International Journal of TROPICAL DISEASE & Health
21(1): 1-10, 2017; Article no.IJTDH.30612
ISSN: 2278–1005, NLM ID: 101632866

SCIENCEDOMAIN *international*
www.sciencedomain.org

Burden of Dengue Virus Infection in Chitwan and Peripheral Districts: An Emerging Disease in Terai Region of Nepal

Sangita Thapa[1*] and Lokendra Bahadur Sapkota[2]

[1]Department of Clinical Microbiology and Immunology, Chitwan Medical College Teaching Hospital, Bharatpur, Nepal.
[2]Department of Biochemistry, Chitwan Medical College Teaching Hospital, Bharatpur, Nepal.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJTDH/2017/30612
Editor(s):
(1) Jorge Paredes Vieyra, Universidad Autonoma De Baja California, Campus Tijuana, Mexico.
(2) Zhiheng Zhou, Thyroid Cancer Research Laboratory, Massachusetts General Hospital, Harvard Medical School, Boston, USA and School of Public Health, Guangzhou University, China.
Reviewers:
(1) Lívia Garcia Bertolacci-Rocha, Universidade Federal de Goiás, Brazil.
(2) Sumit Sohal, Government Medical College, Patiala, Punjab, India.
(3) Scott Halstead, Uniformed Services University of the Health Sciences, USA.
Complete Peer review History: http://www.sciencedomain.org/review-history/17590

Original Research Article

Received 21st November 2016
Accepted 4th January 2017
Published 24th January 2017

ABSTRACT

Background: Dengue has become endemic and subject of concern in Terai regions of Nepal especially Chitwan and its peripheral districts. The evidence of all four serotypes of dengue virus could be a consequence of a sudden resurgence of more severe dengue disease in Nepal.
Study Design: The present study was designed to determine the incidence of Dengue virus infection in clinically suspected patients visiting Chitwan Medical College Teaching Hospital (CMCTH) in a Central part of Nepal.
Methods: A prospective study was conducted from July 2015 to June 2016 at CMCTH, Bharatpur, the fifth largest city of Nepal. Blood specimens from 357 patients from inpatient and outpatient departments, was collected and processed for anti-dengue immunoglobulin IgM by antibody isotype-capture enzyme-linked immunosorbent assay (IgM ELISA).
Results: Among 357 samples processed, 45 (12.60%) were positive for anti-dengue IgM antibodies. The highest number of dengue positive cases was observed in the age group of 21-30

**Corresponding author: Email: drsangitathapa@gmail.com;*

years with greater predilection in males. The gender bias, was statistically significant (p value=0.0004). The dengue positivity was highest in the month of November (51.11%) and majority of dengue infection was recorded in the winter season (86.67%). Dengue was highly prevalent in urban society compared to rural society and the difference is statistically significant (p value<0.0001).

Conclusion: Incidence of dengue infection is increasing and current outbreak in Chitwan and peripheral districts is a serious matter of concern. The higher prevalence of dengue in this study underscore the need for comprehensive surveillance of the disease in order to identify the high risk areas and current disease burden for immediate implementation of preventive measures.

Keywords: Aedes; dengue virus; IgM capture ELISA; Terai.

1. INTRODUCTION

Dengue virus (DENV) is an arthropod vector borne disease caused by a single stranded positive-sense RNA virus which belongs to the genus *Flavivirus*, family *Flaviviridae*. There are four serotypes, DEN-1, DEN-2, DEN-3, DEN-4 which are capable of causing Dengue Fever (DF) [1]. Dengue Fever is characterized by acute febrile illness transmitted by *Aedes* species of the daytime-biting *Stegomyia* family mosquitoes, mainly *Aedes aegypti* (*A. aegypti*) and less frequently *Aedes albopictus* (*A. albopictus*) between natural hosts humans [2]. The dengue virus infection (DVI) causes a spectrum of illness ranging from asymptomatic to mild febrile illness i.e. dengue fever (DF) to severe life threatening forms i.e. Dengue Hemorrhagic fever (DHF) and Dengue Shock Syndrome (DSS). The disease is characterized by high grade fever with headache, retro-orbital pain, skin rash, muscle or joint pain and haemorrhages whereas secondary infection is mainly associated with severe form of disease leading to death [3].

