2022-02

Seroprevalence, knowledge, and practices of Dengue and Chikungunya in Dar es salaam and Zanzibar: selected hospital-based cross-sectional study

Shauri, Suleiman

NM-AIST

https://doi.org/10.58694/20.500.12479/2570

SEROPREVALENCE, KNOWLEDGE, AND PRACTICES OF DENGUE AND CHIKUNGUNYA IN DAR ES SALAAM AND ZANZIBAR: SELECTED HOSPITAL-BASED CROSS-SECTIONAL STUDY

Haliya Suleiman Shauri

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Master's in Life Sciences of the Nelson Mandela African Institution of Science and Technology

Arusha, Tanzania

February, 2022

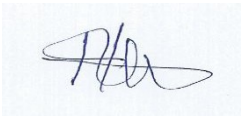
ABSTRACT

The potential shift of significant causes of febrile illnesses from malaria to non-malarial febrile illnesses, including arboviral diseases such as Chikungunya and Dengue, is of great concern. Two cross sectional studies were conducted at Mnazi mmoja in Zanzibar and Temeke hospital in Dar es Salaam Tanzania. The first study assessed the prevalence, knowledge, and practices regarding Chikungunya and Dengue, among individuals attending outpatient departments at Mnazi mmoja in Zanzibar and Temeke hospital in Dar es Salaam Tanzania. The second study involved serological testing of blood samples from the blood bank at Temeke Referral Hospital in Dar es Salaam and the National Blood Bank Unit in Zanzibar was conducted. Seropositive IgM samples from Temeke hospital in Dar es Salaam were 3/101 (2.97%) for Chikungunya and 1/101 (0.9%) for Dengue, while samples from Zanzibar were all IgM negative for both viruses. Chikungunya IgG seropositivity was significantly higher ($p \leq 0.05$) in Temeke hospital in Dar es Salaam 21/101 (21.2%) than Zanzibar 22/180 (12.2%). There was no significant difference in Dengue IgG seropositivity between Temeke hospital in Dar es Salaam 44/101 (43.5%) and Zanzibar 68/180 (37.8%). A total of 332 patients were recruited through a systematic random sampling technique from Zanzibar and Dar es Salaam hospitals. Participants from Dar-es-Salaam had demonstrated lower preventive practices as compared to those from Zanzibar. Only 10.2% of all participants had high knowledge of Dengue and Chikungunya, while only 4.5% were aware of preventive practices. Our results show continuing exposure of Dengue and Chikungunya virus in Tanzania, it associates with low awareness and poor preventive practices. If steps are not taken, may act as a template for big outbreaks when an appropriate condition occurs. Therefore the inclusion of Dengue and Chikungunya in active surveillance program is proposed.

DECLARATION

I, Haliya Shauri, do hereby declare to the Senate of the Nelson Mandela Institution of Science and Technology that this dissertation is my original work and that it has not been, nor will it be presented to any other university for a similar or any other degree award, and is not previously or currently under copyright.

Haliya Shauri



Names and Signature of Candidate Date

The declaration is confirmed by

Prof. Joram Buza

Names and Signature of Supervisor 1 Date

Prof. Sayoki Mfinanga

Names and Signature of Supervisor 2 Date

COPYRIGHT

CERTIFICATION

The undersigned certify that they have read the dissertation titled "Assessment of Seroprevalence, Knowledge, and Practices of Dengue and Chikungunya in Temeke hospital in Dar es Salaam and Zanzibar, a Hospital Base Cross-Sectional Study" and recommend for examination in partial fulfillment of the requirements for the Degree of Master in Life sciences of the Nelson Mandela African Institution of Science and Technology

Prof. Joram Buza

Names and Signature of Supervisor 1 Date

Prof. Sayoki Mfinanga

19/01/2022

Names and Signature of Supervisor 2 Date

ACKNOWLEDGEMENTS

Firstly, I would like to thank the Almighty Allah for giving me the chance to be a part of this programme and bestowed good health during all the time of research and the never-ending blessings that he bestowed on my life.

Secondly, my deepest sense of gratitude goes to my supervisors Prof. Joram Buza (NM-AIST), Prof. Sayoki Mfinanga, Dr. Esther Ngadaya, and Dr. Mbazi Senkoro (NIMRI-Muhimbili), for spending their precious time giving me support and continuous advice and encouragement throughout this research.

I now extend my gratitude to my lovely Parents Ms. Wema Ali and Mr. Suleiman Shauri, and my dear husband, Mr. Sadik Simba, for their support, inspiration, and motivation. They are a living example of working hard to succeed, I couldn't be prouder. Also, I warmly thank Mr. Donatus Mwambete from Muhimbili University, Lab managers from Temeke hospital, Zanzibar Blood Transfusions Services (ZBTS), and Mnazi Mmoja hospital for the time, resources, trust, patience, acceptance, and technical assistance that they provided me.

Not withholding my sincere appreciation to my fellow colleagues in the program for their support and involvement. I thank everyone, including the study participants, who participated from the beginning to the accomplishment of this research. And thanks to all others who helped me blindly and remained there for me when I needed it the most. I can't be more grateful to every one of you. I just want you all to know that I could not have done it without you.

Also, I acknowledg the African Development Bank (AFDB), EDCTP2 grant number RIA 2016E-1609-PANDORA-ID-NET, and EDCTP-RegNet2015-1104 - EACCR2 supported under horizon 2020 (the European Union) and The State of University of Zanzibar (SUZA) for their funding support. I couldn't do this without them. I am very thankful to all lectures and professors at NMAIST for their supportive knowledge and the preparation; they led to this achievement.

DEDICATION

Honestly, I would like to offer my piece of work to my dearest family

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

LIST OF ABBREVIATION AND SYMBOLS

Anti-CHIKV	Anti-Chikungunya Virus
Anti-DENV	Anti-Dengue Virus
APR	Adjusted Prevalence Ratio
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
ELISA	Enzymes Linked Immune Sorbent Assay
KNCHREC	Kibong'oto, Nelson Mandela and Chedah Research Ethical Committee
NSP	Non-Structural Protein
OD	Odds
OR	Odds Ratio
RT-PCR	Real-Time Polymerase Chain Reaction
SD	Standard Deviation
UPR	Unadjusted Prevalence Ratio
WHO	World Health Organization
KAP	Knowledge, Awareness and Preventive practices
MOH & SW	Ministry of Health and Social Welfare

CHAPTER ONE

INTRODUCTION

1.1 Background of the Problem

Dengue and Chikungunya are arboviral diseases that are endemic in tropical and subtropical regions and have been causing sporadic and sometimes large epidemics in humans (Nyamunura *et al.,* 2013 & Salam *et al.,* 2018). The transmission cycle of these diseases begins with a blood meal of *Aedes* Mosquitoes from an infected host (Gubler, 1989). Although environmental causes of the patterns and occurrences of these infections are primarily unidentified (Wolfe *et al.,* 2001), mammals and birds have been reported to serve as hosts or pools for these viruses (Darrigo *et al.,* 2018). Despite being genetically unrelated, Dengue and Chikungunya viruses share similar modes of transmission, same vectors, pathological mechanisms, and clinical presentations (Ciota & Kramer, 2010). These include acute joint pain, high body temperature above 40ºC, pain in muscles and eyes, headache, and backache. Dengue and Chikungunya have become a challenge for health and economic sectors globally, there will be 390 million (95% credible interval 284-528) of dengue infections per year (Bhatt *et al.,* 2013). Many parts of the world, including tropical, sub-tropical countries, and even temperate countries (such as Europe and North America), have been at risk of Chikungunya and Dengue, especially with global warming, unplanned urbanization, and co-circulation of different viruses serotype.

With the declining number of malaria cases, non-malarial febrile illnesses are becoming significant causes of fever (Mweya *et al.,* 2013). In February 2014, about 1017 and 8 confirmed cases of Dengue fever from seven regions in mainland Tanzania and two regions in Zanzibar, respectively, were reported by the Ministry of Health and Social Welfare (MOH & SW) (WHO, 2012). In Zanzibar, many cases of Chikungunya fever have been reported since 2018 though the exact number is unknown. In 2019, Tanzania experienced an outbreak of Dengue fever in its four regions, such that 6873 cases and 13 deaths due to Dengue fever were confirmed. The regions with the most cases were Dar -es- Salaam, Tanga, Dodoma, and Pwani (Mweya *et al.,* 2013). Most of the research on the two diseases in Tanzania has been carried out during outbreaks. However, it is most likely that the two diseases are still prevalent, albeit at lower levels during the inter outbreak periods, because of the presence of the Aedes mosquito vectors and permissible environmental factors in some locations.

Dengue cases associated with blood transfusions and organ transplantations have been reported in some countries (Pozzetto, 2015 & Bianco, 2008). Gasque (2013) reported that during the outbreak

of Arboviral infection in La reunion, blood donations processes were suspended due to fear of transmission. Many Dengue and Chikungunya cases in Tanzania have been reported in different parts of the country (Chipwaza *et al.,* 2014; Mweya *et al.,* 2013; Patrick *et al.,* 2018). However, only one study tried to link the transmission of the two diseases through blood donation (Vairo *et al.,* 2014) and it report detection of Dengue IgG in 50.6% of 500 healthy blood contributors at Zanzibar National blood transfusion services. However, studies are limited that demonstrate evidence of active infection (IgM) or presence of Dengue or Chikungunya viruses in blood banks.

Although the last outbreaks of both Dengue and Chikungunya fevers were reported three years ago in Tanzania, it is not well established whether the diseases disappear after outbreaks or whether they continue to circulate unnoticed. The latter is more likely since the two diseases are slowly becoming endemic in territories they never used to occur. Detection of Dengue and Chikungunya during the inter-outbreak period will alarm the health authorities to include the two diseases in the country surveillance program and disease intervention strategies to prevent future outbreaks. Furthermore, during outbreaks of Dengue and Chikungunya, there are always concerted efforts by the government and other stakeholders to create awareness among communities on the diseases with particular emphasis on how to reduce risks of infection.

These awareness campaigns also enhance the suspicion index among medical personnel for the diseases when they encounter febrile patients. However, with time after outbreaks, the awareness campaigns decrease and eventually disappear altogether, which may result in laxity in communities and reduced suspicion index among medical personnel. This may allow infection and disease propagation with little or no detection with subsequent negative consequences. Some of the consequences may be the maintenance of a pool of virus-infected mosquitoes, which may later become the platform for huge outbreaks when appropriate conditions such as heavy rains occur. Furthermore, because of lack of suspicion, blood donated in hospitals may come from infected asymptomatic individuals and be used for transfusion without screening, leading to unsuspecting recipients' infection. Therefore, the burden of Dengue and Chikungunya must be ascertained, particularly during the inter-outbreak periods, so that appropriate steps are undertaken.

The purpose of this study was to establish the current status of Dengue and Chikungunya in Dar-es-salaam and Zanzibar, three years after the last reported outbreaks, focusing on blood donated in blood banks to investigate the seroprevalence, knowledge, attitude, and practices of the blood donors, and out-patients with febrile illness presenting at Temeke Referral Hospital in Dar es Salaam, the National Blood Bank Unit and Mnazi Mmoja hospital in Zanzibar because of lack of suspicion, blood donated in hospitals may come from infected asymptomatic individuals and be

used for transfusion without screening, leading to unsuspecting recipients' infection (Amarilla *et al.,* 2012).

1.2 Statement of the Problem

Dengue and Chikungunya fevers have been recurring in Tanzania for years though their epidemiological determinants for these diseases are essentially still unknown (Kajeguka *et al.,* 2017a). There is usually enhanced research and public awareness activities during outbreaks, but these activities wane off after outbreaks. Furthermore, the suspicion index of Dengue and Chikungunya among medical practitioners' decreases during the inter-outbreak periods leading to mistaken diagnosis with malaria and overlooking the need for screening blood donated in blood banks. Therefore, there is a need to establish the seroplevalence and understanding awareness among society of the two diseases during the inter-outbreak period to avert possible negative consequences in the future.

