Seroprevalence of Dengue Virus among Febrile Patients Visiting Selected Hospitals in the Western Region of Kenya, 2010/2011

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SUMMARY

Background: **Dengue fever (DF) and Dengue Hemorrhagic fever's (DHF) lack of an effective vaccine and specific treatment promotes the diseases' significance as a public health problem leading to increased morbidity and mortality. The disease is caused by any four closely-related, but antigenically distinct, Dengue virus (DENV) serotypes; DENV-1, DENV-2, DENV-3, and DENV-4 transmitted through mosquito vectors, *Aedes mosquitoes*. Currently, there is scanty information on its incidence and prevalence in populations naturally-exposed to mosquito-borne diseases in western Kenya.**

Methods: **This study was therefore designed to determine the sero-prevalence of DF in patients (n=422, aged>5 years) presenting with fever at three selected health facilities in Kenya; Anderson Medical Centre (in Trans-Nzoia District in Rift Valley Province), and KEMRI/CIPDCR Alupe Clinic and Alupe Sub-district Hospital (in Teso-south District of Western Province). Furthermore, the socio-demographic characteristics associated with potential risk on sero-prevalence of dengue virus were evaluated. Using serum, indirect ELISA was performed as the screening test with Plaque Reduction Neutralization Test (PRNT) as the confirmatory test, while sociodemographic characteristics were evaluated using structured questionnaires. Chi-square tests were used to test for proportionality.**

Results: **Overall, a low sero-prevalence of 1.2% (5/422) was recorded in the two regions. Among the main significant symptoms of classical DF were retro-orbital pain (OR; 7.75, 95% CI; 1.25-48.07, *P*=0.013), muscle ache (OR; 10.89, 95% CI; 1.20-78.50, *P*=0.016) and joint pain (OR; 53.47, 95% CI; 1.22-45.32, *P*=0.009). In addition, walls with cracks (OR; 8.75, 95% CI; 1.43-2.389, *P*<0.001), place of storage of water vessel (OR; 3.20, 95% CI; 2.78-68.10, *P*=0.014), burning of charcoal (OR; 0.06, 95% CI; 0.01-0.38,**

$P<0.001$) and farming (OR; 8.83, 95% CI; 1.97-79.78, $P<0.001$) were significantly associated with DENV-2 sero-positivity. The current study identifies additional factors that may predispose to DF in populations naturally-exposed to mosquito-borne diseases.

<u>**Conclusion:**</u> **The overall seroprevalence was low but non-zero implying that dengue is not a main cause of febrile illness in these study regions, but it may be a potential hazard to public health.**

Key words: sero-prevalence, dengue virus, health facilities, Kenya

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Introduction

Dengue is a mosquito-borne viral infection that in recent decades has become a major international public health concern [1]. Dengue is found in tropical and sub-tropical regions around the world, predominantly in urban and semi-urban areas [1]. For the last 50 years throughout tropical and sub-tropical regions around the world Dengue fever epidemics has reoccurred together with an emergence of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), [2, 3]. The global renaissance of dengue has been implicated with several factors, for instance: - failure to control the Aedes populations, increased airplane travel to dengue endemic areas, uncontrolled urbanization, and an unprecedented population growth [3, 4]. The worldwide incidence is estimated to be 50 to 100 million cases of dengue fever (DF) and several hundred thousand cases of dengue hemorrhagic fever (DHF) per year [1]. DHF is more serious and the fatality rate is about 5% [1].

Arbovirus epidemics have sporadically occurred across regions of Kenya, but limited efforts have been put on the surveillance during these epidemics. Dengue virus serotype 2 was initially isolated from patients along the coast of Kenya in 1982 [5], and later identified in samples from a serology survey in subsequent years suggesting a more extensive circulation of the virus than is currently documented [6]. A study conducted in Kenya between the years 2000-2004, on the serologic evidence of arboviral infections among humans revealed DENV-2 to be the most common dengue serotype in Kenya, followed by DENV-1, DENV-3 and then DENV-4 [7]. As such, the current study focused on DENV-2 since previous observations have demonstrated that it is the most common circulating serotype in Kenya.

However, it is critical to point out that cross-reactivity among flaviviruses prevents conclusive determinants of which dengue serotypes are circulating in Kenya. In addition, information from previous studies has demonstrated that DENV-2 is endemic to Kenya and Somalia, with traces of DENV-1 and DENV-3 being found in nearby Somalia [6] . Given the inter-continental connections routes between Kenya and other countries where dengue is perceived to be endemic, the circulation of multiple DENV serotypes is not unexpected [7].

Despite these observations [7], no documentation on outbreaks has been reported in Rift Valley and Western Provinces of Kenya, two regions predominated with the mosquito vectors *A. Aegypti*, the documented causative agent for dengue and other flavivirus [6], [8].

Global documentation of the prevalence and sero-prevalence studies have been and are still being conducted on dengue worldwide [3] underscoring the health importance of dengue.

Most of these studies suggest that the global prevalence of dengue has grown dramatically in recent decade [9]. In Kenya, fewer studies have been conducted on dengue, for example, a sero-survey conducted during September 1987 for evidence of human arboviral infections in the Coast Province of Kenya [10] revealed a prevalence rate of 1.0% for dengue 2 viruses.

Additional studies conducted in western Kenya in Kisumu, found a prevalence of 1.1% for dengue 2 [11]. These studies demonstrate paucity of knowledge on the actual prevalence of dengue in Kenya, and whether or not it is endemic in Kenya.

In *P. falciparum* holoendemic transmission regions such as in western Kenya, studies have revealed that *A. Aegypti* are found in large populations [12]. However, little efforts have been made in the identification and transmission cycles of these vectors associated with dengue in such holoendemic regions. In addition, there has been limited surveillance and reports on severe forms of dengue since the disease is assumed to be of little health importance.

Furthermore, most DENV human infections are self-limiting and sub-clinical, and are thus, often misdiagnosed in Kenya [13].

Trans-Nzoia District of Rift-Valley Province and Teso-south District region of Western Province are areas that are naturally exposed to arthropod borne diseases. Previous unreported studies carried out in Kenya demonstrated high levels of alpha virus exposure, however, no formal studies have been performed in this region. In addition, despite the fact that the vectors associated with transmission of dengue exist in this region, no studies have been carried out to determine the sero-prevalence of dengue in febrile persons presenting in the two regions. Therefore, the current study used indirect enzyme-linked immunosorbent assay (ELISA) as a screening test and Plaque Reduction Neutralization Test (PRNT) as a confirmatory test, to determine the sero-prevalence of dengue in febrile patients visiting three selected health facilities in Western and Rift Valley Provinces, Kenya.