Dengue virus infection is the major global public health problem responsible for 50-100 million cases and several thousand deaths annually in tropical countries and is expanding endemicity towards new territories. The disease is endemic in Africa, Eastern Mediterranean, Latin America, South East Asia and Western Pacific. In South East Asia and Western Pacific region around 1.8 billion people are at high risk of getting dengue infection [4]. Dengue is a re-emerging mosquito borne disease and significant problem in tropical regions likely due to several factors like; circulating multiple virus serotypes, environmental change, global travel, rapid urbanization, demographic changes, inadequate public healthcare systems such as sewage and waste management, increased frequency of epidemics, expanding geographical distribution of both viruses and vectors [5].

Nepal is bordered by India in the eastern, western and southern belts. Moreover, there is open border between Nepal and India and there is relatively high frequency of travel and trade across the borders. Hence, dengue virus infection is considered to be possible public health threat in Nepal. In Nepal, the first case of dengue virus infection was reported in 2004 in a foreigner and subsequently dengue virus infection has been found to be rapidly spreading across the country within a short period of time. The first outbreak was reported in 2006 which occurred in 9 districts of Terai region with 23 confirmed dengue cases following epidemic outbreak in India in September-October 2006 [6,7]. Nearly four years after its first appearance, massive outbreak occurred in Chitwan in 2010 with 359 confirmed dengue cases [8]. A study conducted in 12 countries of South East Asia region revealed that an annual economic burden of nearly 1 billion dollar incurred due to dengue [9].

Aedes aegypti has been incriminated as the principal vector which is primarily an urban mosquito but sometimes it is also found in the periphery of cities breeding in rain water accumulated in containers. The virus has also been isolated from *A. albopictus*. This species is mainly urban and semi urban, breeding in domestic and peridomestic water storage containers. The disease occurs in epidemic from during late monsoon and post-monsoon season, as an outcome of increase in breeding places and vector population. However, outbreaks of considerable magnitude have also occurred during the summer and pre-monsoon season as a result of water scarcity and consequent storage of water for domestic purposes [10]. Few entomological studies reported the presence of

dengue vectors (*A. aegypti* and *A. albopictus*) in Nepal ranging from Terai lowlands and Siwalik hills to Middle Mountain regions. These vectors are commonly present up to 1350 m in Kathmandu and rarely up to 1750 m to 2100 m in Dhunche, Rasuwa district of Nepal [11].

Immunity in dengue infection is serotype specific. Infection with one serotype provides long-lasting immunity to the same serotype but subsequent infection with other serotypes does not confer protection [12]. All the 4 dengue serotypes are circulating in Nepal, which is matter of serious concern. Unfortunately, there is no specific antiviral therapy or vaccine for dengue virus infection. Symptomatic treatment is mainstay of medical care, treatment is mainly focuses on management of fever and bleeding complications, prevention of dehydration and shock [13].

Due to lack of proper diagnostic facilities in Nepal, diagnosis of dengue is solely based on clinical symptoms. Clinical suspicion is not sufficient to make accurate diagnosis as other infectious diseases may present with similar clinical findings, which underscores the need for laboratory confirmation of dengue infection. Early and proper diagnosis is utmost important and is routinely done by serological test [14]. Anti dengue IgM antibodies appears as early as 3 days after infection and remain in circulation for 1-2 months. IgM-capture enzyme linked immunosorbent assay (ELISA) is used as a standard method for detecting IgM. Anti dengue IgG antibodies appears after 1 week, peaks after 2-3 weeks and remain lifelong in circulation. Serological diagnosis was the mainstay of diagnosis in Nepal during outbreaks of dengue infection [15].

In past few decades, dengue caused significant morbidity and mortality in neighboring countries. Despite, high risk of dengue in Nepal, there are few studies which gives very limited information on the distribution and abundance of mosquito vectors for dengue virus and sero-prevalence of the disease. Dengue infection is firmly established in Terai regions of Nepal, which strongly suggests a need for continuous, surveillance and monitoring of dengue infection. Hence, this study was designed to determine the sero-prevalence of dengue in patients suspected with dengue infection in Terai regions of Nepal.

2. METHODS

2.1 Study Design

This is a prospective study carried out from July 2015 to June 2016 over a period of one year in the department of Microbiology and Immunology, Chitwan Medical College Teaching Hospital (CMCTH), the principal tertiary centre in the city of Bharatpur, Chitwan District, Narayani Zone, Nepal. Samples were collected from all the patients suspected with Dengue infection who visited CMCTH from Bharatpur and locations all over Narayani Zone and its neighbouring districts. A total of 357 samples were collected and processed for anti-dengue IgM by IgM-capture ELISA.

