ORIGINAL ARTICLE

DENGUE INFECTIONS AND CIRCULATING SEROTYPES IN NEGERI SEMBILAN, MALAYSIA

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ABSTRACT

This study observed the pattern of reported dengue infections, clinical manifestations, and circulating dengue serotypes in Negeri Sembilan, Malaysia. The aim of this study was to determine the co-circulation of the four different dengue virus serotypes in Negeri Sembilan. We analyzed the surveillance data (VEKPRO) from Negeri Sembilan State Health Department and National Public Health Laboratory, Malaysia on reported dengue infections from 1st January 2010 to 31st December 2010. There were 1466 reported dengue infections, 1342 (91.5%) cases were dengue fever (DF) and 124 (8.5%) were dengue hemorrhagic fever (DHF). The mean age was 32.2± 15.8 years old and most were young adults, aged 15 years old and older. Males (p < 0.05), and those residing in Seremban district (p < 0.05) were more likely to get dengue infections. Symptoms presented upon admission were fever (100%), headache (99.9%), myalgia and arthralgia (98.8%), rash(24.2%), petechiae (16.0%),bleeding tendencies (7.0%) and neurological deficits(1.2%). All four dengue serotypes (DEN 1 – 4) were present, the pre-dominant serotype was DEN-3, noted in January, then existed together with DEN-2 until around May. DEN-1 was the most pre-dominant circulating dengue serotype afterwards, reaching a peak in December 2010. Dengue affected all age groups particularly young adults and males. Most cases reported were in urban areas and Seremban district. Most of the dengue infections occurred in the first half of the year, with the DEN-2 and DEN-3 serotypes being the most predominant.

***Key words**: Dengue fever (DF), dengue haemorrhagic fever (DHF), socio-demographic factors, dengue serology, dengue serotype.*

INTRODUCTION

Dengue is a global health problem. Dengue is described as 'endemic' in many countries with significant variation between countries and within each country[1]. In Malaysia, an average of 5000 cases were reported dengue cases annually in the early 1990s[2]. The incidence rate also showed an upward trend from 44.3 cases/100,000 population in 1999 to 181 cases/100,000 population in 2007[3]. Areas that experienced rapid development and high population density showed higher number of dengue cases. Negeri Sembilan is one of the states in Malaysia that had been through rapid land development. In 2010, the reported dengue cases in Negeri Sembilan increased dramatically (42.0%) to 1501 with 7 deaths, compared to 1056 with 6 deaths in the previous year[4].

Dengue is caused by a single-stranded RNA virus belonging to the genus *Flavivirus* with serotypes (DEN 1, DEN-2, DEN-3 and DEN-4), classified according to biological and immunological criteria[5,6,7,8]. It is transmitted by the Aedes mosquitoes. The unique structure of the dengue virus and the pathophysiologic responses of the host, different serotypes, and favorable conditions for vector breeding have led to the virulence and spread of the infections.

The manifestations of dengue infections vary from asymptomatic to undifferentiated dengue fever (dengue fever), severe dengue infections (dengue haemorrhagic fever), fatal complications such as dengue shock syndrome and death[3,6,9,10]. The clinical features of classical dengue fever include fever, headache, retro-orbital pain, myalgias and arthralgias, nausea, vomiting, and often a rash. Some patients develop hemorrhagic manifestations, such as hematuria, bleeding gums, epistaxis, hematemesis, melena, and ecchymosis. Dengue haemorrhagic fever patients develop thrombocytopenia and haemoconcentration. Some may progress into dengue shock syndrome, leading to profound shock and death if not properly treated[1,6,7,10]. There are many factors associated

with the occurrence of dengue infection in the community: socio-demographic factors such as age, gender and ethnicity[3,11,12], climate variability[12,13], dengue vector ecology[13], community participation and population distribution[12]. Some of other factors lead to the severity of dengue infections, such as past dengue infection, viral load and viral virulence, host genetic background, T-cell activation and auto-antibodies[14,15,16].

The aim of this research was to determine the sociodemografic factors such as age, gender, ethnicity, population distributions (district, urban and rural area) and common clinical presentations, serological patterns as well as circulating serotypes of dengue virus in Negeri Sembilan, Malaysia. From the litreture we know that each serotype is capable of causing dengue epidemics in tropical and subtropical regions of the world. Negeri Sembilan is located in the west coast of Peninsular Malaysia. It has seven districts; Jelebu, Jempol, Kuala Pilah, Port Dickson, Rembau, Seremban and Tampin. There are few studies in Malaysia that examined the co-circulation of the four different dengue virus serotypes. In this paper we report on the details and findings of dengue infection in Negeri Sembilan, Malaysia. By studying the distribution of dengue infection and the circulating serotypes, have an important implication on dengue control and prevention.

