SEROPREVALENCE OF DENGUE AMONG BLOOD DONORS IN DAR ES SALAAM

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SEROPREVALENCE OF DENGUE AMONG BLOOD DONORS IN DAR ES SALAAM

By

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A Dissertation submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science (Microbiology and Immunology) of

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by Muhimbili University of Health and Allied Sciences a dissertation entitled: **"Seroprevalence of Dengue among Blood Donors in Dar es salaam",** in (partial) fulfillment of the requirements for the degree of the Master of Science (Microbiology/Immunology) degree of Muhimbili University of Health and Allied Sciences.

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Date

DECLARATION AND COPYRIGHT

I, **Colman Evarist Mchau**, declare that this **dissertation** is my own original work and that it has not been presented and will not be presented to any other university for similar or any other degree award.

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DEDICATION

This dissertation is dedicated to my late Mother Mrs. Valeria Morandus and my family who always believed in me and supported me in all that I do. May the good God grant them their due reward on earth and in heaven.

ABSTRACT

Background:

During dengue outbreaks, only symptomatic individuals are screened for dengue infection. However, 75% of all adults infected with dengue virus are asymptomatic and may remain viraemic for up to a week. This increases the risk of transfusion-transmitted dengue if these individuals are asked to donate blood.

Objective: To assess seroprevalence of dengue among blood donors during epidemics.

Methodology: This was a cross sectional study that involved healthy voluntary blood donors. Serum samples were collected and screened for dengue specific antibodies and NS1 antigen using Dengue IgM, IgG+ Ns1 Combo rapid assays. Real time polymerase chain reaction (RT-PCR) was used to confirm active dengue infection among IgM and NS1 positive blood donors. A structured questionnaire was used to collect socio-demographic, environmental and clinical information. Binary logistic regression was used to identify factors associated with dengue seroprevalence. A two sided p-value <0.05 was considered statistically significant.

Results: A total of 421 blood donors were enrolled into the study. The mean age of study participants was 32 ± 10 years, and majorities were males 368/421 (87.4%). The proportion of blood donors with recent exposure to dengue virus (DENV) was low, anti-DENV IgM was detected in 13/421 (3.1%) participants. A quarter of blood donors were previously exposed to DENV, 114/421 (27.1%) of them had anti-DENV IgG antibodies. NS1 antigen was not detected in any of the blood donors, and none of the IgM positive participants had DENV RNA. After adjusted analyses, only age below 30 years was found to be an independent predictor of IgG seroprevalence among blood donors. The odds of previous exposure to dengue were 30% higher among younger blood donors than in participants older than 30 years of age, OR= 1.30 (95% CI; 1.2-5.8), p=0.002.

Conclusion: We found no evidence of viraemia or active dengue infection among blood donors during the 2019 outbreak in Dar es Salaam. The likelihood of transfusion transmitted dengue during epidemics in non-endemic regions is low. There fore it is not compulsory to carry out routine screening of blood donors for DENV virus during epidemics.

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LIST OF ABREVIATIONS

ADE	Antibody dependent Enhancement
DENV	Dengue virus
DF	Dengue Fever
DSS	Dengue Shock Syndrome
DHF	Dengue Hemorrhagic Fever
HIV	Human Immunodeficiency Virus
IgG	Immunoglobin G
IgM	Immunoglobulin M
IRB	Institutional regulatory Board
MOHCDEC	Ministry of Healthy, Community Development, Gender, Elderly and Children
MUHAS	Muhimbili University of Healthy and Allied sciences
NBS	National Bureau of Standards
NIMR	National Institute of medical research
NHLQATC	National health Laboratory Quality Assurance Training Centre
SOP	Standard Operation Procedure
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Dengue is a viral disease caused by infection with dengue virus (DENV). DENV is an Arbovirus belonging to the genus Flavivirus (1). There are four serotypes of DENV; DENV-1, DENV-2, DENV-3 and DENV-4. Infection with one serotype confers protection against the corresponding serotype but only a partial immunity against others (2). Half of the world's population is at risk of dengue infection, with 390 million dengue cases reported each year (3). Southeast Asia is the most affected region, with two-thirds (70%) of the total global burden, followed by Africa 16%, and America 14% (4).

In the tropics, dengue is primarily transmitted by *Aedes aegypti* mosquitoes (5). The spread involves a human-to-mosquito-to-human cycle of transmission, in which female *Aedes aegypti* get infected when they take blood meal from a person infected with DENV, and then infected mosquitoes transmit the virus for the rest of their lives each time they take another blood meal (6).Besides mosquito bites, DENV have also been documented to be transmitted by other means such as from infected mother to her unborn child through the placenta, during organ transplantation,after needle-stick injury and through transfusion of infected blood (7,8,9)

Across the globe, dengue outbreaks occur during the rainy season when mosquitoes multiply in open containers with stagnant water such as tires, buckets, basins, water storage jars, and flower pots (10). These breeding sites increase the degree of contact between mosquitoes and humans, further spreading the DENV, especially in densely populated regions witha large numberof mosquito vectors (11).

Dengue is not endemic in Tanzania. However, two large dengue outbreaks have occurred in Dar es Salaam in 2014 (12) and 2018 (unpublished).Travelers returning from dengue endemic regions, heavy rains, presence of *Aedes aegypti* mosquitoes, discarded containers, tires and a susceptible population are known to be determinants of dengue transmission in the general population in Dar es salaam (13).

Despite the two previous outbreaks, very little has been done to explore whether these outbreaks increase the risk of transmitting the DENV through blood transfusion. This study aimed to assess the existence and magnitude of dengue seroprevalence among blood donors during an epidemic.

1.2 Problem Statement

Unlike HIV, hepatitis and syphilis infections, dengue is not recognized as a transfusion transmissible infection (14,15). However, it is well documented that flaviviruses can be transmitted through blood transfusion. Chikungunya, Zika, West Nile, and dengue viruses have all been detected in blood donors (16,17,18). The incidence of dengue is highest during the rainy season because of a surge in the vector population with subsequent increase in interaction between Aedes mosquitoes and humans (19). In the past four years, Dar es Salaam city has experienced two outbreaks of dengue virus infection during the rainy season, January-June 2014, and 2018 (20). Given that a high proportion of DENV infected adults are asymptomatic, and the virus may replicate in humans for 3-14 days before being neutralized (21), lack of regular screening for dengue during outbreaks may compromise the safety of blood transfusion and put recipients at risk of infection. However, the actual risk of transmission of dengue virus by asymptomatic individuals during blood donation is not known in Tanzania, therefore, this study aimed to establish the odds of transfusion transmitted dengue during outbreaks.

