PanAfrican Medical Journal

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Research

Seroprevalence of Anti-Dengue Virus 2 Serocomplex antibodies in out-patients with fever visiting selected hospitals in rural parts of western Kenya in 2010-2011: a cross sectional study

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Key words: Dengue, Health Facilities, Sero-Prevalence, Kenya

Received: 28/05/2013 - Accepted: 17/10/2013 - Published: 29/10/2013

Abstract

Introduction: There has been a recent increase in the spread of dengue to rural areas. Rural parts of western kenya are naturally prone to mosquito-borne diseases, however, limited research has been documented on infections with dengue. This study therefore investigated the presence of antibodies against dengue virus 2 (denv-2) in a cross-section of febrile out-patients visiting three selected hospitals to assess the level of exposure and to possibly identify the epidemiologic and clinical factors of seropositive participants. **Methods:** In a cross-sectional study, we administered a questionnaire and used indirect elisa to test for the presence of denv-2 antibodies in febrile outpatients (n=422) visiting three selected hospitals in rural western kenya. All positive and borderline samples were re-evaluated by plaque reduction neutralization tests (prnt). **Results:** The prevalence of denv-2 serocomplex antibodies was 8.5% by indirect elisa and 1.2% by prnt. Using bivariable analysis, age (p<0.0001), headache (or, 3.4 (1.6-7.4); p=0.002), retro-orbital pain (or, 3.1 (1.2-7.7); p=0.015), muscle ache (or, 2.6 (1.3-5.2); p= 0.007), joint pain (or, 3.5 (1.7-7.3); p=0.001) and abdominal pain (or, 9.5 (2.44-37.24); p=0.001) were significantly associated with denv-2 seropositivity. **Conclusion:** This study confirms that there is an existence of dengue virus 2 circulating in regions of western kenya. Age, headache, retro-orbital pain, muscle ache, joint pain and abdominal pain were associated with increased denv-2 seropositivity.

Pan African Medical Journal. 2013 16:73. doi:10.11604/pamj.2013.16.73.2891

This article is available online at: http://www.panafrican-med-journal.com/content/article/16/73/full

Introduction

Dengue Virus (DENV), a flavivirus, is the cause of a mosquito-borne viral infection that in recent decades has become a major international public health concern [1]. The virus exists as four closely-related but antigenically distinct single stranded RNA virus serotypes; DENV-1, DENV-2, DENV-3 and DENV-4 [1-3]. The vectors for transmission of these viruses are Aedes (Ae.) mosquitoes; principally *Ae. Aegypti and Ae. Albopictus* [1, 4].

There has been a recent recurrence of dengue infections that has augmented geographically from 9 countries in the past 60 years to more than 100 countries to date [5]. These infections are common in the tropical and sub-tropical regions around the world [1].

It is projected that 2.5-3.0 billion people are at risk of DENV infections worldwide, with 50-100 million dengue fever (DF) infections and 250,000-00,000 of more severe dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) occurring each year [5], accounting to 20,000-70,000 deaths per year [5].

Sporadic cases of DENV infections have been reported in Africa within the past 50 years from 1960-2010 with 20 laboratory confirmed cases reported in 15 countries, most of which were from east Africa [6]. Available data suggest that DENV-2 has caused most epidemics in Africa followed by DENV-1 [5, 7].

In East Africa, there have been reports of DENV-3 outbreaks in Tanzania and Zanzibar Island [8]. A cross-sectional survey reported a seroprevalence of 7.7% in Pemba Island and 1.8% in Tosamaganga[9]. In Kenya, Ministry of Public Health and sanitation reported dengue outbreaks, in Mandera of North eastern Kenya with 1000 cases and four unconfirmed deaths[10]. Recently, health officials and medical experts confirmed at least 58 cases of DF at the Kenyan coast[11].

Data available from the few studies conducted in Kenya reveals that most infections have been caused by DENV-2[12, 13]. Another study revealed DENV-2 to be the most common serotype followed by DENV-1, DENV-3 and DENV-4 [14]. Additional studies conducted in the Kenyan coast [15] and in western Kenya[16] revealed a prevalence rates of 1.0% and 1.1% for DENV-2 respectively. These observations propelled the current study to focus on DENV-2.