MATERIAL AND METHODS

Data for reported cases from 1st January 2010 to 31st December 2010 were retrieved from Negeri Sembilan State Department of Health. The data obtained were from the surveillance data (VEKPRO). These data were based on the mandatory notification, as all physicians attending to dengue cases (based on symptoms) were required by law to notify these cases to the health office.These notification were done online. There were 1501 cases of dengue infections (dengue fever and dengue hemorrhagic fever) documented but only 1466 cases were recruited into the study because the rest had to be excluded due to incomplete data. These data were analyzed for socio-demographic factors: age, gender, ethnicity, occupation, living area (district, urban and rural), time of dengue infections and symptoms of dengue infections such as fever, headache, myalgia and arthralgia, nausea and vomiting, rash, bleeding tendencies – gum and nose bleeding, ecchymosis, haematemesis, melaena, haematuria, petechiae, purpura, rash and neurological involvements – fit, altered consciousness and comatose. Serology results of IgG and IgM were also included. The data for dengue serotype were retrieved from National Public Health Laboratory Sungai Buloh, Selangor to determine the circulating dengue serotypes among dengue cases in Negeri Sembilan for the year 2010. There were 781 samples sent for dengue viral serotyping in 2010 but only 180 samples were positive. The technique used for the dengue serotyping was viral isolation from the serum. The case definition for Dengue Fever (DF) and Dengue Haemorrhagic Fever (DHF) were based on the WHO classification (1997).

Statistical analysis

Data were analyzed using statistical software (SPSS version 18.0; SPSS Inc., Chicago, IL, USA). Descriptive statistics were used to describe the distribution of the demographic data, symptoms of dengue infections and serological data. The categorical variables were analyzed using chi-square and Fisher's exact test.

RESULTS

From 1466 cases of dengue infections in Negeri Sembilan in 2010 analyzed, 1342 (91.5%) cases were dengue fever and the remaining 124 (8.5%) were dengue hemorrhagic fever.

Time of dengue infections

Dengue infections can occur anytime within the study period. Based on the epidemiological week, week 5 and 6 in 2010 had the most cases with 112 and 92 cases respectively. These weeks are in February, the month with the most dengue cases reported, 316 cases with 10 of them were dengue hemorrhagic fever. The dengue infections reduced after that but then increased again in week 23 and 24 with 69 cases and 63 cases respectively, contributing to a total of 252 cases of dengue infections in June (Figure 1).

