Probable primary and secondary dengue viral infections and associated host factors among university undergraduates in Osun State, Nigeria

Waidi F. Sulea, Toluwani O. Fadamitanb, Omotayo A. Lawala, Wasiu O. Adebimpec, Oluyinka O. Opaleyec and Daniel O. Oluwayelud

aDepartment of Microbiology, Faculty of Basic and Applied Sciences, Osun State University, Osogbo, Nigeria; bDepartment of Community Medicine, Faculty of Clinical Sciences, University of Medical Sciences, Ondo, Nigeria; cDepartment of Medical Microbiology and Parasitology, College of Health Sciences, Ladoke Akintola University of Technology, Osogbo, Nigeria; dDepartment of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria

ABSTRACT

Introduction: According to the World Health Organization, global dengue cases have continually increased in recent decades. In resource-poor countries, such as Nigeria, diagnoses are often missed, putting the general population at risk of significant mortality and morbidity. This study investigated exposure to dengue virus (DENV) and probable primary and secondary DENV infections among new undergraduates in Southwestern Nigeria.

Methodology: Institutional-based retrospective study was carried out among 89 eligible undergraduates selected using systematic sampling method. The students were tested for the presence of DENV IgM and IgG antibodies using ELISA kits. Data were statistically analyzed using the SPSS software version 23.0 vis-à-vis their serologic results.

Results: Students aged 15–33 years (mean age: 19.7 ± 2.9 years). Mean age of the 46 female students (19.8 ± 3.2 years) was comparable (p = 0.64) to that of the 43 males (19.5 ± 2.7 years). DENV IgM and IgG prevalence rates were 41.6% and 33.7%, respectively. Unlike DENV IgG prevalence, older age (18–33 years) and feminine gender were, respectively, associated with IgM positivity (p = 0.05 [odds ratio (OR) = 2.7]; p = 0.001 [OR = 4.7]). Probable primary and secondary DENV infections were 22.5% and 33.7%, respectively, with 43.8% of the students being susceptible to DENV infection. Those with primary infections not only stood the risk of secondary heterotypic infections with possibility of severe dengue but they might also be infectious to Aedes mosquitoes, thus further spreading the virus.

Conclusions: The observed high antibody prevalence rates further establish local endemicity of dengue and calls for intensification of prevention efforts targeting the general population.

1. Introduction

Dengue viruses (DENVs) are Flaviviruses that are globally transmitted by Aedes mosquitoes (Aedes aegypti and Ae. albopictus) [1] that are found in tropical and subtropical environments [2]. Four serologically related but antigenically distinct serotypes of DENV exist which are responsible for the most important arboviral disease of humans known as dengue [3]. According to the World Health Organization (WHO) [4], global dengue cases have continually increased in recent decades with about 50–100 million cases annually.

Depending on whether or not there has been a previous exposure, DENV infection is classified into primary (infection of DENV-naïve humans) or secondary (reinfection of DENV-exposed humans) [2,5]. Exposure of DENV antigens to adaptive immunity induces humoral immune response comprising IgM (indicator of recent/acute infection) and IgG (indicator of past infection) against the virus [6]. Usually within a few days (about 4–5 days) after onset of dengue fever, detectable DENV-specific IgM appears in the blood representing initial/primary immune response to primary infection in DENV-naïve person. This antibody reduces the viremia and remains in circulation for about 3–8 months [7,8]. DENV-specific IgG appears (as secondary immune response to the primary infection) in the blood in about 7 days after onset of fever and attains highest titer in about 3 weeks of the disease [9,10]; the titer decreases with time but remains detectable in the blood as immunologic memory [6].