Previous studies report that a large proportion of adults in rural Kenya have been infected with arboviruses at some point in their life [17]. Rural parts of western Kenya are naturally exposed to mosquito-borne diseases and the vectors associated with transmission of dengue exist in these regions. However, limited research has been documented on these infections and no hospital-based studies have been carried out to determine the sero-prevalence of anti-DENV-2 antibodies in these regions. Therefore, the current study used indirect enzyme-linked immunosorbent assay (ELISA) and plaque reduction neutralization test (PRNT) to test for the anti-DENV-2 serocomplex and neutralization antibodies in febrile adult out-patients visiting three selected health facilities.

Socio-demographic and economic factors play key roles for effective communicable disease control [18], however, their role in DENV transmission is still poorly understood since most studies have been inconclusive and inconsistent [18-24]. Other studies have assessed environmental and household factors in other parts of the world [25] but with limited studies in Africa. Since no studies have assessed these factors in Kenya, the current study used a basic questionnaire to obtain the socio-demographic and environmental factors associated with DENV-2 seropositivity. The current study recruited participants who were ≥5 years of age because more severe cases of dengue fever are usually seen in older children[4].

Infection with DENV is normally presented with flu-like symptoms [1, 2, 4] sometimes accompanied by a rash [26, 27]. Minor hemorrhagic manifestations may occur, however, severe hemorrhage is unusual [26, 27]. The current study also determined the clinical characteristics of febrile out-patients seropositive with anti-DENV-2 serocomplex antibodies visiting the three selected health facilities in the two study regions of western Kenya.

Methods

Study design: This was a cross-sectional study, using prospective hospital-based surveillance for cases presenting with fever at the three selected health facilities. This study was conducted between June 2010 and August 2011.

Population

Selected Hospitals in Western Kenya: Participants were enrolled at three health facilities, serving different regions of Kenya: Alupe Sub-district Hospital, KEMRI/CIPDCR Alupe Clinic both of Busia County and Anderson Medical Clinic of Trans-Nzoia County. These health facilities are at proximity and lie along the border belt that connects Kenya to Uganda. They serve both children and adult patients in a primarily rural area of western Kenya. Alupe Sub-district Hospital is a level 3 health facility and is the largest in Teso-south constituency and receives referrals from other health facilities. KEMRI/CIPDCR Alupe Clinic is a level 2 health facility; it serves as a study health facility for clinical studies, provides health care services as well as receiving referrals from other health facilities. Anderson Medical Centre is a level 2 health facility; it serves Cherangani, Kwanza and Saboti constituencies and it receives referrals from other minor health facilities. Therefore, these three health facilities provided a wide coverage of the population in these regions.

Patient Eligibility and Inclusion criteria: Outpatients ?5 years of age with fever ?37.8°C, presenting to one of the participating hospitals who voluntarily provided informed consent/assent to participate in the study were eligible. All those unwilling to provide informed consent/assent for participating in the study and those aged below 5 years were not eligible.

Sample size determination: The formula $n=Z^2PQ/d^2$ was used to derive the desired sample size. Where n is the desired sample size, P is the expected prevalence in the target population, Q is 1-P, Z is 1.96; standard error, d is the level of statistical significance (0.05). A P-value of 50% was used representing maximum uncertainty [28]. Hence, the estimated sample size was 384 with an additional 10% sampled to take care of data inconsistencies [29], providing a total sample size of 422.

Ethical Approval

Ethical clearance for this study was obtained from the Kenya Medical Research Institute (KEMRI) Ethical Review Committee (ERC) and Scientific Steering Committee (SSC) prior to initiation of the study.

Sample collection and processing

Upon presentation to the three health facilities, participants were directed to a study nurse, who recorded socio-demographic, housing, environmental conditions and yellow fever vaccination status data, using a standardized questionnaire. Study physicians performed medical examinations and recorded data systematically on the history of the illness and current symptoms such as; headache, retro-orbital pain, muscle ache, joint pain, vomiting, rash and abdominal pain. Data on any medical tests requested and treatments prescribed was recorded.

About 5 ml of blood was collected in serum separator tubes from each participant by aseptic venipuncture technique by the study clinician or nurse. The sample tubes were centrifuged at 1500rpm for 10 minutes; serum was aliquoted into cryovials and kept at -20°C at the participating health facilities until collection. Once every month, the cryovials were transported within 48 hours, in dry ice to KEMRI/CIPDCR laboratories where they were processed and tested for the presence of DENV-2 antibodies as soon as possible.