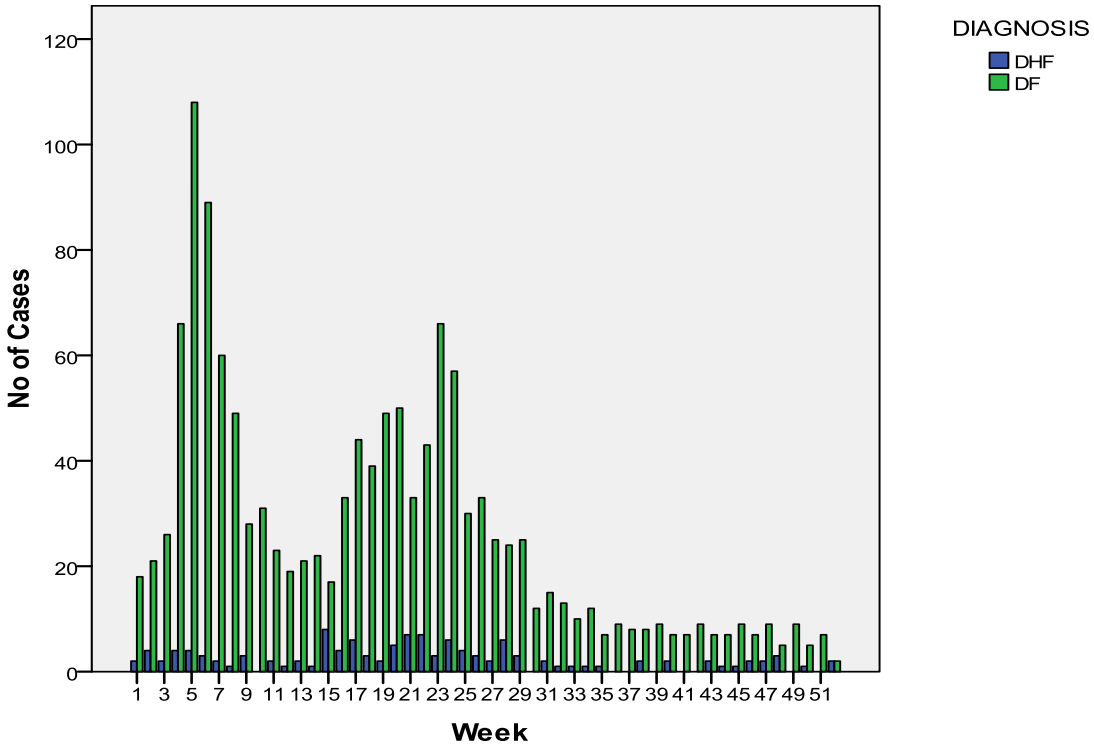

Figure 1. Number of Cases in Negeri Sembilan According to Epidemiological Week in 2010

Socio-demographic factors

All age groups, gender and ethnicity were affected from dengue infections. The youngest was 8 months old and the oldest case was 89 years old. The mean age was 32.2±15.8 years old and most were young adults, aged 15 years old and older (Table 1). For both dengue fever and dengue haemorrhagic fever in this study, there were 860 males compared to 606 females. The ratio of male to female was 1.4 to 1.0. The ratio of dengue fever cases for male to female was 1.4 to 1.0. However, for dengue haemorrhagic fever, the ratio of male to female was 2.2: 1.0.

Table 1. Distribution of socio-demographic factors among the dengue cases

Socio-demographic factors		Diagnosis DHF (n=124)	DF (n=1342)	Test	p value
Age (year)	Mean	32.2 ± 15.8			
	0-14	11 (6.6%)	156 (93.4%)		
	15 and older	113 (8.7%)	1186 (91.3%)	x2=0.853	0.356
Gender	Male	85 (9.9%)	775 (99.1%)		
	Female	39 (6.4%)	567 (93.6%)	x2=5.459	0.019*
Ethnicity	Malay	84 (9.6%)	790 (90.4%)		
	Chinese	18 (5.7%)	299 (94.3%)		
	Indian	13 (6.3%)	192 (93.7%)	x2=7.600	0.055
	Others	9 (12.9%)	61 (87.1%)		
Occupation	Government	15 (9.9%)	136 (90.1%)		
	Private	48 (9.9%)	439 (90.1%)		
	Self employed	13 (9.3%)	137 (90.7%)	x2=4.795	0.309
	Student	27 (8.1%)	306 (91.9%)		
	Unemployed	21 (5.9%)	334 (94.1%)		
District	Jempol	8(14.3%)	48(85.7%)		
	Jelebu	2(9.5%)	19(90.5%)		
	Kuala Pilah	8(32.0%)	17(68.0%)		
	Port Dickson	7(8.8%)	73(91.2%)	x2=28.218**	<0.001
	Rembau	0(0.0%)	33(100.0%)		
	Seremban	99(8.3%)	1101(91.7%)		
	Tampin	0(0.0%)	51(100.0%)		
Living area	urban	109(8.2%)	1224(91.8%)		
	rural	15(11.3%)	118(88.7%)	x2=1.502	0.220

*p value is significant if p < 0.05, **Fisher exact test.

Malay was the most infected ethnicity with 874 (59.6%) cases, followed by Chinese, 317 (21.6%), Indian 205 (14.0%) and others 70 (4.8%). However the differences of type of dengue infection between ethnicity was not significant, p = 0.055.

In this research, majority (75.9%) of the DF and DHF infections occurred in the working groups as compared to the unemployed group. For both DF and DHF, the cases were from the private sectors 487 (33.2%) followed by unemployed 355 (24.2%), students 333 (22.7%), government sectors 151 (9.3%) and self employed 150 (8.3%). However, the differences were not significant, p = 0.309.