2.2 Study Population

This study included a total of 357 patients visiting CMCTH who were clinically suspected of having Dengue viral infection (DVI). Relevant data of the patients were collected from personal interviews using a preformed set of questionnaires.

2.3 Laboratory Tests

About 2 to 3 ml of venous blood was collected from all patients suspected of DVI using strict aseptic precautions and following standard microbiological methods. Aliquots of sera for ELISA were prepared and stored at 2-8℃ for further analysis. Anti-dengue IgM antibodies were detected by a commercially available IgM capture ELISA (SD Bioline, Standard Diagnostic Inc., South Korea). This diagnostic kit provided qualitative detection of IgM antibodies specific to DV in human sera. All the tests were performed following manufacturer's instructions and the test results were recorded and interpreted as either positive or negative on the basis of absorbance with respect to cut-off value.

2.4 Data Analysis

The obtained data were analysed using Statistical Package for Social Science version 20 (SPSS v.200). Comparison of mean values were done using student's t test. p value less than 0.05 was considered to be statistically significant.

2.5 Ethical Issues

Ethical approval was obtained from the Chitwan Medical College Institutional Review Committee

(CMC-IRC) before starting the study and informed consent was obtained from all the patients.

3. RESULTS

Out of 357 samples collected and processed, 45 (12.6%) were found to be positive for anti-dengue IgM antibodies. All dengue positive cases were from Narayani Zone and neighboring districts.

3.1 Age and Gender Wise Distribution

Out of 357 suspected dengue cases, the highest number of dengue suspected cases, 86 (24.08%) belonged to the age group 21-30 years whereas the lowest number, 2 (0.56%) belonged to the age group above 80 years. Among them 54.60% (195/357) were males and 45.40% (162/357) were females. Among 45 anti-dengue IgM antibodies positive cases, maximum number of positive cases, 15 (33.33%) belonged to the age group 21-30 years. In contrast, not a single positive case was reported from the age group ≤ 10 years and > 80 years as depicted in Table 1. Of the total positive cases, 68.89% (31/45) were males and 31.11% (14/45) were females as shown in Fig. 1. This gender bias, a male to female ratio of 2.2:1, was statistically significant (P=0.0004).

3.2 Monthly Distribution of Dengue Cases

Maximum number of Dengue suspected cases were reported in the month of November followed by October and September. Lowest number of Dengue suspects were reported from the month of January as shown in Fig. 2. Similarly, highest number of Dengue positive cases were reported in the month of November 51.11% (23/45) followed by October 40% (18/45). In contrast no positive cases were observed in the following 6 months July, December, January, February, April and June. This variation in the monthly distribution of positive cases was statistically significant (p<0.0001).

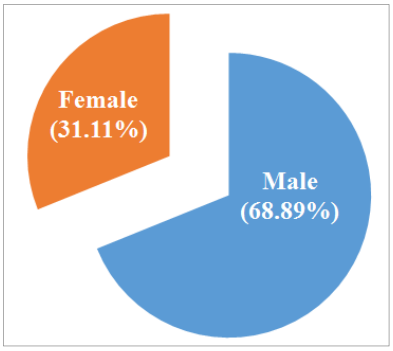

Fig. 1. Male and female positive cases

3.3 Seasonal Variation of Positive Cases

The highest number of Dengue positive cases were reported in the winter season 39 (86.67%) followed by autumn 4 (8.89%) and spring season 2 (4.44%). Not a single positive case was reported in the summer season. This seasonal variation in the occurrence of Dengue was statistically significant (p<0.0001) as depicted in Fig. 3.

3.4 Regional Distribution of Cases

Majority of the cases reported in our study (241/357; 67.5%) were from Chitwan district, with a similar distribution between urban and rural

Table 1. Age and gender wise distribution of dengue cases

Age group	Total suspected cases (n= 357)			Positive cases (n=45)		
Years	Male	Female	Total (%)	Male	Female	Total (%)
≤ 10	10	5	15 (4.2%)	0	0	0 (0%)
11-20	36	27	63 (17.64%)	3	1	4 (8.88%)
21-30	43	43	86 (24.08%)	8	7	15 (33.33%)
31-40	34	34	68 (19.04%)	8	4	12 (26.66%)
41-50	27	18	45 (12.6%)	5	1	6 (13.33%)
51-60	21	19	40 (11.2%)	3	1	4 (8.88%)
61-70	13	11	24 (6.72%)	3	0	3 (6.66%)
71-80	9	5	14 (3.92%)	1	0	1 (2.22%)
>80	2	0	2 (0.56%)	0	0	0 (0%)
Total	195	162	357	31	14	45

settings (133, 37.25% to 108, 30.25%). Remaining cases (116/357; 32.5%) were reported from districts peripheral to Chitwan. Of 200 suspected cases from an urban location, 19.5% were Dengue positive. In contrast, only 6 of 157 suspected cases, 3.82% tested Dengue positive from rural settings. This difference in the detection of dengue between urban and rural settings was statistically significant ($p<0.0001$) as depicted in Table 3.