Laboratory procedures

Viruses and Cell lines: Purified Hawaiian strain of dengue virus (DENV-2 (002ST) 2009; titer (1×10-5 PFU/ml)), obtained from Nagasaki University Institute of Tropical Medicine, was used in all serological tests. African green monkey-derived Vero cells, kindly supplied by Nagasaki University Institute of Tropical Medicine were used as cell lines for viral culture.

Indirect Enzyme-linked Immunosorbent Assay (ELISA): Indirect ELISA screening test was performed to detect virus specific IgA, IgG, and IgM antibodies, according to an in-house kit method as previously described [30] with few modifications to suit the local laboratory settings [31]. Briefly, one half of the 96-well (NUNC) microtiter plate was coated directly with 100µl per well of the DENV-2 antigen diluted 1:500 in 1× Phosphate Buffered Saline (PBS) this were the test wells. The other half was coated with 100µl per well of 1×PBS containing 3% Fetal Calf Serum (PBS-F) constituting the internal control wells. The plates were wrapped in aluminium foil and kept in 4°C overnight.

The following day, the plates were washed four times with 0.05% PBS-T (50µl Tween 20 in 1×PBS) washing buffer and blocked by adding 100µl per well of 3%PBS-F (3ml fetal calf serum in 1×PBS) blocking solution. The plates were incubated for 1 hour at room temperature (23°C). One hundred microliters per well of test sera diluted 1:1000 with PBS-F was added followed by incubation for 1 hour at 37°C and subsequently washed with PBS-T. Secondary antibody (anti-human IgG+IgM+IgA diluted into 1:5000 with PBS-F) was added 100µl per well. The plates were then incubated for another 1 hour at 37°C and washed thereafter. Substrate solution (O-phenylenediamine dihydrochloride (OPD), SigmaFast tablets diluted in distilled water) was added 100µl per well preceded by incubation for 15 minutes at room temperature in the dark. Finally, 100µl of 1N sulphuric acid solution was added into each well to stop the reaction and the plates were read at 492nm.

The Optical Densities (OD) of positive-to-negative (P/N) ratios of >1.0 was considered positive, <0.5 was considered negative and ?0.5 was considered borderline positive. All sera that were positive and border line for DENV indirect ELISA, were further re-evaluated using Plaque Reduction Neutralization Test (PRNT) to confirm infection specific to DENV-2 according to standard methods [32].

Plaque Reduction Neutralization Test (PRNT): Vero cells were propagated at 37°C with 5% CO_2 in an incubator in T175 NUNC flasks using growth media consisting of 1×Eagles Minimum Essential Medium, 10% fetal bovine serum, 1×L-glutamine/Penicillin-streptomycin solution and 1.1g/L $NaHCO_3$. Cell monolayers were prepared by removing growth medium and adding 7ml of trypsin/EDTA solution to cover the cell layer and 37°C and 5% CO_2 for 4 minutes. The trypsin/EDTA solution was poured off and the cells were resuspended in 5ml of fresh growth media. Cells were counted and diluted to 1.2×10^5 cells/ml in Maintenance media (1×Eagles Minimum Essential Medium, 2% fetal bovine serum, 1×L-glutamine/Penicillin-streptomycin solution and 1.1g/L $NaHCO_3$). Two milliliters of Maintenance media was transferred to each well of a 6-well plate (NUNC) to which 1ml of the cell suspension was added. Plates were incubated at 37°C with 5% CO_2 for 1 to 2 days. Test sera were heat inactivated at 56°C for 30 minutes. In an ice bath, 2-fold dilution of the test sera, positive and negative controls beginning with a 1:10 (final 1:20) with virus standard (noted above) diluted to yield 200 plaque forming units per 0.1ml. Virus-serum mixtures was inoculated (0.1ml/well) and adsorbed for 90 minutes at 37°C with 5% CO_2, 4ml per well of overlay media (1.4% methylcellulose, 1×Eagles Minimum Essential Medium, 2% fetal bovine serum, 1×L-glutamine/Penicillin-streptomycin solution and 1.1g/L $NaHCO_3$) was added. Plates were incubated at 37°C with 5% CO_2 for 5 days. Plates were fixed using 1ml/well of 10% formaldehyde in 1×PBS with ultraviolet light. Crystal violet (1% crystal violet solution in water) staining was performed by adding 0.5ml of 1% crystal violet solution. Plates were washed in running water and left to dry overnight at room temperature. Plaques were counted the following day and the percentage reduction calculated by comparing against the positive control virus well (100% plaque forming units). A reduction in plaque count of 90% ($PRNT_{90}$) was used as the neutralizing end point.