District and living area

The district of Seremban contributed most of the dengue cases reported in 2010, with the highest number of dengue cases, 1200 (81.9%). Ninety-nine of them were dengue haemorrhagic fever (8.3%), $p < 0.001$ (Table 1). Distribution of dengue infection across the state of Negeri Sembilan were concentrated in the more urban areas. Majority of the dengue fever and dengue haemorrhagic fever reported in Negeri Sembilan in 2010 came from the urban areas, 1333 (90.9%). For DHF, 109 out of 124 cases (87.9%), resided in urban areas For DF, 1224 out of 1342 cases (91.2%), lived in urban areas. However, this difference was not statistically significant, $p = 0.220$ (Table 1).

IgG and IgM

Serological analysis (IgG and IgM) was important in making the diagnosis of dengue infection in hospital in Malaysia. Out of 1466 dengue cases, 76.9% of them had complete documentation of IgM with positive result was high in dengue infections, 921 cases (dengue fever 866 and dengue haemorrhagic fever 55 cases respectively) (Table 2). However, IgG results were only documented in 25.2% of the cases. The positive results were found in 191 cases of dengue fever and only 9 in dengue haemorrhagic fever cases.

Table 2. Dengue serology analysis (IgM and IgG) among dengue cases

		Diagnosis	
Serology		**DHF**	**DF**
IgM	Positive	55 (6.0%)	866 (94.0%)
	Negative	23 (11.1%)	184 (88.9%)
	Nil	46 (13.6%)	292 (86.4%)
IgG	Positive	9 (4.5%)	191 (95.5%)
	Negative	8 (4.7%)	161 (95.3%)
	Nil	107 (9.8%)	990 (90.2%)

In this study, the patients commonly presented with fever upon admission 1466(100.0%), followed by headache 1464(99.9%), myalgia and athralgia 1449(98.8%), and nausea and vomiting 758(54.4%). The less common symptoms were rash 355(24.2%), petechiae 234(16.0%), bleeding tendencies 103(7.0%), and neurological deficits 17(1.2%).

Dengue serotypes

All four dengue serotypes (DEN 1 – 4) were documented in Negeri Sembilan in 2010. The commonest was DEN-1 (43.9%), followed by DEN-3 (31.1%), DEN-2 (20.0%) and DEN-4 (5.0%). The pattern of dengue serotype infections can be observed, with the pre-dominant serotype was DEN-3, starting in January, then followed by DEN-2 together with DEN-3 until around May 2010. DEN-1 was the most dominant circulating dengue serotype afterwards, reaching a peak in December 2010 (Figure 2). The dengue serotypes affected all age groups, gender and ethnicity in Negeri Sembilan. Young adults and adults aged older than 15 year old groups were more affected (Table 1). Male and female gender are all affected by all serotypes notably with DEN-1 and DEN-3. Malay ethnic had been infected by DEN-1 and DEN-3 the most, and this pattern was the same for the rest of other ethnics.