1.3 Rationale of the Study

Establishing the Dengue and Chikungunya disease current burden, and Knowledge, Awareness and Preventive practices (KAP) during the inter- outbreak period will alert health authorities to include the two diseases in surveillance and intervention programs, including screening of blood donors to make sure the donated blood is free from these arboviral infections/diseases.

1.4 Research Objectives

1.4.1 General Objectives

To determine the seroprevalence of antibodies for Dengue and Chikungunya among blood donors, and knowledge and prevention practices toward Dengue and Chikungunya virus infections among febrile patients at Mnazi Mmoja and Temeke hospitals.

1.4.2 Specific Objectives

(i) To determine the seroprevalence of antibodies for Dengue and Chikungunya in the selected blood samples in Temeke referral hospital and Zanzibar national blood bank.

(ii) To determine awareness of knowledge and prevention practices regarding Dengue and Chikungunya infections among outpatients in selected health facilities in Dar es Salaam and Zanzibar.

1.5 Research Questions

(i) What is the seroprevalence of Dengue and Chikungunya among blood donors in Dar-es-salaam and Zanzibar three years after the last reported outbreak?

(ii) What is the knowledge and practices regarding Dengue and Chikungunya among febrile patients attending outpatient departments in Dar-es-salaam and Zanzibar three years after the reported outbreak?

1.6 Significance of the Study

Information obtained from this study will be useful in many ways; establishment of the presence of infections, three years after the reported outbreak, will help alert the health authorities to the fact that Dengue and Chikungunya are becoming endemic in Tanzania and therefore be accorded appropriate procedures; which include incorporating the two viral infections into the national surveillance program, and raising public awareness on prevention and control, and vigilance by medical personnel in diagnosing the two viral diseases. Evidence of possible transmission of Dengue and Chikungunya through blood transmission will lead to policies and procedures for screening blood donors before transmission.

1.7 Delineation of the Study

This study was carried out to understand the seroprevalence of antibodies against Chikungunya and Dengue fever and perceived knowledge and prevention practices toward preventing and controlling the two viral infections. The methodological approaches used, include collection of blood samples from Temeke referral hospital in Temeke district, Dar es Salaam and Zanzibar national blood bank and Zanzibar archipelago. In this study febrile outpatients seeking health services at Temeke and Mnazi mmoja hospitals were interviewed.

CHAPTER TWO

LITERATURE REVIEW

2.1 Disease and Etiology

2.1.1 Chikungunya

Chikungunya is a mosquito-borne viral infection transmitted to humans through biting mosquitoes belonging to the genus Aedes (Chhabra *et al.,* 2008). The virus responsible for causing Chikungunya fever belongs to the genus Alphavirus and the family Togaviridae (Issac *et al.,* 2014). The word Chikungunya originated from the Makonde tribe in the Southern part of Tanzania, it means the bending position due to joint pain experienced by people suffering from Chikungunya fever (Robinson, 1955). Aedes species serve as an important vector for Chikungunya fever and Dengue fever (Kraemer *et al.,* 2015).

2.1.2 Dengue

Dengue is a mosquito-borne viral infection similar to Chikungunya, and is as well transmitted through biting by mosquitoes belonging to genus Aedes (Kraemer *et al.,* 2015). The virus responsible for causing Dengue fever belongs to the Flaviviridae family (Gubler, 1997). The word Dengue has its root in the Kiswahili language; ka- dinga pepo, which was thought to be caused by evil spirits (Srinivas & Srinivas, 2015).

2.2 Mode of Transmission and Associated Risk Factors

The important transmission mode of Chikungunya and Dengue is through biting by Aedes mosquitoes (Kraemer *et al.,* 2016). The transmission occurs when the mosquito feeds on the blood meal from an infected individual. During viremia, the virus migrates to the mosquito's salivary gland, and when the mosquito bites another individual, the virus gets transmitted to that individual. The dynamics of transmission is associated with various factors such as climatic and environmental factors, the interaction between human and pathogen, and the interaction between host and pathogen (WHO, 2009). For Dengue and Chikungunya, the areas with heavy rains followed by flooding, high temperature, and coastal regions are most favorable for vector breeding sites, enhancing circulation and survival. It is notably challenging to avoid mosquito bites and control mosquito populations, especially in tropical climates (Rana & Lunia, 2015). Non-vector transmission of arbovirus has been reported by vertical transmission, sexual transmission, blood transfusion, and in nosocomial settings (Lenglet *et al.,* 2006). *Aedes aegypti* and *A. albopictus* are

the main Zika, Chikungunya, and Dengue, but most Aedes species are likely vectors in Africa and Asia (Gubler, 1989). The *A. aegypti*, which live very near to humans, are the most capable vectors for the transmission of these infections, and they primarily feed on humans with more bites in a single meal and have particular unnoticeable bites. The *A. albopictus* is well distributed in subtropical and temperate regions. The *A. albopictus* is very aggressive and resilient and can survive in both rural and urban environments for a fairly long-life span of about 4-6 weeks. In addition, the mosquito can survive in cold winters (Wolfe *et al.,* 2001). Both common vectors have activities that peak during the day, hindering the use of treated insecticide nets at night (Kay *et al.,* 2002).

2.3 Clinical Signs

2.3.1 Clinical Signs of Chikungunya

Chikungunya is associated with a sudden onset of illness and pain in joints (Strauss). Generally, the symptoms start within four to seven days after being bitten by infected mosquitoes. In the critical stage, Chikungunya's symptoms range from mild to severe. The symptoms are high fever (38.5^0-40^{o}C), skin rashes, headache, eyes pain, weakness, and nausea (Burt *et al.,* 2012). A Chikungunya disease at the first stage is associated with high peaks of viremia (Sourisseau). Generally, the symptoms and their occurrence may vary from one patient to another (Munasinghe *et al.,* 1966). Normally, patients recover fully, yet joint pain can persist for many years, thus the reason for high morbidity (Singh *et al.,* 2012). Also, some complications have been reported, such as neurological and heart problems (Staikowsky *et al.,* 2006). The disease sometimes can be one of the courses of death in older people (Clark *et al.,* 2018).

2.3.2 Dengue Clinical Signs

Primary symptoms of Dengue include High body temperature (38.8-40.5^{o}C), pain on the front of the head, pain behind the eyes, maculopapular rash, arthralgia, myalgia, nausea, and vomiting (Guzmán *et al.,* 2002). Usually, the symptoms end within two to seven days (Nimmannitya *et al.,* 1987). A form of severe Dengue is more dangerous and associated with plasma leakage, fluid accumulation, severe bleeding, and organ failure (Almas *et al.,* 2010). At the clinical stage, it is difficult to differentiate Dengue fever from other fever-like diseases. Also, the assessment of whether the disease is in extreme or non-serious form is difficult.

2.4 Epidemiology

2.4.1 Chikungunya Epidemiology

Chikungunya is a global problem with the regular expansion of outbreaks around the globe. Recent reports from Centers for Disease Control and Prevention (CDC) show that Chikungunya outbreaks have spread in all parts of the world, including Asia, the Pacific, and Europe (Kendrick *et al.,* 2014). In Africa, Chikungunya was first reported in Tanzania in 1952 and then in Central, Southern, and Western African countries (Wahid *et al.,* 2017). In Eastern Africa, regular outbreaks have been reported in Lamu, Kenya. In 2004 more than half-million cases were reported, and seroprevalence of antibodies against the virus of 70% was recorded (Sergon *et al.,* 2008). Seroprevalence of antibodies against the virus of 75% was later reported in 2005 due to migration of natives between Lamu-Kenya and islands such as Mauritius, Seychelles, Comoros, and Réunion Island of Indian ocean (Wahid *et al.,* 2017).

2.4.2 Epidemiology of Dengue

It is well known that the burden of Dengue has increased drastically in recent years (Murray *et al.,* 2019) and is widespread in over 132 countries (Hammond *et al.,* 2005). Earlier in 1970, it was in a few countries, but the number has increased more than fourfold (Murray *et al.,* 2013). It has been reported that in 2001 alone, more than 609 000 were new cases, out of which approximately 15 000 were severe Dengue cases (Cavalcanti *et al.,* 2011). In 2012, the Dengue outbreak occurred in Madeira island in Europe and spread into interior parts of Portugal (Gasparetti *et al.,* 2007). In 2006, India was reported to have the worst outbreak, with about 15 000 cases of Dengue in Delhi (Gupta *et al.,* 2012). Dengue outbreak has also been reported in over 22 countries in Africa (Oyero *et al.,* 2014).

2.5 Chikungunya and Dengue Outbreaks in Tanzania

Tanzania has experienced many cases of Dengue outbreaks in the last three years (Mweya *et al.,* 2013). As the prevalence of malaria is decreasing, many cases of non-malaria febrile illnesses are increasing (Mtove *et al.,* 2011). In recent years, many cases of Dengue and Chikungunya have been reported in different parts of Tanzania (Kajeguka *et al.,* 2016). According to WHO report (2014), Dengue outbreak was confirmed in seven regions of Tanzania mainland (Dar es Salaam, Tanga, Singida, Dodoma, Pwani, Iringa, Morogoro) and most regions of Zanzibar Island, 1018 dengue cases were confirmed, respectively (Ward *et al.,* 2017). Again in 2019, Tanzania was reported to have an outbreak of Dengue in four regions of Tanzania, such as Dar es Salaam, Tanga Dodoma,

and Singida, with about 1222 cases confirmed and about 39 people died (Chipwaza *et al.*, 2021). Chikungunya fever was reported in different study but not an outbreaks (Mboya *et al.,* 2020; Patrick *et al.,* 2018). Since both dengue and chikungunya are haboured in the same vactors (Saleh *et al.,* 2018), there is high chance of outbreak of chikungunya to happen in future.

2.6 Laboratory Diagnosis

2.6.1 Dengue

The main diagnostic test for Dengue is serological (Ooi & Gubler, 2011). Most use enzymes linked immune Sorbent assay (ELISA), hemagglutination inhibition test, and neutralization test. However, the precise one, measures the Non Structural protein (NSP) 1 antigen normally high in the first stage of infection with any serotype of Dengue using the ELISA technique (WHO, 2009). Real-time Polymerase Chain Reaction is the only technique that can detect the virus during the viremic phase (Faria *et al.,* 2017). The viral culture is the standard test and can be performed using a cell from monkey kidneys or newborn mice (WHO, 2009). Serologic methods are most commonly employed, especially IgM capture ELISA (Wilson & Chen, 2010). Serologic confirmation of infection requires demonstration of a fourfold rise in antibody titer between acute and convalescent-phase sera or by demonstration of Immunoglobulin M (IgM) antibodies specific for the virus (Padhi *et al.,* 2011). Expectedly, after disease onset, IgM antibodies are detectable in 50% by days 3–5, by day five are detectable in 80%, and 99% by ten after initial symptoms. Also, IgM antibodies may persist for months. Hence are reliable markers of recent but not necessarily acute infection. The IgG antibody response develops a few days after the onset of IgM antibodies, and IgG may persist for many years (Anastacio *et al.,* 2012).

2.6.2 Chikungunya

Chikungunya can be diagnosed by serology, virus isolation, or nucleic acid amplification depending on the timing of the patient's blood specimen with the onset of symptoms. Convalescent sera may be tested for IgG/IgM by ELISA, hemagglutination inhibition, or neutralization tests (Hasebe *et al.,* 2002). The viral culture is the standard test and can be performed using a cell from monkey kidneys or newborn mice (Gasque, 2013). Using Real-Time Polymerase Chain Reaction (RT-PCR) also is the right choice in the determination of the virus (Hasebe *et al.,* 2002).

2.7 Treatment

2.7.1 Chikungunya and Dengue Treatment

No available treatment for Chikungunya (Gallian *et al.,* 2017). What is done is to give patients anti-pyretic optimal analgesic and fluids with more rest to alleviate the pain (Gasque, 2013). Treatment for Dengue infection is supportive; no effective antiviral agents are used to treat Dengue. The most important issue is the maintenance of body fluid (Jahan, 2011). Currently, there is an approved Dengue vaccine that has been in use in about twenty countries (Ferguson *et al.,* 2016).