Seropositivity was defined as positivity to all anti-DENV-2 serocomplex antibodies (IgA+IgM+IgG).

Data Management and Analysis

Data obtained from the questionnaires were entered and managed in Excel spreadsheets. All data were imported to STATA v10.0 (Stata Corp, College Station, Texas) prior to analysis. The main outcomes of interest were IgA, IgM and IgG serocomplex antibodies and neutralizing antibodies against DENV-2. From each outcome, we calculated the overall seropositivity rates. Serocomplex antibodies (Indirect ELISA test) and neutralization antibody (PRNT) status were recorded and analyzed as dichotomous variables (positive or negative). The X^2 tests were used to evaluate associations and statistical significance of the distribution of the outcomes among the different variables. Using bivariable logistic regression, we examined the effects of the socio-demographic, housing conditions, environmental, Yellow Fever Vaccine (YFV) vaccination status and clinical factors individually on the odds of seropositivity to DENV-2.

Results

Prevalence of anti-DENV-2 serocomplex antibodies (IgM, IgG and IgA) based on indirect ELISA tests and PRNT by health facilities: A total of 422 blood sera samples were obtained from febrile out-patients visiting Alupe Sub-district Hospital (ADH), KEMRI/CIPDCR Alupe Clinic (KAC) and Anderson Medical Centre (AMC). The distribution of the participants by each health facility is summarized in **Table 1**. Only 36 (8.5%) sera were positive for DENV-2 serocomplex antibodies, however, the distribution of seropositivity within the three health facilities was comparable (P=0.236). Additional analyses demonstrated that only five samples (1.2%) were PRNT positive with significant distribution of the PRNT results within the three health facilities (P=0.033; **Table 2**).

Distribution of febrile out-patients with anti-DENV-2 antibodies based on indirect ELISA tests by Health Facility: Table 3 summarizes the distribution of febrile out-patients to each study hospital stratified by gender and age based on DENV-2 seropositivity. A total of 240 (57%) female and 182 (43%) male febrile out-patients participated in this study from all selected hospitals, however, the distribution of males vs. females in the three health facilities were comparable (KAC, P=0.712; ADH, P=0.304; AMC, P=0.369). Additional analyses showed significant differences in the distribution of individuals in each age category in those positive vs. negative for KAC (P<0.0001) and ADH (P<0.0001) but not AMC (P=0.408), with a serological evidence of infection with DENV-2 being higher in those 11 years and above in the populations.

The demographic, socio-economic and environmental factors associated with a positive anti-DENV-2 antibody titer: Table 4 summarizes the association between socio-demographic and environmental factors of the study participants with anti-DENV-2 seropositivity using bivariable logistic regression analysis. The seroprevalence of anti-DENV-2 was comparable across the three health facilities (P=0.236; X^2 test) with a distribution of 7.4% in AMC, 12.1% in ADH and 6.8% KAC. Age was a significant factor in the distribution of seroprevalence in the studied population (P<0.0001; X^2 test).

Seroprevalence in males vs. females was comparable (P=0.604; X^2 test), even though 9% (17/182) of males and 8% (19/240) of female were seropositive with anti-DENV-2.

Finally, the level of education, type of house, walls with eaves/openings, dumping site with cans, tins, broken pots and/or old tyres near the house and Yellow Fever Vaccine in the past 10 years did not alter the proportions of those seropositive in this population (P=0.344, P=0.101, P=0.454, P=0.932 and P=0.451, respectively; X^2test).

Clinical characteristics of febrile out-patients with anti-DENV-2 antibodies: Table 5 summarizes the association between seropositive for anti-DENV-2 antibodies and specific clinical presentation using bivariable logistic regression. Those who reported to have a headache (OR 3.4 (1.56-7.41), P=0.002), retro-orbital pain (OR 3.09 (1.24-7.67), P=0.015), muscle ache (OR 2.59 (1.30-5.19), P=0.007), joint pain (OR 3.53 (1.71-7.28), P=0.001) and abdominal pains (OR 9.53 (2.44-37.24), P=0.001) had higher odds of DENV-2 seropositivity than those with neither of these symptoms.

Lower seroprevalence of DENV-2 antibodies was noted among those who reported to be having a rash when compared to those without a rash (OR, 0.397 (0.169-0.929), P=0.03). Vomiting did not alter the distribution of seroprevalence in the current study (OR, ∞, P=0.354; X^2 test).