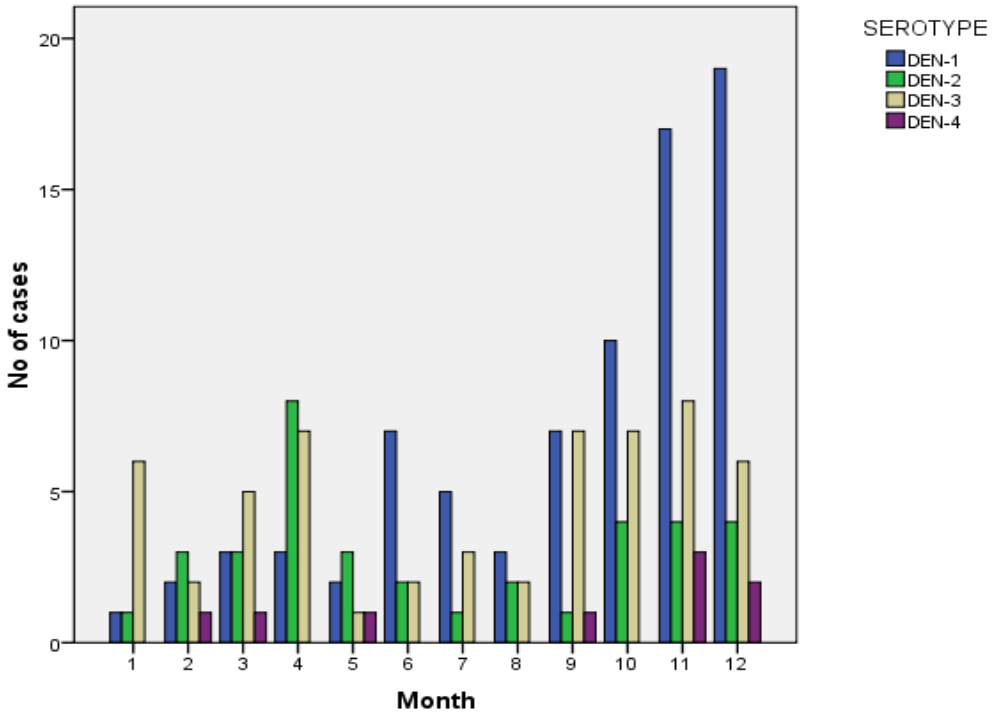

Figure 2. Circulating dengue serotypes according to month in 2010

Data collected had revealed only five out of seven districts reported and complete serotyping result. DEN-1 was found to be present in all districts. DEN-3 was the dominant dengue serotypes circulating in Seremban, 46 (39.7%) cases (Table3 and Figure 3).

Table 3. Circulating dengue serotypes in Negeri Sembilan 2010 according to age, gender and ethnicity

		Dengue Serotype (n=180)			
		DEN-1	**DEN-2**	**DEN-3**	**DEN-4**
Age (year)	Mean		33.3 ± 15.6		
	0 – 14	5(50.0%)	2 (20.0%)	3 (30.0%)	0 (0.0%)
	15 and older	74(43.5%)	34(20.0%)	53(31.2%)	9(5.3%)
Gender	Male	46 (45.1%)	21 (20.6%)	34 (33.3%)	1 (1.0%)
	Female	33 (42.3%)	15 (19.2%)	22 (28.2%)	8 (10.3%)
Ethnic	Malay	56 (45.5%)	21 (17.1%)	41 (33.3%)	5 (4.1%)
	Chinese	13 (46.4%)	7 (25.0%)	6 (21.4%)	2 (7.1%)
	Indian	9 (37.5%)	7 (29.2%)	7 (29.2%)	1 (4.2%)
	Others	1 (20.0%)	1 (20.0%)	2 (40.0%)	1 (20.0%)
District	Jelebu	34(79.1%)	5(11.6%)	3(7.0%)	1(2.3%)
	Jempol	7(53.8%)	0.(0.0%)	6(46.2%)	0(0.0%)
	Kuala Pilah	1(50%)	1(50.0%)	0(0.0%)	0(0.0%)
	Seremban	34(29.5%)	29(25.0%)	46(39.7%)	7(6.0%)
	Tampin	3(50.0%)	1(16.7%)	1(16.7%)	1(16.7%)