1.3 Rationale

The demand for blood components is always high, and dengue virus has potential to cause serious complications in recipients of infected blood. Findings from this study will be useful in improving the safety of blood transfusions during epidemics and periods of heavy rainfall. Additionally, it will assist in development of interventions to prevent transfusion transmitted dengue. It will also raise clinical awareness during epidemics and reduce dengue associated morbidity and mortality in the community.

1.4 Research question

What are the chances of transmitting dengue virus through blood transfusion during outbreaks?

1.5 Objectives

1.5.1 Broad Objective

To assess seroprevalence of dengue among blood donors in Dar es salaam

1.5.2 Specific Objective

1. To determine seroprevalence of DENV specific IgM and IgG antibodies among blood donors
2. To determine factors associated with the presence of anti-DENV IgM and IgG antibodies among blood donors

1.7 Conceptual framework

Studies show that risk of dengue infection is higher in children under 15 years and old people above 50 years of age than in other groups in the general population (22). People living in overcrowded areas, are at higher risk of acquiring dengue virus infection because of poor drainage system, and collection of stagnant water around their homes which serve as mosquito breeding sites (23). Travelers returning from dengue-endemic regions account for 2% of all dengue cases in non-endemic countries (24). People with low levels of education and those working in rice fields have a higher incidence of dengue than others (25). Presence of uncovered water storage containers in the households increase the risk of dengue transmission as they become vector breeding sites (26).Also blood transfusion has been associated with infection in dengue endemic areas(27).

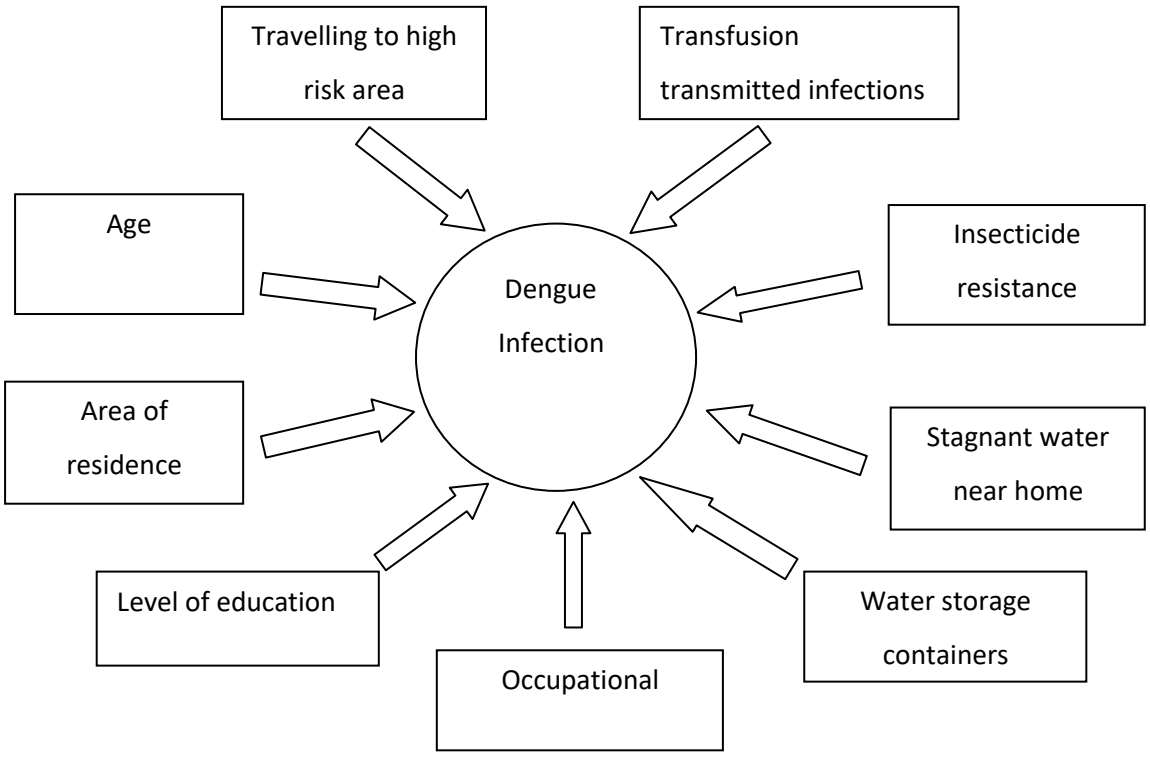


Figure 1: Conceptual framework of factors contributing to dengue infections

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction

2.1.1 Virology

Dengue viruses (DENV) are single-stranded ribonucleic acid (RNA) viruses of the genus *Flavivirus* (28). The viral genome encodes for three structural and seven non-structural proteins (29). The envelope protein sinduce protective neutralizing antibodies where as non-structural proteins have been associated with development of severe dengue disease (29). There are four serotypes of DENV; DENV-1, DENV-2, DENV-3 and DENV-4. Infection with one serotype induces sero type specific protection and confers partial immunity against other serotypes (30). Therefore, an individual can be infected by DENV more than once during his/her life (31) .

2.1.2 Global distribution of dengue virus

Currently dengue is endemic in 112 countries with estimation of 100 million cases of dengue fever worldwide with dengue hemorrhagic fever occurring in one and a half million people around the world and most affected age are children under fifteen years of age (32). By the end of 2016, a total of 291,964 outbreak-associated dengue cases had been reported in the literature, mainly from China (27.9%), Singapore (27.0%) and Malaysia (15.1%). The majority (72.4%) of dengue patients were reported in the Western Pacific region, followed by the American region (19.4%), Southeast Asia Region (4.8%), Eastern Mediterranean region (1.5%), European region (1.5%) and African region (0.3%) (33). In Tanzania review from 10 seroprevalence studies was done based on acute confirmed infections population. The estimated seroprevalence of past dengue infection ranged from 50.6% in a health facility-based study to 11% in a population based study. Acute confirmed infections of dengue were estimated to be as high as 38.2% of suspected cases (34).