Discussion

Prevalence of anti-DENV-2 serocomplex antibodies (IgM, IgG and IgA) based on indirect ELISA and PRNT tests by health facility

This study assessed the prevalence of antibodies against DENV-2 in a cross-section of febrile out-patients visiting three selected hospitals in western Kenya and for the first time report a DENV-2 prevalence of 1.2% by PRNT. By indirect ELISA, a seroprevalence of DENV-2 antibodies was found in 8.5% of the participants. This prevalence is higher than that found in a recent study conducted in western Kenya (1.1%) in which serum was obtained from healthy afebrile children and tested for the presence of anti-DENV-2 IgG antibodies [16]. Additionally, a second study using population-based, cross-sectional study design and ELISA test on blood of healthy adults from the three rural districts in Kenya, demonstrated a prevalence of 14.4% from all the three districts [17]. In the previous study, seropositivity of 1.96% was shown in Busia district of Western Province Kenya, 34.17% from Malindi district of Coast Province and 1.72% from Samburu District of Rift-valley Province, Kenya [17]. The disparity of the prevalence from the previous studies vs. those observed in the current study is mainly because of differences in the specific focus in the different studies. Whereas the current study focused on the detection of serocomplex antibodies (i.e. IgA, IgM and IgG) from febrile participants, the previous two studies focused mainly on detection of IgG antibodies from healthy participants. There was considerable discrepancy between indirect ELISA (8.5%) and PRNT results in our study. As much as the PRNT revealed a prevalence of more than 90% neutralizing antibodies in 1.2% of the participants, this prevalence was much lower than that found by indirect ELISA. Although ELISA is known to be the method of choice for the detection of DENV-specific antibodies in serum [5], our study shows that use of ELISA as the sole serologic diagnostic method when testing blood sera may be insufficient. However, it would be worthwhile to point out that the high ELISA anti-DENV-2 antibody titers, maybe reasonably real given that DENV-2 infections may exist prior to the production of neutralizing antibodies detectable by PRNT.

Distribution of febrile out-patients with anti-DENV antibodies based on indirect ELISA tests by Health Facility

The distribution of anti-DENV positive individuals among the three health facilities was comparable. This suggests that the risk of infection from DENV-2 is relatively homogenous within the populations from each health facility. However, our findings were quite inconsistent with previous observations in Tanzania and Zanzibar Island, in which a significantly higher seroprevalence from adult patients from a hospital in Pemba Island in Zanzibar compared to those from a health facility in Tanzania was shown [9].

Additional analyses demonstrated a comparable distribution of anti-DENV-2 antibodies between males and females in each of the three health facilities. These findings are comparable with a study conducted in Tanzania and Zanzibar Island [9] as well as a survey carried out in Nigeria [24]. In these studies, it was demonstrated that dengue infection was comparable between females and males. Consistency of these findings could be because all studies used febrile patients to test for the presence of DENV antibodies.

The current study also showed a significant homogeneous age differences in presentation with respect to anti-DENV-2 antibodies among the participants from KAC and ADH but not AMC. This could be attributed to the fact that KAC and ADH had majority of participants between ages 5 to 20 years whereas AMC had few participants falling in this age group. Collectively, these suggest that there could be more stable rates of infection with DENV-2 among the population tested. However, the absence of association observed between age and DENV-2 seropositivity among the out-patients visiting AMC may suggest that the leading cause of seropositivity in populations visiting this health facility may be sporadic exposure to infected mosquitoes.

The demographic, socioeconomic and environmental factors associated with a positive anti-DENV-2 antibody titer

There was no difference detected in the proportion of individuals with evidence of DENV-2 infection within the three study hospitals. This finding suggests that the risk of infection with DENV-2 is homogenous in this population, an observation congruent with that of a previous study [17]. There was, however, a significant presence of anti-DENV-2 antibodies among adult out-patients compared to out-patients who were 10 years and below. This finding express a higher seroprevalence rate in adults compared to children, an observation consistent with those of other studies carried out in Tanzania and Zanzibar Island [9], in Somalia [33] and in India [19-21]. Reasons provided for this kind of phenomenon relate to the fact that antibodies tend to remain in circulation for extended periods of time and can therefore accumulate, resulting in the highest titers in older individuals [34].

