Final technical report:

Project ID No.: **SGS14/34**

Project title: **Entomological and socioeconomic determinants affecting Dengue prevalence in Kassala area, Sudan**

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Summary

Dengue, a mosquito-borne viral disease, is becoming a major health problem in some parts of the world [1, 2]. Dengue haemorrhagic fever and dengue shock syndrome are responsible for heavy morbidity and mortality every year, it's burden have been rising in the recent years and become a public health problem of global importance [3]. Sudden onset of dengue epidemics can be greatly mitigated using rapid and reliable diagnostic methods for the control and management of this disease [4].

Kassala locality, eastern Sudan has witnessed increasing incidence of dengue outbreaks during the last few years according to the clinical services records [5, 6]. The problem of dengue in Kassala area is compounded by the huge population, cross broader movement, poor medical and diagnostic facilities, inadequate mosquito control activities and all the ground conditions that favor expansion of the vector breeding sites [6, 7]. Surveillance of vulnerable people and mosquitoes transmit dengue viruses can help monitor the epidemiological picture of local human populations and mosquito populations harboring specific serotype and provides an early warning sign to predict an impending epidemic [3, 6].

Several risk factors are associated with the presence of both dengue and its vector in Kassala area. A cross-sectional study was conducted during summer season to determine the entomological, behavioral, social and economic risk factors associated with dengue fever prevalence in Kassala area. The research project was done in collaboration with local health authorities to build the capacity for Dengue entomological/sero-prevalence surveys in Kassala area, eastern Sudan.

Introduction

Dengue is a vector-borne virus infection, which is transmitted to humans by infected *Aedes* mosquitoes. It is a disease of tropical and sub-tropical areas [1, 2]. Dengue burden have been rising in the recent years and became a public health problem of global importance. The infections with dengue has increased by a 30-fold over the past 50 years, making dengue the most rapidly spreading mosquito-borne viral disease in the world [11]. The annual estimated deaths due to dengue are around 25,000 deaths annually [4]. The causes of dengue increasing spread are attributed to geographic expansion of the vector, the *Aedes aegypti* mosquitoes, leading to the increased co-circulation of the virus mainly in urban areas [7]. The conditions of uncontrolled urbanization, with inadequate housing, water distribution systems, sewer and waste management were favorable for the *Aedes aegypti* breeding and transmission of the DENV serotypes [13]. Despite the lack of epidemiological surveillance, reports from different African countries have revealed that outbreaks of the four serotypes have increased dramatically since 1 980, although they are still rare when compared with South East Asia and the Americas [14]. Dengue epidemics are mainly occurring in eastern Africa and to a smaller extent in western Africa [15]. Dengue is one of the causes of febrile illness in Sudan [16]. In 1984 dengue outbreak caused by serotype 2 and 1 was confirmed in Port Sudan. That outbreak was treated first as Malaria [17]. A dengue outbreak attributed to DEN-2 was reported to have occurred in Sudan in 1986 [16, 18] and recently

in Kassala 2010, Portsudan 2014 and Alfashir2014 dengue outbreaks were reported (Ministry of Health personal communication) [19].

Kassala witnessed increasing of dengue cases during the last three years according to the clinical services records [20]. A key strategy of the dengue control program relies on vector control to reduce viral transmission [21]. But, in different complex epidemiological settings, various factors that can influence dengue transmission dynamics remain to be established. This is because of more diverse socio-cultural contexts and changes in socio-political, socio-economic, demographic, technologic, and environmental conditions as well as ineffective management of household-level information and improper implementation of those control strategies [22].

The history of previous outbreaks of dengue, poor health system coverage, poor economic situation of the community, high number of refugees from Eritrea and Ethiopia, and the local gold mining with high numbers of internally displaced people (IDPs), the high illiteracy rates, limited financial resources, natural disasters such as the floods from El-Gash river, geographical inaccessibility of some areas specially during the rainy season and free movement between Kassala state and Red Sea state which is a known as an endemic area of dengue fever [23]. The study of biological, social and economic determinants associated with dengue transmission in Kassala locality will identify the potential risk factors responsible for disease transmission, and will attract the attention to the probable hidden factors that may be responsible for successive epidemics with high prevalence measurements in order to apply this knowledge for attraction the local and global financial support necessary to improve the health care system, vector management and delivery of vector control services, assessing the efficacy of insecticides application to administrate interventions used to control dengue epidemics, establishing of sustainable surveillance system, emergency preparedness and response.