2.1.3 Transmission of dengue virus

The principal vectors of dengue are *Aedes aegypti* and *Aedes albopictus* mosquitoes, which are mainly found in Asia and Pacific regions, respectively (35). There are two known cycles of dengue transmission, the Sylvatic cycle, which involves transmission of the virus between non-human primates (monkeys) and jungle mosquitoes (36), and the urban cycle, in which infection circulates between humans and *Aedes aegypti* mosquitoes (36). The urban cycle is the one which usually leads to huge outbreaks in the tropics (37). The viruses are passed on to humans through the bites of an infected female *Aedes* mosquito, which mainly acquires the virus while feeding on the blood of an infected person (37). Infected humans are the main carriers and multipliers of the virus, and a source of the virus for uninfected mosquitoes (37). The virus circulates in the blood of an infected person for 2-7 days, at approximately the same time that the person develops fever. Infected patients can transmit the virus to *Aedes* mosquitoes up to 5 days after the onset of symptoms (37). Recovery from dengue infection provides specific immunity to that serotype. However, this immunity confers only partial and transient protection against subsequent infection by the other three serotypes of the virus (38). Evidence shows that sequential infection increases the risk of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) through antibody dependent enhancement (ADE) (38). Upon secondary infection with different DENV serotype, preexisting non protective DENV antibodies are used as receptors for viral entry into monocytic cells (39) .

2.1.3Dengue infection among blood donors

Dengue infection can also spread through other modes of transmission such as mother to child transmission, organ transplantation, needle stick injuries, and blood (40). Dengue asymptomatic individuals can pose as potential source of virus infection in the community through blood donation (41). Recent findings in Guangzhou, China show that anti-dengue IgG antibody was detected in 3.4% of blood donors and dengue RNA was also detected in blood samples suggesting that dengue virus can be risky for blood safety during dengue outbreak in epidemic areas (42). The virus has also been recognized as potential threat on the supply of blood products in non-endemic areas with cases being reported in Hong-Kong, Singapore and Puerto Rico (43). Studies in New Delhi show high seroprevalence of

dengue infection in blood donors where anti-dengue IgG was positive in 58% and IgM was seen in 13.5% of screened blood donors (44).

2.1.4 Factors associated with transmission of dengue through blood transfusion

Among factors that increase the risk of transmitting dengue through blood transfusion is the absence of quantitative or molecular and serological methods for screening for DENV and/or its antibodies in asymptomatic blood donors (45). Environmental factors are the main contributors to spread of mosquito-borne diseases due to their effects on vector transmission cycles (46). Temperature, rainfall and humidity, poverty, poor public health infrastructure, uncontrolled and expansion of urban population, faster modes of transportation, globalization of trade and increased international travel are factors which also associated with rapid spread of dengue(47). Rapid population growth in the urban area with poor water supply leads to the use of containers to store water, creating breeding sites for *Aedes aegypti* mosquitoes. Also poor drainage and sewage systems with inadequate vector control programs, and vector resistance to insecticides are some of the current factors which support the contribution of the mosquito vector(48). Together with environmental factors, ecological status of the local area contribute to the spreading of dengue (49).

2.1.5 Laboratory detection of dengue virus

Laboratory diagnosis of dengue virus infection can be done using either molecular techniques that detect viral nucleic acids or through serological detection of viral antigens or/ and antibodies (50). After the onset of illness, the virus can be detected in serum, plasma, circulating blood cells and other tissues for 4–5 days (50). During the early stages of the disease, virus isolation, nucleic acid or antigen detection can be used to diagnose the infection. At the end of the acute phase of infection, serology is the method of choice for diagnosis (50).The four dengue serotypes share some epitopes on the envelope protein, and this may result in cross-reactivity in serologic tests (50). IgM antibodies are detectable in 50% of patients by days 3-5 after onset of illness, increasing to 80% by day 5 and 99% by day 10. IgM levels peak about two weeks after the onset of symptoms and then decline generally to undetectable levels over 2–3 months (50).

Serological tests are readily available at low cost, easy to use and to store, and provide early detection of infection (51). The rapid immunoassays detect IgM antibodies that develop 4-5 days after the onset of symptoms, and IgG-specific antibodies are a long lasting marker of past infection (52). Inendemic areas, infection by other flaviviruses may cause cross-reactivity leading to false-positive results (53).DENV can also be diagnosed by antigen tests. Rapid immunoassays can detect DENV NS1 protein during the first 5 days after onset of symptoms (54).The sensitivity of NS1 test is optimal during primary infection, however, negative NS1 test does not mean the absence of dengue, especially in secondary infection (54)

The gold standard for the diagnosis of recent infections is the detection of DENV genome (54). Molecular tests are very sensitive and specific, and are positive within the first 5 days of disease (54). Molecular technologies used for the diagnosis of DENV infection include ereal time polymerase chain reaction (RT-PCR), transcription-mediated amplification (TMA) and other isothermal amplification assays (55).

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study design and study sites

This was across sectional study that involved 421 voluntary blood donors. Serum samples were collected from blood donation centers at Muhimbili National Hospital, Mwananyamala, Amana and Temeke regional hospitals and National Blood Transfusion Service in Dar es Salaam. The centers receive an average of 2,000 blood units for transfusions every month (56) .

3.3.1 Inclusion criteria

All voluntary blood donors aged 18 to 65 years,weighing 50 kilograms or more with Hb>12.5g/dl and normal blood pressure who agreed to participate in the study and provided written informed consent.

3.3.2 Exclusion criteria

Any healthy voluntary blood donor who had been on treatment two weeks before blood donation, and those with chronic illinesses like diabetes, asthma, were excluded from the study.

3.4 Sampling procedure

Proportional probability to size sampling technique was used to get the number of blood donors from each site. At the respective sites, systematic sampling was employed to select blood donors. The sampling interval was calculated by dividing average monthly number of blood donors at each center by site-specific sample size.

3.5 Sample size calculation

Sample size at the required absolute precision level was calculated using the following formula

$$N = \frac{z^2 \mathrm{p}(1-\mathrm{p})}{\varepsilon^2}$$

Where:

N = sample size

p = is 3.4% reported prevalence of dengue IgM in blood donors in Brazil(57)

Z = standard normal deviate corresponding to the critical region (α) of 1.96 and

ε = Margin of error estimated to be 1.7%

$$n=\frac{1.96^2 \text{ x } 3.4\text{x } (100-3.4)}{1.7^2}$$

The minimum required sample size was 487

3.6 Data collection

Structured questionnaire was administered to the participants by the trained researcher assistants to obtain socio-demographic details such as age, sex, residence marital status, level of education, and behavioral factors that decreased or increased the probability of getting bitten by mosquitoes for example use of insecticide spray and mosquito nets, presence of stagnant water around their houses, occupational and travel history. Clinical information on dengue status and history of previous dengue infection were also recorded.