Demographic, economic, behavioral and social factors play key roles for effective communicable disease control and underpin successful public health programs [14], however, the role of these factors in predisposing to dengue virus transmission is still poorly understood since most studies performed in the context of dengue have been inconclusive and inconsistent [14]. For example, studies conducted in Asia, demonstrated that severe forms are mostly experienced in children under 15 years of age [15], [16], whereas in the Americas, the dengue syndromes are experienced in all age groups with most fatalities being experienced in children [3]. Some population-based studies have also shown dengue to be more severe and common in females than males [17]; [18] . Other socio-demographic characteristics such as education, occupation, marital status and place of habitat have also been assessed in some studies [19] but limited to Africa. Since no studies have been carried out in Africa to assess the risk factors associated with dengue, the current study used a basic questionnaire to obtain the individual level risk factors, household-level risk factors, socioeconomic activities, use of insect treated nets (ITNs), use of other vectors control-level risk

factors and yellow fever vaccination in the past ten years to identify the potential risk factors on sero-prevalence of DENV in two regions in Kenya.

The clinical picture of dengue fever in infants and young children is often associated with a non-specific febrile illness that can hardly be differentiated from other viral illnesses [3].

However, more severe cases of dengue fever are usually seen in older children and are characterized by a rapidly rising temperature (≥ 39°C) that lasts 5-6 days [20]. Hence, the current study recruited participants who were ≥5 years of age. The febrile period is usually accompanied by severe headache, retro-orbital pain, myalgia, arthralgia, nausea and vomiting [1], [3], [20]. Over half of the infected people report a rash during the febrile period that is initially macular or maculopapular and becomes diffusely erythematous [13], [21]. Minor haemorrhagic manifestations like petechae, epistaxis and gingival bleeding do occur, however, severe haemorrhage is unusual [13], [21]. Therefore, the current study also determined the clinical characteristics of people with dengue antibodies in the two study regions.

Methods

Study design: This was a descriptive study using prospective hospital-based surveillance for cases presenting with fever at the three selected health facilities; Alupe Sub-district Hospital,

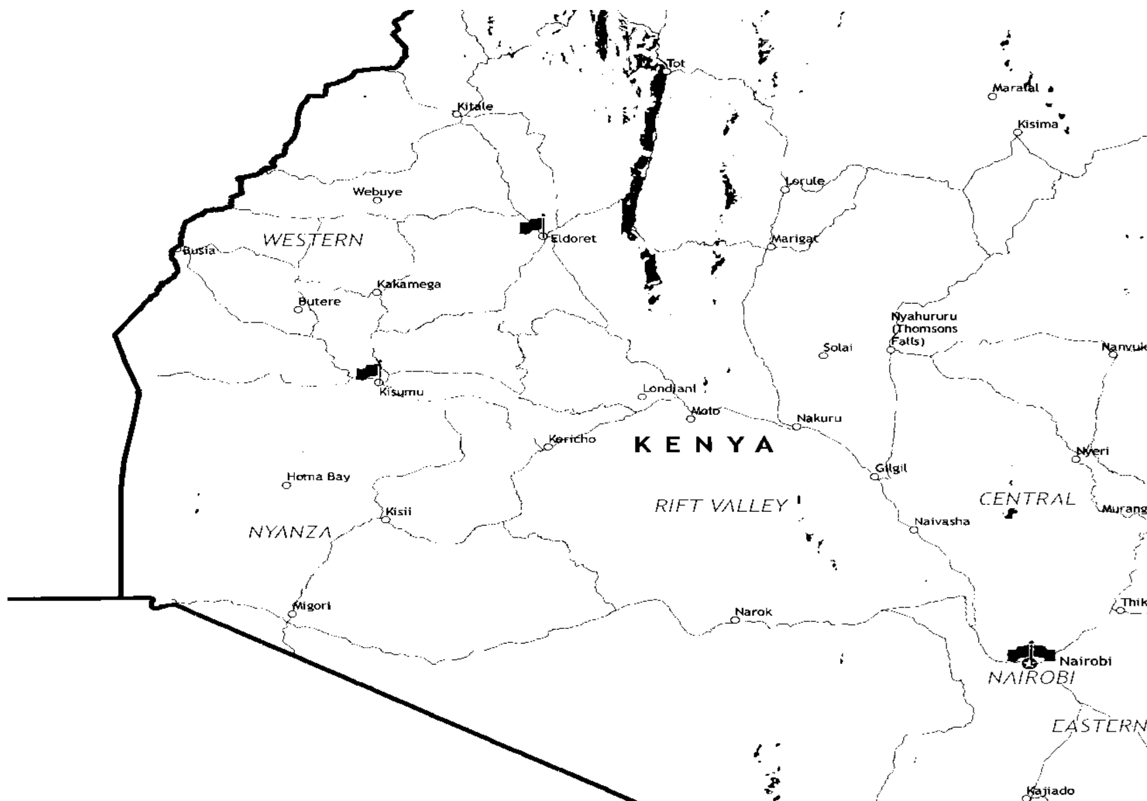
KEMRI/CIPDCR Alupe Clinic both of Teso-south District and Anderson Medical Clinic of Trans-Nzoia District, in western Kenya. The surveillance and laboratory testing was done to identify antibodies associated with DENV-2 infection and characterize the epidemiological and clinical characteristics of DENV-2 infection at participating hospitals.

Population

Selected Hospitals in Western Kenya: Participants were enrolled at three health facilities, serving different regions of Kenya: Alupe Sub-district Hospital and KEMRI/CIPDCR Alupe

Clinic both of Teso-south District serve a primarily rural area of western Kenya, and Anderson Medical Clinic of Trans-Nzoia District serves a wider area which includes Cherangani, Kwanza and Saboti Constituencies in Rift Valley Province, of Western Kenya. These three health facilities are at a close proximity to each other and lie along the border belt that connects Kenya to Uganda (Figure 1). The three health facilities serve both children and adults patients.

Figure 1. Map of Western Kenya (Source: USAID 2009) showing the study sites



Teso-South District: In Teso-South District of Western Kenya, two health facilities were involved; Alupe Sub-district Hospital (which is the primary health care facility) and KEMRI/CIPDCR Alupe Clinic (is the secondary heath care facility). Both health facilities are in Alupe Division of Teso-south District in Western Province. Geographically, they fall on the rural Western part of Kenya along the Border belt which borders Kenya and Uganda. The population area belongs predominantly to the Ateso ethnic group, many of whom practice subsistence farming. Alupe Sub-district Hospital is a level 3 and is the largest in the District and receives referrals from smaller facilities and other facilities from Kenya. KEMRI/CIPDCR Alupe Clinic is a level 2 health facility; it serves a study health facility for clinical studies and also provides health care services to the rural population of this region. Being a KEMRI health facility, it receives referrals from other health facilities in different regions of Western Kenya as well. All the laboratory clinical evaluations, virological and serological tests were performed at the KEMRI/CIPDCR. Medical care was provided to the study participants as per Ministry of Health (MoH) guidelines.