2.8 Knowledge and Practices Regarding Dengue and Chikungunya

Preventive and control measures of the disease depend on the knowledge, attitude, and practices of that population in relation to the particular disease (Alves *et al.,* 2016). Understanding of community, in particular of the awareness of febrile illnesses, is of much importance, especially in control and management of such illnesses (Chipwaza *et al.,* 2014). Meanwhile, over-treatment with either antimalaria or antibiotics without any evidence of the present pathogens could lead to drug resistance, and other treatment costs (Rafique *et al.,* 2015). Knowledge, attitude, and practices are widely used in enhancing people understating of the nature of various diseases, which in turn result in changing practices, attitudes, and behaviors to minimize diseases burden (Kajeguka *et al.,* 2017). Regardless of their importance, only a few studies have been carried out on awareness of febrile illnesses in Tanzania (Chipwaza *et al.,* 2014; Kajeguka *et al.,* 2017). Again, those studies demonstrated insufficient knowledge regarding these arboviral diseases (Alves *et al.,* 2016; Itrat *et al.,* 2008). One study conducted in Tanzania, investigating the knowledge and practices regarding Chikungunya and Dengue fever to community and health care workers revealed that; among community members who took part in the study, 15.2% had good knowledge about Dengue, while of all community members only 3.2% had heard about Chikungunya (Kajeguka *et al.,* 2017). Insufficient knowledge of these diseases among the societal members may lead to misinterpretation with other common febrile illnesses and finally adverse health problems (Kajeguka *et al.,* 2017).

2.9 Current Knowledge Gap for Dengue and Chikungunya in Tanzania

Although Tanzania has experienced a number of Dengue and Chikunganya outbreaks, the epidemiological determinants for these diseases, such as hotspots, related socioeconomic and demographic factors, cultures, co-infection, prevalence, prevalence to diseases, incidence rate, attack rate, mortality rate strain variability and associated risk factors of the two diseases are largely still unknown at national level (Kajeguka *et al.,* 2017a). Furthermore, most reports on Dengue and

Chikungunya in Tanzania are associated with outbreak periods. However, as has been happening in new areas across the world where the diseases are extending, the diseases are becoming endemic. It is now about three years since the last outbreak of the two diseases were reported, but there is currently little information in Tanzania detailing the disease state during the -post-outbreak period, particularly prevalence, risk factors, areas where virus-infected mosquitoes are concentrated, knowledge, awareness and practice of medical personnel and the general public which can perpetuate the disease such as failure to screen blood before transfusion. The current study will attempt to shed light on the status of Dengue and Chikungunya during the post-outbreak period in Tanzania.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site and Study Design

3.1.1 Study Site

This study was conducted in Temeke and Mnazi Mmoja hospitals located in Dar es Salaam and Zanzibar, respectively. Mnazi Mmoja is located in the archipelago of Zanzibar, which is a semi-autonomous region of Tanzania, situated in the Indian Ocean off the east coast of mainland Tanzania. The annual rainfall in Zanzibar is about 1600 mm in Unguja Island and 1900 mm in Pemba Island. Annual temperatures are high throughout the year. The daily temperature ranges from 29ºC to 33ºC. Mnazi Mmoja hospital is the only referral hospital in the Island where many people from different sites come to seek medical treatment, therefore by selecting Mnazi Mmoja hospital as study site it of important since people from all part of Zanzibar could be recruited in sample hence will be representative sample.

Temeke Hospital is located in Dar es Salaam, which is among the coastal regions of Tanzania which lie 16 m above sea level with an average temperature of 26.1ºC/ 79.1ºF and annual precipitation of 1150 mm. During previously outbreaks many dengue cases were reported from Temeke hospital there selecting as study site probable will give insight of previously outbreak (According to personal communication).

Figure 1: Map that shows the geographical location of the study sites (Dar es Salaam and Zanzibar)

3.1.2 Study Design

This is a cross-sectional, hospital-based study design through which the analysis of Dengue and Chikungunya seroprevalence, their associated risk factors together with assessment of KAP were carried out.

3.2 Objective One: Seroprevalence of Antibodies against Dengue and Chikungunya in Blood Banks in Dar-es-Salaam and Zanzibar three years after Last Reported Outbreak

3.2.1 Sample Size

Based on previous publications in Tanzania, both Dengue and Chikungunya seroprevalence are 15.5% hospital-based (Kajeguka *et al.,* 2017). To estimate the sample size, the following formula as described by (Arya *et al.,* 2012) was used.

$$n = \frac{z2p\ (1-p)}{d^2}$$

N= Minimum sample size

Z= Standard normal deviate

P= prevalence as seen from previous studies

D=allowed margin of error

Sample Size

$$n = \frac{(1.96^2)0.155\ (1-0.155)}{0.05^2} = 201.26$$

With 10% addition led the sample size to reach 221 blood samples. The addition of 10% is to allow adjustment of other factors such as withdrawals and missing data which may reduce the sample size.

3.2.2 Blood Sample Collection

The blood samples were retrieved from the blood bank in Temeke referral hospital and Zanzibar national blood bank. The blood samples used under this study were collected from donors between the 5th and 6th of May, 2020. About 3 mL of blood sample was taken from the stored blood bank and then put into a plain vacutainer tube. Then, the plain vacutainer tubes with 3 mL of blood were

centrifuged for 5 minutes at 5000 rpm to get the clear serum. About 1.5 mL of the serum was aliquoted into the cryo-vial tube and stored under 2-4ºC in the cool box and transported to Muhimbili National Hospital in microbiology laboratory where they were stored under -20ºC until when were analyzed using ELISA.

3.2.3 Enzyme-Linked Immunosorbents Techniques for Determination of Dengue and Chikungunya IgM and IgG antibodies

All anti-Dengue and anti-Chikungunya were detected using indirect Enzyme Linked Immunosorbent assays ELISA (Euro immune). The indirect ELISA technique is two-step process that involves two binding processes of primary and labelled secondary antibodies. All samples were diluted in a ratio of 1 to 1000 with the sample diluent provided with the kits. The sample diluent from the kit was pre-coated with the detection antibodies. Thus after being added to the blood sample, a complex with antigen was formed. The presence of a specific antibody that binds to antigen was done by a secondary labelled antibody that was linked to an enzyme through bio conjugation. Between each step, the plate was washed to remove the unbounded antibodies. After the final wash step, the enzymatic substrate was added to produce a visible signal, and then the intensity was measured using a spectrophotometer at the wavelength of 540 nm. Briefly, for the Anti-Dengue IgM/IgG and IgM anti-Chikungunya ELISAs, the diagnostic cutoff value was calculated as the average OD of negative controls + 0.300. For the IgG Chikungunya ELISA, the threshold for positivity was based on the OD cutoff value of the cutoff control + 10 %.

3.2.4 Statistical Analysis

All data were collected /extracted from the computer system by entering the sample codes to the computer enabling donor's information to be extracted and recorded in a notebook. The following variables were extracted: Sex, age, occupation, and marital status. The retrieved variables were entered and validated into an excel sheet, then re-grouped and analyzed using Stata version 15. During analysis, the χ^2 test with 95% confidence interval was performed to compare the results of categorical data, the prevalence of Dengue and Chikungunya together with co-infection among the same individuals. The association between seroprevalence with demographic variables was done using simple logistic regression. The odds ratio (OR) with 95% confidence intervals were as well estimated. Differences were considered to be statistically significant if P value was ≤ 0.05.