When a DENV-exposed individual is reinfected by the virus, transient and low titer IgM is produced from DENV-naïve B cells that last a few days (representing primary immune response to secondary DENV infection); the virus-specific IgG (immunologic memory) against reinfection by DENV increases rapidly and becomes readily detectable in about 5–7 days post-reinfection (equivalent to about the first day of symptoms) when the titer is highest (usually higher than DENV IgG produced as secondary humoral immune response of the convalescent phase of primary infection) [6]. It is...
noteworthy, however, that DENVs display antibody epitopes that are unique to each serotype and epitopes that are shared between serotypes, such that most DENV-specific antibodies in human immune sera are weakly neutralizing and bind to multiple DENV serotypes [6]. Hence, the acute phase IgG antibodies (IgG1 and IgG3) in secondary infection do not cross-protect against different serotypes [10,11].

The severe forms of dengue (dengue hemorrhagic fever and/or dengue shock syndrome) ensue when the secondary anti-DENV IgG response binds to but fails to neutralize a secondary DENV serotype that is different from the serotype of primary infection (i.e. heterotypic DENV infection). From the epidemiologic (and clinical) view point, the foregoing explains the reason for conducting serology to classify humoral immune response to DENV infection [12]. Also, diagnosis of dengue or screening of humans for exposure to DENV depends on serology. Thus, the detection and classification of immune response to DENV infections are important to monitor dengue epidemic and to identify humans at risk of secondary dengue with its attendant severe disease phenotype [6]; these entail detection of DENV-specific IgM and IgG [13].

Interpretation of DENV ELISA results and their implications require careful consideration [14]. In fact, Wahala and de Silva [6] opined that the dominance of cross-reactive antibodies precludes the use of simple antigen binding assays to identify a Flavivirus responsible for infection. Although IgG avidity assay and IgM/IgG ratio are supportive [12,15,16], several workers [13,17] have used patterns of antibody reactivity to DENVs (in ELISAs) for classifying patients as having primary/recent or secondary/past dengue. In the previous studies, De Souza et al. [18] and Blacksell et al. [19] considered detection of DENV IgM without detectable DENV IgG as clear indication of primary DENV infection, while dual DENV antibody positivity (IgM+IgG+) (though with high IgG avidity) identified secondary infection. Another study [16], however, observed that, in the absence of data on the timing of specimen collection in relation to onset of symptoms, a dual antibody positivity pattern (with high avidity) can only be considered a marker of probable secondary DENV infection.

Dengue is neglected, under-recognized, and underreported in Nigeria due to poor awareness by health-care providers and its non-prioritization by public health authorities [14]. This study was therefore undertaken to investigate exposure of apparently healthy new undergraduate students of Osun State University (UNIOSUN), Osogbo, Nigeria, to DENV and determine whether they had had primary or secondary infection with the virus.

### 2. Materials and methods

#### 2.1. Study setting and design

The study was conducted between November 2014 and August 2015 in the Osun State University, Osogbo, Osun State (latitude 7.5876°N and longitude 4.5624°E), southwest Nigeria (Figure 1). The vegetation in the state ranges from derived savannah to rainforest [20]. The study participants were apparently healthy, newly admitted undergraduate students

![Figure 1. Map of Nigeria showing Osogbo, wherein lies Osun State University (Wikipedia).](attachment:figure1.png)
from all the six campuses of the university in the 2014/2015 academic session who, by the university policy, presented for medical tests in the College of Health Sciences. The prevalence of dengue infection in the study area is not known, with diagnostic facilities to diagnose or manage dengue cases available in the only teaching hospital. The university admits students from different parts of Nigeria. This is a point-prevalence, tertiary education institution-based retrospective study. Only registered undergraduates who have presented for the admission screening exercises were recruited into the study.

2.1.1. Sampling methodology
Students were randomly selected based on their states of residence in order to have a nationwide representative sample. For each of the three applicable geopolitical zones in Nigeria, a systematic sampling of one in three students on the sampling frame or list of students was selected having selected the first at random. Eighty-nine plasma samples were randomly selected from the archived samples.

2.1.2. Data collection
Data collection was done using a validated checklist containing of dengue-related parameters. The designated laboratory assistant also helped in synthesizing demographic data such as age and gender from laboratory records of the students.

