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## Burden estimation of dengue at National Public Health Laboratory, Kathmandu

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## PEER REVIEW

## Peer reviewer

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## Comments

In this paper, authors determined the burden of dengue in the patients visiting National Public Health Laboratory, Kathmandu. It is a good basic study and arrives at a meaningful conclusion on the improvement of surveillance and vector control.

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## ABSTRACT

**Objective:** To determine the burden of dengue in the patients visiting National Public Health Laboratory, Kathmandu.

**Methods:** A cross sectional study was carried out at National Public Health Laboratory, Kathmandu from May to December, 2013. Serum samples were collected from patients suspected of dengue virus infection and tested by ELISA.

**Results:** Among 266 patients suspected of dengue virus infection, 45 (16.9%) showed anti-dengue immunoglobulin M antibodies in serum. Males and economically active people were more infected and the maximum number of cases was during the month of October.

**Conclusions:** This study revealed that the proportion of dengue was more in Kathmandu, especially among the economically active males. So, the control measures should be initiated targeting these groups of people.

## KEYWORDS

Dengue, Infection, ELISA, Kathmandu

## 1. Introduction

Dengue is an arthropod borne febrile illness that is transmitted mainly by the bite of infected *Aedes* mosquitoes. Dengue virus (DENV) belongs to the family Flaviviridae and consists of four serotypes (DENV 1-4)[1] that cause a disease that may result in

a mild dengue fever with or without warning signs or severe dengue[2]. Dengue fever usually presents with high fever, severe headache, myalgia, arthralgia, skin rashes, retro-orbital pain, leucopenia and thrombocytopenia which lead to respiratory distress, hemorrhage, organ impairment. The first case of dengue was identified in Nepal in 2004[3]. Since then, it has been

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transmitting by the form of all the serotypes.

Nepal is at a higher risk of dengue transmission as the Terai Region is surrounded by India from the east, south and west and frequent outbreaks have been reported from India[4]. The disease has been circulating in southern of lowlands; the region has already experienced two dengue outbreaks in 2006 and 2010[5]. The occurrence of DENV-1, DENV-2, DENV-3 and DENV-4 serotypes in the territory of Nepal augment the chances for the epidemic to be flourished in the country[6-8]. Due to the change in climate, the vector mosquitoes have been expanding in their geographical areas and *Aedes aegypti* have already been identified in Kathmandu[9]. Therefore, we attempted to study the burden of dengue cases in Kathmandu, their distribution among different age groups and gender, and seasonal variation of the cases.

## 2. Materials and methods

The study was carried from May to December, 2013. A suspected case was defined as a febrile case with at least two clinical symptoms suggestive of dengue infection and excluded, if the case was provisionally diagnosed as infection, other than dengue and being treated for bacterial and parasitic infections[10]. The suspected cases, visiting National Public Health Laboratory, Teku were included in the study. Patients' personal details about the symptoms, age, sex, etc. were obtained through a questionnaire method by direct interview. Verbal consent was obtained from all the responding individuals. The blood samples (5 mL from adult and 3 mL from children) were collected from each suspected cases in sterile, clean, dry and labeled test tube. The collected blood in the test tube was allowed to clot at room temperature. Then the blood in test tube was centrifuged at 3000 r/min for 5 min and the serum was separated. The serum was then collected in vial and then screening test for dengue was done by rapid immunochromatographic test (Standard Diagnostics Inc., Korea) to get a preliminary idea about dengue. Finally, both rapid test positive and negative samples aliquots were stored at 2-8 °C until tested by Panbio Dengue immunoglobulin M (IgM) capture ELISA.

## 3. Results

A total of 266 samples were analyzed. Among them, 45 (16.9%) cases were found to possess anti-dengue IgM antibodies in serological testing by ELISA. In this study, the positive cases in ELISA have been termed as dengue cases as the sensitivity and specificity of ELISA is more rapid than immunochromatographic tests. The distribution of cases was more males (21.3%) than females (9.3%) (Table 1). Age wise distribution of the cases revealed that the disease was more among the economically

active age group of 15-50 years (18.8%) than in the children of less than 15 years (15.5%) and old aged people of more than 50 years (11.6%) (Table 2).