3.7 Laboratory procedures

A total of 5ml of venous blood was collected from each participant in plain vacutainer tube and left for 30 minutes to clot. Sample was then centrifuged at 1250 revolution per minutes (RPM) for 10 minutes within 6 hours of blood collection to obtain serum. Serum samples were then stored at -20^0C at each site and then transported to MUHAS Microbiology Laboratory for testing.

Dengue IgM, IgG and Ns1 rapid test was done using CAMP Medica Dengue IgG/IgM+NS1 antigen Combo (Bought from Medical Supply Department) followed by Real time Polymerase chain reaction (RT-PCR) for all IgM and Ns1 positive samples. RT-PCR was performed at the National Healthy Laboratory Quality Assurance and training Centre (NHLQATC) for confirmation of infection and serotyping. A flow chart is provided in fig 1 below.

3.7.1 Dengue rapid assay

The CAMP Medica Dengue IgM, IgG+ Ns1 Combo detecting both NS1 and IgM/IgG in single cassette was used to screen blood donors. The rapid assay has sensitivity of 78.4% for DENV IgM/IgG and 90.2% for NS1 and specificity of 100% as compared to reference rapid dengue test for both antigen and antibodies (manufacturer insert). Serum reacted with dengue antigen-coated particles in the test cassette and the mixture flowed through the membrane chromatographically by capillary action then reacted with anti-human IgG and IgM line regions. A test was considered reactive if two lines appear at T" band and "C" band within the result window. In the NS1 sample flows through the membrane and mobilize the gold anti-NSI conjugate that is coated on the conjugate pad. If NS1 is present, then it results in formation of colored band at the test T band region. Absence of a line at T band indicates a negative result, and the test results would be invalid if the control line (C) failed to appear.

3.7.2 Identification of dengue serotype

PCR test was used for confirmation and serotyping using Applied Biosystem 7500 Fast Dx Real-Time PCR Instrument. This is nucleic acid amplification assay that detects DENV serotypes 1,2,3,and 4 RNA from human sera (58). The assay included a set of oligonucleotide primers and dual-labeled hydrolysis (Taqman®) probes for in vitro qualitative detection of DENV serotypes 1, 2, 3 or 4. The assay involved transcription of targeted regions of viral RNA into complementary (cDNA) which were then amplified by the polymerase chain reaction (PCR). The fluorescently labeled probes were annealed to the amplified DNA fragments and the fluorescent signal intensity was detected by the ABI 7500 Fast Dx instrument during each PCR cycle. Amplification of target was recorded as an increase of fluorescence over time in comparison to background signal. A positive control virus mix which consisted of heat-inactivated DENV-1 Haw, DENV-2 NGC, DENV-3 H87, and DENV-4 H241 was always included in the assay. A non-infectious cultured human cell material (Human Specimen Control) provided a positive signal in the assay and demonstrated successful recovery of RNA as well as the integrity of the RNA extraction reagent. The human RNase P RNA (RP) present in cultured cell material and in most clinical samples were detectable by RT-PCR using the primers and probes provided as part of assay control. The CDC DENV-1-4 Real-Time RT-PCR Assay was run in

multiplex the four DENV serotypes are run in the same reaction (59) This test was done at NHL-QATC Laboratory.

3.7.3 RT-PCR assay results interpretation

All samples with RP target value as ± and either DENV-1,-2,-3 or -4 value as + had to be reported as positive for the specific DENV serotype (e.g. RP ± and DENV-2 + had to be reported as positive for DENV serotype 2).All samples with RP target value as + and all DENV-1,-2,-3 and -4 values as – were reported as DENV negative. Any sample with RP target value as - and all DENV-1,-2,-3 and -4 values as – had to be considered inconclusive and efforts done to re-extract and retest the RNA from that donor's specimen. Sample that tested positive for two different serotypes, the test would be repeated and if results would be repetitively reactive for both two different serotypes this would be resulted as dual infection and would have to be confirmed by the CDC-Dengue branch.

3.7.4 Study data dissemination

The information generated from this study will be disseminated through journal publication.