4. DISCUSSION

Dengue is one of the emerging disease and has become major public health problem, particularly in tropical and sub-tropical regions of the world. The WHO currently estimated that epidemic outbreak of dengue infection occurs in more than 100 countries each year [16]. South East Asia most seriously affected, which bear nearly 75% of current global burden of dengue infection likely because the countries share common features as increasing population, rapid urbanization, monsoon rains and development activities. Urban populations and travellers constitute the reservoir and predominant factor for the spread of viruses [17].

Although dengue infection has been found in our neighbouring country over a long period of time there was no documented case of dengue infection in Nepal prior to 2004. Since, then Nepal has encountered several dengue fever outbreaks in various Terai regions of Nepal. All four confirmed dengue serotypes (DEN 1-4) are circulating, which could be a consequence of sudden resurgence of a more severe dengue outbreak in Nepal [18]. The burden of dengue is under reported due to lack of knowledge regarding the disease and proper laboratory diagnostic facilities.

Table 2. Month-wise distribution of dengue suspected cases and positive cases

Month	Dengue suspects	Positive cases
July	8	0
August	26	1
September	28	1
October	73	18
November	107	23
December	23	0
January	6	0
February	15	0
March	15	2
April	19	0
May	19	0
June	18	0
Total	357	45

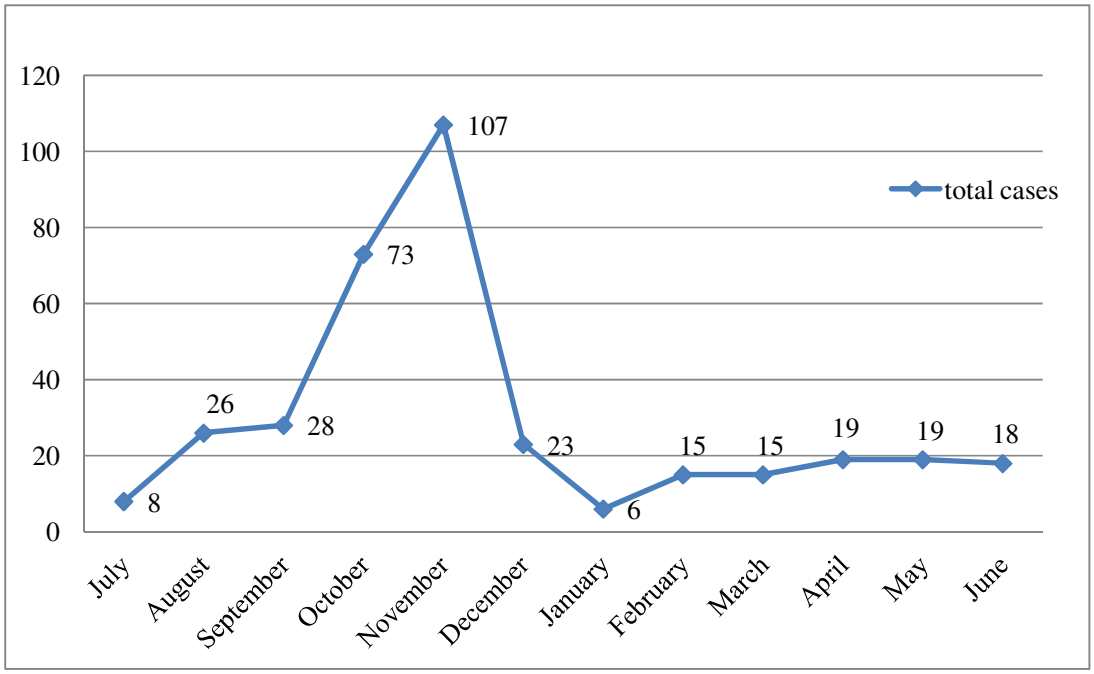

Fig. 2. Monthly distribution of clinically suspected cases

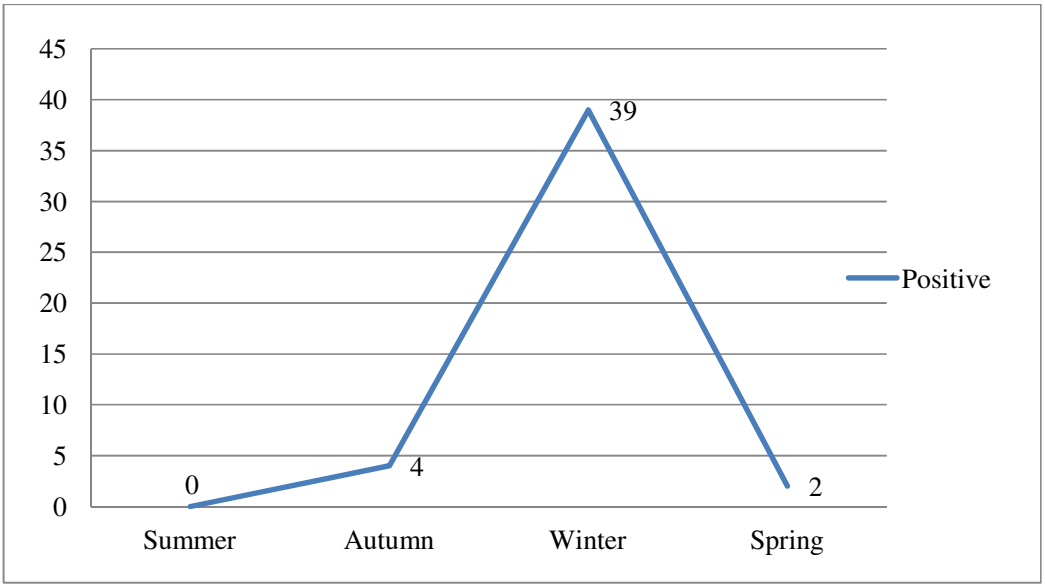

Fig. 3. Seasonal distribution of dengue positive cases attending CMCTH

Table 3. Regional distribution of dengue cases

District	Residency	Suspected cases	Dengue positive cases (%)	Positive/Total (%)
Chitwan	Urban	133	27 (20.3)	28 (11.61)
	Rural	108	1 (0.9)	
Nawalparasi	Urban	32	6 (18.7)	7 (12.28)
	Rural	25	1 (4.0)	
Tanahun	Urban	6	2 (33.3)	2 (25.0)
	Rural	2	0	
Makawanpur	Urban	7	1 (14.2)	2 (16.66)
	Rural	5	1 (20.0)	
Parsa	Urban	7	1 (14.2)	1 (5.88)
	Rural	10	0	