Trans-Nzoia District: In this District, only one health facility was involved, as it lies on the border belt of Kenya and Uganda; Anderson Medical Centre. It serves primarily the Mount Elgon region in Trans-Nzoia District. The hospital receives referrals from other facilities as it is well equipped both in workmanship and medical equipment. Trans-Nzoia District is an administrative District of Rift Valley Province, Kenya. The district has three constituencies: Cherangani, Kwanza and Saboti.

Patient Eligibility and Number of participants Persons ≥5 years of age who had fever ≥37.8°C presenting to one of the participating hospitals were eligible for the study, according to the inclusion criteria which involved willingness by the participants or the parents/guardians of minors to provide informed consent for their children to participate in the study. All persons who were unwilling to give consent and assent for participating in the study and those who were below 5 years were excluded from participation.

Based on a retrospective review of hospital treatment logs, it was expected that approximately half of the participants to be children ≤13 years of age; the remainder being older children and adults. The clinical picture of dengue fever in infants and young children is often associated with a non-specific febrile illness that can hardly be differentiated from other viral illnesses (3). However, more severe cases of dengue fever are usually seen in older children and are characterized by a rapidly rising temperature (≥ 39°C) that lasts 5-6 days (20). The prevalence of DENV-2 in the two study regions of Western Kenya is not known; therefore a P-value of 50% was used to yield a maximum sample size [22]. The sample size used in this study was dependent on the desired degree of precision and the anticipated prevalence; hence the formula n=Z2PQ/d2 was used to derive the desired sample size. Where *n* is the desired sample size, *P* is the expected prevalence in the target population with the characteristic being measured, *Q* is 1-P, *Z* is 1.96; standard error (95% confidence level of the standard deviation from the mean), *d* is the level of statistical significance (0.05).

Based on this formula, the estimated sample size was 384. An additional 10% was sampled to take care of missing or inconsistent data collections [23], providing a total sample size of 422. As such, a total sample size of 140 individuals was to be drawn from each facility. There was bias towards KEMRI/CIPDCR Clinic as most patients were always referred to this facility hence a larger proportion of the participants were recruited from KEMRI/CIPDCR ~ 176.

Sample collection and processing

Upon presentation to the two study hospitals, participants were directed to a trained study nurse, who recorded their socio-demographic information including gender, age in years, marital status, occupation, education and place of habitat, tympanic temperature using a standardized questionnaire. Data on housing conditions, yellow fever vaccination status, and use of bednets were also recorded. Study physicians performed a medical examination and recorded data systematically on the history of the illness and current symptoms, consisting of 20 variables including headache, retro-orbital pain, joint pain, vomiting, lower and upper respiratory symptoms, gastrointestinal symptoms, urinary tract symptoms, musculoskeletal pain, rashes and hemorrhagic manifestations. Data on any medical tests requested and treatments prescribed was recorded.

Blood samples were collected by venipuncture using aseptic technique by the study clinician or study nurse. At each venipuncture, about 5 ml of blood was collected from study participants. These specimens were collected in vacutainer serum tubes and centrifuged at 1500 rpm for 10 minutes. The tubes containing the serum were kept at -20ºC at the participating health facilities until collection. Once every month, the tubes containing the serum were transported in dry ice to KEMRI/CIPDCR laboratories where they were processed and tested as soon as

possible. The total time taken to transport samples from the field to KEMRI did not exceed 48 hours. In situations where an urgent diagnosis was necessary, for instance in a patient with suspected hemorrhagic fever, the serum sample was sent to KEMRI/CMR P3 labs immediately.

All laboratory procedures were performed under the same experimental conditions. Quality assurance was maintained at high standards for each test performed. Screening indirect ELISA tests were performed in 2 months while the PRNT confirmatory tests were performed in 4 months.

Laboratory procedures

Viruses and Cell lines: Purified Hawaiian strain of dengue virus [Dengue type II (DEN-2) (002ST) 2009; titre (1×10-5 PFU/ml)], obtained from Nagasaki University Institute of Tropical Medicine, was used in all serological tests. In addition, the 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 according to an in-house kit method as previously described (24) with few modifications to suit the local laboratory settings (25). The IgA, IgG, and IgM were measured for the determination of the sero-prevalence of dengue virus in febrile patients visiting the three selected health facilities. In order to make the test cells, the DENV-2 antigen was diluted 1:500 in 1× Phosphate Buffered Saline (PBS) and 100μl of the DENV-2 antigen solution added into a 96-well plate in rows A, B, E, F. For the internal control wells, 100μl of 1×PBS containing 3% Fetal Calf Serum (PBS-F) was added into each of the 96-well plates in rows C, D, G, H. These wells were used in the experiment to distinguish between clear backgrounds after substrate reaction. 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% Tween in 1×PBS (PBS-T) washing buffer, using an ELISA plate washer (Thermoscientific Well Washer, Ratastie 2, Ratastie 2, F1-01620 Vantaa, Finland). A 100μl of PBS-F solution was then added as blocking solution into every well of the plate, and the plates covered with parafilm and incubated for 1 hour at room temperature. The test sera were then diluted 1:1000 with PBS-F and kept on ice. After the first 1-hour incubation period, the plates were gently tapped on blotting papers to discard the blocking solution completely, then 100μl of the diluted test serum was added in duplicates into virus antigen and PBS-F pre-coated wells. The plates were then incubated for another 1 hour at 37°C in an incubator. Secondary antibody; antihuman IgG+IgM+IgA was diluted 1:5000 in PBS-F and kept on ice. After the second 1-hour incubation, the plates were washed four times with PBS-T. Then 100μl of the diluted secondary antibody solution was added into each well. The plates were then incubated for another 1 hour at 37°C in an incubator. The substrate solution [O-phenylenediamine dihydrochloride (OPD), SigmaFast tablets] was diluted in distilled water (1 tablet each of the buffer and OPD into 20ml of distilled water and allowed to dissolve completely in the dark). After the third 1-hour incubation above, the plates were washed 4 times and blotted to dry. A 100μl of the OPD solution was then added into each well, preceded by incubation for 15 minutes at room temperature in the dark. Finally, a 100μl of 1N sulphuric acid solution was added into each well to stop the enzyme-substrate reaction.

The plates were then read on an ELISA plate reader (Thermoscientific Multiskan ex. Version, Tokyo, Japan) at a wavelength of A492nm (Ascent software version 2.6-Deafulte.See, Shangai, China). 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-tested using the Plaque Reduction Neutralization Test (PRNT) to confirm the diagnosis according to standard methods (26).