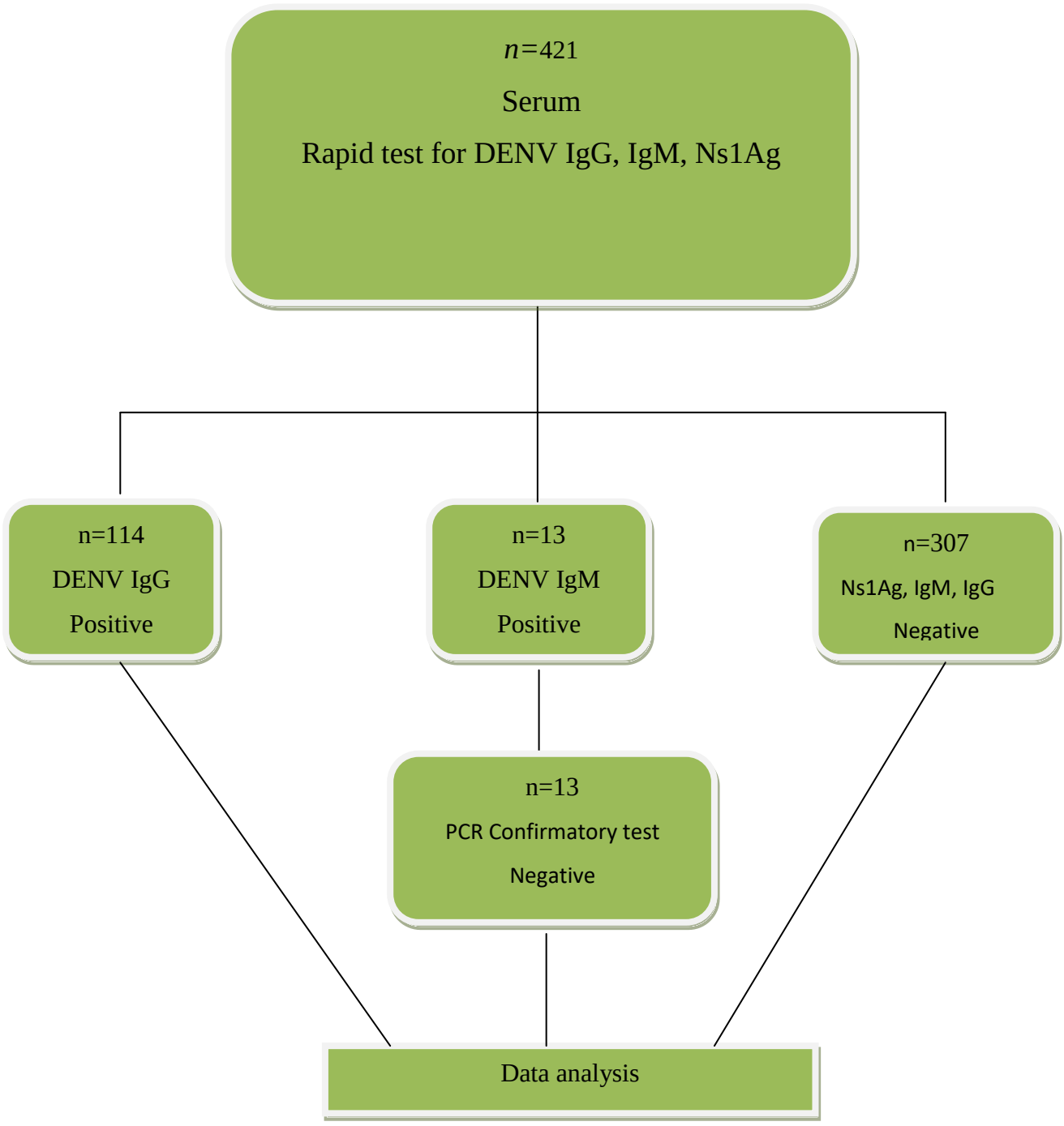


Figure 2: Laboratory Flow chart showing the expected testing process

3.8 Data analysis

Statistical Package for Social Science version 23 was used for analysis. Data was entered and cross checked to ensure completeness, accuracy and reliability of data. Mean and standard deviation was used in analysis of continuous variables, whereas, proportions was used to describe categorical variables. Binary logistic regression was used to identify factors associated with dengue seroprevalence in blood donors. A two sided p-value <0.05 was considered statistically significant.

3.9 Ethical considerations

Ethical approval was obtained from MUHAS senate Research and Publication Committee. Permission to conduct study was given by Director of National Blood Transfusion Centre and Site Research Officers (RO) of each municipality and medical officers in charge of the respective sites. Written informed consents were obtained from each participant prior to enrolment and benefits and risks associated with the study were explained to participants. Participants' names did not appear on any of questionnaires, only identification numbers were used to ensure confidentiality of the received data. Volunteers were free to decline participation in the study, and those who refused to join were not denied health services.

CHAPTER FOUR

4.0 RESULTS

4.1 Socio-demographic characteristics of study population

Of the 421 blood donors were enrolled into the study, majority were males 368/421 (87.4%), older than 35 years 161/421 (38.2%), unmarried 257/421 (61.0%), had at least secondary education 185/421 (43.9%) and 269/421 (63.9%) were not businessmen. Two-thirds of blood donors lived in non-crowded households 281/421 (66.7%), and one-third of the study participants were residents of Kinondoni district in Dar es Salaam145/421 (34.4%), Table 1.

Table 1: Socio-demographic characteristics of study population (N=421)

Variables	**Frequency n (%)**
Sex	
Male	368(87.4)
Female	53(12.6)
Age (years)	
<25	71(16.9)
25-30	99 (23.5)
31- 35	90(21.4)
>35	161(38.2)
Marital status	
Married	164 (39.0)
Unmarried	257 (61.0)
Level of education	
Primary	142 (33.7)
Secondary	185 (43.9)
College/University	94(22.3)
District of Residence	
Kinondoni	145(34.4)
Ubungo	43 (10.2)
Ilala	107(25.4)
Temeke	126(29.9)
Occupation	
Businessmen	152 (36.1)
Other professions	269(63.9)
Number of people in a household	
1-4	281 (66.7)
5-8	133 (31.6)
>9	7 (1.7)

4.2 Behavioral characteristics of study population

Most of the participants used insecticide treated nets 389/421 (92.4%) and indoor residual spraying 280/421 (66.5%) to protect themselves from mosquito bites. Although few blood donors 104/421 (24.7%) lived in places surrounded with stagnant water ponds, majority 323/421 (76.7%) stored water in open containers at home. Few participants had travelled out of the country two months before blood donation 42/421 (10.0%) Table 2

Table 2: Behavioral characteristics of study population (N=421)

Variables	Frequency n (%)
Use of bed nets	
Yes	389 (92.4)
No	32 (7.6)
Indoor residual spraying	
Yes	280 (66.5)
No	141 (33.5)
Water storage in open containers	
Yes	323(76.7)
No	98 (23.3)
Water ponds around home	
Yes	104 (24.7)
No	317 (75.3)
Travelled out of the country in the past two months	
Yes	42 (10.0)
No	379(90.0)

4.3 Seroprevalence of DENV specific IgM and IgG among blood donors

Proportion of blood donors with recent exposure to DENV was low, only 13/421 (3.1%) of study participants had anti-DENV IgM antibodies. However, a quarter 114/421 (27.1%) of blood donors had previous exposure to DENV (Fig 3). There was no evidence of viraemia or active dengue infection among blood donors. NS1 antigens were not detected in any of the blood donors, and DENV RNA was not detected in any of the recently exposed blood donors.