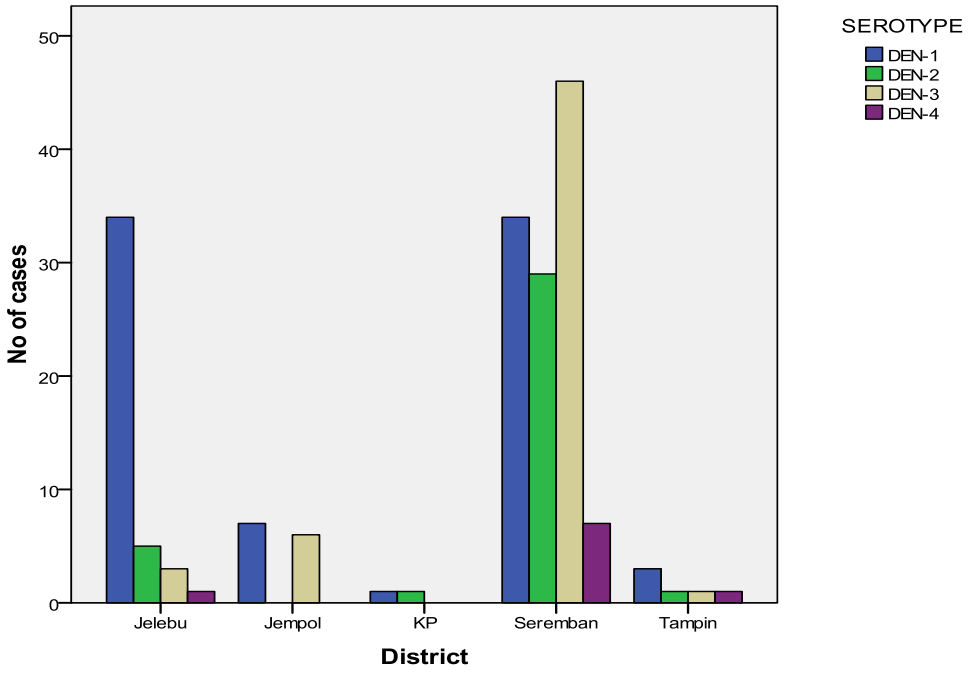

Figure 3. Circulating dengue serotypes in different district in Negeri Sembilan

DISCUSSION

According to WHO 2002, in the South East Asia region, dengue infections is more severe in the less than 15 year old age group giving rise to dengue haemorrhagic fever, shock and death[1,7,10]. In Malaysia, the incidence of dengue cases increased in the age group of 15 and above particularly for the past two years with the highest incidence rate is among the working and school-going age groups.

In this study, the mean age of the cases of dengue infections was 32.2 ± 15.8 years old, which can be categorised as young adults. It was also found that working and student age group were the most infected. This was supported by other studies done in Singapore, Bangladesh and Indonesia[11,12]. In this research, most cases (81.9%) came from district of Seremban and 90.9% of the dengue cases reported were from urban areas where there are high density of populations and rapid development activities factors, which are favourable for dengue transmission[3].

Dengue infection presents as a wide spectrum of illnesses. It ranges from dengue fever, dengue haemorrhagic fever, dengue shock syndrome or dengue death[1,3,7,10]. The symptoms of dengue infection help clinicians in diagnosing the dengue infections according to the World Health Organization Classification of dengue fever and dengue haemorrhagic fever (1997). Fever, headache, myalgia and arthralgia, nausea and vomiting, rash, petechiae, bleeding tendencies and/or neurological deficits were the main symptoms documented upon admission to the hospitals in Negeri Sembilan in 2010. All these symptoms presented in dengue infections in Negeri Sembilan were also reported by previous studies around the world[9,17-22].

The IgM capture enzyme-linked immunosorbent assay (ELISA) is the most widely used serological test. This antibody titre is significantly higher in primary infections, compared to secondary infections. Once the IgM is detectable, it rises quickly and peaks at about 2 weeks after the onset of symptoms, and it reduced to undetectable levels by 60 days and up to 90 days in some patients[3,18]. In primary and secondary dengue infection, dengue IgG was detected in 100% of patients after day 7 of onset of fever[3,14,18]. In this study, 62.8%–of cases were IgM positive and 13.0% were IgG positive.

In Thailand, researchers found that DEN-2 is the most severe serotype which is usually associated with secondary infections (91.8%) and the occurrence of DHF (87.2%), especially DSS (32.9%). DEN-3 and DEN-4 had more degree of liver involvement as demonstrated by a higher percentage of patients with elevation of liver enzyme (AST and ALT) and DEN-1 seemed to be the mildest serotype[22,23]. Fried et al (2006) also noted that DENV-2 appears to be marginally associated with more severe dengue disease as evidenced by a significant association with DHF grade I when compared to DEN-1. In addition, the secondary infection is more severe can lead to DHF/DSS. They found that DEN-2 and -3 to be twice as likely to result in DHF as DEN-4 and DEN-1[24]. Few other previous studies done in India also found that DEN-2 and DEN-3 were causing more severe dengue infections[25,26] and supported by Guzman et al (2002, 2008), noted that Asian genotypes of both DEN-2 and DEN-3 were more virulent in term of DHF outbreaks[27,28].

In Malaysia, all four serotypes can be isolated at any one time but the predominant circulating dengue virus will show a sinusoidal pattern. For example, DEN-3 was the predominant serotype in the early 90s with a peak in 1993, and then subsequently declined. It then re-emerged, reaching the peak in 2001. Since then DEN-4 was predominant until year 2006. In 2007 the reemergence of DEN-2 as the predominant serotype[3]. In this research it was found that all four circulating serotypes were also present in 2010 in Negeri Sembilan with DEN-1 was the predominant dengue serotype. The predominant serotype was found to be DEN-3 in January and together with DEN-2 until around May in which DEN-1 started to be pre-dominant until reaching the peak in December. This was consistent with the rise of dengue infections in Negeri Sembilan in 2010 particularly DHF and death reported with the more severe circulating DEN-2 and DEN-3[21-27], particularly the first six month of the study period.