2.2. Plasma samples and serological assays
Plasma samples prepared from students’ blood samples and stored at −20°C were retrieved and allowed to completely thaw at room temperature prior to serologic tests. The plasma samples were qualitatively tested with commercial ELISA kits for the qualitative/semi-quantitative determination of IgM and IgG antibodies to DENV serotypes 1, 2, 3, and 4 in human plasma and sera (DIA.PRO Diagnostic Bioprobes Srl. Via G. Carducci, no. 27 20,099 Sesto San Giovanni, Milano, Italy). The sensitivity and specificity of both kits were >98.0%. The assays were conducted and interpreted according to the manufacturer’s instructions.

2.3. Statistical analysis
The SPSS software version 23.0 was used in data analysis. Data were presented with descriptive statistics (mean ± standard deviation, percentages, and 95% confidence intervals). Differences in ages of participants were compared using independent Student’s t-test while associations between participants’ variables and serologic results were established with Pearson’s Chi-square test. The strength of association was further evaluated by estimating odds ratio (OR). The students with equivocal results for DENV IgM antibody were considered negative for estimation of proportion. A two-tailed test was used and level of statistical significance was set at p ≤ 0.05. The statistical package, SPSS version 16.0 for Windows (SPSS Inc., Chicago, IL), was used for the data analysis.

2.3.1. Ethical issues
Ethical approval to conduct this study on archived plasma samples of the students was obtained from the Health Research Ethics Committee of UNIOSUN.

3. Results
3.1. Characteristics of study participants
Eighty-nine newly admitted students participated in the study with their ages ranging from 15 to 33 years (average of 19.7 ± 2.9 years). There were 46 females (16–33 years; average of 19.8 ± 3.2 years) and 43 males (15–27 years; average of 19.5 ± 2.7 years). There was no significant difference (p = 0.64) in the average age of the male and female students. However, the 65 adult students (18–33 years; average of 20.8 ± 2.6 years) were significantly older (p = 0.001) than the 24 young ones (15–17 years; average of 16.6 ± 0.6 years).

3.2. Prevalence rates of DENV IgM and IgG antibodies
Of the 89 plasma samples tested for DENV IgM, 37 (41.6%) were positive while 10 gave equivocal results (herein considered seronegative). For the DENV IgG, 30 (33.7%) were positive. The group-specific prevalence rates for DENV IgM and IgG antibodies with the associated risk factors are presented in Table 1. Only 19.1% of the students had dual antibody positivity (IgM+IgG+) while 43.8% were negative for both DENV IgM and IgG antibodies (Table 2).

4. Discussion
This retrospective study was conducted to investigate exposure of apparently healthy new undergraduate students to DENVs and determine whether there had been primary or secondary DENV infections among them. These participants are apt for the study as they were young adults (15–33 years) who stay mostly outdoors for schooling, work, or pleasure, thereby becoming exposed to mosquito vectors of DENV or other arboviruses [22]. In addition, based on the socioeconomic impact of dengue, the WHO had recommended the introduction of dengue epidemiology into school curriculum as well as public health education in dengue-endemic countries in order to encourage patients and their families to seek prompt medical care [1].
Expectedly, the female and male participants were statistically comparable in mean age as they were all newly admitted students. However, adult students were significantly older than the young ones. Moderately high seroprevalence rates of 41.6% and 33.7% for DENV IgM and IgG, respectively, further confirm the dengue-endemic status of Nigeria. Oyinloye et al. [13] earlier reported much higher DENV IgM and IgG prevalence rates of 74.4% and 90.0%, respectively, among antenatal subjects and nonpregnant women/males with febrile complaints in Maiduguri, Nigeria. A likely reason for the wide disparity in antibody prevalence rates reported by these workers and those of the current study might be the occurrence of febrile illness (probably due to ongoing DENV or related flaviviral infections) among some of their study participants. In a similar study conducted using the ELISA on a large number of febrile patients aged less than 1–101 years in Barbados, Kumar and Nielsen [17] reported lower prevalence rate of DENV IgM (36.6%, \( n = 8296 \)) but higher IgG prevalence rate (75.7%, \( n = 7227 \)) than our findings. The disparity in the seroprevalence rates might be due to the fact that they used a larger sample size and many participants in their study were also suspected of having acute dengue (febrile illness).