Bara	Urban	9	1 (11.1)	1 (8.33)
	Rural	3	0	
Sarlahi	Urban	3	0	1 (20.0)
	Rural	2	1 (50.0)	
Rautahat	Urban	0	0	1 (50.0)
	Rural	2	1 (50.0)	
Gulmi	Urban	2	1 (50.0)	1 (50.0)
	Rural	0	0	
Dang	Urban	1	1 (100.0)	1 (100.0)
	Rural	0	0	
Total	Urban	200	39 (19.5%)	p value <0.001
	Rural	157	6 (3.82%)	

p value < 0.05 is significant

In our study IgM capture ELISA method was used which is the most commonly used serological test for laboratory diagnosis of dengue. This technique has sensitivity and specificity of approximately 96.4% and 98.9% respectively. This method is reliable even if there is co-infection with Japanese encephalitis (JE) [19]. Patients with prior history of JE immunization were not included in this study so cross-reactivity with other flaviviruses and false positive results were not reported.

The present findings showed that among 357 sera tested, 45(12.6%) were seropositive for anti-

dengue IgM. The findings of our study concurs with results of Gupta et al. [20] who reported the positivity for anti-dengue IgM to be 29.09%. There is increasing trend of dengue infection based on reports of previous studies. A study conducted in Nepal by Sah et al. [21] reported sero-positivity of and 30%. Various studies conducted in Bangladesh [22] and India [23] reported prevalence of 13.7% and 19.7% respectively. Similarly, a study conducted from the Dang and Chitwan districts in the year 2014 reported 19.31% prevalence of Dengue [24]. The findings in the present study shows increased rate of dengue virus infection when compared with results of Poudel et al. [25] who reported the positivity for anti-dengue IgM to be 12.17%. Nevertheless, the possible justification might be due to regional differences of enrolled patients. The other confounding factors for the results might be the geographical and demographic variations, social and environmental changes, urbanization and effectiveness of vector control programmes in endemic regions. Probably, other predisposing factor might be increased rate of migration due to open border as Terai belt of Nepal is bordered with India.