The use of secondary data certainly produced some limitations to the research done such as the data collection and documentation particularly the clinical presentations of the patients including serological analysis reported. The use of viral isolation in dengue serotyping has generally poorer result when compared to the more expensive technique such as polymerase chain reaction (PCR) technique[3,18,28]. It is most probably due to the viability of the virus and the quality of the sample sent for serotyping including time, storage and transportation[3]. Another limitation of this study is that from the way the data is collected, it was not possible to link up between the serotype results to the individual patients seen in the government health clinics. Therefore we were unable to determine whether there were any reinfection among the cases.

CONCLUSION

Dengue infection is one of the most deadliest vector borne disease in the world affecting millions of people every year from all age groups, gender and ethnicity, causing considerable morbidity and mortality. In this research, the pattern of the disease in Negeri Sembilan for the year 2010 was discussed in term of socio-demographic factors such as age, ethnicity, locality (district, urban and rural), clinical presentations, including serological analysis (IgG and IgM) and dengue serotypes (DEN-1, DEN-2, DEN-3 and DEN-4). In Negeri Sembilan, Malaysia for the year 2010, most of the dengue cases occurred in young adults, male working class affected compared to female, and Malay ethnicity. Seremban district contributed most of those dengue cases and more concentrated in urban areas. Wide spectrum of dengue illnesses ranging from undifferentiated dengue fever to dengue haemorrhagic fever, dengue shock syndrome and death. Among symptoms of dengue infections presented upon admission were fever, headache, myalgia and arthralgia, nausea and vomiting, rash, petechiae, bleeding tendency and/or neurological deficits. Dengue serology (IgM and IgG) were important tools in supporting the diagnosis of the dengue infections in Malaysia. All dengue serotypes (DEN-1, DEN-2, DEN-3 and DEN-4) were present in 2010 in Negeri Sembilan. DEN-3 was the pre-dominant serotype noted in January and existed together with DEN-2 until around May. After that DEN-1 was the pre-dominant serotype.

From the data analyzed in this research, dengue infection is and will continue to be a major public health issue in Negeri Sembilan. Therefore, it is vital that the public health services are optimized to meet these challenges. Epidemiological, serological, virological as well as entomological surveillance should be strengthened. Public awareness as well as community commitment and participation should be enhanced to improve the overall public health management of dengue fever and dengue haemorrhagic fever.

ACKNOWLEDGEMENT

We wish to acknowledge the generous co-coperation that we have received from the National Public Health Laboratory, Sungai Buloh and Negeri Sembilan State Health Department. The authors would also like to thank the National University of Malaysia for ethics approval and funding this project (Grant code: FF-345-2011) and the Ministry of Health NMRR No: 10758.

REFERENCES

1. WHO. Dengue guidelines for diagnosis, treatment, prevention and control- new edition, 2009. Geneva: World Health Organization, 2009.

2. Ministry Of Health Malaysia. Annual Report, 2004.

3. Ministry Of Health. Clinical Practice Guidelines on Management of Dengue Infection in Adults (revised 2nd ed), 2010.

4. Ministry of Health Malaysia. Annual Report. Available from: http:www. moh.gov.my. (accessed 13 January 2012).

5. Balmaseda B, Hammond S, Perez L, et al. Serotype-specific differences in clinical manifestations of Dengue. *Am. J. Trop. Med. Hyg.* 2006; **74**(3): 449–56.

6. Kalayanarooj S, Nimmannitya S. Clinical and Laboratory Presentations of Dengue Patients with Different Serotypes. *Dengue Bulletin.* 2000; **24**: 53-59.

7. WHO. Clinical and laboratory guidelines for dengue fever and dengue haemorrhagic fever/dengue shock syndrome for health care providers, 2000. Geneva: World Health Organization, 2000.

8. Abu Bakar S, Shafee N. Outlook of dengue in Malaysia : a century later. *Malaysian J Pathol* 2002; **24**(1): 23–7.

9. Rahim MA, Sikder MS. Clinicopathologic manifestations and outcome of dengue fever and dengue haemorrhagic fever. *Bangladesh Med Res Counc Bulletin* 2005; **31**: 36-45.

10. WHO. Prevention and control of dengue and dengue hemorrhagic fever: comprehensive guidelines, 1999. Geneva: World Health Organization, 1999.