The moderately high antibody prevalence rates in the present study further establish endemicity of dengue in Nigeria. This might be attributable to the fact that environmental factors that favor breeding of the mosquito vectors, such as poor drainage system, improper waste disposal that results in the presence of stagnant water bodies, and water collection in waste metal containers and used vehicle tires are common in most Nigerian cities [14,23]. Higher prevalence rates were observed for both DENV IgM and IgG among older (18–33 years) and female students. While these were not significantly associated with DENV IgG prevalence, older and female students had significantly higher DENV IgM prevalence rates (\( p = 0.05 \) and 0.001, respectively). The computed OR further strengthened these associations as older and female students were approximately three and five times more likely to be DENV IgM-positive than younger and male students, respectively. The fact that female gender was apparently associated with DENV IgM antibodies in this study is consistent with the observations of Vasconcelos et al. [24] and Idris et al. [23] who reported significantly higher DENV-2 and DENV-3 IgM prevalence rates among females in Tocantins, Brazil, and Maiduguri, Nigeria, respectively. On the other hand, da Silva-Nunes et al. [25] reported a higher prevalence of DENV IgG among males while Reiskind et al. [26] and Siqueira et al. [27] observed that both sexes were equally affected. It has, however, been suggested that high exposure of females to infected mosquitoes in households was responsible for the higher seroprevalence observed [28]. In addition, although not

### Table 1. Age- and gender-specific prevalence rates of DENV IgM and IgG antibodies among newly admitted undergraduates, UNIOSUN, Osogbo, Nigeria.

| Variables            | Number tested | Number positive (%) | Odds ratio (95% CI) | \( p \)-Value |
|----------------------|---------------|---------------------|---------------------|--------------|
| **DENV IgM antibody** |               |                     |                     |              |
| Age (years)          |               |                     |                     |              |
| 15–17                | 24            | 6 (25.0)            | 2.7 (1.0–7.8)       | 0.05         |
| 18–33                | 65            | 31 (47.7)           |                     |              |
| Gender               |               |                     |                     |              |
| Female               | 46            | 27 (58.7)           | 4.7 (1.9–11.8)      | 0.001        |
| Male                 | 43            | 10 (23.3)           |                     |              |
| **DENV IgG antibody** |               |                     |                     |              |
| Age (years)          |               |                     |                     |              |
| 15–17                | 24            | 6 (25.0)            | 1.8 (0.6–5.0)       | 0.29         |
| 18–33                | 65            | 24 (36.9)           |                     |              |
| Gender               |               |                     |                     |              |
| Female               | 46            | 19 (41.3)           | 2.0 (0.8–5.0)       | 0.12         |
| Male                 | 43            | 11 (25.6)           |                     |              |

### Table 2. Interrelationship/interpretation of DENV IgM and IgG antibody results among newly admitted undergraduates, UNIOSUN, Osogbo, Nigeria.

| Serological group | Sample size | Proportion (%) | Suggested interpretation | Probable implication |
|-------------------|-------------|----------------|--------------------------|----------------------|
| 1                 | + –         | 20 (22.5)      | Probable primary/recent DENV infection | Susceptible to secondary heterotypic DENV infection with possibility of severe dengue infection |
| 2                 | + +         | 17 (19.1)      | Convalescent phase of primary DENV infection or acute phase of secondary DENV infection (probable secondary DENV infection) | Apparently protected against secondary/tertiary DENV infection |
| 3                 | – +         | 13 (14.6)      | Past DENV infection (probable secondary DENV infection) | Susceptible to secondary heterotypic DENV infection but may be protected against secondary homotypic infection |
| 4                 | – –         | 39 (43.8)      | No evidence of current/past exposure to DENVs | Susceptible to primary infection by all DENV serotypes with possibility of dengue fever |
| Total             | 89          | 100            |                          |                      |

Note: + (positive); – (negative). Adapted from Lima et al. [21].
significantly higher, females in this study were twice more likely to be DENV IgG-positive. We therefore recommend further studies to investigate the association of female gender with higher prevalence of DENV infection.