In our study, dengue positive cases was highest 33.33% among the age group 21-30 years. Observations from Nepal, India and Bangladesh supports the prevalence at the age group 21-30 [26-28]. Of the total positive cases, 68.89% were males and 31.11% were females. This gender bias, a male to female ratio of 2.2:1, was statistically significant (P=0.0004). Our finding was supported by Shah et al. [29] and Neeraja et al. [30] which has also reported higher prevalence rate in males. Whereas, more females were found to be affected in comparison to males in a study conducted by Chakravarti et al. [31]. However, the result is in harmony with the results obtained during dengue outbreak in Nepal in the year 2006 in which dengue positivity was found commonly in the age group greater than 15 years [32].

Several factors are associated with seasonal outbreak of disease such as humidity, rainfall, air temperature, type of land, mosquito bite rate and mosquito density [33,34]. Highest number of Dengue positive cases were reported in the month of November 51.11% and October 40%. In contrast, no positive cases were observed in the following 6 months July, December, January, February, April and June. Statistically, there was significant difference ($p<0.0001$) between monthly distribution and seropositive dengue cases. In present study dengue was reported all year round, majority of dengue positive cases were reported in the winter season 86.67% followed by autumn 8.89% and spring season 4.44%. Not a single positive case was reported in the summer season. This seasonal variation in the occurrence of Dengue was statistically significant ($p<0.0001$). The occurrence of dengue infection is consequent to the monsoon season in June to September every year, which would trigger the *A. aegypti* abundance and transmission. This result is in agreement with findings by Hari et al. [35] and report from Bangladesh [36]. During this time of year, especially adult males are involved in outdoor activities and mobility of people is high, thereby exposure to mosquitoes. In addition, major Nepalese religious and cultural festivals, Dashain, Tihar, Losar, New year coincides in October to December which is in winter and autumn season. The occurrence of dengue is more likely due to festival and holiday season, there is increased frequency of movement of people across the border either for economic or educational purposes.

Majority of the cases reported in our study 67.5% were from Chitwan district, with a similar distribution between urban and rural settings (37.25% to 30.25%). Remaining cases 32.5% were reported from districts peripheral to Chitwan. Highest seropositivity from Chitwan might be due to endemicity of dengue outbreaks reported in this region [37] and more cases were reported from Chitwan as compared to districts peripheral to Chitwan. In present study, dengue positivity was highest 19.5% from an urban location and 3.82% from rural settings. This difference in the detection of dengue between urban and rural settings was statistically significant ($p<0.0001$). Various other investigators reported that dengue is essentially a disease of urban environments [38,39]. In contrast, studies from India, Sri Lanka, Thailand and Cambodia revealed higher prevalence of dengue in rural settings [40-42]. Urbanization is often less managed with relatively high density of population and also provide numerous artificial breeding sites for mosquitoes which facilitate vector breeding and its transmission especially in city than in the countryside [43].

There is no specific treatment for DF, management is mainly supportive. Currently Sanofi's Pasteur dengue vaccine is introduced against dengue virus infection but it is not available in Nepal. Therefore, there is urgent

need for early diagnosis and vector control strategy to prevent future dengue outbreaks. This study indicate that dengue infection is firmly established in Chitwan and its peripheral districts. Hence, this strongly suggests a need for a continuous, surveillance and monitoring of dengue virus infection in Terai regions of Nepal.