11. Koh BKW, Ng LC, Kita Y, et al. The 2005 dengue epidemic in Singapore: epidemiology, prevention and control. *Ann Acad Med Singapore*. 2008; **37**: 538-45.

12. Guha-Sapir D, Schimmer B. Dengue Fever : New Paradigms for a Changing Epidemiology. Emerging Themes in Epidemiology 2005; 2:1. Available from: http: www.ete-online:com/content/2/1/1(accessed 15 November 2011).

13. Bangs MJ, Larasati RP, Corwin AL, et al. Climatic factors associated with epidemic dengue in Palembang, Indonesia: implications of short term meteorological events on virus transmission. *Southeast Asian J Trop Med Public Health* 2006; **37**(6): 1103-16.

14. Byron M, Koraka P, Osterhaus ADME. Dengue virus pathogenesis: an integrated view. *Clinical Microbiology Reviews* 2009; **22**(4): 564-81.

15. Halstead SB, Simasthien P. Observations related to the pathogenesis dengue haemorrhagic fever. II antigenic and biologic properties of dengue viruses and their association with disease response in the host. *Yale J Biol Med* 1970; **42**: 276-92.

16. Lei HY,Huang KJ, Lin YS, et. al. Immunopathogenesis of dengue hemorrhagic fever. *American Journal of Infectious Diseases* 2008; **4**(1): 1-9.

17. Buchy P, Yoksan S, Peeling RW, Hunsperger E. Laboratory tests for the diagnosis of dengue virus infection. Working paper for the Scientific Working Group on Dengue Research, convened by the Special Programme for Research and Training in Tropical Diseases, Geneva, 1-5 October, 2006. WHO on behalf of the Special Programme for Research and Training in Tropical Diseases 2007.

18. Qiu FX, Gubler DJ, Liu J C, Chen QQ. Dengue in China: A clinical review. *Bull World Health Organ.* 1993; **71**: 349-59.

19. Khan AH, Hayat AS, Masood N, et al. Frequency and clinical presentation of dengue fever at tertiary care hospital of Hyderabad/Jamshoro. *JLUMHS* 2010; **9**(2): 88-94.

20. Wichmann O, Hongsiriwon T, Bowonwatanuwong C, et al. Risk factors and clinical features associated with severe dengue infection in adults and children during the 2001 epidemic in Chonburi, Thailand. *Tropical Medicine and International Health* 2004; **9**(9): 1022–9.

21. Anantapreecha S, Chanama S, A-Nuegoonpipat A, et al. Serological and virological features of dengue fever and dengue haemorrhagic fever in Thailand from 1999 to 2002. *Epidemiol Infect*. 2005; **133**: 503–07.

22. Fried JR, Gibbons RV, Kalayanarooj S, et al. Serotype differences and dengue hemorrhagic fever: an analysis of data collected in Bangkok, Thailand from 1994 to 2006. Available from: http:www.plosntds.org. (accessed 22 October 2010).

23. Nisalak A, Endy TP, Nimmannitya S, et al. Serotype-specific dengue virus circulation and dengue disease in Bangkok, Thailand from 1973 to 1999. *Am J Trop Med Hyg*. 2003; **68**:191-202.

24. Bharaj P, Chahar HS, Pandey A, et al. Concurrent infections by all four circulating dengue serotypes during outbreak of dengue in 2006 in Delhi, India. *Virology Journal* 2008; **5**: 1.

25. Gupta E, Dar L, Kapoor G, Broor S. The changing epidemiology of dengue in Delhi, India. *Virology Journal* 2006; **3**: 92.

26. Guzman MG, Kouri G, Valdes L, et al. Enhance severity of secondary dengue-2 infection: death rates in 1981 and 1997 Cuban outbreaks. *Pan-American Journal of Public Health* 2002; **11**: 223-7.

27. Guzman MG, Perez AB, Fuentes O and Kouri G. Dengue, Dengue Haemorrhagic Fever. PAHO/WHO Collaborating Centre for the Study of Dengue and it's Vector, 'Pedro Kouri' Tropical Medicine Institute, Havana, Cuba. 2008. Available from: http://www.sciencedirect.com/science/article/pii/B9780123739605005645 (accessed 22 September 2012).

28. Zhao B, Mackow E, Buckler-White A, Markoff L, Chanock RM, Lai CJ, et al. Cloning full-length dengue type 4 viral DNA sequences: Analysis of genes coding for structural proteins. *Virology* 1986; **155**: 77-88.