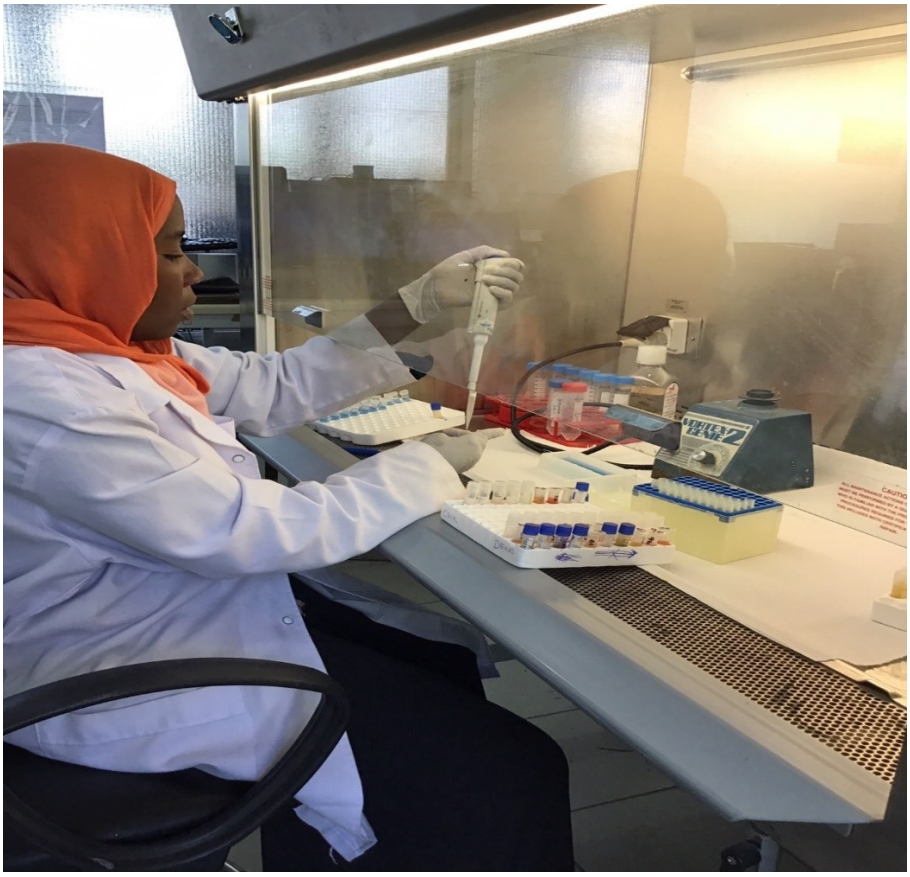


Plate 1: **Mixing sample with sample diluent**

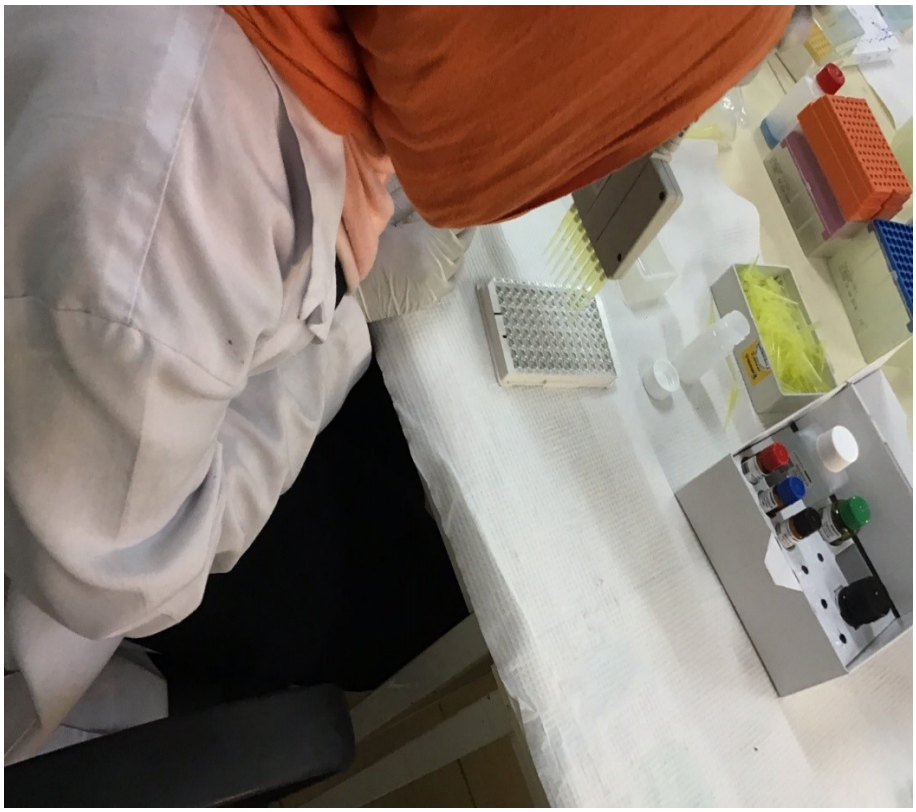


Plate 2: **Addition of enzymatic substrate in the microtiter plate**

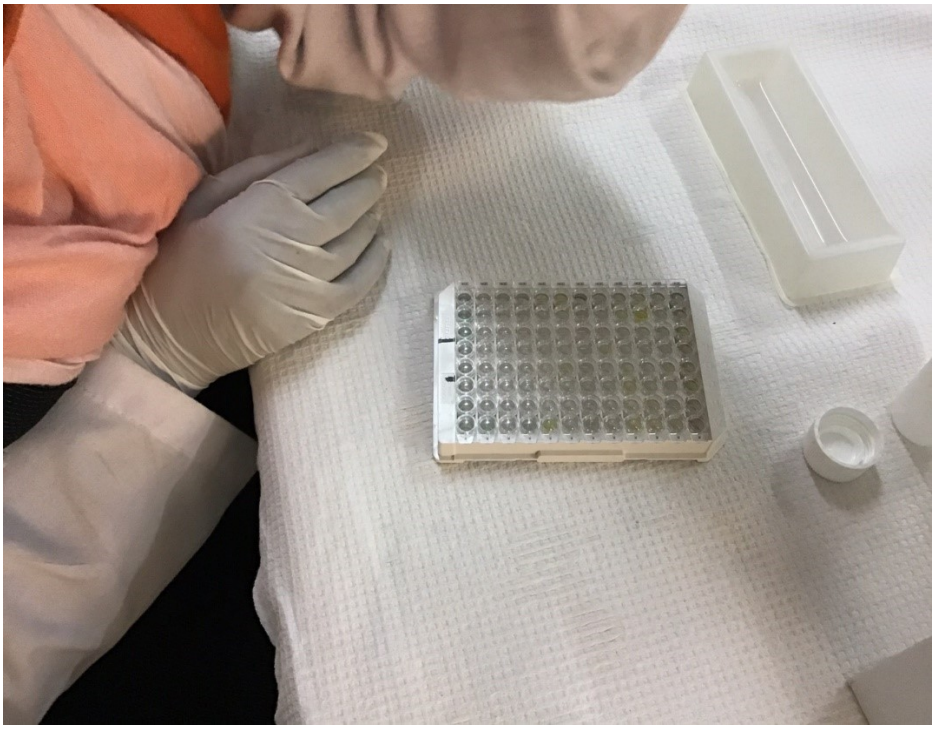


Plate 3: Microtiter plate with some positive reaction of ELISA test

3.3 Objective two: Determination of Knowledge and Practices Regarding Dengue and Chikungunya among Outpatients at Temeke Hospital in Dar es Salaam and Mnazi Mmoja, Zanzibar

3.3.1 Sample Size

Based on previous publications in Tanzania, Dengue knowledge and practice among community members was 15.2%, while Chikungunya was 3.2% (Kajeguka *et al.,* 2017). To estimate the sample size, the following formula as described by Arya *et al.* (2012) was used.

$$n = \frac{z2p\ (1-p)}{d^2}$$

N= Minimum sample size

Z= Standard normal deviate

P= prevalence as seen from previous studies

D=allowed margin of error

Sample Size

$$n = \frac{(1.96^2)0.152\ (1-0.152)}{0.05^2} = 198$$

To maximize the sample size, we add samples to 330 for both areas.

3.3.2 Data Collection Procedure

Systematic random sampling was used to select participants for the study. About 330 were included, of which 166 were from Temeke Referral hospital and 166 were from Mnazi mmoja referral Hospital. Before taking part in the study, participants above 18 years old were informed about the study objectives and were invited to participate voluntarily. In addition, parents/guardians were asked to assist them, specifically those under the custody of parents/guardians.

3.3.3 Selection Criteria and Participants' Recruitment

For patients to be included in the study, they were supposed to attend the outpatient department on that particular day and seek medical care. The Criteria used to recruit study participants included an age range from 1 to >50 years. Any patient who was observed to have an adverse health condition and needed immediate care and who did not provide the consent was excluded.

Participants were recruited by the time they came to the hospital at the registry desk. The researcher gave an introduction and aims of the study before asking participants for recruitment. Systematic random selection was done for those patients who were ready to participate. This was done by randomly picking one individual from the registry as number one and then proceeded to select every third individual by jumping two individuals from the registry list. In addition, written consent forms were given to randomly selected participants to read, and for those who were unable to read, the research read before them. After understanding and being satisfied with what was in the consent forms, they were asked to sign them. Both questionnaires and interviews were used to assess the knowledge and practices regarding Dengue and Chikungunya fever experiences. From health facilities, also some of the information of stored samples was retrieved. These included: Age, sex, occupation and marital status which were used to assess risk factors.

3.3.4 Knowledge and Practice Assessment

Self-administered questionnaires constructed in Swahili language were used to assess participants' information based on socio-demographic and knowledge in awareness and practices regarding Dengue and Chikungunya fever. The questions were intended to assess individual knowledge (on disease patterns, causative agents, sign and symptoms), associated risk factors (time of day) and preventive measure (mosquito net, repellents, remove water receptacles that keeps water during the rain). After completion, all questionnaires were checked for completeness. Modified Bloom's cut-off points were used for the assessment of knowledge with few modifications. The knowledge score

was calculated by adding all positive responses of items. Each item was assigned 10 for a positive response and 0 for a negative response and then all items were included by applying a 100 points scale. The total knowledge scores went from 0 to 100. The scores <50 were considered being inadequate while scores ≥ 50 were considered being adequate.

3.3.5 Data Management and Statistical Analysis

(i) Data Management

The plan for data management was as follows: Well-trained and qualified staffs were involved during samples collection, processing and analysis. Complete documentation of all processes and protocols with proper language together with clearly defined procedures were done. Frequency cross-checking of each tool used during the study was done to ensure no unauthorized changes to the data. All of the records were stored securely and confidentially.

(ii) Statistical Analysis

All completed questionnaires were double-checked and verified for completeness and consistency. The dependent variables were knowledge and practices while independent variables were age, sex, education level, employment status, marital status, and economic status. Descriptive statistic was used to present socio-economic information. The main outcome variables were levels of knowledge and Practices while the Independent variables were Gender, Age, educational level and marital status, employment status, and income level. All categorical variables were cross-tabulated and calculated using the chi-square test. The association between levels of knowledge with socio-economic demographic variables was done using logistic regression, and the prevalence ratio with 95% confidence intervals was estimated. Knowledge differences were considered to be statistically significant if P was ≤ 0.05, and if the 95% confidence did not include one cross-tabulation. For multivariate analysis, an unadjustable prevalence ratio and adjusted prevalence of more than 1 were considered to be significant. All data analysis was done using STATA v. 15.

3.4 Ethics Approval and Participants Consent

The study protocol was approved by the Northern Zone Ethical Research Committee Kibong'oto, Nelson Mandela and Chedah Research Ethical Committee with Approval Research Proposal Number KNCHREC0019. Additional ethical approval was obtained from Zanzibar Health Research Ethical Committee. The study objectives were being explained to the participants and finally, they were asked to sign the informed consent forms. Additional permission from the respective hospital was sought before beginning study procedures. Informed consent was sought

from each participant, and for participants who were less than 18 years old, Informed consent was sought from a parent or caregiver.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Results for Seroprevalence of Dengue and Chikungunya among Blood Donors in Temeke Hospital in Dar es Salaam and Mnazi Mmoja in Zanzibar

(i) Descriptive Demographic Characteristics of Blood Donors of the Study Samples

A total of 281 blood samples were tested, of which 180 (64%) were from Mnazi Mmoja in Zanzibar, and 101 (35.9%) were from Dar es Salam. However, only demographic information of blood donors' samples was retrieved from Mnazi Mmoja in Zanzibar. Out of 180 samples from Mnazi Mmoja in Zanzibar, 171 (95%) blood samples were from male donors. About 106(58.9%) were in the age of < 30 years with a mean (SD) age of 37 (12.97) years. About 91 (50.6%) were unemployed people. The demographic characteristics of Mnazi Mmoja in Zanzibar donors are shown in Table 1.

Table 1: Demographic characteristics of the blood donors of study Samples from Mnazi Mmoja in Zanzibar

Variables	Category	Frequency (Percentage) N= 180
Age	19 to 30	106(58.9)
	31 to 40	35(19.4)
	41 to 61	39(21.7)
Mean age years (SD)	37 (12.97)	
Sex	Male	171(95.0)
	Female	9(5.0)
Marital status	Married	84(46.7)
	Divorced	4(2.2)
	Single	92(51.1)
Occupation	Work	89(49.4)
	Not work	91(50.6)

(ii) Seroprevalence of antibodies against Dengue and Chikungunya in Zanzibar and Dar-es-salaam

The Dengue and Chikungunya prevalence are summarized in Table 2. Dengue IgG seropositivity was detected n 43.6% (44 /101) and 37.8% (68/180) of samples from Dar-es-salaam and Zanzibar, respectively, but prevalence was not significantly different. However, the Chikungunya IgG prevalence of 21/101 (20.8%) of samples from Temeke hospital in Dar-es-salaam was significantly (*p*-value = 0.047) than the prevalence of 22/180 (12.2%) of samples from Mnazi Mmoja in Zanzibar. Neither Dengue nor Chikungunya seropositive IgM was observed in Mnazi Mmoja in Zanzibar. In contrast, both Chikungunya IgM 3/101(2.97%) and Dengue IgM 1/101(0.9%) were detected in samples from Temeke hospital in Dar-es-salaam. Chikungunya and Dengue co-infection was demonstrated by IgG prevalence of 13/101(12.9 %) for samples from Temeke hospital in Dar-es-salaam and 11/180 (6.1%) in Mnazi Mmoja in Zanzibar. However, the prevalence was not statistically different (*p*-value = 0.052).

Table 2: Seroprevalence of antibodies for Dengue and Chikungunya in Mnazi Mmoja in Zanzibar (N=180) and Dar-es-salaam (N=101)

Test	Location	Positive n (%)	Confidence intervals	P-value[a]
Dengue IgG	Mnazi Mmoja, Zanzibar	68(37.8)	31.0-45.0	0.342
	Temeke, Dar-es-salaam	44(43.5)	34.0-53.0	
Chikungunya IgG	Mnazi Mmoja Zanzibar	22(12.2)	7.0—17	0.047*
	Temeke Dar-es-salaam	21(21.2)	13-29	
Dengue IgM	Mnazi Mmoja, Zanzibar	0(0)	NA	NA
	Temeke Dar-es-salaam	1(0.9)	-1.0-3.0	
Chikungunya IgM	Mnazi Mmoja, Zanzibar	0(0)	NA	NA
	Temeke, Dar-es-salaam	3(2.97)	-1.0-3.0	
Co-infection IgG	Mnazi Mmoja,Zanzibar	11(6.1)	3.0-10	0.052
	Temeke, Dar-es-salaam	13(12.9)	6.0-20	

a = Chi-square test comparing prevalence between Zanzibar and Dar-es-salaam
*P<0.05, **P<0.01, ***P<0.001.

4.1.2 Results for Knowledge and Practices Regarding Dengue and Chikungunya Infection among the Individual Attending Outpatient Department Mnazi Mmoja in Zanzibar and Temeke Hospital in Dar es Salaam in Tanzania

(i) Characteristic of the Knowledge, Awareness and Preventive Practices Study Participants

A total number of participants was 332 of which 166 (50%) were from Tanzania's main land and 166 (50%) were from Mnazi Mmoja in the Zanzibar archipelago. Out of the total participants, 177 (53.3%) were females. Their mean age was 34.2 with a standard deviation of 0.8 years. The majority 202 (60.9%) had secondary education and above 174 (52.4%) were married. About 73 (22%) of

the participants were housewives followed by 68 (20.5%) who were entrepreneurs (Business class) and the least were fishermen 4 (1.2%). Among all participants, 163 (49.1%) were in a medium class of economic status (Table 4).