With respect to age, similar to the findings of Idris et al. [23] in Maiduguri, Nigeria, we observed a higher DENV IgM prevalence rate among 18–33-year-old participants in the current study. In addition, Oyero and Ayukekong [29] documented a high DENV IgG seroprevalence among adults >40 years of age compared to younger persons. A possible reason for this observation is the suggestion by Ayukekong [14] that persons at greatest risk of DENV infection are those with regular exposure (supposedly young adults) to mosquito vectors, probably as a result of their occupation or other outdoor activities.

The two antibody isotypes were used to classify the students into primary (recent) and secondary (past) DENV infections. Our findings revealed that 22.5% of the students had DENV IgM only (Table 2) and these represent persons with probable primary DENV infection. The students in this category were susceptible to secondary heterotypic DENV infection with possibility of severe dengue. From the foregoing, it is clear that appreciable proportion of the students had probable primary DENV infection. It has been reported [16] that in the absence of data on the timing of specimen collection in relation to onset of symptoms, a dual IgM+IgG+ positivity pattern (with high avidity) could only be considered a marker of probable secondary DENV infection. In this work, information on the timing of specimen collection and onset of symptoms were unavailable; the students with dual positivity for DENV IgM and IgG and those IgM−IgG+ were therefore grouped together as having probable secondary DENV infection. While those with dual antibody positivity might be immunologically protected against secondary/tertiary DENV infection, the students with IgM−IgG+ might be susceptible to secondary/tertiary heterotypic DENV infection. It should be borne in mind that the students were apparently healthy at the time of sample collection; so whether or not any one developed clinical dengue afterward was not known as they were not followed up for the occurrence of febrile illness. Considering that students positive for DENV IgM were not clinically ill during blood sample collection, it is possible that they might be infectious to biting female Aedes mosquitoes, thus making such vectors capable of transmitting the virus to susceptible humans. A study in Osogbo also reported DENV IgM prevalence rate of 2.2% among 91 intending blood donors [30]. Furthermore, we observed that about 40.0% of the undergraduates had no detectable DENV antibodies. While this showed that they were not having active DENV infection (i.e. they were DENV-naïve) at the time of gaining admission into the University, it also implied that they were susceptible to all DENV serotypes.

The finding in this study that new university undergraduates (aged 15–33 years) had moderately high prevalence rates of recent and past DENV infections partly corroborates the report of Kumar and Nielsen [17] who found that the age of DENV infection was likely to shift to younger adults and children who are more likely to have severe dengue in the future. The earlier report of Beatty et al. [22] that the average dengue case fatality rate of about 5% was mainly among children and young adults also supports the focus on children and young adults as dengue-risk groups. While this is consistent with the fact that dengue is endemic and a cause of febrile illness in Nigeria, the disease is, however, neglected, under-recognized, and underreported in the country due to lack of awareness by health-care providers and the fact that it is not prioritized by public health authorities [14].

We concluded that the high DENV antibody prevalence rates obtained among adolescents/young adults in this study further establish endemicity of the infection in Nigeria. These findings therefore provide a platform for public enlightenment about dengue with a view to encouraging prevention and control measures against mosquito vectors of the virus among university students and the Nigerian populace in general.

