 through AG5, representing 0–10, 11–20, 21–30, 31–40, and >40 yr. An attempt was made to collect equal number of samples from each representative age group. It was ensured that all settlements of the villages were included for sampling.

Ethical considerations

Ethical clearance and approval (KEMHRC ID. No 1047 dated October 24, 2010 and 110(01)EC-I/1054 dated February 15, 2011) for the study protocol was obtained from the Institutional Ethics Committees of the National Institute of Virology, Pune and the KEM Hospital Research Centre, Pune, India. The consent forms were prepared in English and the regional language, Marathi. Written informed consent was obtained from the participants or their parents/legal guardians.

Inclusion/exclusion criteria

All residents of the Vadu area were eligible for inclusion in the study; however, migrants were not included. Individuals who had been vaccinated for Japanese encephalitis or yellow fever were excluded from the study. Individuals with known history of bleeding disorders were also excluded.

Serological assay

Dengue virus (DENV) specific IgG antibodies were assessed in 702 samples from village A and 153 samples from village B using an indirect IgG ELISA kit (E-DEN01G, Panbio Inc., Brisbane, Queensland, Australia). The Panbio units were calculated as per the manufacturer’s Instructions. The Panbio <9 were considered negative, between 9 and 11 equivocal; and >11 were considered positive for IgG.

Statistical analysis

Vadu health and demographic surveillance system data is managed in MySQL format. Study data were exported in MS Excel. Simple proportional analyses with confidence intervals for community prevalence were calculated with the STATA-11 software. The community prevalence for age groups and genders were compared based on confidence intervals. The estimated percentage of people exposed to dengue for each age group (dengue exposure) was calculated based on formula: Dengue exposure = Increase in prevalence × 100/(100–Prevalence in previous age group).

Comparative analyses (Odds ratios) were performed with Epi Info-7 software.

RESULTS

Characteristics of the villages

The characteristics of the two villages in Vadu area selected for the study are shown in Table 1. As village A (Pimple Jagtap) had no matching criteria of urbanization it was considered rural. Village B (Koregaon Bhima), which is situated along the highway had two matching criteria, and was considered as urbanized (though it was under the process of urbanization). Water supply in both villages depended on wells and bore-wells.

Anti-DENV IgG positivity

In the rural village A, 702 residents were tested and

<p>| Table 1. Characteristics of the villages used in the study |
|-------------------------|-------------------------|-------------------------|</p>
<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Village A</th>
<th>Village B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population</td>
<td>2621</td>
<td>15253</td>
</tr>
<tr>
<td>Population density</td>
<td>159/km²</td>
<td>779/km²</td>
</tr>
<tr>
<td>Distance from National Highway</td>
<td>7.4 km</td>
<td>0 km</td>
</tr>
<tr>
<td>Population engaged in agriculture</td>
<td>80%</td>
<td>50%</td>
</tr>
<tr>
<td>Seroprevalence (based on present study results)</td>
<td>42.8%</td>
<td>58.8%</td>
</tr>
</tbody>
</table>
in the urbanized village B, 153 residents were included. The anti-DENV IgG positivity for village A was 42.8% and in village B, it was 58.8%. Seropositivity was higher in village B compared to village A ($\chi^2 = 6.46, p = 0.011$).

The age group dependent analysis of seroprevalence in the rural village A showed that 13.7% of the population was exposed to DENV before the age of 10 (AG1) (Table 2). Within AG1, children aged 0–5 yr had significantly lower seroprevalence (8%) than children aged 6–10 yr (24%) (OR = 0.28; 95% CI: 0.09–0.84). The frequency of seropositive samples increased with age. Each of the age groups, AG1 (0–10) through AG4 (30–40) differed significantly from each other (Fig. 1). Age group, AG5 (>40 yr) showed maximum seroprevalence. The male : female ratio among the participants was 1 : 1.29. A comparison of seroprevalence among males and females across age-groups revealed no significant difference up to the age of 30 yr. However, in AG4 and AG5, males showed higher seroprevalence than females (Table 3).