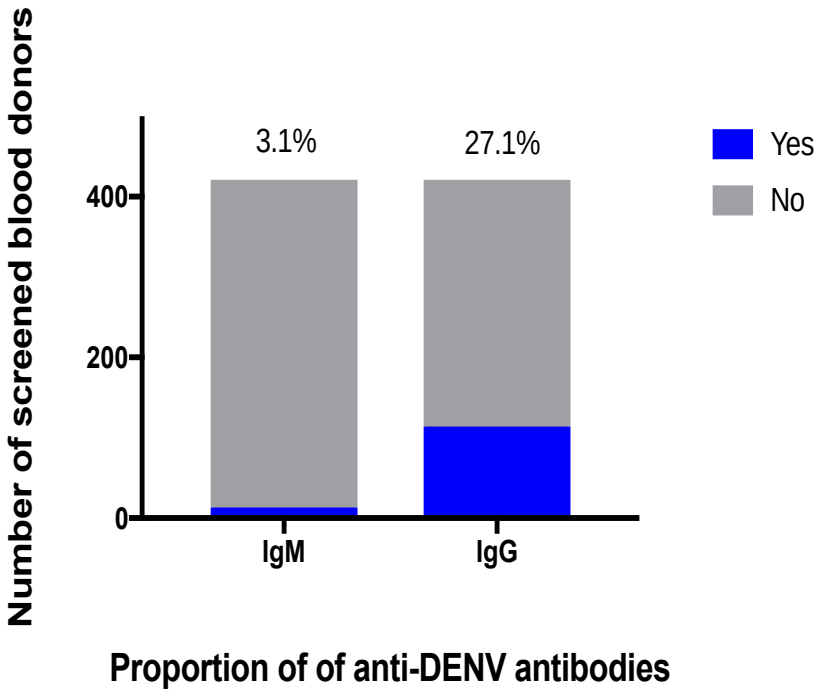


Figure 3: Seroprevalence of dengue antibodies among blood donors

4.4 Predictors of IgM seroprevalence among blood donors

In univariable and multivariable analysis, socio-demographic and behavioral factors could not independently predict recent exposure to DENV among blood donors. Results are summarized in table 3.

Table 3: Factors associated with IgM seroprevalence among blood donors

Variable	IgM Negative	IgM Positive	COR (95%CI)	P-value	AOR 95%CI)	P-value[b]
Sex						
Female	53(13.0)	0(0.0)	1.1 (0.1-3.3)	0.649		
Male	355(87.0)	13(100.0)	**1**			
Age (years)						
<30	153(37.5)	4(30.8)	1.35 (0.4-4.5)	0.622	0.8(0.1-8.1)	0.906
≥30	255(62.5)	9(69.2)	**1**			
Marital status						
Married	155(38.0)	7(53.8)	**1**			
Unmarried	253(62.0)	6(44.2)	1.90(0.5-1.7)	0.255	0.8(0.1-3.2)	0.874
Level of Education						
Primary	138(33.8)	4(30.8)	1.15(0.4-3.8)			
At least secondary	270(66.2)	9(69.2)	**1**			
District of residence						
Kinondoni	141(34.6)	4(30.8)	1.18(0.4-3.9)	0.777	3.3(0.6-10.1)	0.613
Ubungo	40(9.8)	3(23.1)	3.08(0.6-15.8)	0.179		
Ilala	104(25.5)	3(23.1)	1.21(0.2- 6.0)	0.839	2.7(0.8-4.7)	0.126
Temeke	123(30.1)	3(23.1)	**1**			
Number of people in household						
<5	273(66.9)	8(61.5)	**1**			
≥5	135(33.1)	5(38.5)	1.3(0.4- 3.9)	0.686		
Use of bed nets						
Yes	378(92.6)	11(84.6)	**1**			
No	30(7.4)	2(15.4)	0.4 (0.1-2.1)	0.295		
In door residual spraying						
Yes	270(66.2)	10(76.9)	**1**			
No	138(33.8)	3(23.1)	1.7(0.5-6.3)	0.424	2.5(0.7-16.2)	0.342
Water storage in open container						
Yes	96(23.5)	2(15.4)	0.6(0.1-2.7)	0.273	0.8(0.2-4.3)	0.227
No	313(76.5)	11(84.6)	**1**			
Water ponds around home						
Yes	103(25.2)	1(7.7)	4.1(0.5-31.5)	0.181	3.4(1.3-26.5)	0.09
No	305(74.8)	12(92.3)	**1**			
Travelled out of country						
Yes	39(9.6)	3(23.1)	0.35(0.1-1.3)	0.26		
No	369(90.4)	10(76.9)	**1**			

COR=crude odds ratio, AOR= adjusted odds ratio, p-value[b] = p-value for adjusted odds ratio.

4.5 Predictors of IgG seroprevalence among blood donors

Young age was a significant predictor of previous exposure to dengue among blood donors. In adjusted analyses, the odds of previous exposure to dengue were 30% higher among blood donors younger than 30 years of age compared to their older colleagues, OR= 1.30 (95% CI; 1.2-5.8), p=0.002. Sex, marital status, level of education, overcrowding, use of bed nets, indoor residual spraying, storage of water in open containers, and history of travelling outside of Tanzania were not associated with IgG seroprevalence in blood donors (Table 4).

Table 4: Factors associated with IgG seroprevalence among blood donors

Variable	IgG Negative	IgG Positive	COR (95%CI)	P- value	AOR(95%CI)	P-value[b]
Sex						
Female	42(13.7)	11(9.6)	1.48(0.7-3.0)	0.268		
Male	265(86.3)	103(90.4)	**1**			
Age (years)						
<30	119(38.8)	38(33.3)	1.16(1.1-6.3)	0.042	1.3(1.2-5.8)	0.002
≥30	188(61.2)	76(66.7)	**1**		**1**	
Marital status						
Married	121(39.4)	41(36.0)	**1**		**1**	
Unmarried	186(60.6)	73(64.0)	0.7(0.4-1.2)	0.196	0.9(0.5-1.7)	0.918
Level of Education						
Primary	107(34.9)	35(30.7)	1.8(0.7-1.8)	0.477		
From Secondary	200(65.1)	79(69.3)	**1**			
District of residence						
Kinondoni	101(32.9)	44(38.6)	01.5(0.8-2.65)	0.13		
Ubungo	34(11.1)	9(7.9)	0.89 (0.4-2.1)	0.78		
Ilala	74(24.1)	33(28.9)	1.55 (0.9-2.8)	0.148		
Temeke	98(31.9)	28(24.6)	**1**			
Number of people in household						
<5	202(65.8)	79(69.3)	**1**			
≥5	105(34.2)	35(30.7)	0.9(0.6-1.4)	0.644		

Use of bed nets						
Yes	282(91.9)	107(93.9)	**1**			
No	25(8.1)	7(6.1)	1.35(0.6-3.2)	0.492	1.1(0.4-2.8)	0.827
Indoor residual spraying						
Yes	197(64.2)	83(72.8)	**1**		**1**	
No	110(35.8)	31(27.2)	1.49(0.9-2.4)	0.096	1.3(0.8-2.3)	0.83
Water storage in open container						
Yes	228(74.3)	95(83.3)	0.6(0.3-1.0)	0.052	0.62(03-1.1)	0.133
No	79(25.7)	19(16.7)	**1**			
Water ponds around home						
Yes	81(26.4)	23(20.2)	1.4(0.8-2.4)	0.191	1.3(0.7-2.3)	0.068
No	226(73.6)	91(79.8)	**1**		**1**	
Travelled out of country						
Yes	26(8.5)	16(14.0)	**1**			
No	281(91.5)	98(86.0)	0.56(0.29-1.10)	0.09		

COR=crude odds ratio, AOR= adjusted odd ratio, p-value[b] = P-value for adjusted odds ratio

CHAPTER FIVE

5.0 DISCUSSION

This study aimed to determine the seroprevalence of dengue virus IgM and IgG antibodies among blood donors during an outbreak. Despite the epidemic, the proportion of recently exposed blood donors was low, 3.1%, however, a substantial proportion of blood donors had been previously exposed to DENV, anti-DENV IgG antibodies were detected in 27.1% of the participants. There was no evidence of viraemia among blood donors, and DENV RNA was not detected in any IgM positive blood donors. Young age was a significant predictor of previous exposure to DENV among blood donors.