Acknowledgments

We thank the laboratory technologists (Mr. Olaniyi Olayinka and Mr. Adeleke Ismail) in College of Health Sciences, UNIOSUN for retrieving the archived plasma samples from –20°C; and Mrs. Olusola Akanbi, Mr. Gbolabo Odewale, and Mr. Johnson Ojo of Medical Microbiology and Parasitology, College of Health Sciences, Ladoke Akintola University of Technology, Osogbo, Nigeria, for assisting in conducting the ELISA.

Disclosure statement

No potential conflict of interest was reported by the authors.

Notes on contributors

Waidi F. Sule (DVM., M.Sc., PhD) is presently a Senior Lecturer in Microbiology, Osun State University, Osogbo, Nigeria. His expertise is in Animal Virology.

Toluwani O. Fadamitan is a graduate in Microbiology (B.Sc. Microbiology), Osun State University, Osogbo, Nigeria.

Omotayo A. Lawal is a graduate in Microbiology (B.Sc. Microbiology), Osun State University, Osogbo, Nigeria.

Wasiu O. Adebimpe (FWACP, PhD, MACE, MNIM) is an associate Professor of Community Medicine and Public Health, he is the Acting Head of Department of Community Medicine, Faculty of Clinical Sciences, University of Medical Sciences, Ondo Nigeria.

Olayinka O. Opaleye (DVM., M.Sc., PhD) is currently an Associate Professor of Virology, Medical
Microbiology, College of Health Sciences, LAUTECH, Osogbo, Nigeria. His area of expertise is Animal Virology.

Daniel O. Oluwayelu (DVM, M.Sc., PhD) is a Professor of Virology, Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Ibadan, Nigeria. He is presently the Head of Department. His area of expertise is Animal and Human Virology.