Study Objectives

- Detection of dengue sero-prevalence using Enzyme-linked immunosorbent assay (ELISA) among humans in study area.
- Detection of the dengue virus circulation among collected adult *Aedes* mosquito using real-time RT-PCR.
- Monitoring of aquatic stages indices (*stegomyia* indices) of the dengue vector (house index, pupal index, Breteau index) in the study area.
- Investigation of the behavioral, social and economic factors that affect the dengue fever among vulnerable populations.

Materials and Methods

Study area

Kassala state is the one of the eastern Sudan states encompassing 12 localities. Kassala state is bordered by Eritrea and Ethiopia to the east, Red Sea state to the north; Khartoum and River Nile states to the west and Gedaref state to the south west. Kassala state land space is 42.282 km² [6]. In the northern parts of the state the climate is the red sea climate, while in the other parts the environment is a desert, semi desert, and valley and savannah climate with large fruit farms inside Kassala locality. The average rainfall is 350 to 911 ml and the temperature is 33˚C to 92˚C. Kassala locality is the locality of the state capital. Kassala locality contains Kassala town, which is the capital of the state. It is location10°12′N 34°19′E. The land space of Kassala locality is 1.115 km². Kassala locality is located along the main Khartoum-Port Sudan highway which makes it a main trading center besides the connections with Eritrea and Ethiopia. Added to this is the airport in the town which facilitates travelling from and to the town. Kassala locality population is 298,529 inhabitants living in 97 neighborhoods. The total number of households in Kassala locality is

52,853 according to 2008 census [6]. The average family size is 6.3. The drinking water sources are superficial, ground and deep ground waters. In the urban areas the percentage of the population getting clean drinking water is 68% and in the rural areas this drops to 40%.

Study surveys and study population

The study was a community-based survey with two cross-sectional surveys during summer and winter seasons in Kassala area; the two stage cluster sampling technique has been used for households' selection using a systematic random sampling technique. Within the household, participants aged more than 5 years old who signed an informed consent and agreed to be interviewed was randomly selected for blood collection. The area has been stratified into high and low socioeconomic strata (urban and peri-urban).

Sampling size and sampling method

A total of 252 households has been surveyed on each survey (252×2 cross-sectional survey=504). The formula for the calculation of the sample size was applied with OpenEpi software [8]. Only one person per house has been asked to donate the blood sample, all water containers have been inspected by entomological inspectors and one room has been selected for spraying for mosquito adult collection. Ten districts from the area has been chosen to represent urban and peri-urban areas (5 districts on each strata and 25 households for each district).

Sampling Framework

Activity	Sep 2015	Oct 2015	Nov 2015	Dec 2015	Jan 2016	Feb 2016	Mar 2016	Apr 2016	May 2016	Jun 2016	Jul 2016	Aug 2016	Sep 2016
Installment of fund													
Training and selection sites													
Ethical clearance from targeted community authorities													
Pilot survey													
Entomological and household surveys													
Identification of Dengue vectors													
Detection of Dengue infections in mosquitoes (rt-PCR)													
Blood samples analysis (ELISA)													
Questionnaire analysis													
Reports writing													

Data collection tools

The study variables are; recent dengue infection will be used as dependent variable (outcome). The entomological and socioeconomic factors will be used as independent variables.

Entomological investigation

In larval/pupal survey, the inspectors has been equipped with sieves, large-mouth pipettes, white enamel pans, small shell vials to examine all artificial containers indoor and outdoor for mosquito vector aquatic stages. Containers have been classified according to type, source of water, capacity, presence of a proper lid, proximity to shrubbery, and presence of larval control measures. Number of larvae in each container has been recorded. All detected pupae and 4^{th} instars larvae were pipetted in labeled vials (cluster code – household number – team number - date), transferred to the lab, where a senior entomologist has conducted the identification of mosquito species. An observation recording form was utilized. The following entomological indices have been calculated for Kassala area for each cluster:

House Index (HI) = number of positive houses for *Ae. aegypti*/ total number of houses inspected in the cluster.

Container Index = number of positive containers for *Ae. aegypti*/ total number of containers inspected in the cluster.

Breteau Index (BI) = number of positive containers for *Ae. aegypti*/ 100 houses inspected in the cluster.

Pupal demographic Index (P/D) = total number of *Ae. aegypti* pupa in the cluster's positive houses/total number residents in the cluster's positive houses.

Pupal children index (P/C) = total number of *Ae. aegypti* pupa in the cluster's positive houses/ total number under 5 years old children residents in the cluster's positive houses.