The odds of having a positive anti-DENV-2 antibody titer among the males and females in this population were comparable. These findings are however inconsistent with previous observation in Mexico in which increased risk was observed among women than in men [35]. Other studies carried out in Rural Amazonia [23], Asia [18] and India [19-22] suggested that infections with DENV-2 were more frequent in men. There could be other factors other than gender affecting DENV-2 infection in African population relative to other populations. Our laboratory is currently exploring these additional factors in the context of a wider African population.

Additional results showed a higher odds of DENV-2 seropositivity among participations who reported to have no education relative to the educated ones. This is not surprising given that education is generally associated with increased socio-economic status, increased access to preventive and curative measures and good habitation, all of which would presumably lead to a lower risk of exposure to infected mosquitoes [36]. Though not significant in this population, housing type may be an important risk factor in exposure to infected mosquitoes [17]. Studies carried out in Thailand have shown that infection with dengue is likely to be dependent on quality of housing and use of prevention measures [37]. Previous studies have reported that the environment surrounding an individual?s place of habitat can also act as a good breeding site for mosquitoes [38]. Despite these previous observations, walls with eaves or openings and dumping site with cans, tins, broken pots, old tyres near the house, were not associated with DENV-2 seroprevalence in the current study.

Yellow Fever Virus (YFV) vaccination did not affect DENV-2 antibody titers in the current study population. This observation is contrary to the norm shown in previous studies suggesting that cross-reactivity among flaviviruses could affect DENV antibody measurements in populations exposed to or immunized against YFV [32]. Another study in Brazil also demonstrated that participants with antibodies to the vaccine strain of YFV (17DD) detected by Heamagglutination Inhibition Assay (HIA) [39] were considerably more likely to have DENV IgG detected by ELISA. More studies on how YFV vaccination affect DENV-2 antibody titers in African populations need to be further explored.

Clinical characteristics of febrile out-patients with anti-DENV-2 antibodies

Even though, those who reported to have a headache, retro-orbital pain, muscle ache, joint pain and abdominal pains had higher odds of DENV-2 seropositivity than those with neither of these symptoms by bivariable logistic regression, associating these features as presenting characteristics to DENV-2 infection was challenging. A review by Potts and Rothman was also unable to draw any clear conclusions on the signs and symptoms that can clinically distinguish dengue from other febrile illnesses [40]. However, it is important to note that data obtained from the current study, provides insights into the difficulties in differential diagnosis of dengue infections which has been challenging due to its presentation with non-specific clinical symptoms.

Limitations: There was lack of follow-up serological data. This information would have been useful in comparing the acute vs. convalescent antibodies. Plans are underway to expand this paradigm in future studies. Capturing fever of less than 5 days posed as a challenge in antibody detection because DENV antibodies are usually detectable after day 5 of infection. In addition, we were unable to attribute the causal relationship between total anti-DENV-2 antibody seropositivity and the associated clinical manifestations due to lack of healthy controls. Nonetheless, these findings may provide baseline data on DENV-2 seroprevalence in the two counties of western Kenya. Finally, omission of individuals less than 5 years was a constraint, as pediatric population also plays an important role in epidemiology of dengue. Therefore, future studies should explore beyond this age group and have a more representative sample size from each age category.

Conclusion

This study confirms that there is likely an existence of DENV-2 circulating in Busia and Trans-Nzoia counties of western Kenya. The study emphasizes need for careful evaluation of serological tests when interpreting results in the clinic. Seropositivity was comparable between males and females from all the three selected health facilities. There was a homogeneous distribution of anti-DENV-2 antibodies in young adults and adult patients than patients below 11 years in ADH and KAC health facilities. The study recommends that well-designed hospital-based and targeted studies on population distributions and improved classification and diagnosis of dengue syndromes should be carried out to give more insights on biological, socio-demographic, environmental and economic factors that drive disease patterns in a community.

Competing interests

There is no conflict of interest generated by this investigation.

Authors' contributions

JAA carried out laboratory assays and shipped samples from the study sites to KEMRI/CIPDCR and KEMRI/CMR laboratories. MM designed and optimized in house based kits for the assays, shipping and purification of virus antigens and participated in the drafting of the manuscript. CO and JRO performed the statistical analyses and participated in the drafting of the manuscript. All authors read and approved the final manuscript.