**Table 1**  
Sex-wise distribution of positive DENV cases.

| Sex    | No. of samples tested | No. of positive samples [n (%)] | Positive cases in total (%) | Statistics     |
|--------|-----------------------|---------------------------------|-----------------------------|----------------|
| Male   | 169                   | 36 (21.3)                       | 13.5                        | $\chi^2=5.577$ |
| Female | 97                    | 9 (9.3)                         | 3.4                         | $P=0.018$      |
| Total  | 266                   | 45 (16.9)                       | 16.9                        |                |

**Table 2**  
Age-wise distribution of positive DENV cases.

| Age   | No. of samples tested | No. of positive cases [n (%)] | Positive cases in total (%) | Statistics     |
|-------|-----------------------|-------------------------------|-----------------------------|----------------|
| <15   | 58                    | 9 (15.5)                      | 3.4                         | $\chi^2=1.348$ |
| 15-50 | 165                   | 31 (18.8)                     | 11.6                        | $P=0.510$      |
| >50   | 43                    | 5 (11.6)                      | 1.9                         |                |
| Total | 266                   | 45 (16.9)                     | 16.9                        |                |

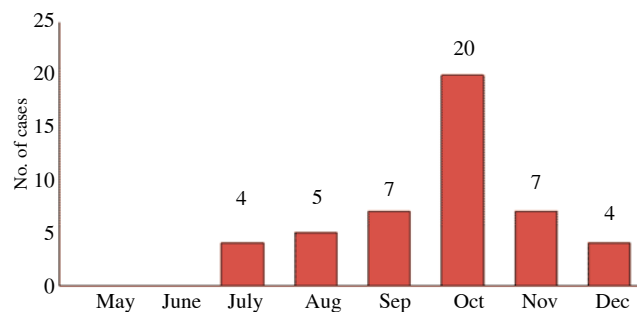
Month-wise distribution of the cases showed that the maximum number of cases were during October (20), followed by September and November (7 each). Maximum cases (80%) were found during the post monsoon period (Figure 1 and Table 3). Maximum number of dengue cases were during 4-7 d after the onset of fever (Table 4).

**Table 3**  
Season-wise distribution of DENV cases [n (%)].

| Season       | ELISA positive cases | ELISA negative cases | Total tested cases |
|--------------|----------------------|----------------------|--------------------|
| Monsoon      | 9 (20)               | 69 (31)              | 78 (29)            |
| Post monsoon | 36 (80)              | 152 (69)             | 188 (71)           |
| Total        | 45 (100)             | 221 (100)            | 266 (100)          |

**Table 4**  
Relation between dengue and onset of fever [n (%)].

| Fever onset day (at the time of blood collection) | ELISA positive cases | ELISA negative cases | Total tested cases |
|---|----------------------|----------------------|--------------------|
| <3 d  | 7 (15)               | 61 (28)              | 68 (26)            |
| 4-7 d   | 21 (47)              | 84 (38)              | 105 (39)           |
| >7 d  | 17 (38)              | 76 (34)              | 93 (35)            |
| Total   | 45 (17)              | 221 (83)             | 266 (100)          |



**Figure 1.** Season-wise distribution of dengue positive cases.

#### 4. Discussion

This study showed higher prevalence of the disease in Kathmandu. The result is higher than some previous studies[11-13], but some other studies report still higher prevalence[14,15]. The difference might be due to the change in the geographical distribution. The previous studies have been done mostly in the Terai Region of Nepal which has been experiencing the disease for a longer period.

The distribution of cases was more in the males of the population and among the economically active age group of 15-50 years. As the number of suspected male cases was higher and the number of positive cases was also high in males than in female. The ratio of dengue positive cases in male to female was 4:1. Statistically, there is significant relationship between sex and the occurrence of the disease ( $P=0.018$ ). The result was also in harmony with the previous studies done in Nepal in which the number of DENV cases was more in males[13,14]. However, the finding was not in accordance with study[11,14]. The number of cases was generally more in males because males are more likely to be exposed to mosquitoes during their outdoor activity. Although dengue vectors can bite both indoor and outdoor people, generally the places inside houses are kept clean, the presence of stagnant water and small bushes in the kindergarden where usually males work has increased their exposure to the vectors.