In adult *Ae. aegypti* survey, adults were collected using simple aspiration and pyrethrum spray catch (PSC). The collected adults will be preserved in specimens' tubes containing Qiazol and transported to the lab for identification and for viral RNA extraction and detection by real time-RT- PCR. An observation recording form has been utilized. RNA will be extracted from adult mosquito specimens using the QIAamp® RNA viral kit according to the manufacture instructions. Extracted RNA will be assessed for quality and quantity, then detection of Dengue virus will be using Liferiver ^{TM} real time RT-CR kit following manufacture instructions. The DENV-1-4 Real-Time RT-PCR Assay will be used in rRT-PCR on an ABI 7500 Fast Dx Real-Time PCR Instrument. The DENV-1-4 Real-Time RT-PCR Assay includes a set of oligonucleotide primers and dual-labeled hydrolysis (Taqman®) probes for in vitro qualitative detection of DENV serotypes 1, 2, 3 or 4 from collected mosquitoes. The targeted regions of viral RNA will be transcribed into complementary (cDNA) and amplified by the polymerase chain reaction (PCR). The fluorescently labeled probes anneal to amplified DNA fragments and the fluorescent signal intensity will be monitored by the ABI 7500 Fast Dx instrument during each PCR cycle. Amplification of target will be recorded as increase of fluorescence over time in comparison to background signal. A positive control virus mix will be included; then the DENV-1-4 Real-Time RT-PCR Assay will be run in multiplex (the four DENV serotypes will be run in the same reaction).

Household questionnaire to assess determinants factors of Dengue in Kassala

After pilot testing, a household structured questionnaire in each site has been introduced by interviewers to obtain information on interviewees about the demographic characteristics, living

conditions; occupation, educational degrees, average family income, household members, purpose of building, number of floors, construction material, protection of windows, characteristics of the peridomestic area; water supply and storage, container management, toilets, waste disposal, access to health care resources, structural conditions, sewage system, and their perceptions and attitudes towards dengue risk and current dengue prevention efforts, and other environmental factors (trees or bushes around the house).

Household's sero-survey

Individuals have been asked to provide a blood sample to measure IgM, IgG and neutralizing antibodies against dengue fever virus. Two milliliters (mls) of blood have been collected from adults and children above 5 years by venipuncture. Kits of enzyme-linked immunosorbent assay (ELISA) will be used to detect dengue-specific IgM and IgG antibodies in all blood samples, the IgM/IgG ratio will be calculated to distinguish primary from secondary dengue virus infections.

The collected blood samples have been centrifuged, the serum has been separated in labeled cryovials, stored at icebox, and transported to the Institute of Endemic Diseases, University of Khartoum. Dengue IgM Capture ELISA will be performed for detection of primary dengue infection "Serum IgM antibodies can be detected from dengue patients as early as three to five days after the onset of fever and generally persist for 30 - 41 days". Dengue IgG Capture ELISA also will be performed for the detection of secondary dengue infection. According to the manufacturer "the Panbio Dengue IgG Capture ELISA is for the qualitative presumptive detection of elevated IgG antibodies to dengue virus (serotypes 1-4) in patients with secondary infection". High IgG levels indicative of secondary dengue are detectable on the Panbio Dengue IgG Capture ELISA as early as 3 days post onset of illness. However, the peak detection window for accurate secondary diagnosis is 6-15 days following

onset of illness. All the test kits will be Panbio® kits, manufactured by Invernes Medical Innovations Australia (http://panbiodengue.com/). The test procedure will be done according to the manufacturer instructions using the ELISA machine, washer and an incubator [9, 10].

Meteorological data

Meteorological data (rainfall, temperature, humidity, sun shine, wind speed and river flooding) will be obtained from Meteorological Authority. The rooms' internal temperature and humidity have been measured by thermo-hygrometer device during field trips. All screened houses have been geo-referenced using GPS receivers.

Results

1. **Training activities and capacity building**

   Training workshop was held in the last two weeks of October 2015 (18-30) at Institute of Endemic Diseases, University of Khartoum to train the research teams on: the entomological inspection including (aquatic stages collection and identification, adult collection and identification), filling the data record sheets for entomological inspection, and filling household questionnaire.

2. **Selection of study areas and households**

   Ten districts from Kassala city have been selected representing 5 urban (New Khatmia town) and 5 peri-urban areas (Old Khatmia town) with help from Kassala Ministry of Health officers for summer season survey. **NB;** 5 district in each strata and 25 households for each district. Hundred (100) households has been visited upon the time of this report submission out of 252 households among the first cross-section survey of summer season in March 2016.