Acknowledgments

This project reflects the support and contribution of many persons at Nagasaki University Institute of Tropical Medicine, Jomo Kenyatta University of Agriculture and Technology (JKUAT), as well as at Kenya Medical Research Institute (KEMRI). These data have been published with the approval of the Director, KEMRI. This study was funded partly by Nagasaki University Institute of Tropical Medicine (NUITM), Nagasaki, Japan and Consortium for National Health Research (CNHR), Nairobi, Kenya.

Tables

Table 1: Prevalence of anti-DENV-2 serocomplex antibodies (IgM, IgG and IgA) based on indirect ELISA tests by health facilities, Western Kenya, 2010 (n=422)

Table 2: Prevalence of anti-DENV-2 neutralizing antibodies by Plaque Reduction Neutralization Tests (PRNT) by health facilities, Western Kenya, 2010 (n=422)

Table 3: Distribution of febrile out-patients with anti-DENV-2 antibodies based on indirect ELISA tests by Health Facility and as stratified according to gender and age, western Kenya, 2010

Table 4: Factors associated with anti-DENV-2 seropositivity among febrile out-patients visiting selected health facilities in Western Kenya, 2010

Table 5: Clinical characteristics of febrile out-patients with anti-DENV-2 antibodies, Western Kenya, 2010

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TABLE 1: PREVALENCE OF ANTI-DENV-2 SEROCOMPLEX ANTIBODIES (IGM, IGG AND IGA) BASED ON INDIRECT ELISA TESTS BY HEALTH FACILITIES, WESTERN KENYA, 2010 (N=422)			
HEALTH FACILITY, N (%)	**POSITIVE, N (%)**	**NEGATIVE, N (%)**	***P*-VALUE**
ALUPE SUB-DISTRICT HOSPITAL 124 (100)	15 (12.1)	109 (87.9)	0.236[A]
KEMRI/CIPDCR ALUPE CLINIC 176 (100)	12 (6.8)	164 (93.2)	
ANDERSON MEDICAL CENTRE 122 (100)	9 (7.4)	113 (92.6)	
TOTAL SAMPLE SIZE 422 (100)	36 (8.5)[*]	386 (91.5)	
DATA ARE PRESENTED AS N (%). [A]=X^2 TEST. [*]8.5% PREVALENCE BY INDIRECT ELISA TEST			

Table 2: Prevalence of anti-DENV-2 neutralizing antibodies by Plaque Reduction Neutralization Tests (PRNT) by health facilities, Western Kenya, 2010 (n=422)			
Health Facility, n (%)	**Positive, n (%)**	**Negative, n (%)**	***P*-value**
Alupe Sub-district Hospital 124 (100)	1 (0.8)	123 (99.2)	0.033[a]
KEMRI/CIPDCR Alupe Clinic 176 (100)	0 (0)	176 (100)	
Anderson Medical Centre 122 (100)	4 (3.3)	118 (96.7)	
Total sample size 422 (100)	5 (1.2)[**]	417 (98.8)	
Data are presented as n (%). [a]=χ^2 test. [**]1.2% prevalence by neutralizing antibodies that neutralized with more than 90% Plaque reduction rate			

Table 3: Distribution of febrile out-patients with anti-DENV-2 antibodies based on indirect ELISA tests by Health Facility and as stratified according to gender and age, western Kenya, 2010									
Health Facility	**KAC n=176 (42%)**			**ADH n=124 (29%)**			**AMC n=122 (29%)**		
	Positive, n (%)	**Negative, n (%)**	**P-value**	**Positive, n (%)**	**Negative, n (%)**	**P-value**	**Positive, n (%)**	**Negative, n (%)**	**P-value**
Gender									
Female	6 (6.2)	91 (93.8)	0.712[a]	6 (9.2)	59 (90.8)	0.304[a]	7 (9)	71 (91)	0.369[a]
Male	6 (8)	73 (92)		9 (15.3)	50 (84.7)		2 (4.5)	42 (95.5)	
Age (yrs.)									
5-10	1 (1)	115 (99)	<0.0001[a]	0 (0)	77 (100)	<0.0001[a]	0 (0)	10 (100)	0.408[a]
11-20	6 (16.2)	31 (83.8)		4 (11.8)	30 (88.3)		0 (0)	13 (100)	
21-30	1 (1)	6 (99)		6 (100)	0 (0)		3 (5.8)	49 (94.2)	
31-40	2 (28.6)	5 (71.4)		3 (100)	0 (0)		3 (11.5)	23 (88.5)	
41-50	0 (0)	3 (100)		1 (100)	0 (0)		2 (20)	8 (80)	
51+	2 (33.3)	4 (66.7)		1 (33.3)	2 (66.7)		1 (1)	10 (99)	
Data are presented as n (%). [a]=χ^2 test.									