Table 3: Socio-economic demographic characteristics of study respondents N=332 (%)

Variables		n (%)
Sex	Male	155(46.7)
	Female	177(53.3)
Age	<18	23(6.9)
	18-30	164(49.4)
	>30	145(43.7)
Mean (SD)		32.4(0.8)
Education	No education	31(9.3)
	Primary	99(29.8)
	Secondary and above	202(60.9)
Marital status	Married	174(52.4)
	Single	130(39.2)
	Divorced	11(3.3)
	Widow	14(4.2)
	Cohabiting	3(0.9)
Occupation	Employed	46(13.9)
	Self-employed	48(14.5)
	Fishing	4(1.2)
	Business	68(20.5)
	Farmer	22(6.6)
	Student	55(16.6)
	Housewife	73(22.0)
	Others	16(4.8)
Economic status	Low	111(33.4)
	Medium	163(49.1)
	High	58(17.5)

(ii) Current Illnesses

Participants were asked whether the signs and symptoms they had resembled to those of Dengue and Chikungunya. About 157 (47.3%) had a fever, 82 (24.7%) had muscles pain, again about 157 (47.3%) had a headache and 135 (40.7%) had joint pain (Table 5).

Table 4: Current illness N=332

Variables	n (%)
Fever	
Yes	157(47.3)
No	175(52.7)
Muscles pain	
Yes	82(24.7)
No	250(75.3)
Joint pain	
Yes	135(40.7)
No	197(59.3)
Back pain	
Yes	94(28.3)
No	238(71.7)
Rashes	
Yes	24(7.2)
No	308(92.8)
Stomach pain	
Yes	142(42.8)
No	190(57.2)
Headache	
Yes	157(47.3)
No	175(52.7)
Swelling of Joint	
Yes	42(12.7)
No	290(87.3)
Vomiting/Nausea	
Yes	63(19.0)
No	269(81.)

(iii) Knowledge and Practices Regarding Dengue and Chikungunya among Outpatients Participated in the Study

Of all participants in three quarters 252 (75.9%) had heard of Dengue while only 102 (30.7%) had heard Chikungunya. About 73 (28.5%) had heard through radio followed by 63 (24.6%) who had heard through magazines. Most participants recognized fever as a symptom of Dengue and Chikungunya, followed by joint pain 62 (18.7%) and headache 60 (18.1%). About 10 (3%) recognized bleeding as a symptom of Dengue and Chikungunya. It was found that 159 (47.9%) knew mosquitoes were the transmitting vectors but didn't know the mode of transmission. Only 88 (26.5%) knew that the mosquitoes transmit Dengue and Chikungunya are day biters. Few participants 16 (4.8%) had mentioned car tires to be breeding site for mosquitoes while the majority 125 (37.6%) didn't know the breeding sites of the mosquitoes. Regarding preventive practices about 79 (23.8%) used bed net while only 9 (2.9%) had window screen (Table 6).

Overall, only 10.2% of all participants had good knowledge of Dengue and Chikungunya while 89.8 had insufficient knowledge. In preventive practices, only 4.5% of all participants had good preventive practices and 95.5% had poor practices regarding Dengue and Chikungunya (Fig. 1).

Table 5: Awareness, Knowledge and Practices regarding Dengue and Chikungunya among outpatients in Mnazi Mmoja in Zanzibar and Temeke hospital N=332 (%)

Variables	n (%)
Have you heard about Dengue fever?	
Yes	252(75.9)
No	80(24.1)
Have you heard about Chikungunya fever?	
Yes	102(30.7)
No	230(69.3)
If Yes where have you heard (n=256)	
Radio	72(28.5)
T. V	45(17.6)
Health facility	24(9.4)
Family member	33(12.9)
Neighbors	11(4.3)
Magazine	63(24.6)
I don't know	7(2.7)
Sign and Symptom	
Fever	105(31.6)
Headache	60(18.1)
Joint pain	62(18.7)
Nausea/Vomiting	24(7.2)
Bleeding	10(3.0)
Mode of Transmission	
Mosquitos (true is mosquitos)	159(47.9)
Flies	2(0.6)
Air	12(3.6)
I don't know	159(47.9)
Mosquitos biting behavior	
Afternoon (true is Afternoon)	88(26.5)
Evening	19(5.7)
Night	41(12.4)
Morning	7(2.1)
I don't know	177(53.3)
Breeding site	
Used car tires (true answer)	16(4.8)
Clear water in bucket/tank (true answer)	31(9.3)
Dirty water	101(30.4)
Waste	89(26.8)
I don't know	125(37.6)
Preventive practice	
Use bed net (true answer)	79(23.8)
Mosquito repellent (true answer)	46(13.9)
Window screen (true answer)	9(2.7)
Clearing ponds	71(21.4)
Cutting down bushes/grasses near homes	48(14.5)

(iv) Association between Socio-demographic and Economic Factors with Knowledge, and Practices of the Participants

It was observed that participants from Temeke hospital in Dar es Salaam were strongly associated with insufficient (low knowledge) knowledge and poor practices regarding Dengue and Chikungunya as compared with participants from Mnazi Mmoja in the Zanzibar archipelago at *p*-value of 0.01 and 0.001, respectively. No/primary education, self-employed and being in low economic status increased the likelihood of insufficient knowledge and poor practices regarding Dengue and Chikungunya with a statistically significant *p*-value of 0.01 and 0.001, respectively.

Table 6: Association between socio-demographic and economic factors with knowledge, and Practices of the participants

Variable		n (%)	Low knowledge n (%)	Poor practices n (%)
Sites	Temeke hospital, Dar es Salaam	166(50.0)	157(94.6)**	166(100)***
	Mnazi Mmoja, Zanzibar	166(50.0)	141(84.9)	151(91.0)
Sex	Male	155(46.7)	140(90.3)	50(96.8)
	Female	177(53.3)	158(89.3)	167(94.4)
Age (years)	<30	174(52.4)	153(87.9)	163(93.7)
	30+	158(47.6)	145(91.8)	154(97.5)
Education	Non/Primary	130(39.2)	125(96.2)**	130(100)**
	Secondary/above	202(60.8)	173(85.6)	187(92.6)
Marital status	Married	177(53.3)	159(89.8)	168(94.9)
	Not married	155(46.7)	139(89.7)	149(96.1)
Occupation	Employed	46(13.9)	35(76.1)	40(87.0)
	Self-employed*	142(42.8)	137(96.5)***	142(100)***
	Others	144(43.4)	126(87.5)	135(93.7)
Economic status	Low	111(33.4)	107(96.4)***	108(97.3)*
	Medium	163(49.1)	147(90.2)	158(96.9)
	High	58(17.5)	44(75.9)	51(87.9)

**P-value<0.05, **P-value<0.01 & ***P-value<0.001*

(v) Association between Socio-demographic and Economic Factors and Low Knowledge, and Poor Practices of the Participants using Multivariable Analysis

It was observed that 1.1 (95% CI 1.03-1.2) participants from Temeke hospital in Dar es Salaam were more likely to have low knowledge than those from Mnazi Mmoja in Zanzibar, and this was

also true when looked at poor practices. Results also showed that level of education was associated with low knowledge among those having none or primary education, and also being more likely to have low knowledge compared to those with secondary and above education level APR 1.1(1.05-1.2). Economic status was another factor that was found to be associated with having low knowledge and also poor practices (Table 8).

Table 7: Association between socio-demographic and economic factors and low knowledge, and poor practices of the participants using multivariable analysis

Variable	UPR, 95%CI	APR, 95%CI	UPR, 95%CI	APR, 95%CI
Hospital				
Temeke hospital,	1.1(1.03-1.2)	1.1(1.03-1.2)	1.1(1.05-1.2)	1.1(1.03-1.2)
Dar es salaam				
Mnazi Mmoja, Zanzibar	Ref	Ref	Ref	Ref
Sex				
Male	Ref		Ref	
Female	1.0(0.9-1.1)		1.0(0.9-1.02)	
Age (years)				
<30	1.0(0.9-1.03)		1.0(0.9-1.01)	1.0(0.9-1.1)
30+	Ref		Ref	Ref
Education				
Non/Primary	1.1(1.05-1.2)	1.1(1.001-1.1)	1.1(1.04-1.1)	1.1(1.0-1.1)
Secondary/above	Ref	Ref	Ref	Ref
Marital status				
Married	1.0(0.9-1.1)		1.0(0.9-1.03)	
Not married	Ref		Ref	
Occupation				
Employed	Ref	Ref	Ref	Ref
Self-employed*	1.3(1.1-1.5)	1.2(1.01-1.4)	1.2(1.03-1.3)	1.2(1.01-1.4)
Others	1.2(1.0-1.4)	1.1(0.9-1.3)	1.1(1.0-1.2)	1.1(0.9-1.3)
Economic status				
Low	1.3(1.1-1.5)	1.2(1.04-1.4)	1.1(1.0-1.2)	1.2(1.04-1.4)
Medium	1.2(1.02-1.4)	1.2(1.02-1.4)	1.1(1.0-1.2)	1.2(1.02-1.4)
High	Ref	Ref	Ref	Ref

Unadjusted Prevalence Ratio (UPR) & Adjusted Prevalence Ratio (APR)

4.2 Discussion

The decline of malaria as a major cause of febrile illnesses has made the importance of other febrile diseases; the so-called "non-malaria febrile illnesses" which include Dengue and Chikungunya, amongst others. It is now more than three years since the last Dengue and Chikungunya outbreaks were reported in Tanzania. This brings out a question as to what is the disease situation during the interepidemic period. The present study was conducted to compare the seroprevalence of anti-

DENV and anti-CHIKV IgM and IgG and their co-circulation in Temeke hospital in Dar es Salaam and Mnazi Mmoja in Zanzibar in addition to investigating the knowledge, attitude and practice towards the two diseases.

Generally, lack of routine diagnosis of Dengue and Chikungunya fever could be misdiagnosed as malaria and prescribed wrong treatment and hence could lead to adverse health effects, especially for Dengue. Since complications from DENV infection can lead to life-threatening; Dengue hemorrhagic fever and Dengue shock syndrome, thus both of which necessitate careful management to prevent death (WHO, 2009).

In this study, it was observed Dengue and Chikungunya IgM seropositivity of 0.9% and 2.97% respectively from samples collected from Dar-es-salaam, while IgM for Dengue or Chikungunya was not detected observed in samples from Mnazi Mmoja in Zanzibar. The presence of IgM indicates that infections of Dengue and Chikungunya are ongoing in the mainland, Tanzania and may be predictive of a future epidemic. The impact of large-scale epidemics is tangible in terms of direct and indirect medical costs (Suaya *et al.,* 2009) as well as the risk of long-term sequelae in Chikungunya (Gasque, 2013) or death in the case of Dengue (WHO, 2009).

Therefore, there is a need for continuing screening for these infections since the presence of Ig E could either recent infection or remnant of those outbreaks of three years back. Epidemics of Dengue, Chikungunya, and other arboviral diseases in the coming decades as climate change are projected to favor *A. aegypti* proliferation and viral transmission. So, this necessitates a call for improved vector control and disease surveillance. Again, as outbreaks of these viruses continue in various parts of the world, pose a transmission risk via blood donations as blood is not routinely screened for Chikungunya or Dengue during the transfusion process (Faria *et al.,* 2017; Vairo *et al.,* 2014).The lower Chikungunya and Dengue IgM seropositivity in this study are similar to the result obtained from a study conducted at Kilombero district in the South-Eastern part and Bondo district, the Northern part of Tanzania by Kajeguka *et al.* (2016) and Ndosi *et al.* (2016).

In another study conducted in Tanzania by Patrick *et al.* (2018), the prevalence of Chikungunya IgM was found to be (3.8%), which is relatively higher compared to this study. The differences could be attributed to many variables including the type of samples used. However, the samples used in the current study were collected from asymptomatic blood donors who could have given different results if blood samples were taken directly from febrile patients or a random population samples. The random sample would provide unbiased samples representing the entire population in contrary to a sample of blood donors which have a high probability of being biased or unrepresentative of the population. Therefore, there is high probability for results to be different.