Plaque Reduction Neutralization Test (PRNT)

Cell Culture and Resuscitation of frozen Vero cell lines: The Vero cells lines were revived and maintained at BSL-3 laboratory, in culture media. Growth Medium (1×EMEM, 1.1g/L NaHCO3, 10%FCS, L-glut/P/S) and Maintenance Medium (1xEMEM, 1.1g/L NaHCO3, 2%FCS, L glut/P/S) were prepared. The T175 NUNC cell culture flasks were labeled according to cell line, passage number and date of preparation. The vials containing cells (stored in liquid nitrogen) was collected and placed in water bath at 37°C. The cells were allowed to thaw for 1-2 minutes until a small amount of ice remained in the vial. The vial was then transferred to a safety cabinet where it was sterilized and the cap loosened. Cells were slowly pipetted drop-wise in centrifuge tubes containing 10ml of pre-warmed growth medium to dilute the Dimethyl sulphoxide (DMSO). The cells were span at 1500rpm for 5 minutes at room temperature to separate DMSO. The supernatant was removed and the cells re-suspended in 10ml of pre-warmed growth medium. The cells were then cultivated in T175 culture flasks containing 50ml of pre-warmed growth medium, after which they were monitored under inverted microscope to ensure even distribution prior to incubating at 37°C and 5% CO2 for 24 hours to allow cell growth.

Sub-culture of adherent Vero cell lines: Cultures were viewed on an inverted microscope to assess degree of confluence and to monitor that bacterial and fungal contaminations do not occur. Supernatants harvested from the growing cells was removed and placed in centrifuge tubes. The cell monolayer formed was washed twice with approximately 10ml of 1×PBS. Approximately 7ml of trypsin/EDTA solution was added onto the washed cell monolayer, ensuring that the flask was gently rotated for the trypsin to cover the monolayer evenly. The flask was then placed in an incubator at 37°C and 5% CO2 for 4 minutes to allow for trypsin incorporation by the cells. The cells were then examined on an inverted microscope to ensure that all the cells are detached and floating. The side of the flask was then gently tapped to detach monolayer completely and the cells re-suspended with the rest of the spent growth medium to inactivate trypsin. Cells were then transferred into centrifuge tubes and spun at 1600rpm for 5 minutes at room temperature. The supernatant was discarded and the cells were resuspended in 5ml of fresh growth medium, and approximately 100μl of cell mixture placed in a cell count chamber for counting. Approximately 1×106cells/ml to 2×105cells/ml were transferred to a newly-labeled flask containing pre-warmed medium and observed under inverted microscope to ensure even distribution and then placed in an incubator at 37°C and 5% CO2 for ~24-48 hours for them to grow further. These sub-cultures were performed for about 3-4 days to allow the cells to stabilize enough for PRNT assays.

The Plaque Reduction Neutralization Test (PRNT): In order to perform PRNT test, 2μl of maintenance

medium (MM) was added into each of the 6 well plates (Nunc), followed by addition of Vero cells at a concentration of 1.2×105 cells/ml to allow seeding in each of the wells. Cells were cultured in MM for 1-2 days at 37°C and 5% CO_2 and observed on an inverted microscope to assess for 80% confluence. All test sera were heat-inactivated at 56°C in a water bath for 30 minutes. Two-fold serial dilution for test sera, positive and negative controls beginning with 1:10 (final 1:20) were prepared in MM and added to equal volume of virus standards diluted to yield 200PFU/0.1ml. Virus-serum dilution mixtures of 0.5ml total volume were incubated for 60 minutes at 37°C. Six-well plates containing 80% confluent Vero cell monolayer were labeled in readiness for inoculation. One milliliter of the culture medium was aspirated from each well and enough residual fluid of ~1ml left to prevent the monolayer from becoming dry. One hundred microlitres of each of the virus serum mixture was added and inoculated into each of the two wells of the properly prepared 6-well plates. The virus-serum mixture was then evenly distributed by rocking the plates back and forth and sideways. The virus-serum mixtures together with the cells were incubated for 90 minutes at 37°C and 5% CO_2 for virus absorption to take place. After the incubation period, 4ml of overlay medium was added into each well and plates were incubated at 37°C and 5% CO_2 for 5 days (DENV-2) to allow plaques to develop. On day 5, the overlay medium was aspirated completely using a plastic sero-pipette attached to a pipette aid. The aspirated overlay medium was discarded in 2% Sodium Dodecyl Sulphate (SDS) solution and later autoclaved. One milliliter of 10% formaldehyde in 1×PBS (1/10 diluted formalin with 1×PBS) was added over the cells and the plates incubated for 1 hour at room temperature in a safety cabinet with ultraviolet light to fix the plates. Formaldehyde was completely aspirated and properly discarded. The surface of the plates was wiped to sterilize before taking them out of the safety cabinet for washing. Plates were washed gently with running water and absorbed on blotting paper. Half milliliter of 1% crystal violet solution in water-staining solution was added to each well. The plates were placed at room temperature for 10 minutes for the cells to absorb the dye. The excess dye was discarded and the plates were washed gently with tap water, followed by absorption on blotting paper and a final air-drying at room temperature. Plaques were counted for each set of duplicate wells and the percentage reduction calculated by comparing against the positive control virus well (100% plaque forming units). More than 90% plaque reduction was regarded as positive.

Data Management and Analysis: Data obtained from the questionnaires were entered and managed in Excel spreadsheets. Uniquely-coded samples were noted as either DENV negative or DENV-positive. The results were categorized as per the DENV antibody status. All data were imported in SPSS software v19.0 prior to analyses. Data obtained from the PRNTs were also categorized as PRNT-positive or PRNT-negative. Proportionality tests were used to determine sero-prevalence of DENV-2. A logistic regression analysis was carried out to identify socio-demographic characteristics, clinical characteristics and risk factors associated with sero-prevalence. Statistical significance was determined at $P \leq 0.05$.

Results

The sero-prevalence of DENV-2 among study participants: A total of 422 serum samples were available for DENV serotyping. The DENV serotype

test detects the presence of IgM, IgG and IgA DENV antibodies. The sera were first subjected to indirect ELISA screening test as per WHO recommendations [26]. Out of the 422, 14.7% (n=62) were positive for DENV antibodies. Screening test positives per health facility was as follows: Alupe Sub district Hospital, 24.2% (30/124), KEMRI/CIPDCR Alupe Clinic, 13% (23/176) and Anderson Medical Clinic, 7.4% (9/122) (Table 1).

A chi-square test demonstrated that proportions of those serologically positive across the three different sites was statistically significant (P=0.0007) (Table 1). These differences were attributed to the highest proportions of those serologically positive in Alupe Sub-district Hospital (24.2%), with the least being observed from Anderson Medical Centre (7.4%).

Following the establishment of the sero-status, serologically-positive samples (n=62) were transported in dry ice to Kenya Medical Research Institute/Centre for Medical Research (KEMRI/CMR) P3 level laboratory, where they were further tested by PRNT for confirmation. Results revealed that only 1.2% (n=5) were positive by PRNT confirmatory test (Table 2). In the current study, sero-positivity was based on the presence of neutralizing antibodies specific for DENV-2. A chi-square test revealed that the distribution of those testing positive with the confirmatory test was significantly higher in those individuals that tested positive with the serological test (P=0.020, Table 2).