3. Household sero-survey

Ethical clearance was obtained from the following institutions;

- Department of Parasitology and Medical Entomology, Institute of Endemic Diseases, University of Khartoum for conducting the study.
- Kassala Ministry of Health, Epidemic Department of Kassala Ministry of Health
- University of Kassala, Faculty of Medicine.

An agreement to participate in the sero-survey and to keep serum blood samples at the University of Kassala lab, while the field work is ongoing, was obtained from Faculty of Medicine, University of Kassala

During the first cross-section survey (summer season) and after getting verbal acceptance from the participants to participate in the study, 100 blood samples (2 mls) were collected from 100 selected houses, after signed the informed consents. The blood samples were immediately separated and serum samples were kept in -20ºC in Faculty of Medicine, University of Kassala.

4. Household questionnaire survey

According to the pilot survey, the modified questionnaire has been submitted to 100 selected households in both urban and peri-urban sites during the summer cross-sectional survey. The general information of the participants are summarized in the table below:

Information	New Khatmia town Urban sites	Old Khatmia town Peri-urban sites
Total no. of houses visited	50	50
No. of people living in the households	Men (99) 38.4% Women (197) 48.2% Children<5 years (18) 40.9%	Men (159) 61.6% Women (212) 51.8% Children<5 years (26) 59.1%
Educational level	Never attend schools(6) 33.3% Informal education (4) 44.4% Primary school (5) 33.3% Secondary school (16) 45.7% Graduates (17) 65.4%	Never attend schools (12)66.7% Informal education (5) 55.6% Primary school (10) 66.7% Secondary school (19) 54.3% Graduates (9) 34.6%
Monthly income in SP	Range of 300 – 1000 SD	Range of 100 – 500 SP
Permanent residence in Kassala	Yes (50)	Yes (50)
Knowledge about Dengue fever	Yes (50)	Yes (50)
Travelled to Port-Sudan city	Yes (4)	Yes (2)
General house construction	Flat, bricks with cement houses with iron sheets/concrete roofs	Flat, muddy with poles houses with grass roofs

5. Entomological survey

The first cross-sectional survey during summer season has been conducted, the 4th mosquito's larvae and pupae stages have been collected from 100 households using appropriate equipment. No adult mosquitoes were found all selected rooms in both urban

and peri-urban sites. The entomological data for collected mosquitoes are summarized in the table blew:

Information	**New Khatmia town Urban sites**	**Old Khatmia town Peri-urban sites**
No. of 4th larvae collected	1244 (25%)	3724 (75%)
No. of pupae collected	122 (35.3%)	224 (64.7%)
No. of adult collected	0	0
No. of room inspected	50	50
Type and no. of positive containers	Clay pot (24) 39.3% Iron Barrel (1) 33.3% Plastic Barrel (2) 22.2% Plastic Pocket (0) Others (0)	Clay pot (37) 60.7% Iron Barrel (2) 66.7% Plastic Barrel (7) 77.8% Plastic Pocket (0) Others (0)
Type and no. of negative containers	Clay pot (54) 30% Iron Barrel (20) 34.5% Plastic Barrel (18) 24.7% Plastic Pocket (14) 29.9% Others (1) 33.3%	Clay pot (126) 70% Iron Barrel (38) 65.5% Plastic Barrel (55) 75.3% Plastic Pocket (33) 70.2% Others (2) 66.7%

6. Meteorological data

The entire temperatures were recorded from the rooms selected for mosquito adult spray sheet collection, the temperature range was 31ºC - 48ºC. Also the humidity was recorded in each selected room and the range of humidity was 10 - 34 %.

Discussion

This study is aimed at identifying the entomological and socioeconomic factors that may cause dengue outbreaks at Kassala area in eastern Sudan. During the cross-sectional survey of summer season, a total of 100 households have been visited out of 252 households upon the time of this report writing, and the survey is still ongoing, till April 2016.

In the sero-survey, 100 serum samples have been collected for ELISA detection to done after completion of sample size (252 samples).

Questionnaire survey data shows that the general life style in New Khatmia town (urban sites) was higher than that of Old Khatmia in terms of family income, educational level, house construction, and water container management.

In the entomological survey, no adult mosquitoes were found in the selected rooms. This finding may be attributed to thermal fogging of mosquitoes by the Kassala Ministry of Health and as a result of high temperature (48ºC) during summer days.

Conclusion

The cross-sectional survey was conducted in the urban and peri-urban areas in Kassala, and the results of the sero-survey, questionnaire survey, entomological survey and meteorological data collected from the selected households are presented and discussed.

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