We observed Dengue and Chikungunya IgG seroprevalence in both study facilities in Dar es Salaam and Zanzibar. This may be a consequence of ongoing infections as signified by IgM responses observed in this study but also previous outbreaks (Chipwaza *et al.,* 2021; Sindato *et al.,* 2019). This is because the IgG can be detected many years post-infection (Luo *et al.,* 2018). The anti- Dengue and anti–Chikungunya IgG antibodies detected in this study are in agreement with many other studies in various parts of Tanzania (Budodo *et al.,* 2020; Patrick *et al.,* 2018; Vairo *et al.,* 2014) suggesting the diseases are becoming endemic in the country.

The presence of Chikungunya and Dengue dual antibodies has been reported previously (Chipwaza *et al.,* 2014). However, the reported estimates were lower compared to the prevalence reported in this study, which was 6.1% for Mnazi Mmoja in Zanzibar and 12.9% for Temeke hospital in Dar es Salaam. The observed dual antibodies for Chikungunya and Dengue in the same individual in the study sites indicate that the two viruses are prevalent among blood donors. This may result in illness with overlapping signs and symptoms, which lead to difficulties in treatment and diagnosis.

Because samples from blood banks did not provide sufficient demographic information, we conducted a separate study to estimate knowledge, awareness, and practices on the two diseases at Temeke hospital in Dar-es-salaam and Mnazi Mmoja Hospital in Zanzibar. The study showed that there is a lack of knowledge and poor practices about Dengue and Chikungunya among the study participants. The Dengue and Chikungunya average knowledge scores were 10.2% which is relatively lower than a study conducted in Kilimanjaro, Tanzania which found that 15.2% had good knowledge scores (Kajeguka *et al.,* 2017).

Furthermore, it was found that only 4.5% were reported to have good preventive practices about Dengue and Chikungunya. It is more than 3 years since the last Dengue or Chikungunya outbreak was reported in Tanzania. Therefore, the difference may reflect decay in awareness and preventive practices which occurs with time after reported outbreaks. For example at the moment, awareness and preventive practices will be higher for COVID19 as compared to Dengue and Chikungunya, because COVID19 is still very fresh in people's minds. This result is contrary to previous studies conducted in various countries which show high knowledge compared to our study. For instance, Malaysia indicated that 64.3% and the Philippines 61.45% of all participants had good knowledge (Al-Zurfi *et al.,* 2015; Yboa & Labrague, 2013). The difference may be due to differences in the effectiveness of public sensitization campaigns in those countries. It may also be that the studies in Malaysia and Philippines occurred immediately after the disease outbreaks, as compared to this study which was done three years after reported outbreaks (Al-Zurfi *et al.,* 2015; Yboa & Labrague, 2013). Also, being with no/primary education, self-employed, and low economic status increased

the likelihood of low knowledge regarding Dengue and Chikungunya and poor practices toward preventive practices. This confirms results from other study which show that lower education levels and social-economic status have a negative influence on awareness and preventive practices to diseases (Kajeguka *et al.,* 2017; Saringe *et al.,* 2019).

No association was observed between seroprevalence and demographic characteristics. This might be due to a number of factors which in a way represent the limitation of the study. To begin with, only a few demographics information was available from the blood bank register as compared to what is normally collected during disease surveillance. Secondly, only samples from Zanzibar had the associated demographic information available for analysis, therefore, denying us the opportunity to compare the two locations. It is, however, important to note that blood collection register centers only pay attention to demographic information which is important for their purpose but not necessarily disease surveillance.

Another limitation is Chikungunya and Dengue viruses are not readily differentiated serologically due to the cross-reactivity of their serocomplexes, so there is a need for molecular detection methods. We acknowledge the limitations in this study such as sample size which is small to generalize the entire population of Zanzibar and Dar es Salaam though the results are similar to other studies (Chipwaza *et al.,* 2014; Kajeguka *et al.,* 2017). Therefore, a large survey is required to cover the entire population regarding the understanding of Dengue and Chikungunya knowledge and practice in general populations. However, the information obtained from this study will help to flag the potential danger of transmission of the two diseases through blood transfusion and also collaborate other studies suggesting that Dengue and Chikungunya may be endemic in Tanzania.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Detection of IgM for Dengue and Chikungunya in samples from Temeke hospital in Dar es Salaam indicates recent or ongoing transmissions of the two viruses in the absence of a reported outbreak. These findings suggest the possibility of transmission of the two viral infections through blood transfusion, hence the need to screen for the two viruses before blood donation. Detection of IgG antibodies for Dengue and Chikungunya viruses in this study might be contributed by both the ongoing infections and residual responses caused by the preceding infections in the country. This study shows that there is insufficient knowledge and low preventive practice regarding prevention and control of Dengue and Chikungunya among patients attending outpatients departments in the study facilities in Dar es Salaam and Zanzibar and this may help perpetuate the two diseases.

5.2 Recommendations

(i) The results from the blood banks may represent the tip of the iceberg. Further studies are needed, using a more representative sample of the population to gain insight into the two diseases' true burden in Tanzania.

(ii) It is recommended for screening for both Dengue and Chikungunya at blood donation centers to avoid infection via transfusion which would require viral detection in the form of RNA or antigen example NS1.

(iii) Also, we recommend a bigger sample size should be considered in both Dar es Salaam and Zanzibar to get a full picture of the current disease burden in the country.

(iv) Therefore, government through Ministry of health and Social welfare and other public health stakeholders should arrange campaigns regarding knowledge and preventive practices of chikungunya and dengue should be raised to public awareness.

(v) The findings obtained from this study will help the Ministry of health and other stakeholders to take necessary precautions and have in place good preparedness strategies to prevent future outbreaks

(vi) The KAP approach should be applied to another community will help in improving the awareness among individuals in about the disease's signs and symptoms, mode of transmission, and improved preventive practices.

(vii) The health system is at immediate risk of being overburdened by the influx of patients, while critical societal functions risk disruption due to disease-related absenteeism and required emergency activities. Zanzibar and coastal areas of Tanzania including Dar es Salaam, are likely to see intensified and more frequent

REFERENCES

Al-Zurfi, B. M. N., Fuad, M. D. F., Abdelqader, M. A., Baobaid, M. F., Elnajeh, M., Ghazi, H. F., Ibrahim, M. H., & Abdullah, M. R. (2015). Knowledge, Attitude and Practice of Dengue Fever and Heath Education Programme among Students of Alam Shah Science School, Cheras, Malaysia. *Malaysian Journal of Public Health Medicine*, *15*(2), 69–74.

Almas, A., Parkash, O., & Akhter, J. (2010). Clinical Factors Associated with Mortality in Dengue Infection at a Tertiary Care Center. *Southeast Asian Journal of Tropical Medicine and Public Health*, 41(2), 333-40.

Alves, A. C., Fabbro, A. L. D., Passos, A. D. C., Carneiro, A. F. T. M., Jorge, T. M., & Martinez, E. Z. (2016). Knowledge and Practices Related to Dengue and Its Vector: A Community-Based Study From Southeast Brazil. *Revista Da Sociedade Brasileira De Medicina Tropical*, 49, 222-226.

Arya, R., Antonisamy, B., & Kumar, S. (2012). Sample Size Estimation in Prevalence Studies. *The Indian Journal of Pediatrics*, 79, 1482-1488.

Bhatt, S., Gething, P., Brady, O., Messina, J., & Farlow, A. (2013). The Global Distribution and Burden of Dengue. *Nature*, 496(7446), 504-507.

Bianco, C. (2008). Dengue and Chikungunya Viruses in Blood Donations: Risks to the Blood Supply? *Transfusion,* 48(7), 1279-1281.

Budodo, R. M., Horumpende, P. G., Mkumbaye, S. I., Mmbaga, B. T., Mwakapuja, R. S., & Chilongola, J. O. (2020). Serological Evidence of Exposure to Rift Valley, Dengue and Chikungunya Viruses among Agropastoral Communities in Manyara and Morogoro Regions In Tanzania: A Community Survey. *Plos Neglected Tropical Diseases,* 14(7), E0008061.

Burt, F. J., Rolph, M. S., Rulli, N. E., Mahalingam, S., & Heise, M. T. (2012). Chikungunya: A Re-Emerging Virus. *The Lancet*, 379(9816), 662-671.

Yboa, B. C., & Labrague, L. J. (2013). Dengue Fever, Dengue Preventive Practices, Dengue Knowledge, Samar Province. *American Journal Of Public Health Research,* 1(2), 47-52.

Cavalcanti, L. P., Vilar, D., Souza-Santos, R., & Teixeira, M. G. (2011). Change in Age Pattern of Persons with Dengue, Northeastern Brazil. *Emerging Infectious Diseases*, 17(1), 132.

Chahar, H. S., Bharaj, P., Dar, L., Guleria, R., Kabra, S. K., & Broor, S. (2009). Co-Infections with Chikungunya Virus and Dengue Virus In Delhi, India. *Emerging Infectious Diseases*, 15(7), 1077.

Chhabra, M., Mittal, V., Bhattacharya, D., Rana, U. V. S., & Lal, S. (2008). Chikungunya Fever: A Re-Emerging Viral Infection. *Indian Journal of Medical Microbiology*, 26(1), 5-12.

Chipwaza, B., Mugasa, J. P., Mayumana, I., Amuri, M., Makungu, C., & Gwakisa, P. S. (2014). Community Knowledge and Attitudes and Health Workers' Practices Regarding Non-Malaria Febrile Illnesses in Eastern Tanzania. *Plos Neglected Tropical Diseases,* 8(5), E2896.

Chipwaza, B., Mugasa, J. P., Selemani, M., Amuri, M., Mosha, F., Ngatunga, S. D., & Gwakisa, P. S. (2014). Dengue and Chikungunya Fever among Viral Diseases in Outpatient Febrile Children in Kilosa District Hospital, Tanzania. *PloS Neglected Tropical Diseases*, 8(11), E3335.

Chipwaza, B., Sumaye, R. D., Weisser, M., Gingo, W., Yeo, N. K.W., Amrun, S. N., Okumu, F. O., & Ng, L. F. P. (2021). Occurrence of 4 Dengue Virus Serotypes and Chikungunya Virus In Kilombero Valley, Tanzania, During the Dengue Outbreak In 2018. *Open Forum Infectious Diseases*, *8*(1), 1–6. Https://Doi.Org/10.1093/Ofid/Ofaa626

Ciota, A. T., & Kramer, L. D. (2010). Insights into Arbovirus Evolution and Adaptation from Experimental Studies. *Viruses*, *2*, 2594–2617. Https://Doi.Org/10.3390/V2122594

Clark, M. H., Warimwe, G. M., Di Nardo, A., Lyons, N. A., & Gubbins, S. (2018). Systematic Literature Review of Rift Valley Fever Virus Seroprevalence in Livestock, Wildlife and Humans in Africa from 1968 To 2016. *PloS Neglected Tropical Diseases*, 12(7), E0006627

Darrigo, L. G., De Sant'Anna Carvalho, A. M., & Machado, C. M. (2018). Chikungunya, Dengue, and Zika in Immunocompromised Hosts. *Current Infectious Disease Reports*, 20, 1-10.

Dias, L. L., Amarilla, A. A., Poloni, T. R., Covas, D. T., Aquino, V. H., & Figueiredo, L. T. M. (2012). Detection of Dengue Virus in Sera of Brazilian Blood Donors. *Transfusion,* 52(8), 1667-1671.

Jahan, F. (2011). Dengue Fever (DF) In Pakistan. *Asia Pacific Family Medicine*, 10, 1-4.

Faria, N. R., Da Costa, A. C., Lourenço, J., Loureiro, P., Lopes, M. E., Ribeiro, R., Alencar, C. S., Kraemer, M. U. G., Villabona-Arenas, C. J., Wu, C. H., Thézé, J., Khan, K., Brent, S. E.,

Romano, C., Delwart, E., Custer, B., Busch, M. P., Pybus, O. G., Sabino, E. C., & Glynn, S. A. (2017). Genomic and Epidemiological Characterisation of A Dengue Virus Outbreak among Blood Donors in Brazil. *Scientific Reports*, *7*(1), 1–12.