References

[1] Pierson TC, Diamond MS. Flaviviruses. In: editors, Knipe DM, Howley PM. Fields virology. 6th ed. Philadelphia: Lippincott William and Wilkins; 2013. p. 747–758.
[2] Sun P, Kochel TJ. The battle between infection and host immune responses of dengue virus and its implication in dengue disease pathogenesis. Sci World J. 2013. Article ID 843469.
[3] Guzman MG, Harris E. Dengue. Lancet. 2015;385:453–465.
[4] WHO. Dengue control. 2017. Available from: http://www.who.int/denguecontrol/en/.
[5] Chakravarti AM, Kumar MA. Discrimination between primary and secondary dengue virus infection by using an immunoglobulin G avidity test. Dengue Bull. 2008;32:67–72.
[6] Wahala WMPB, de Silva AM. The human antibody response to dengue virus infection. Viruses. 2011;3:2374–2395L.
[7] Chow L, Hsu ST. MAC-ELISA for the detection of IgM antibodies to dengue type I virus. Chin J Microbiol Immunol. 1989;22:278–285.
[8] Chen WJ, Hwang KP, Fang AH. Detection of IgM antibodies from cerebrospinal fluid and sera of dengue fever patients. Southeast Asian J Trop Med Public Health. 1991;22:659–663.
[9] WHO. Dengue haemorrhagic fever: diagnosis; treatment and control. Geneva, Switzerland: World Health Organization; 1997.
[10] Gubler DJ. Dengue and dengue hemorrhagic fever. Clin Microbiol Rev. 1998;11:480–496.
[11] Koraka P, Suharti C, Setiati TE, et al. Kinetics of dengue virus-specific serum immunoglobulin classes and subclasses correlate with clinical outcome of infection. J Clin Microbiol. 2001;39(12):4332–4338.
[12] Matheus S, Deparis X, Labeau B, et al. Discrimination between primary and secondary dengue virus infection by an immunoglobulin G avidity test using a single acute-phase serum sample. J Clin Microbiol. 2005;43(6):2793–2797.
[13] Oyinloye SO, Wajiroko M, Lawan AM, et al. Dengue virus infection in northeast Nigeria: case study of a squatters’ Camp. Int J Perceptions Public Health. 2016;1(1):59–65.
[14] Ayukbekong JA. Dengue virus in Nigeria: current status and future perspective. Br J Virol. 2014;1(3):106–111.
[15] Vaughn WD, Nisalak A, Solomon T, et al. Rapid serologic diagnosis of dengue virus infection using a commercial capture ELISA that distinguishes primary and secondary infections. Am J Trop Med Hyg. 1999;60(4):693–698.
[16] Prince H, Yeh C, Lapé-Nixon M. Utility of IgM/IgG ratio and IgG avidity for distinguishing primary and secondary dengue virus infections using sera collected more than 30 days after disease onset. Clin Vaccine Immunol. 2011;18:1951–1956.
[17] Kumar A, Nielsen AL. Trends in the patterns of IgM and IgG antibodies in febrile persons with suspected dengue in Barbados, an English-speaking Caribbean country, 2006—2013. J Infect Public Health. 2015;8:583–592.
[18] De Souza VA, Tateno AF, Oliveira RR, et al. Sensitivity and specificity of three ELISA-based assays for discriminating primary from secondary acute dengue virus infection. J Clin Virol. 2007;39:230–233.
[19] Blacksell SD, Mammen MP, Jr Thongpaseuth S, et al. Evaluation of the PanBio dengue virus nonstructural 1 antigen detection and immunoglobulin M antibody enzyme-linked immunosorbent assays for the diagnosis of acute dengue infections in Laos. Diagn Microbiol Infect Dis. 2008;60:43–49.
[20] Sofoluwe NA, Tijani AA, Baruwa OI. Farmers’ perception and adaptation to climate change in Osun State, Nigeria. Afr J Agric Res. 2011;6:4789–4794.
[21] Lima JRC, Rouquayrol MZ, Callado MRM, et al. Interpretation of the presence of IgM and IgG antibodies in a rapid test for dengue: analysis of dengue antibody prevalence in Fortaleza City in the 20th year of the epidemic. Rev Soc Bras Med Trop. 2012;45(2):163–167.
[22] Beatty M, Letson W, Edgil D, et al. Estimating the total world population at risk for locally acquired dengue infection. Abstract presented at the 56th annual meeting of the American society of tropical medicine and hygiene. Am J Trop Med Hyg. 2007;77(5):170–257.
[23] Idris AN, Baba MM, Thairu Y, et al. Sero-prevalence of dengue type-3 Virus among patients with febrile illnesses attending a tertiary hospital in Maiduguri, Nigeria. Int J Med Med Sci. 2013;5(12):560–563.
[24] Vasconcelos PF, Travassos Da Rosa ES, Travassos Da Rosa JF, et al. Outbreak of classical fever of dengue caused by serotype 2 in Araguaiana, Tocantins, Brazil. Rev Inst Med Trop São Paulo. 1993;35:141–148.
[25] da Silva-Nunes M, de Souza VA, Pannuti CS, et al. Risk factors for dengue virus infection in rural Amazonia: population-based cross-sectional surveys. The American Journal of Tropical Medicine and Hygiene 2008; 79: 485–494.
[26] Reiskind MH, Baisley KJ, Calampa C, et al. Epidemiological and ecological characteristics of past dengue virus infection in Santa Clara, Peru. Trop Med Int Health. 2001;6:212–218.
[27] Siqueira JB, Martelli CM, Maciel IJ, et al. Household survey of dengue infection in central Brazil: spatial point pattern analysis and risk factors assessment. Am J Trop Med Hyg. 2004;71:646–651.
[28] Kohn MA. Survey on indoor resting mosquito species in Phnom Penh, Kampuchea. Folia Parasitol (Praha). 1990;37(2):165–174.
[29] Oyero OG, Ayukbekong JA. High dengue NS1 antigenemia in febrile patients in Ibadan, Nigeria. Virus Res. 2014;191:59–61.
[30] Muhibi MA, Adeleke MA, Shittu BT. Jeremiah ZADengue virus infection among voluntary blood donors in Osogbo, Southwestern Nigeria. Am J Biomed Sci. 2017;9(3):113–118.