The age-wise analysis of participants from village B indicated that AG1 had significantly lower seroprevalence (17.2%) compared to AG2 [72.2%; OR=0.0801, 95% CI:0.0195–0.3287]. Seroprevalence in AG2 and the older age groups (AG3–70.9%, AG4–79.1%, and AG5–70.5%) was almost similar. The four-fold increase in positivity from AG1 to AG2 was in contrast to the stepwise increase observed among the different age groups in village A (Fig. 1). In the urban village B, the male : female ratio was 1 : 2.06. The percentage positivity was 58% among males and 62% among females. The seroprevalence did not vary significantly between males and females in different age groups.

The prevalence and the risk of exposure for village A are shown in Table 2. The highest increase in prevalence was observed from AG2 to AG3 compared to the increase from AG4 to AG5. Assuming that the disease was endemic in village A, and both dengue seropositive and seronegative populations are at equal risk of being bitten by a DENV infected vector, percentage calculated for exposure to dengue virus was found to be highest in AG3 (20–30) and AG4 (30–40), and least in AG5 (>40).

### Table 3. Seroprevalence of dengue in males and females of different age groups of rural village A

<table>
<thead>
<tr>
<th>Age groups (yr)</th>
<th>E* (–)</th>
<th>(+)</th>
<th>Total</th>
<th>% positivity</th>
<th>Population</th>
<th>Binomial Ex CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>AG1 (10–20)</td>
<td>118</td>
<td>19</td>
<td>138</td>
<td>13.7</td>
<td>421</td>
<td>10.6–17.4</td>
</tr>
<tr>
<td>Female</td>
<td>51</td>
<td>8</td>
<td>60</td>
<td>13.3</td>
<td>192</td>
<td>9–19.2</td>
</tr>
<tr>
<td>Male</td>
<td>67</td>
<td>11</td>
<td>78</td>
<td>14.1</td>
<td>229</td>
<td>9.7–19.1</td>
</tr>
<tr>
<td>AG2 (21–30)</td>
<td>89</td>
<td>41</td>
<td>136</td>
<td>30.1</td>
<td>424</td>
<td>25.8–34.8</td>
</tr>
<tr>
<td>Female</td>
<td>44</td>
<td>23</td>
<td>71</td>
<td>32.3</td>
<td>184</td>
<td>25.8–39.8</td>
</tr>
<tr>
<td>Male</td>
<td>45</td>
<td>18</td>
<td>65</td>
<td>27.6</td>
<td>240</td>
<td>22.3–34</td>
</tr>
<tr>
<td>AG3 (31–40)</td>
<td>75</td>
<td>65</td>
<td>144</td>
<td>51.2</td>
<td>566</td>
<td>41–49.2</td>
</tr>
<tr>
<td>Female</td>
<td>52</td>
<td>45</td>
<td>99</td>
<td>45.5</td>
<td>255</td>
<td>39.4–51.6</td>
</tr>
<tr>
<td>Male</td>
<td>23</td>
<td>45</td>
<td>68</td>
<td>44.4</td>
<td>311</td>
<td>38.7–50</td>
</tr>
<tr>
<td>AG4 (40+)</td>
<td>53</td>
<td>83</td>
<td>136</td>
<td>45.4</td>
<td>410</td>
<td>54.5–64.3</td>
</tr>
<tr>
<td>Female</td>
<td>46</td>
<td>86</td>
<td>134</td>
<td>45.4</td>
<td>195</td>
<td>46.0–60.6</td>
</tr>
<tr>
<td>Male</td>
<td>13</td>
<td>37</td>
<td>50</td>
<td>69.8</td>
<td>215</td>
<td>63.1–75.8</td>
</tr>
<tr>
<td>AG5 (&gt;40)</td>
<td>49</td>
<td>93</td>
<td>142</td>
<td>64.1</td>
<td>800</td>
<td>60.6–67.4</td>
</tr>
<tr>
<td>Female</td>
<td>37</td>
<td>42</td>
<td>79</td>
<td>52.5</td>
<td>391</td>
<td>47.3–57.5</td>
</tr>
<tr>
<td>Male</td>
<td>12</td>
<td>51</td>
<td>65</td>
<td>78.4</td>
<td>409</td>
<td>73.9–82.1</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>384</td>
<td>301</td>
<td>42.8</td>
<td>2621</td>
<td>40.9–44.7</td>
</tr>
</tbody>
</table>

*Equivocal results; considered as ‘Negative’ for analyses. *Source: Vadu Health and Demographic Surveillance System-Health and Demographic Surveillance System (HDSS), Vadu, Pune, India.