Ferguson, N. M., Rodríguez-Barraquer, I., Dorigatti, I., Mier-Y-Teran-Romero, L., Laydon, D. J., & Cummings, D. A. (2016). Benefits and Risks of The Sanofi-Pasteur Dengue Vaccine: Modeling Optimal Deployment. *Science*, 353(6303), 1033-1036.

Gallian, P., Leparc-Goffart, I., Richard, P., Maire, F., Flusin, O., Djoudi, R., Chiaroni, J., Charrel, R., Tiberghien, P., & De Lamballerie, X. (2017). Epidemiology of Chikungunya Virus Outbreaks in Guadeloupe And Martinique, 2014: An Observational Study in Volunteer Blood Donors. *PloS Neglected Tropical Diseases*, 11(1), E0005254.

Gasparetti, M. V., Castro, R., Castro, S. A., Maria, G. S., & Cotait, A. (2007). Dengue Incidence in ABCD Region. *The Arquivos Brasileiros De Cardiologia,* 32, 70-73.

Gasque, P. (2013). Chikungunya Virus Infection. *Viral Infections of the Human Nervous System*, *61*(2), 295–315. Https://Doi.Org/10.1007/978-3-0348-0425-7_12

Gubler, D. J. (1989). *Aedes Aegypti* and *Aedes Aegypti*-Borne Disease Control in The 1990s: Top Down Or Bottom Up. *American Journal of Tropical Medicine and Hygiene*, 40, 571-578.

Gupta, N., Srivastava, S., Jain, A., & Chaturvedi, U. C. (2012). Dengue in India. *The Indian Journal of Medical Research*, *136*(3), 373.

Guzmán, M. G., & Kouri, G. (2002). Dengue: An Update. *The Lancet Infectious Diseases*, 2(1), 33-42.

Hammond, S. N., Balmaseda, A., Pérez, L., Tellez, Y., Saborío, S. I., Mercado, J. C., Videa, E., Rodriguez, Y., Pérez, M. A., Cuadra, R., Solano, S., Rocha, J., Idiaquez, W., Gonzalez, A., Harris, E., Silva, S., & Rocha, C. (2005). Differences in Dengue Severity in Infants, Children, and Adults in A 3-Year Hospital-Based Study in Nicaragua. *American Journal of Tropical Medicine and Hygiene*, *73*(6), 1063–1070. Https://Doi.Org/10.4269/Ajtmh.2005.73.1063

Hasebe, F., Del, M., Parquet, C., Pandey, B. D., Hasebe, F., Parquet, M. C., Pandey, B. D., Mathenge, E. G. M., Morita, K., Balasubramaniam, V., Saat, Z., Yusop, A., Sinniah, M., Natkunam, S., & Igarashi, A. (2002). Combined detection and genotyping of Chikungunya virus by a specific reverse transcription-polymerase chain reaction. *Journal of Medical*

Virology, 67(3), 370-374.

WHO, D. (2012). *Severe Dengue. Fact Sheet No 117*. Https://Scholar.Google.Com/

Issac, T. H. K., Tan, E. L., & Chu, J. J. H. (2014). Proteomic Profiling Of Chikungunya Virus-Infected Human Muscle Cells: Reveal The Role of Cytoskeleton Network in CHIKV Replication. *Journal of Proteomics*, 108, 445-464.

Itrat, A., Khan, A., Javaid, S., Kamal, M., Khan, H., Javed, S., Kalia, S., Khan, A.H., Sethi, M.I., & Jehan, I. (2008). Knowledge, Awareness and Practices Regarding Dengue Fever among The Adult Population of Dengue Hit Cosmopolitan. *Plos One*, 3(7), E2620.

Kajeguka, D. C., Desrochers, R. E., Mwangi, R., Mgabo, M. R., Alifrangis, M., Kavishe, R. A., Mosha, F. W., & Kulkarni, M. A. (2017). Knowledge and Practice Regarding Dengue and Chikungunya: A Cross-Sectional Study among Healthcare Workers and Community in Northern Tanzania. *Tropical Medicine and International Health*, *22*(5), 583–593. Https://Doi.Org/10.1111/Tmi.12863

Kajeguka, D. C., Kaaya, R. D., Mwakalinga, S., Ndossi, R., Ndaro, A., Chilongola, J. O., Mosha, F. W., Schiøler, K. L., Kavishe, R. A., & Alifrangis, M. (2016). Prevalence of Dengue and Chikungunya Virus Infections in North-Eastern Tanzania: A Cross Sectional Study among Participants Presenting with Malaria-Like Symptoms. *BMC Infectious Diseases*, *16*(1), 1–9. Https://Doi.Org/10.1186/S12879-016-1511-5

Kay, B. H., Sinh Nam, V., Van Tien, T., Thi Yen, N., Vu Phong, T., Bich Diep, V. T., Uyen Ninh, T., Bektas, A., & Aaskov, J. G. (2002). Control of Aedes Vectors of Dengue in Three Provinces of Vietnam by use of Mesocyclops (Copepoda) and Community-Based Methods Validated by Entomologic, Clinical, and Serological Surveillance. *American Journal of Tropical Medicine and Hygiene*, *66*(1), 40–48. Https://Doi.Org/10.4269/Ajtmh.2002.66.40

Kendrick, K., Stanek, D., Blackmore, C., & Centers For Disease Control and Prevention (CDC). (2014). Notes from The Field: Transmission of Chikungunya Virus in The Continental United States: Florida, 2014. *Morbidity and Mortality Weekly Report*, 63(48), 1137.

Kraemer, M.U., Sinka, M.E., Duda, K.A., Mylne, A.Q., Shearer, F.M., Barker, C.M., Moore, C.G., Carvalho, R.G., Coelho, G.E., Van Bortel, W., & Hendrickx, G (2015). The Global Distribution of the Arbovirus Vectors *Aedes Aegypti* and *Aedes Albopictus. Elife*, 4, E08347.

Lenglet, Y., Barau, G., Robillard, P. Y., Randrianaivo, H., Michault, A., Bouveret, A., Gérardin, P., Boumahni, B., Touret, Y., Kauffmann, E., & Schuffenecker, I. (2006). Chikungunya Infection In Pregnancy: Evidence for Intrauterine Infection in Pregnant Women and Vertical Transmission in the Parturient. Survey of the Reunion Island Outbreak. *Journal De Gynecologie, Obstetrique Et Biologie De La Reproduction,* 35(6), 578-583.

Luo, S., Cui, W., Li, C., Ling, F., Fu, T., Liu, Q., Ren, J., & Sun, J. (2018). Seroprevalence of Dengue Igg Antibodies in Symptomatic and Asymptomatic Individuals Three Years After an Outbreak in Zhejiang Province, China. *BMC Infectious Diseases*, *18*(1), 1–5.

Mboya, L. B., Nkya, E. S., Matemba, L. E., & Kinimi, E. (2020). Evidence of Chikungunya but Not Dengue Virus Circulating among Febrile Patients During Low Transmission Period in Morogoro Municipality, Tanzania. *International Journal of Tropical Disease & Health*, *40*(4), 1–8. Https://Doi.Org/10.9734/Ijtdh/2019/V40i430236

Mtove, G., Amos, B., Nadjm, B., Hendriksen, I. C., Dondorp, A.M., Mwambuli, A., Kim, D. R., Ochiai, R. L., Clemens, J. D., Von Seidlein, L., & Reyburn, H. (2011). Decreasing Incidence of Severe Malaria and Community-Acquired Bacteraemia among Hospitalized Children in Muheza, North-Eastern Tanzania, 2006-2010. *Malaria Journal*, 10(1), 1-11.

Munasinghe, D. R., Amarasekera, P. J., & Fernando, C. F. O. (1966). An Epidemic of Dengue-Like Fever in Ceylon (Chikungunya)-A Clinical and Haematological Study. *Ceylon Medical Journal*, 11(4), 129-42.

Murray, N. E. A., Quam, M. B., & Wilder-Smith, A. (2013). Epidemiology of Dengue: Past, Present and Future Prospects. *Clinical Epidemiology*, 299-309.

Mweya, C. N., Kimera, S. I., Stanley, G., Misinzo, G., & Mboera, L. E. (2016). Climate Change Influences Potential Distribution of Infected *Aedes Aegypti* Co-Occurrence with Dengue Epidemics Risk Areas in Tanzania. *Plos One*, 11(9), E0162649.

Mweya, C. N., Kimera, S. I., Kija, J. B., & Mboera, L. E. (2013). Predicting Distribution of *Aedes Aegypti* and *Culex Pipiens Complex*, Potential Vectors of Rift Valley Fever Virus in Relation to Disease Epidemics in East Africa. *Infection Ecology & Epidemiology*, 3(1), 21748.

Ndosi, R., Kwigizile, E., Ibrahim, U., Dossajee, U., Rwiza, J., Kabanyana, C., Ndaro, A., & Chilongola, J. (2016). Risk Factors for Concurrent Malaria and Arbovirus Infections in Handeni, Northeastern Tanzania. *International Journal Of Tropical Disease & Health*, *20*(4),

1–7.

Nimmannitya, S., Thisyakorn, U., & Hemsrichart, V. (1987). Dengue Haemorrhagic Fever with Unusual Manifestations. *The Southeast Asian Journal of Tropical Medicine and Public Health*, 18(3), 398-406.

Mweya, C. N., Kimera, S. I., Kija, J. B., & Mboera, L. E. (2013). Predicting Distribution of *Aedes Aegypti* and *Culex Pipiens Complex,* Potential Vectors of Rift Valley Fever Virus in Relation to Disease Epidemics in East Africa. *Infection Ecology & Epidemiology*, 3(1), 21748.

Ooi, E. E., & Gubler, D. J. (2011). Dengue and Dengue Hemorrhagic Fever. *Tropical Infectious Diseases*, 504–510. Https://Doi.Org/10.1016/B978-0-7020-3935-5.00075-6

Oyero, O. G., & Ayukekbong, J. A. (2014). High Dengue NS1 Antigenemia in Febrile Patients in Ibadan, Nigeria. *Virus Research*, 191, 59-61.

Patrick, B. N., Angwenyi, S., Kinimi, E., Shayo, M., Hugo, M., & Kasanga, C. J. (2018). Evidence of Anti-Chikungunya Virus Igg and Igm Antibodies among Patients Seeking Treatment in Different Health Facilities in Kyela District, Tanzania. *Journal of Bio Innovation,* 7 (4), 585-598.

Pozzetto, B. (2015). Is Transfusion-Transmitted Dengue Fever A Potential Public Health Threat? *World Journal Of Virology*, *4*(2), 113. Https://Doi.Org/10.5501/Wjv.V4.I2.113

Rafique, I., Nadeem Saqib, M. A., Siddiqui, S., Arif Munir, M., Malik, I. A., Rao, M. H., Ahmed, J., Bashir, S., Khan, O., Firdous, R., & Ahmed, S. (2015). Dengue Knowledge and Its Management Practices among Physicians of Major Cities of Pakistan. *Journal of The Pakistan Medical Association*, *65*(4), 392–396.

Rana, S., & Lunia, P. (2015). Reemergence and Global Spread of Chikungunya. In 3rd International Conference. *Journal of Biological Chemistry and Environmental Sciences,* 2015, 69-74.

Robinson, M. C. (1955). An Epidemic of Virus Disease in Southern Province, Tanganyika Territory, in 1952–1953. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 49(1), 28-32.

Salam, N., Mustafa, S., Hafiz, A., Chaudhary, A. A., Deeba, F., & Parveen, S. (2018). Global Prevalence and Distribution of Coinfection of Malaria, Dengue and Chikungunya: A Systematic Review. *BMC Public Health*, 18, 1-20.