5. CONCLUSION

Dengue is becoming an emerging problem in Terai regions of Nepal. The incidence of dengue is dramatically increasing in Chitwan and its neighboring districts. The clinical symptoms of dengue are similar with other febrile illness. Although acute dengue infection is generally asymptomatic or mild, sometimes it may present with severe form of dengue such as DHF/DSS. If early diagnosis and prompt appropriate management of the disease is not undertaken sometimes it can be fatal. Hence, reliable diagnostic accuracy is essential for diagnosis of dengue in Nepal and developing countries. Probably, dengue is endemic disease in Terai regions of Nepal, so it strongly suggests that concerned authority should initiate surveillance of dengue infection and commence integrated vector control program to abate epidemic outbreak of dengue in the future. Moreover, it is essential that health care providers should enhance their clinical knowledge about dengue infection and its geographical distribution so that sudden and unexpected surge of dengue infection can be well managed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Normile D. Surprising new dengue virus throws a spanner in disease control efforts. Trop Med. Science. 2013;342(6157):415. DOI: 10.1126/science.342.6157.415
2. Gubler DJ. Dengue and dengue haemorrhagic fever. Clin. Microbiol Rev. 1998;119(3):480-96.
3. Rothman AL. Dengue: Defining protective versus pathologic immunity. J Clin Invest. 2004;113:946-51.
4. Guzman MG, Kouri G. Dengue: An update. Lancet Infec Dis. 2002;2:33-42.
5. Gibbons RV, Vaughn DW. Dengue: An escalating problem. Brit Med J. 2002;324: 1563-6.
6. Pandey BD, Rai SK, Morita K, Kurane I. First case of dengue virus infection in Nepal. Nep Med Coll. J. 2004;6:157–9.
7. Epidemiology and Disease Control Division (EDCD). Annual report 2006/2007. Department of Health Services (DoHS), Ministry of Health, Government of Nepal; 2008.
8. Gupta BP, Mishra SK, Manandhar KD, Malla R, Tamarakar CS, Raut PP, et al. Seroprevalence of dengue virus infection in Nepal. Int J Appl Sci Biotechnol. 2013; 1:224.
9. Murray NE, Quam MB, Wilder-Smith A. Epidemiology of dengue: Past, present and future prospects. Clin. Epidemiol. 2013;5:299–309. [PubMed].
10. Nepal Health Research Council. Environmental conditions associated with vector of dengue and corrective actions for its prevention in Nepal. Ramshah Path, Kathmandu, Nepal. 2009;2.
11. Shrestha R, Pant ND, GC G, Thapa S, Neupane B, Shah Y, et al. Serological and entomological study of dengue in Dang and Chitwan Districts of Nepal. PLoS ONE. 2016;11(2): 1-10.
12. Simmons CP, Farrar JJ, Nguyen VV, Wills B. Dengue. N Engl J Med. 2012;366:1423–32.
    DOI: 10.1056/NEJMra1110265
    PMID: 22494122
13. Gupta BP, Manandhar KD, Malla R, Tamrakar CS, Mishra SK, Rouniyar R. Emergence of dengue virus infection in Nepal. Int J Appl Sci Biotechnol. 2013; 1(3):79-84.
14. Malla S, Thakur GD, Shrestha SK, Banjeree MK, Thapa LB, Gongal G, et al. Identification of all dengue serotypes in Nepal. Emerg Infect Dis. 2008;14:1669-70.
15. Pandey BD, Morita K, Khanal SR, Takasaki T, Miyazaki I, Ogawa T, et al. Dengue virus, Nepal. Emerg Infect Dis. 2008;14:514-5.
16. World Health Organization. Dengue: Guidelines for diagnosis, treatment, prevention and control. New Ed. 2009, Geneva, World Health Organization; 2009.
17. Mahmood BAI, Mahmood SAI. Emergence of dengue in Bangladesh a major international public health concern in recent years. J Environ Manage. 2011;2: 35-41.
18. Malla S, Thakur GD, Shrestha SK, Banjeree MK, Thapa LB, Gongal G, et al.