Table 2: Sero-prevalence of DENV Sero-positivity based on PRNT

Test, n (%)	Confirmatory +ve n (%)	Confirmatory –ve n (%)	P-value
Screening serologically +ve (62)	5(8.1)	57(91.9)	**0.020** [a]
Screening serologically –ve (362)	0(0)	360(100)	
Total sample size 422 (100)	**5(1.2)** *	**417(98.8)**	

Data are presented as n (%).[a]=χ^2 test. *Only 5 individuals tested positive with PRNT confirmatory test. +ve=positive, -ve=negative

Socio-demographic and Clinical characteristics of the study participants

Socio-demographic characteristics: The socio-demographic characteristics of the study participants who presented with fever at the three selected facilities and who tested positive with the serological tests are shown in Table 3. A total of 261 children between ages 5-12 years, 14 older children between ages 13-17 years and 147 adults, aged 18 years and above, were included in the study. Out of these, 182 (43.13%) were males while 240 (56.87%) were females. When stratified by gender, the DENV sero-prevalence was 1.7% (4/240) in female and 0.5% (1/182) in male patients, however, the sero-prevalence in males *vs.* females was comparable (P=0.294, Table 3).

Even though the age for the participants ranged from <20 years to > 60 years, those confirmed to be having dengue infections were between the ages of 21 to 60 years. Age was a significant factor in the distribution of sero-prevalence in the studied population (P=0.001, Table 3).

Of the total study population, 133 (31.5%) were married, 1 (0.2%) was widowed, 13 (3.1%) were single or divorced or separated and the remaining 275 (65.2%) did not provide marital status (NB: Out of this figure 261 were children). All of the 5 individuals confirmed to be sero-positive by PRNT were married. None of the school-going persons were sero-positive for dengue infection. Employed persons were 33 (7.82%), self-employed 47 (11.14%) and unemployed were 73(17.3%). Of the sero-positive individuals, only 1(3%) was employed, 3(6.4%) were self-employed, while 1(1.4%) was unemployed. The prevalence of those seropositive was significantly higher in the self-employed group (6.4%) relative to any other occupation (*P*=0.002, Table 3). Finally, the level of education and place of residence did not alter the proportions of those sero-prevalent in this population (*P*=0.475 and *P*=0.337, respectively, Table 3).

Table 3: Sero-prevalence of DENV according to socio-demographic factors

Socio-demographic factor	Positive, n (%)	Negative, n (%)	*P*-value	OR (95% CI)	*P*-value
Gender					
Females	4 (1.7)	236 (98.3)	0.294[a]	3.06 (0.34-27.68)	NS[b]
Males	1 (0.5)	181 (99.5)		1.00	
Age in years (range)					
<20 (n=287)	0 (0.0)	287 (100.0)	**0.001**[a]	∞	NS[b]
21-30 (n=65)	1 (2.0)	64 (98.0)		1.000	
31-40 (n=36)	2 (6.0)	34 (97.0)		3.76 (0.32-42.03)	
41-50 (n=14)	1 (7.0)	13 (93.0)		0.20 (0.01-3.46)	
51-60 (n=10)	1 (10.0)	9 (90.0)		0.14 (0.08-2.45)	
>60 (n=10)	0 (0.0)	10 (100.0)		∞	
Marital status					
Married	5 (3.8)	128 (96.2)	**0.012**[a]	1.000	NS[b]
Single/divorced/separated	0 (0.0)	13 (100.0)		∞	
Widowed	0 (0.0)	1 (100.0)		∞	
No marital status given	0 (0.0)	275 (100.0)		∞	
Occupation					
Attending school	0 (0.0)	269 (100.0)	**0.002**[a]	∞	NS[b]
Employed	1 (3.0)	32 (97.0)		2.25 (0.13-37.11)	
Self-employed	3 (6.4)	44 (93.6)		4.90 (0.49-48.67)	
Unemployed	1 (1.4)	72 (98.6)		1.000	
Education					
Educated	2 (1.8)	108 (98.2)	0.475[a]	1.000	NS[b]
Not educated	3 (1.0)	309 (99.0)		1.908 (0.315-11.569)	

Place of habitation					
Town	0 (0.0)	65 (100.0)	0.337[a]	∞	NS[b]
Rural	5 (1.4)	352 (98.6)		1.000	

Data are presented as *n* (%).[a]=χ^2 test. [b]= logistic regression analyses. ∞=infinity. NS=not significant.

Clinical characteristics and DENV seropositivity: Fever was the main inclusion criteria into the current study i.e. all the study participants had to present with fever to be included in the study. Table 4 presents other clinical characteristics of the study participants at the time of enrolment. When the clinical characteristics were evaluated against sero-prevalence in the population, retro-orbital pain (*P*=0.010), muscle ache (*P*=0.008), and joint pain (*P*=0.011) significantly altered distribution of sero-prevalence in the current study. However, headache (*P*=0.167), vomiting (*P*=0.740), presence of rashes (*P*=0.447), bleeding diathesis (*P*=0.755) and abdominal pain (*P*=0.740) did not alter the distribution of sero-prevalence in the population (Table 4). Further logistic regression analyses demonstrated that retro-orbital pain (OR; 7.75, 95% CI; 1.25-48.07, *P*=0.013), muscle ache (OR; 10.89, 95% CI; 1.20-78.50, *P*=0.016) and joint pain (OR; 53.47, 95% CI; 1.22-45.32, *P*=0.009) were significantly associated with DENV seropositivity (Table 4). These results demonstrate that retro-orbital pain, muscle ache, and vomiting may be clinically associated with sero-prevalence in the present study.