Table 4: Factors associated with anti-DENV-2 seropositivity among febrile out-patients visiting selected health facilities in Western Kenya, 2010

Factor	Positive, n (%)	Negative, n (%)	P-value	OR (95% CI)	P-value
Health Facility					
KAC	12 (6.8)	164 (93.2)	0.120[b]	0.53 (0.24-1.18)	0.236[a]
ADH	15 (12.1)	109 (87.9)	Ref	Ref	
AMC	9 (7.4)	113 (92.6)	0.217[b]	0.579 (0.24-1.38)	
Age (yrs)					
5-10	1 (0.5)	202 (99.5)	Ref	Ref	<0.0001[a]
11-20	10 (12)	74 (88)	0.002[b]	27.30 (3.44-216.93)	
21-30	10 (15.4)	55 (84.6)	0.001[b]	36.73 (4.60-293.14)	
31-40	8 (22)	28 (78)	<0.0001[b]	57.71 (6.96-478.95)	
41-50	3 (21.4)	11 (78.6)	0.001[b]	55.09 (5.29-573.70)	
51+	4 (20)	16 (80)	0.001[b]	50.5 (5.33-478.96)	
Gender					
Female	19 (8)	221 (92)	0.604[b]	0.834 (0.421-1.655)	0.604[a]
Male	17 (9)	165 (91)	Ref	Ref	
Education					
Formal education	7 (6.4)	103 (93.6)	Ref	Ref	0.344[a]
No education	29 (9.3)	283 (90.7)	0.347[b]	1.51 (0.64-3.55)	
Type of House					
Permanent	14 (12.2)	101 (87.8)	Ref	Ref	0.101[a]
Semi-permanent	22 (7.2)	285 (92.8)	0.105[b]	0.56 (0.27-1.13)	
Walls with eaves/openings					
Yes	7 (10.9)	57 (89.1)	0.454[a]	1.39 (0.58-3.33)	0.454[a]
No	29 (8)	329 (92)	Ref	Ref	
Dumping site with cans, tins, broken pots, old tyres near the house					
Yes	15 (8.7)	158 (91.3)	0.932[b]	1.03 (0.52-2.06)	0.932[a]
No	21 (8.4)	228 (91.6)	Ref	Ref	
Yellow fever vaccinationin the past ten years					
Yes	0 (0)	6 (100)	∞	∞	0.451[a]
No	36 (8.6)	380 (91.4)	Ref	Ref	

Data are presented as n (%). [a]=χ^2 test. [b]=P-value calculated using logistic regression analysis

Table 5: Clinical characteristics of febrile out-patients with anti-DENV-2 antibodies, Western Kenya, 2010				
Clinical Characteristic	**Positive, n (%)**	**Negative, n (%)**	**P-value**	**OR (95% CI)**
Headache				
Yes	**27 (13)**	**181 (87)**	**0.002**	**3.40 (1.56-7.42)**
No	**9 (4)**	**205 (96)**	**Ref**	**Ref**
Retro-orbital pain				
Yes	**7 (20)**	**28 (80)**	**0.015**	**3.09 (1.24-7.67)**
No	**29 (8)**	**358 (92)**	**Ref**	**Ref**
Muscle ache				
Yes	**17 (15)**	**99 (85)**	**0.007**	**2.59 (1.30-5.19)**
No	**19 (6)**	**287 (94)**	**Ref**	**Ref**
Joint pain				
Yes	**14 (19.2)**	**59 (80.8)**	**0.001**	**3.53 (1.71-7.28)**
No	**22 (6)**	**327 (94)**	**Ref**	**Ref**
Vomiting				
Yes	**0 (0)**	**9 (100)**	**∞**	**∞**
No	**36 (9)**	**377 (91)**	**Ref**	**Ref**
Rash				
Yes	**7 (5)**	**146 (95)**	**0.03**	**0.40 (0.17-0.93)**
No	**29 (11)**	**240 (89)**	**Ref**	**Ref**
Abdominal pain				
Yes	**4 (44)**	**5 (56)**	**0.001**	**9.53 (2.44-37.24)**
No	**32 (8)**	**381 (92)**	**Ref**	**Ref**
Data are presented as n (%).				