Identification of all dengue serotypes in Nepal. Emerg Infect Dis. 2008;14:1669-70.
19. Pun R, Pant KP, Bhatta DR, Pandey BD. Acute dengue infection in the Western Terai region of Nepal. J Nepal Med Assoc. 2011;51:11-4.
20. Gupta BP, Mishra SK, Manandhar KD, Malla R, Tamarakar CS, Raut PP, et al. Seroprevalence of dengue virus infection in Nepal. Int J Appl Sci Biotechnol. 2013; 1:224–7.
21. Sah OP, Subedi S, Morita K, Pandey BD. Serodiagnosis of dengue by particle agglutination assay. J Nepal Health Res Counc. 2009;7(14):29–32.
22. Dahal EB, Banu D, Talukder KR, Chowdhury MJH, Bangali AM, et al. Sero epidemiological study of dengue/dengue haemorrhagic fever in a metropolitan hospital in Bangladesh. Dengue Bull. 2002; 26:1-6.
23. Garg A, Garg J, Rao YK, Upadhyay GC, Sakhuja S. Prevalence of dengue among clinically suspected febrile episodes at a teaching hospital in North India. J Infect Dis Immunol. 2011;3:85-89.
24. Gaire B, Rijal KR, Neupane B, Paudyal P, Gautam I, Banjara MR, et al. Prevalence of dengue vector in relation to dengue virus infection in central region of Nepal. Dengue Bulletin. 2014;38:96–107.
25. Poudel A, shah Y, Khatri B, Joshi DR, Bhatta DR, Pandey BD. The burden of dengue infection in some vulnerable regions of Nepal. Nepal Med Coll J. 2012; 14(2):114–7.
PMID: 23671960
26. Sah OP, Subedi S, Morita K, Inone S, Kurane I, et al. Serological study of dengue virus infection in Terai region, Nepal. Nepal Med Coll J. 2009;11:104-6.
27. Gupta E, Dar L, Kapoor G, Broor S. The changing epidemiology of dengue in Delhi. India. Virol J. 2006;3:92.
28. Rahman M, Rahman K, Siddque AK, Shoma S, Kamal AH, et al. First outbreak of dengue hemorrhagic fever, Bangladesh. Emerg Infect Dis. 2002;8:738-740.
29. Shah Y, Khadka G, Gupta GP, Adhikari N, Poudel A, et al. Serodiagnosis of dengue virus in different hospitals of Nepal. Int J Infect Microbiol. 2012;1:58-62.
30. Neeraja M, Lakshmi V, Teja VD, Umabala P, Subbalakshmi MV. Serodiagnosis of dengue virus infection in patients presenting to a tertiary care hospital. Indian J Med Microbiol. 2006;24:280-2.
31. Chakravarti A, Roy P, Malik S, Siddiqui O, Thakur P. A study on gender-related differences in laboratory characteristics of dengue fever. Indian J Med Microbiol. 2016;34:82-4.
32. World Health Organization. Outbreak investigation of DF in Nepal. New Delhi; WHO Regional Office for Southeast Asia; 2006.
33. Nakhapakorn K, Tripathi NK. An information value based analysis of physical and climatic factors affecting dengue fever and dengue haemorrhagic fever incidence. Int J Health Geogr. 2005; 4:13.
34. Bartley LM, Donnelly CA, Garnett GP. The seasonal pattern of dengue in endemic areas: Mathematical models of mechanisms. Trans R Soc Trop Med Hyg. 2002;96:387-97.
35. Nepal HP, Ansari S, Gyawali N, Gautam R, Paudel R, et al. Detection of IgM against dengue virus in clinically suspected patients presenting at a TertiaryCare Centre, Narayani Zone, Nepal. J Trop Dis. 2014;2:139.
DOI: 10.4172/2329-891X.1000139
36. Subedi D, Andrew W. Taylor-Robinson. epidemiology of dengue in Nepal: History of incidence, current prevalence and strategies for future control. J Vector Borne Dis. 2016;53:1–7.
37. Dahal EB, Banu D, Talukder KR, Chowdhury MJH, Bangali AM, et al. Sero epidemiological study of dengue/dengue haemorrhagic fever in a metropolitan hospital in Bangladesh. Dengue Bull. 2002;26:1-6.
38. Mulligan K, Elliott SJ, Schuster-Wallace C. The place of health and the health of place: Dengue fever and urban governance in Putrajaya, Malaysia. Health Place. 2012;18:613-20.
39. Padmanabha H, Durham D, Correa F, Diuk-Wasser M, Galvani A. The interactive roles of aedesaegypti super-production and human density in dengue transmission. PLoS Negl Trop Dis. 2012; 6(8):e1799.
DOI: 10.1371/journal.pntd.0001799
40. Vong S, Khieu V, Glass O, Ly S, Duong V, et al. Dengue incidence in urban and rural Cambodia: Results from population-based

active fever surveillance, 2006-2008. PLoS Negl Trop Dis. 2010;4(11):e903. DOI: 10.1371/journal.pntd.0000903

41. Reller ME, Bodinayake C, Nagahawatte A, Devasiri V, Kodikara- Arachichi W, et al. Unsuspected dengue and acute febrile illness in rural and semi-urban Southern Sri Lanka. Emerg Infect Dis. 2012;18:256-63.
42. Chareonsook O, Foy HM, Teeraratkul A, Silarug N. Changing epidemiology of dengue hemorrhagic fever in Thailand. Epidemiol Infect. 1999;122:161-6.
43. Kumar A, Sharma SK, Padbidri VS, Thakare JP, Jain DC, et al. An outbreak of dengue fever in rural areas of northern India. J Commun Dis. 2013;3: 274-81.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/17590