Table 4. Sero-prevalence of DENV according to clinical characteristics

Clinical characteristic	**Positive** n (%)	**Negative** n (%)	***P*-value**	**OR (95%CI)**	***P*-value**
Fever					
Yes	5 (1.2)	417 (98.8)	§	§	§
No	0 (0.0)	0 (0.0)			
Headache					
Yes	4 (1.9)	204 (98.1)	0.167[a]	4.17 (0.46-37.68)	**NS[b]**
No	1 (0.5)	213 (99.5)		1.00	
Retro-orbital pain					
Yes	2 (5.7)	33 (94.3)	**0.010[a]**	1.00	**0.013[a]**
No	3 (0.8)	384(99.2)		7.75 (1.25-48.07)	
Muscle ache					
Yes	4 (3.5)	112 (96.5)	**0.008[a]**	10.89 (1.20-78.50)	**0.016[a]**
No	1 (0.3)	305 (99.7)		1.00	
Joint pain					
Yes	3 (4.1)	70 (95.9)	**0.011[a]**	7.43 (1.22-45.32)	**0.009[a]**
No	2 (0.6)	347 (99.4)		1.00	
Vomiting					
Yes	0 (0.0)	9 (100.0)	0.740[a]	1.00	**NS[b]**

No	5 (1.2)	408 (98.8)		∞	
Rash					
Yes	1 (0.7)	152 (99.3)	0.447[a]	0.43 (0.04–3.93)	**NS**[b]
No	4 (1.5)	409 (98.5)		1.00	
Bleeding diathesis					
Yes	0 (0.0)	8 (100.0)	0.755[a]	1.00	**NS**[b]
No	5 (1.2)	409 (98.8)		∞	
Abdominal pain					
Yes	0 (0.0)	9 (100.0)	0.740[a]	1.00	**NS**[b]
No	5 (1.2)	408 (98.8)		∞	

§no statistics are computed because fever is a constant. [a]=χ^2 test. [b]= logistic regression analyses. ∞=OR could not be computed due to small sample sizes. NS=not significant.

Associations between household factors and DENV seropositivity: The potential risk factors associated with dengue virus infection in patients with fever visiting the three selected health facilities in Trans-Nzoia and Teso-south Districts: Based on previous studies [1], household level (housing conditions and surrounding), socio-economic, ITN use, other vector control activities and Yellow Fever vaccination were the main factors considered in the current study. Results revealed that walls with cracks (P=0.005) and place of storage of water vessel (P=0.002) in the households significantly altered DENV sero-positivity. Additional logistic regression analyses demonstrated that walls with cracks (OR; 8.75, 95% CI; 1.43-2.389, P<0.001), and place of storage of water vessel (OR; 3.20, 95% CI; 2.78- 68.10, P=0.014) were significantly associated with DENV-2 sero-positivity (Table 5).

Table 5: Household factors associated with DENV infection

Risk factor	Positive n (%)	Negative n (%)	*P*-value	OR (95%CI)	*P*-value
HOUSE HOLD-LEVEL RISK FACTORS					
Type of house walls					
Bricks/stones/cement	1 (0.9)	114 (99)	0.922[a]	1.00	
Wood	0 (0)	2 (100)		∞	
Mud	4 (1.3)	301 (98.7)		1.51 (0.16-13.69)	NS[b]
Walls with cracks					
Yes	3 (4.7)	61 (95.3)	**0.005**[a]	8.75 (1.43-53.47)	**<0.001**[b]
No	2 (0.6)	356 (99.4)		1.00	
Dumping site near house					
Yes	1 (0.6)	172 (99.4)	0.337[a]	0.35 (0.03-3.21)	NS[b]
No	4 (1.6)	245 (98.4)		1.00	

Water storage vessel					
Covered	5 (1.2)	410 (98.8)	0.770[a]	1.00	
Not covered	0 (0)	7 (100)		∞	
Place of storage of the vessel					
Inside house	1 (0.3)	320 (99.7)	**0.002[a]**	1.00	
Outside house	2 (9.1)	20 (90.9)		3.20 (2.78–68.10)	**0.014[b]**
Both	2 (2.5)	77 (97.5)		8.31 (0.74–92.85)	NS[b]
Water bodies surrounding the house					
Yes	1 (0.5)	183 (99.5)	0.284[a]	0.18 (0.02–1.65)	NS[b]
No	4 (1.7)	234 (98.3)		1.00	
Vegetation surrounding the house					
Yes	4 (2.0)	198 (98.0)	0.148[a]	4.42 (0.49–39.91)	NS[b]
No	1 (0.5)	219 (99.5)		1.00	

Data are presented as *n* (%). [a]=χ^2 test. [b]= logistic regression analyses. ∞=OR could not be computed due to small sample sizes. NS=not significant.

Associations between socio-economic factors and DENV seropositivity: Results on the socio-economic activities showed that burning of charcoal (P<0.0001) and farming (P=0.020) were significantly associated with DENV sero-positivity. However, the use of ITN (P=0.478), routine use of other vector control activities (P=0.767), types of vector control activities used (P=0.996) and Yellow Fever vaccination in the past 10 years (P=0.787) were not associated with DENV sero-positivity in this study population (Table 6).

Table 6: Socio-economic factors associated with DENV infection

Risk factor	Positive; n (%)	Negative; n (%)	*P*-value	OR (95% CI)	*P*-value
SOCIO-ECONOMIC ACTIVITIES-LEVEL RISK FACTORS					
Herd cattle					
Yes	1 (1.5)	64 (98.5)	0.775[a]	1.00	NS[b]
No	4 (1.1)	353 (98.9)		1.37 (0.15–12.53)	
Burn charcoal					
Yes	2 (11.1)	16 (88.9)	<0.0001[a]	0.06 (0.01–0.38)	**<0.001[a]**
No	3 (0.7)	401 (99.3)		1.00	
Fetch water					
Yes	1 (1.4)	68 (98.6)	0.824[a]	0.77 (0.08–7.08)	NS[b]

No	4 (1.1)	349 (98.9)		1.00	
Play near forest					
Yes	0 (0)	84 (100)	0.528[a]	1.00	NS[b]
No	5 (1.5)	333 (98.5)		3.52 (0.38-32.01)	
Farming					
Yes	4 (3.0)	130 (97.0)	0.020[a]	8.83 (1.97-79.78)	**0.024[a]**
No	1 (0.3)	287 (99.7)		1.00	
ITN use-level risk factor					
Yes	4 (1)	388 (99)	0.478[a]	3.59 (0.38-33.27)	NS[b]
No	1 (3.6)	27 (96.4)		1.00	
No response	0 (0)	2 (100)		0.34 (0.01-88.41)	
Use of other vector control-level risk factors					
Never	3 (1.1)	262 (98.9)	0.767[a]	1.39 (0.23-8.46)	NS[b]
Daily	2 (1.6)	125 (98.4)		1.00	
Occasionally	0 (0)	30 (100)		∞	
Type of vector control activities					
Herbs	0 (0)	6 (100)	0.996[a]	∞	NS[b]
Insect repellants	0 (0)	14 (100)		∞	
Insecticide sprays	1 (1.4)	70 (98.6)		0.71 (0.07-6.99)	
Mosquito coils	1 (1.6)	63 (98.4)		0.79 (0.08-7.76)	
No control	3 (1.1)	264 (98.9)		1.00	
Yellow fever vaccination in the past 10 years					
Yes	0 (0)	6 (0)	0.787[a]	∞	NS[b]
No	5 (1.2)	411 (98.8)		1.00	

Data are presented as *n* (%).[a]=χ^2 test. [b]= logistic regression analyses. ∞=OR could not be computed due to small sample sizes. NS=Not significant.