The absence of active dengue infection among recently exposed blood donors observed in our study is supported by findings previously reported from a dengue epidemic in Rio De Janeiro, Brazil (60). During the 2012 Dengue epidemic in Rio De Janeiro, DENV specific IgM antibodies were detected in 8.8% of blood donors, however, DENV RNA was detected in less than one percent (0.5%) of IgM positive blood donors. Similarly, Vairo F, et al, (61) did not find any active dengue virus infection among healthy blood donors in Zanzibar. The low probability of transmitting dengue virus through blood transfusion could be explained by early onset of DENV specific IgM antibodies that neutralize the virus within the first two weeks after infection (50).

The proportion of blood donors who had been previously exposed to DENV virus was much lower in our study compared to previous reports from Brazil(60) and Puerto Rico (62). An IgG seroprevalence of 90% and 92% were reported in Brazil and Puerto Rico, respectively. The fact that dengue is not endemic in Dar es Salaam may explain the observed differences in proportions between the three cities. The prevalence of dengue among general populations in Brazil and Puerto Rico is much higher than in Dar es salaam (63).

In our study, the likelihood of a blood donor younger than 30 years to have a past exposure to DENV was 30% higher than their counterparts. This is consistent to observations in Zanzibar, where, the odds of past exposure to DENV were three times higher among blood donors aged 26-35 years than other age groups (61). Additionally, the chances of past

exposure to dengue were significantly higher among blood donors from urban than rural parts of Zanzibar (61).

Other demographic and social factors were not found to be associated with IgG seroprevalence among blood donors in Zanzibar (61) . In the 2014 dengue outbreak in Dar es Salaam, risk of infection for >15 years of age was twice that for patients <15 years of age (odds ratio 2.54, 95% CI1.10–5.87; p = 0.029) and majority 48.7%of infected patients were coming from Kinondoni, meaning that living in Kinondoni and being >15 years of age increased the likelihood of contracting dengue compared to other groups in the general population(64) .

5.1 Study limitation

Delay in delivery of test kits compelled us to perform testing on the stored samples. The samples were tested a month after collection, meaning that participants could not receive their test results in time. This would have affected management of the participants if they had active dengue infection. Also add the statement about anti-dengue IgG.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

In Conclusion, we found that there was no active dengue infection among blood donors during the 2019 epidemic in Dar es Salaam. This means that outbreaks do not necessarily increase the chances of transmitting dengue through blood transfusion in non-endemic countries.

6.2 Recommendation

According to the findings of this study, we are suggesting that there is probably no need of regular screening of blood donors for DENV during epidemics in Dar es Salaam. But there is need for wider study covering all phases of a future dengue epidemic, to follow up the evolution and dynamics of DENV serological and viraemia parameters among blood donors.

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APPENDICES

Appendix I: Consent Form-English Version

MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES

DIRECTORATE OF RESEARCH AND PUBLICATIONS, MUHAS

CONSENT FORM.

ID NO ☐ ☐ ☐

Consent Form

Greetings!

My name is COLMAN EVARIST MCHAU; I am a postgraduate student at MUHAS, doing research on

SEROPREVALENCE OF DENGUE VIRUS AMONG BLOOD DONORS IN DAR ES SALAAM

Purpose of the study

To assess seroprevalence of dengue virus among blood donors in Dar es salaam.

Information on seroprevalence of dengue virus among blood donors in Dar es salaam will show how blood is safe from risk of causing Dengue infection and will be used to make decision on whether dengue testing should be included in the blood screening procedure before transfusion, so that transfusion transmission risk could not occur.

Also these findings will be beneficial in the community from regional to country level because blood safety will be certain and blood recipients will be protected from Dengue virus infection. Since reduced infections saves time and cost which occur in hospitalized dengue infected cases, treatment costs and production potentials in daily work. So impact of Dengue on the community will be resolved.

What Participation Involves

If you agree to participate in the study, you will be required to answer questions during interview and to provide blood sample for laboratory investigation.

Confidentiality

All collected information will be handled carefully, study identification number will be used instead of names, Confidentiality will be provided and unauthorized persons will have no access to the data collected.

Potential Risks

No harm will be expected to happen to you because of your participation in this study however during collection of blood sample one may feel some pain which is self limited after sometime.

Rights to Withdraw and Alternatives

Participating in this study is voluntary. You can choose not to participate in this study and even if you have already accept to participated in the study you can quit at any time if you feel so. Refusal to participate or withdrawal from the study will not involve penalty or loss of any benefits.

Benefits

If you agree to take part in this study, you will benefit by getting tested for Dengue carriage which can lead to early detection and lead to the clinical management of patients, however another benefit will be indirect by protecting other community member from getting Dengue infection through blood transfusion.

Payment

No payment will be requested from you as a fee to participate in the study

In Case of Injury

We do not anticipate that any harm will occur to you or your child as a result of participation in this study. However, if any physical injury resulting from participation in this research occurs, we will provide you with medical treatment according to the current standard of care in Tanzania

Who to contact

If you ever have questions about this study, you should contact the Principal Investigator

COLMAN EVARIST MCHAU, from Muhimbili University of Health and Allied Sciences, P.O .Box 65001, Dar-es-salaam. If you have any question about your rights as a participant, you may call DR BRUNO SUNGUYA Chairman of the College Research and Publications Committee, P.O. Box 65001, Dar es Salaam. Tel: 2150302-6.

Signature:

Do you agree?

Participant agrees…….Participant does not agree ……

I _____ have read the contents in this form. My questions have been answered. I agree to participate in this study.

