Dengue Surveillance in Colombo, Sri Lanka: Baseline seroprevalence among children


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Abstract

Dengue virus (DENV) infection has been endemic to Sri Lanka and reliable estimates of burden of disease are limited. Such information is crucial for prevention and control of the disease and for vaccine introduction. We are conducting a community based enhanced passive surveillance study to estimate burden of DENV infection and disease. The study is based on a one year follow-up cohort of 800 randomly-selected children aged <12 years in a municipality ward of Colombo, Sri Lanka. The baseline blood samples tested by IgG capture ELISA indicate that the flavivirus seroprevalence in the cohort is 52% (ranges from 14% below 1 year to 72% at 12 years). These results suggest endemic transmission of DENV among children in Colombo.

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1. Introduction

Dengue virus (DENV) infection has become the most common mosquito-borne viral disease in humans and is a major international public health concern, with millions of people living in tropical and subtropical parts of the world [1, 2] It is estimated that 50-100 million infections occur yearly, including 500,000 Dengue hemorrhagic fever (DHF) cases and 22,000 deaths, mostly among children spread throughout over 100 countries [3]. The current method of prevention of dengue is mostly limited to the control of mosquito vectors in endemic areas. Safe and effective vaccines against all four serotypes of dengue virus are under development and several vaccine candidates are reaching large scale clinical trials while other candidates are expected to reach clinical trials at least in the next five years [4].

Sri Lanka, an island off the southern coast of India with an estimated population of about 20 million for 2009 [5], is one of the Asian countries where dengue has become endemic and a major public health problem. Dengue is a notifiable disease in Sri Lanka and the number of reported cases of Dengue in the national surveillance system has increased substantially from 15463 (80/100,000 population) in the year 2004 to 30,251 (142/100,000 population) in 2009 [6]. It has been shown that even though all four dengue serotypes have been circulating in Sri Lanka for over 40 years, the emergence of new clades of dengue 3 coincided with increasing numbers of DHF cases after 1989 [7].

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A prospective community-based fever surveillance with a household enrolment model was established in September 2008 in Colombo, Sri Lanka with the broad aim to generate estimates of the burden associated with clinical dengue and dengue infection in the paediatric population. The study will determine the incidence of symptomatic dengue infection and annual seroconversion, and it will help in laying the necessary groundwork for a future vaccine introduction. This paper will focus only on the seroprevalence of dengue in this cohort of children at the time of recruitment.

2. Materials and methods

**Study site & cohort:** The surveillance site is located in the city of Colombo, the commercial capital of Sri Lanka. Colombo city has a population of 647,100 and is the most densely populated area in the island with 17,353 persons per square kilometer enumerated in 2001[5]. The city is divided into 47 municipal wards and one such ward – municipal council ward 33, was selected with a stable population with mixed socio-economic status and is representative of the population base of the entire Municipal Council area. The ward is endemic for dengue with the catchment population seeking healthcare in the tertiary care institution situated within its boundaries. Since the last decennial census has been done in 2001, a house-to-house population census was carried out at the beginning to gather information on the size of the latest child population in the Ward, household details, current socio-demographic and health seeking pattern of this population. All households within the ward were divided up into a grid with 42 Census-blocks and visited by research assistants employed and specially trained by the investigators. A database of households with eligible children – being permanent residents and less than 12 years of age was created using the census information.

A sample of 800 children under 12 years of age stratified by census blocks and sample allocated probability proportional to the population of same age in each census block was generated using the census database. All children less than 12 years in a randomly selected household meeting the eligibility criteria were recruited based on written informed consent of parent/legal guardian. Each participating child was given a personal identification card with a unique ID Number.

**Sample collection and processing:** A baseline finger prick blood sample was collected on to a protein saver card (Whatman & ID Biological systems, USA) labeled with the participant’s ID at initial recruitment, in order to determine the dengue seroprevalence. These samples were transported in specially designed containers to the testing laboratory where they were left over night at room temperature for air drying and then were stored at -20 °C temperature in labeled Bitran airtight re-sealable storage bags containing desiccants and a humidity indicator until testing was done in batches.

**Serologic tests: IgG capture ELISA:** An in-house IgG assay developed at University of North Carolina, USA was done in order to measure IgG levels in all baseline samples. The anti-dengue 4G2 mouse monoclonal antibody (ATCC, USA) was used as the capture antibody. The plates were washed (Wash buffer-1% TBS and 0.2% Tween 20) and non-specific binding was blocked at 37 °C with blocking buffer (1x TBST, 3% Normal Goat Serum). Then the antigen mixture (1:1:1:1 D1-D4) was added for 1 hour. Following washes the serum/or eluted filter paper sample was added at 1:50 dilution and incubated for 1 hour. Filter papers were punched and eluted using elution buffer (1x TBST, 0.5% Tween20, 5% Non fat dry milk) and incubating at 37°C for 2 hours. Then the secondary antibody (IgG –Fc specific – Alkaline phosphatase antibody- Sigma A9544) was added and the plate was finally developed by adding substrate (p-Nitrophenyl Phosphate-Sigma). Each sample was duplicated in test and the average of the two optical density values (OD) was used. Samples which gave an OD of \( \geq 0.3 \) after background subtraction were considered positive/flavivirus immune.

Ethical approval for this research was obtained from the Ethical Review Committee of the Faculty of Medicine, University of Colombo, Sri Lanka and the Institutional Research Board of the International Vaccine Institute, Seoul, Korea.

3. Results

A total of 797 baseline samples were tested. Of these, 412 were seropositive, giving a baseline seroprevalence of 52% among children in the study cohort. There was no significant difference between male (46%) and female (54%) participants. By 5 years of age 51% of the children were seropositive. Further, by the age of 9 years almost around 70% were seropositive, as shown in Figure 1.
4. Discussion

This is the first community based follow-up study to estimate burden of dengue among children in Sri Lanka. With the continuation of the project over a period of few years depending on funding support, we expect to collect significant amounts of data on the burden of dengue in this cohort of children. Our preliminary data is in agreement with a study done in 1980-85 using a cohort of 5-7 year old children from Colombo, which showed a seroprevalence of 50% in this age group [8]. This suggests that despite a significant increase in the number of dengue fever and DHF cases, there seems to be no difference in DENV transmission over this period [10]. It is also important to note that among the infants 14% were seropositive, indicating the possibility of a high level transfer of maternal antibodies during pregnancy from a majority seropositive adult population. The proportion of seropositives were even higher (25%) among the under 6 month of age group in the cohort further justifying this hypothesis.

We expect to conduct a repeat seroprevalence assessment of the same cohort after one year to determine the sero-conversion rate among the study population. In addition, we plan to estimate age specific incidence among the children helping us to identify target age groups for vaccination, once a vaccine is available.

5. Acknowledgements

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6. References