**DISCUSSION**

The present cross-sectional study compared dengue prevalence in two demographically well-defined villages in Vadu in Pune district, western India. The two villages differed in the level of their urbanization. Based on the criteria of Census, India (2011), they were categorized as rural and urban. Data from both the villages, specifically the
rural village indicated that dengue is endemic in that area. Variable prevalence of dengue infection was observed in the two villages. The urbanized village B showed significantly higher seroprevalence than the rural village A. The higher prevalence in village B could be attributed to higher population density, proximity to a major highway, and greater commercial activity; indicated by the number of restaurants, schools, a major bus station and weekly market that attracts thousands of people from neighbouring villages. The association of higher incidence of dengue with population density has been reported in a study by Roriz-Cruz et al. Spread of DENV and probably its vector is also favoured by the high intensity and frequency of private and public transportation, a characteristic of urbanized village B.

An interesting finding was the age-dependent gradual increase in seroprevalence in the rural village as compared to the one-step increase in the urban village from AG1 to AG2. The abrupt increase in seroprevalence may indicate a very high intensity of transmission during the past decade. This abrupt increase was also observed in a seroprevalence study from Chennai, a metropolitan city of India. However, the seroprevalence was still lower in the age group of 0–10 in the urbanized village of the present study compared to the Chennai, implicating urbanization as a major factor influencing the seroprevalence of dengue. More number of villages, representative of A and B, need to be surveyed to determine if this difference in age-wise seroprevalence is common to other rural and urban villages. In AG1, the 6–10 yr-old group showed significantly higher seropositivity than the 0–5 yr group, indicating that exposure to DENV is not common in younger age group as reported in Thailand.

In the rural village, the percentage exposure was higher in the 20–40 yr age groups, which is the dynamic age group. Significantly, higher seroprevalence was observed among males in the age groups >30 yr, the age at which women in this area probably tend to remain in houses for long periods. Though, *Ae. aegypti* is considered a ‘domestic’ vector, it is not strictly limited to households, and is found in workplaces and public places as well. An inquiry into the time spent in households vs workplaces by the males and females will help understand the exact reasons of difference in seropositivity.

The results must be considered in the context of the limitations of the serological surveys. More number of female subjects than the male subjects participated in this study. This was because the households were visited during working hours. The data is limited as the study included only two villages. Seroprevalence of dengue is influenced by vector density and transmission capability of the vector. The present study lacks data on vector density for the two villages. However, studies have shown that urbanization increases the larval habitats of *Aedes* mosquitoes there by leading to increased vector density, larval development rate and adult survival rate, which ultimately increases the vector capacity to transmit the virus.

The present study had implications for introducing dengue vaccine. Recently, Dengvaxia, a vaccine for dengue has been approved in certain endemic countries for use in subjects aged between 9 and 45 yr. A World Health Organization (WHO) position paper has reported that introduction of Dengvaxia should be considered only in geographical settings where in the seroprevalence of targeted age group is ≥ 70%. It is not recommended when seroprevalence is < 50%. In the present study, seroprevalence was 49.9% in the age group >10 yr in the rural village, while it was >70% in the urban village. This suggests that even in countries endemic for dengue, the seroprevalence rates might vary drastically among metropolitan, urban, semi-urban and rural setups.

**CONCLUSION**

On the basis of this preliminary study, it can be concluded that dengue prevalence is probably associated with level of urbanization and population density in rural areas of Pune district, western India. The disease burden and longitudinal ‘disease and risk factor surveillance’ studies along with vector related investigations are required to understand the disease dynamics in a better way.

**Conflict of interest**

The authors declare that they have no conflict of interest.

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