Signature of Participant_____

Signature of Research Assistant_____

Date of signed consent _____

Appendix II: Fomu Ya Ridhaa Ya Kujiunga Katika Utafiti

Nakala ya kiswahili

Namba ya utambulisho ☐ ☐ ☐

Ridhaa ya kushiriki kwenye utafiti .

Hujambo!

Ninaitwa COLMAN EVARIST MCHAU mwanafunzi wa shahada ya uzamili katika chuo kikuu cha Afya na Sayansi yaTiba cha Muhimbili.Dares Salaam.

Madhumuni ya Utafiti

Utafiti unalenga kujua kama kuna virusi vya homa ya dengu kwa wachangiaji damu.
Taarifa itakayopatikana kuhusu uwepo wa virusi vya homa ya dengu katika wachangiaji wa damu Dar es salaam utaonyesha ni kiasi gani damu ipo salama dhidi ya maambukizi ya homa ya dengu na taarifa hiyo pia itatumika kwenye uamuzi wa ama kuambatanisha vipimo vya dengu wakati waku wapima wachangiaji damu na hivyo kuzuia maambukizi ya homa ya dengu kwa njia ya kuchangia damu ya sitokee.

Pia taarifa za utafiti huu zitakuwa na faida kwa jamii kuanzia kimkoa mpaka nchi nzima kwa sababu usalama wa damu utakuwa umehakikiwa na wanaowekewa damu watakuwa wanalindwa dhidi ya maambukizi ya homa ya dengu. Hiyo pia itaokoa muda na gharama na athari ambazo zinatokea kwa wagonjwa wa dengu ikiwamo kukosekana kwa nguvu kazi kwa jamii.

Nini kinahitajika ili kushiriki.

Iwapo unakubali kushiriki katika utafiti,utaombwa kujibu maswali katika dodoso utakayoulizwa na pia kutoa kiasi kidogo cha damu ya kwenye mshipa kwa ajili ya uchunguzi wa kimaabara.

Usiri

Taarifa zitakazokusanywa kupitia dodoso hili zitakuwa ni za siri na hakuna mtu yeyote atakaeambiwa ulichosema,taarifa hizi zitaingizwa kwenye kumbukumbu kwa kutumia namba ya utambulisho

Athari

Hakuna athari yoyote itakayoweza kutokea kwa kushiriki kwako katika utafiti huu.

Haki ya kushiriki

Ushiriki wako katika utafiti huu ni wa hiari. Kutoshiriki au kujitoa kutoka kwenye utafiti hakutakuwa na adhabu yeyote na hutapoteza stahili zako, endapo utaona ni vema kufanya hivyo.

Faida

Kama ukikubali kushiriki katika utafiti huu,utanufaika kwa kufanyiwa vipimo vya homa ya dengu ambapo kama utakuwa na maambukizi itapelekea kugundulika mapema na hivyo kupata matibabu mapema. Vilevile utakuwa umeikinga jamii dhidi ya homa ya dengu kwa kupitia kuongezewa damu.

Gharama

Hakuna malipo kutoka kwenu kama ada ya kushiriki katika utafiti huu.

Endapo utapata madhara

Nakuhakikishia kwamba sitarajii kama kuna hatari yeyote yaweza kutokea kwa sababu ya ushiriki wako katika utafiti huu.Pengine unaweza kuhisi maumivu kidogo tu wakati wa kutoa damu kwenye mshipa kwa ajili ya uchunguzi wa kimaabara.

Nani wa kuwasiliana naye

Kama una maswali kuhusiana na utafiti huu , unaweza kuwasiliana na Mtafiti mkuu wa utafiti huu COLMAN EVARIST MCHAUwa Chuo Kikuu cha Afya na Sayansi yaTiba Muhimbili, SLP 65001, Dar es salaam. Kama una swali kuhusu staili zako kama mshiriki unaweza kumpigia, Mwenyekiti wa kamati ya utafiti na uchapishaji Dr BRUNO SUNGUYA, S.L.P 65001, Dar es salaam, Simu 2150302-6

Sahihi:

Je umekubali?

Mshiriki amekubali .....…… Mshiriki hajakubali .....…….....

Mimi_____nimesoma maelezo ya fomu hii na kuielewa.Maswali yangu yamejibiwa.Nakubali kushiriki katika utafiti huu

Sahihi ya mshiriki…….....

Sahihi ya shahidi (Kama Mshiriki hawezi kusoma/kuandika

…….Sahihi ya mtafiti msaidizi……

Appendix III: Questionnaire English Version

TITLE: ASSESSMENT OF SEROPREVALENCE OF DENGUE VIRUS AMONG BLOOD DONORS IN DAR ES SALAAM

Serial number ☐☐☐

Date of interview ☐☐☐☐

Registration number ☐☐☐

Name of Blood donation site…….Phone number ……..

Part A

1. Age…..….yrs

2. Sex…….…….

3. Address……...

4. Marital status…….

5. Residence……

6. Level of education…….

7. Occupation……

8.No. of people in household…….

9. Bed net use……

10. Insecticide spraying at home……

11. Water storage…….

12. Standing water near home……

13. Travel out of Dar es salaam……

14. if yes where……..

15. How long……..

Part B

Laboratory Investigations

S/N	Test	Results
1	NS 1 Antigen	
2	IgM Antibodies	
3	IgG Antibodies	
4	PCR test	

Appendix IV: Dodoso Kwa Lugha Ya Kiswahili

UTAFITI WA MAAMBUKIZI YA HOMA YA DENGU KWA WACHANGIAJI DAMU DAR ES SALAAM

Namba ya fomu ☐☐☐

Tarehe ya usaili ☐☐☐☐

Namba ya mshiriki ☐☐☐

Kituo cha uchangiaji damu…… Namba ya simu ……..

Sehemu A

1. Umri…..…. …… 2. Jinsia…….……

3. Anuwani…….

4. Ndoa……..……

5. Makazi……..... 6. Kiwango cha Elimu……

7. Kazi……..…… 8. Idadi ya waliopo nyumbani…….

9. Matumizi ya neti……

10. Unyunyuziaji dawa ya mbu……..

11. Vyombo vya kuhifadhi maji…….12.Maji yaliyotuama nyumbani……

13. Ulisafiri nje ya Dar es salaam……

14. Ulisafiri kwenda wapi……15. Ulisafiri kwa muda gani…….

Sehemu B

Laboratory Investigations

S/N	Test	Results
1	NS 1 Antigen	
2	IgM Antibodies	
3	IgG Antibodies	
4	PCR Test	