Consistent with these observations, a further logistic regression analyses demonstrated that burning of charcoal (OR; 0.06, 95% CI; 0.01-0.38, $P<0.001$) and farming (OR; 8.83, 95% CI; 1.97-79.78, $P<0.001$) were significantly associated with DENV-2 sero-positivity (Table 6).

Discussion

Sero-prevalence of DENV in patients with fever visiting the study sites

A sero-prevalence rate of 1.2% was obtained among the patients with febrile illness who visited the study health facilities. This suggests low prevalence of dengue in this population from Rift Valley and Western Provinces of Kenya. These findings are in agreement with other investigations of DENV conducted in Kenya [10], [11]. In these two studies, a seroprevalence rate of 1.0% (19/1624) was achieved [10] in eastern coast of Kenya, however, the serum was collected on healthy participants in the region. In the second survey [11], a seroprevalence rate of 1.1% (4/354) was obtained from healthy afebrile children in Kisumu of western Kenya. Despite the fact that study individuals had fever, a low sero-prevalence was recorded, indicating that DENV was not the main cause of febrile illness in this region. In yet another study carried out in Kilifi and Malindi districts in coastal Kenya [5] on febrile patients, higher prevalence rate of 20% (3/15) was obtained. However, these observations may not reflect on the true prevalence since the authors regarded their results as inconclusive due to cross-reactivity among flaviviruses [5], hence this made it difficult for them to determine the true sero-prevalence rate at that point in time.

The current study only evaluated the presence of circulating DENV-2 and not the other three serotypes (DENV-1, DENV-3 and DENV-4). The focus of the current study was on DENV-2 since there was fear of an outbreak of this particular serotype following previous similar epidemic in the coastal region of Kenya in 1982 [5], [10], [11]. Moreover, a study carried out in Kenya on the serological evidence of arboviral infections among humans in Kenya [7], revealed that the evidence of DENV-2 was most common, followed by evidence of DENV-1, then DENV-3 and DENV-4. Furthermore, DENV-2 is now believed to be endemic in Kenya and Somalia [6].

Socio-demographic and clinical characteristics of patients with DENV

The socio-demographic characteristics of febrile patients with dengue antibodies, visiting the three selected health facilities were also determined in the current study. Even though females had a higher sero-positivity (1.7%; 4/240) compared to males (0.5%; 1/182), these were not statistically different. These findings are comparable to a survey carried out in Nigeria [18] on the serological evidence of acute dengue virus infection among febrile patients attending Plateau State Specialist Hospital Jas, Nigeria, in which dengue infection was shown to be comparable between females and males. However, the current findings were inconsistent with previous observation in Mexico in which an increased risk was observed among women than in males [17]. A couple of other studies carried out in Rural Amazonia [27] demonstrated that the risk of past DENV infection at baseline and that of subsequent infections during the follow-up remained significantly higher among men, after controlling for migration patterns and travel history [27]. Furthermore, hospital-based studies in Asia have suggested that infections are more frequent in men [14]. Other studies carried out in India [9, [28-30] also suggest a higher prevalence of dengue infection among males than females. However, the biological bases for male-female differences in DENV infection rates remained undetermined [27]. It is hypothesized that such sex differences may be attributed to differences in immune responses elicited by DENV in

men and women patients [31] and that such sex-related differences may reflect differential health care seeking behavior in males versus females. We hypothesize that lack of differences in sero-positivity in our population could be due to DENV patients having the same health-seeking behavior irrespective of the gender. This hypothesis is currently being investigated in our population.

The current study also showed significant age differences in DENV infection rates (P=0.001) with most infections occurring in the age 21 to 60 years. This suggests a higher seroprevalence rate in adults compared to children. These observations were consistent with those of other studies carried out in Somalia [32], and in India (28-30). In contrast, other investigators revealed that dengue is a pediatric public health problem [15], [16], [33], [34]. In Americas, DENV syndromes occur in all age groups, however, majority of fatalities during epidemics occur in children [3]. In a previous study [9], it was concluded that the true endemicity of dengue is reached when the adult infection declines and occurs only through the new entrants into the population, that is, the children are affected more by the disease. However, in a longitudinal study on age-specificity of clinical dengue during primary and secondary infections [35], it was suggested that the estimated age-specific risk of clinical dengue increases as a function of age for both primary and secondary infections. The study further concluded that age is an important modulator of clinical dengue and further explains the recent increase in dengue notifications in aging countries such as in Southeast Asia [35]. Moreover, there is a paradox in the increase in adult patients resulting from a decline in the force of infection, which may be caused by various factors including time-dependent variations in epidemiological, ecological and demographic dynamics. It would be worthwhile to explore these hypotheses in the current study population.

A DENV infection rate as stratified according to marital status was not found to be significant in this study. It is rather difficult to associate marital status in regard to DENV infections because DENV infections are vector-borne and not sexually transmitted. In addition, no studies have the association between marital status and DENV infection. However, in the context of the current study population demographics, we demonstrate that all those individuals confirmed by PRNT to be sero-positive for DENV were married.

The difference in occupation was found to be statistically significant (P=0.002), with majority of the infected persons being either employed or self-employed, however, no evidences so far can exactly explain this outcome. Since self-reported responses to the structured questions were used to obtain the socio-demographic characteristics in this study, no detailed information on the type of work and its association with the exposures to DENV could be assessed properly. Therefore, the study could only determine these characteristics but could not clearly associate them to DENV infections.

The current study also assessed place of habitat as a socio-demographic factor. Individuals were divided into rural or town habitation. However, the distribution of those living in rural versus urban and DENV sero-positivity were comparable. Notwithstanding the insignificance, all the positive individuals hailed from the rural areas (1.4%). Historically, dengue has been reported as occurring predominantly among urban populations where density of dwellings and short flying distance of the vector create the right conditions for

transmission [2], [14], while other studies reveal that, dengue transmission and outbreaks occur in rural setting in both Asia, Latin America [14] and Amazonia [27]. The reasons for spread of dengue to rural areas have been highly associated with increased transport contact, mobility and spread of peri-urbanization [36]. The main symptoms associated with classical dengue fever [1] were used to determine the clinical characteristics in this study. Fever was the main entry point into the study. Majority of the participants who were sero-positive presented with headache (80%; 4/5), muscle ache (80%; 4/5), joint pain (60; 3/5), retro-orbital pain (40%; 2/5) and rash (20%; 1/5) in decreasing frequency of presentation. In addition, none of the sero-positives had vomiting, abdominal pain and bleeding diathesis as presenting symptoms. However, associating these symptoms as presenting features specific to dengue 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 [37]. Most developing countries have epidemics of febrile illnesses which can be confused with dengue fever [38].

Collectively, the data obtained from the current study, provides insights into the difficulties in differential diagnosis of dengue infections which have been challenging due to its presentation with non-specific clinical symptoms.