The highest number of anti-dengue IgM positive cases was from the productive age group of 15-50 years, followed by below 15 years group and the least were from age group above 50 years. The result is in harmony with the data obtained in a study in Nepal in the year 2009 in which more adults of 20-40 years were infected[6] and the other study from southern lowlands of Nepal[11]. Statistically, there is no significant relationship between age and the occurrence of the disease ( $P=0.51$ ). The active group is involved in outdoor activities. So there is increased risk of vector contact with this age group. Being economically more significant group, the possibility of attending to hospital is high in this age group than other. The reason for lower number of isolates in the younger age group could be due to improper selection of clinical cases. In children, DENV manifests as undifferentiated illness, pre-adolescent children exhibit a DENV-like illness but are not as severely incapacitated as adults. The disease in adults is severe enough that patients feel sick and demand medical attention. This seems to be the reason why adult patients are particularly apparent during dengue epidemics.

Roughly, the collection covers the part of the monsoon and part of the post monsoon period. The highest number of cases was recorded in the month of October followed by September,

November and August. This data is consistent with the result obtained during outbreak of dengue in Nepal in which 75% of DENV cases were reported in month of October and few positive cases in September and November[6]. High number of dengue cases was recorded in the month of October and November in the epidemic of 2010[13]. Dengue has been circulating more during the post monsoon period as the conditions are favorable for the growth and multiplication of *Aedes* mosquitoes. The rate of transmission is pronounced as the distribution of *Aedes aegypti* larval indices is the highest during this period[16]. The factors that promote vector proliferation includes environmental conditions (temperature, humidity and altitude), poor sanitation or availability of potential breeding sites are important constituents of spreading dengue in the post monsoon season[10].

In Nepal, many people lack knowledge about dengue[17,18]. In such areas, the results of this study can be of importance for the hospitals in early screening of dengue cases, which has been a major problem in less equipped hospitals of our country. But, there are some limitations. During selection of cases, some dengue cases might have been missed due to exclusion of cases with provisional diagnosis of another infection. The qualitative serological tests reported the cases as either positive or negative, but could not classify them as primary or secondary infection. Further, the use of advanced molecular techniques as PCR for diagnosis could be more reliable in such studies. This study is an attempt to study the burden of dengue in our society.

This study states that the proportion of dengue is higher in Kathmandu. The disease was confined to the southern lowlands, but now it has been expanding to new areas. So the concerned authority should initiate extensive surveillance of dengue virus infection and commence an integrated vector control program in such areas.

#### Conflict of interest statement

We declare that we have no conflict of interest.

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## Comments

### Background

A cross sectional study was carried out at National Public Health Laboratory, Kathmandu from May to December, 2013 to determine the burden of dengue. Serum samples were collected from patients suspected of dengue virus infection and tested by ELISA.

### Research frontiers

Burden estimation of dengue is an appropriate topic. The disease was confined to the southern lowlands, but now it has been expanding to new areas. So the concerned authority should initiate extensive surveillance of dengue virus infection and commence an integrated vector control program in such areas.

### Related reports

This study showed higher prevalence of the disease in Kathmandu. The result is higher than some previous studies by Neupane B *et al.*, 2013, Shah Y *et al.*, 2012, Pun R *et al.*, 2011, but some other studies by Sah O *et al.*, 2009 and Pandey BD *et al.*, 2010 still reported higher prevalence.

### Innovations & breakthroughs

It is certainly a good approach to estimate the true burden of the disease.

### Applications

The study can contribute to burden estimate of dengue in Nepal. The study has mentioned ways to improve the surveillance of dengue.

### Peer review

In this paper, authors determined the burden of dengue in the patients visiting National Public Health Laboratory, Kathmandu. It is a good basic study and arrives at a meaningful conclusion on the improvement of surveillance and vector control.

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