Risk factors associated with DENV infection

Age (P=0.001) was the only individual risk factor significantly associated with seropositivity. Majority of the sero-positive individuals were between ages of 21 to 60 years. Studies carried out in India, have reported 15 to 45 years as the most affected age group [28]-[30]. In Somalia, dengue has also been highly regarded as an adult disease [32]. Another study on age-specific risks of clinical dengue attack [35], revealed that adolescents and young adults are more likely to develop symptomatic dengue than younger individuals (such as primary school children).

Infection with dengue is likely to be dependent on the quality of housing and use of prevention measures [39], [40]. In this study, the differences in housing conditions such as walls with cracks (P=0.005), place of storage of water vessels (P=0.002) were significantly associated with risks to dengue infections. In comparison to studies carried out in Thailand, housing conditions have greatly been shown to be a risk factor to dengue infection [39]. In the same study, it was also reported that storing of water vessels outside the house without covering displayed a higher risk for acquiring dengue, as they act as breeding sites for *A. Aegypti* [39]. In another study, dengue sero-positivity and environmental factors were linked through location of households [40]. Despite findings in these previous studies, type of house walls (P=0.922), dumping site near the house (P=0.337), water storage vessel (P=0.770), water bodies surrounding the house (P=0.284) and vegetation surrounding the house (P=0.148) were not associated with risk to DENV sero-positivity in the current study.

Studies carried out on the spatial patterns of dengue infection have also shown that persons living in houses surrounded by natural and agricultural vegetation had a lower risk of infection [40]; this is because some vegetation such as forests are not a favourite breeding place for *Aedes* mosquitoes [40]. The environment surrounding a person's place of habitat can also act a good breeding site for *Aedes* mosquitoes [4]. However, contrary to these observations, these factors did not

alter DENV sero-positivity in our population. Most studies conducted on socio-economic factors, are normally ardent on the rich/poor indices [14]. The current study assessed individual socio-economic activities of the participants; herding of cattle (P=0.775), fetching water (P=0.824), playing near the forest (P=0.528) which were not associated with dengue sero-positivity. However, burning of charcoal (P<0.0001) and farming (P=0.020) were significantly associated with dengue seropositivity in the current study. Reasons for our observations could be attributed to the fact that these two (burning of charcoal and farming) are day time activities and *Aedes* mosquitoes bite mainly during day time [1]. Furthermore, sylvatic and peri-domestic cycles of DENV vectors have been reported [8] and activities such as herding of cattle, fetching water, playing near the forest even though not significant in this study, may act as risk factors subjecting people to infections with DENV.

The use of insecticide-treated bed nets (ITN) was not significantly associated with dengue infections (P=0.478), in addition the routine use of other vector control activities (P=0.767) such as herbs, insects repellants, insecticide sprays, mosquito coils and lack of a vector control (P=0.996) did not significantly affect dengue sero-positivity. Use of ITN is normally mentioned as a prevention measure for dengue infection, nevertheless, this measure is not effective for dengue infection [39] as *Aedes* mosquitoes bite mainly during daytime [1]. Although studies have shown that the major mosquito vector for transmission of DENV is prevalent in the sylvatic and peri-domestic regions of western Kenya [8], the low seroprevalence (1.2%) of DENV in the rural settings, further supports the hypothesis of little autochthonous transmission in rural settlement. This observation has also been pointed out in other studies carried out on the rural Amazonian regions in the Americas such as Ramal do Granda [27] and Peruvian Amazonia [41] making vector control to be inappropriate for DENV control in rural areas where little autochthonous transmission occurs. Cross-reactivity among flaviviruses could affect DENV antibody measurements in populations exposed to or immunized against Yellow Fever Virus (YFV) [26]. For instance, participants with antibodies to the vaccine strain of YFV (17DD) used in Brazil, detected by Haemagglutination Inhibition Assay (HIA) [42], were considerably more likely to have DENV IgG detected by ELISA. However, the current study was based on the confirmatory test results of PRNT and only 6 (1.42%) of the study participants reported YFV vaccination over the past 10 years. DENV neutralizing antibodies were similarly prevalent in nonvaccinated individuals. This finding suggests that YFV vaccination has not substantially affected DENV neutralizing antibody measurements in the current study population (P=0.787).

Conclusions

Even though the current study reported a low sero-prevalence rate of DENV, it still remains a potential hazard for public health in the study areas, as it may be an emerging circulating viral disease in the population [43]. It is therefore important to include DENV and possibly other endemic arboviruses in the differential diagnosis of febrile illness in Kenya. Even though standardized diagnostic criteria (IgM and IgG capture ELISA) are widely used in Kenya, the diagnosis of DENV infection remains inaccurate because several locally prevalent febrile illnesses may be misdiagnosed as dengue fever. For instance, in this study, the screening tests performed by indirect ELISA

designating the presence of IgM, IgG and IgA DENV antibodies found 62 of 422 serum samples to be positive, but only 5 sera were confirmed positive by PRNT. These findings underscore the need for laboratory confirmation of DENV infections for outbreak investigation and disease surveillance.

Socio-demographic characteristics should be put into consideration when conducting dengue studies as they give an insight of the exposures and risk factors associated with dengue infections. Determining sex differences, both in infection and severity of disease, requires well-designed and targeted studies to capture both biological and social factors that drive disease patterns in a community. Hospital-based studies on population distributions and improved classification and diagnosis of dengue syndromes should be carried out to give more insight on age shift in dengue. Risk factors to dengue should be of keen interest as they predict the high and low risks regions in a country. Standard epidemiological techniques such as spatial studies of cases and careful patient histories could shed further light into risk factors and transmission of dengue. Furthermore, more studies should be carried out on DENV in Kenya using longitudinal study designs. The findings from this study could act as hypothesis to be tested further in order to obtain a clear picture of DENV prevalence in Kenya. Surveillance with good laboratory services serves as an 'early warning system' against impending outbreak of arbovirus infections. In addition, absence of a specific treatment for dengue fever management is mainly supportive, and since there are no vaccines currently available in the market, early diagnosis and vector control is the only method by which dengue can be effectively controlled.

Limitations of the study

This study only ascertained the evidence of DENV-2 circulating in the study areas and not the other three serotypes (DENV-1, DENV-3 and DENV-4) due to limited funding and resources. Furthermore, most of our study population was drawn from KEMRI/CIPDCR Alupe clinic as most patients prefer to go for medical services in the Teso-South District region. Finally, Anderson Medical Centre was the only health facility that could be selected in Trans-Nzoia as it falls on the border belt between Kenya and Uganda and also samples that met our recruitment criteria of fever could only be found from this health facility. Future studies should explore beyond these health facilities and have a more representative sample size from each locality sampled.

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Research Institute (KEMRI) Ethical Review Committee (ERC) and Scientific Steering Committee (SSC) was awarded the protocol SSC No.1833 (Revised).

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