Saleh, F., Kitau, J., Konradsen, F., Alifrangis, M., Lin, C. H., Juma, S., Seif Mchenga, S., Saadaty, T., & Linda Schiøler, K. (2018). Habitat Characteristics for Immature Stages of *Aedes Aegypti* in Zanzibar City, Tanzania. *Journal of the American Mosquito Control Association*, *34*(3), 190–200. Https://Doi.Org/10.2987/17-6709.1

Saringe, S., Kajeguka, D. C., Kagirwa, D. D., Mgabo, M. R., & Emidi, B. (2019). Healthcare Workers Knowledge and Diagnostic Practices: A Need for Dengue and Chikungunya Training in Moshi Municipality, Kilimanjaro Tanzania. *BMC Research Notes*. Https://Doi.Org/10.1186/S13104-019-4074-X

Sergon, K., Njuguna, C., Kalani, R., Ofula, V., Onyango, C., Konongoi, L. S., Bedno, S., Burke, H., Dumilla, A. M., Konde, J., Njenga, M. K., Sang, R., & Breiman, R. F. (2008). Seroprevalence of Chikungunya Virus Infection on Lamu Island, Kenya, October 2004. *American Journal of Tropical Medicine and Hygiene*, *78*(2), 333–337.

Sindato, C., Karimuribo, E., Mboera, L., Njenga, K., Choby, C., & Karsan, Y. (2019). *Dengue Outbreaks in Tanzania: Recent Trends and Importance of Research Data in Disease Surveillance.* Https://Www.Google.Com

Singh, P., Mittal, V., Rizvi, M. M. A., Chhabra, M., Sharma, P., Rawat, D. S., Bhattacharya, D., Chauhan, L. S., & Rai, A. (2012). The First Dominant Co-Circulation of Both Dengue and Chikungunya Viruses During the Post-Monsoon Period of 2010 In Delhi, India. *Epidemiology & Infection*, 140(7), 1337-1342.

Srinivas, V., & Srinivas, V. R. (2015). Dengue Fever: A review article. *Journal of Evolution of Medical and Dental Sciences*, *4*(29), 5048–5058. Https://Doi.Org/10.14260/Jemds/2015/736

Staikowsky, F., Le Roux, K., Schuffenecker, I., Laurent, P., Grivard, P., Develay, A., & Michault, A. (2008). Retrospective Survey of Chikungunya Disease in Reunion Island Hospital Staff. *Epidemiology & Infection*, 136(2), 196-206.

Vairo, F., Nicastri, E., Yussuf, S. M., Cannas, A., Meschi, S., Mahmoud, M. A. A., Mohamed, A. H., Mohamed Maiko, P., De Nardo, P., Bevilacqua, N., Castilletti, C., Di Caro, A., Racalbuto, V., & Ippolito, G. (2014). Igg Against Dengue Virus in Healthy Blood Donors, Zanzibar, Tanzania. *Emerging Infectious Diseases*, *20*(3), 465–468.

Wahid, B., Ali, A., Rafique, S.,Idrees, M. (2017). Global Expansion of Chikungunya Virus: Mapping the 64-Year History. *International Journal of Infectious Diseases*, *58*, 69–76.

Https://Doi.Org/10.1016/J.Ijid.2017.03.006

Ward, T., Samuel, M., Maoz, D., Runge-Ranzinger, S., Boyce, R., Toledo, J., Velayudhan, R., & Horstick, O. (2017). Dengue Data and Surveillance in Tanzania: A Systematic Literature Review. *Tropical Medicine & International Health*, 22(8), 960-970.

Wolfe, N. D., Kilbourn, A. M., Karesh, W. B., Rahman, H. A., Bosi, E. J., Cropp, B. C., Andau, M., Spielman, A., & Gubler, D. J. (2001). Sylvatic Transmission of Arboviruses among Bornean Orangutans. *American Journal of Tropical Medicine and Hygiene*, *64*(5), 310–316. Https://Doi.Org/10.4269/Ajtmh.2001.64.310

World Health Organization, Special Programme for Research, Training in Tropical Diseases, World Health Organization. Department of Control of Neglected Tropical Diseases, World Health Organization. Epidemic, & Pandemic Alert. (2009). *Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control*. World Health Organization. https://www.google.com

APPENDICES

Appendix 1: Recruitment

1. RECRUITMENT PROCEDURE

The recruitment of the participants will be when the patients come to the hospital, will be explained about the study. A written agreement will be attainable to everyone ready to participate in the study, an interview will be done to assess the associated risk factors including socioeconomic, demographic and environmental information.

STANDARD OPERATION PROCEDURE FOR CONDUCTING ELISA TEST

SPECIMEN COLLECTION

Centrifuge whole blood to get serum. (Do not leave sera at room temperature for prolonged periods. Separated serum should remain at 20-25ºc for 8 hours. If test is not done within 8 hours, serum should be refrigerated at 2-8 ºc. For longer storage (more than 48 hours), serum should be frozen at or below -20 ºc.

PRINCIPLE OF THE TEST

IgM/G Capture ELISA is a type of sandwich immunoassay. In this test, IgM/G negative control, IgM/G positive control and unknown serum samples are diluted with sample diluents, then incubated in microtiter well. If IgM/G antibodies are present in sample, it combines either mouse monoclonal anti-human IgM/G antibodies which is coated on well and then is bound to mixture of antigen and mouse monoclonal anti- HRP Conjugate (MAb). After incubation, the micro wells are washed and a colorless substrate, tetramethylbenzidine (TMB) is added. Substrate is hydrolyzed by enzyme and the substrate changes to blue color. The enzyme activity found in the well will be directly proportional to the IgM/G antibody concentration in patient serum. After adding the stop solution with acid, the TMB becomes yellow. Color development is indicative of the presence of anti-Dengue or Chikungunya IgM antibody in the test sample.

2. TEST PROCEDURE

a. PRECAUTION

Bring all kit reagents and sample to room temperature (25 ºc) before use. Thoroughly mix the reagents and samples before use. After washing the strips, the next reagents must be added immediately before the well become dry.

b. PROTOCOL

1. Take the required number of micro wells from the foil pouch. Number the strips as 1, 2, 3……
2. Dilute Negative control, Positive control and Patient's serum with sample diluent in 1:100 ratios.

3. Prepare 1X wash buffer. (Dilute wash buffer concentrates to 1X with distilled water).
4. Prepare Dengue antigen and Anti-Dengue HRP conjugate.
    i. Dilute a bottle of antigen powder using 1500µl of the conjugate diluent.
    ii. Mix it well.
    iii. Dilute the Anti- Dengue HRP conjugate with diluted Dengue antigen in 1:1 ratio.
    iv. Mix it and leave at room temperature (15-30ºc) for 60 minutes.
5. Add 100µl of diluted controls and patient's samples into their respective wells of micro-plate. Five micro-wells are required for negative control in triplicate positive control in

duplicate.

6. Cover the micro-plate with adhesive plate sealer.
7. Incubate the plate at 37 ºc for 60 minutes.
8. Wash the wells 5 times with 300µl diluted wash buffer. Tap the plate after last wash on a tissue paper.
9. Transfer 100µl of diluted Anti-Dengue HRP conjugate solution into each wells of microplate.
10. Cover the plate with adhesive plate sealer. Incubate the plate at 37 ºc for 60 minutes.
11. Wash the wells 5 times with 300µl diluted wash buffer.
12. Add 100µl of working tetramethylbenzidine (TMB) into each well.
13. Cover with aluminium foil and incubate at room temperature for 10 minutes. The blue color will develop.
14. Pipette 100µl of stopping solution into each well to stop the reaction. Blue color will change to yellow.
15.
16. Within 30 minutes, read the absorbance of each well at 450nm with a reference filter of 620nm.**Interpretation of Results**

Evaluation

Calculate the mean absorbance of negative controls, and then calculate the cutoff value by adding 0.300.

Average of negative control= x.

Cut-off= 0.3 +x.

Test results

Sample < cut-off= anti-Dengue/Chikungunya IgM negative

Sample >cut-off= anti-Dengue/Chikungunya IgM positive

Result interpretation

Negative results: No detectable IgM antibody.

Positive results: Presence of detectable antigen of Dengue and Chikungunya

Appendix 2: Informed Consent Form

Researcher: **HALIYA SULEIMAN SHAURI**

Title of Project: **DETERMINATION OF SEROPREVALENCE OF DENGUE AND CHIKUNGUNYA INFECTIONS AND ASSOCIATED RISK FACTORS AMONG FEBRILE PATIENTS AT MNAZIMMOJA AND TEMEKE HOSPITAL IN TANZANIA.**

I'm asking for your voluntary participation in my science fair project. Please read the following information about the project. if you would like to participate, please sign in the appropriate area below

Purpose of the project:

To understanding the Dengue and Chikungunya infections seroprevalence together with main factors that contribute to its distribution

If you participate, you would be asked to:

Answer some question that related to assessing the knowledge and practices regarding Dengue and Chikungunya infection

Time required for participation**: about 15-25 minutes**

Potential Risks of the study:

There is no and potential risk in this study

Benefits:

The study expects to give an overview of the knowledge status and practices of these diseases.

How confidentiality will be obtained

All of the records will be stored in a secure and confidential way

If you have any questions about this study, feel free to contact:

Individual: **Hahiya Suleiman Shauri Phone number/ Email +255 718 632382/ Email shaurih@nm-aist.ac.tz**

Voluntary Participation:

Participation in this study is completely voluntary, if you decide not to participant there will not be and negative consequence. Please be aware that if you decide to participate, you may stop participating at any time and you may decide not to answer any specific question.

By signing this form, I am attesting that I have read and understand the information above and I freely give my consent/assent to participate or permission for my child to participate

Adult Informed Consent or Minor Assent Date Reviewed and signed

Signature

Parent/Guardian Permission Name	Date Reviewed and signed

Appendix 3: Questionnaire

KNOWLEDGE AND PRACTICES REGARDING DENGUE AND CHIKUNGUNYA

SEHEMU YA 1: TAARIFA ZA MSINGI

REGION

DISTRICT

WARD

STREET/VILLAGE

NAME OF THE HOSPITAL

LEVEL OF THE HOSPITAL

DATE

SECTION 2: MAELEZO YA MHUSIKA YA AWALI

2,1 Jinsia

a. Mme b. Mke

2.2 UMRI WAKO

2.6 UMEPATA ELIMU KIWANGO GANI?

1. Sijasoma)

2. (Msingi)

3. Sekondari)

4. Sekondari ya juu)

5. Vyuo vya kati

6. Vyuo vya juu

2.3 HALI YAKO YA NDOA?

a. (Nimeolewa/oa)

b. (Sijaolewa/oa)

c. (Nimeachika/acha)

d. Mjane/Mgane

e. Tunaishi Pamoja

2.7 KAZI

1. Ajira za serikali
2. mashirika binafsi
3. Mvuvi
4. Mjasiliamali (Pamoja na machinga)
5. Mfanyabiashara
6. Mkulima
4. Mwanafunzi
5. Dereva
6. Mama wa nyumbani

SEHEMU YA 3: MASWALI YA YAHUSUYO MARADHI YALIYOKULETA HOSPITALI

3.1 Katika maswali yafuatayo ningependa unijibu ndio au hapana kama una au hauna dalili zifuatazo

a. Homa
   1. Ndio 2. Hapana

b. Maumivu ya misuli
   1. Ndio 2. Hapana

c. Maumivu ya Viungo
   1. Ndio 2. Hapana

d. Maumivu ya mgongo
   1. Ndio 2. Hapana
e. Vipele vinavyotokea kwenye Ngozi huwa rangu nyekundu huambatana maambukizi ya virusi
   1. Ndio 2. Hapana
f. Maumivu ya tumbo
   1. Ndio 2. Hapana

g. Kuumwa na kichwa
   1. Ndio 2. Hapana

h. Kuvimba kwa viungo
   1. Ndio 2. Hapana
i. Kutapika/nausea
   1. Ndio 2. Hapana

SEHEMU YA NN UFAHAMU NA MATENDO DHIDI YA MAGONJWA YA DENGU NA CHIKUNGUNYA

A. UELEWA KUHUSU DENGUE NA CHIKUNGUNYA

a) Umewahi kusikia

1. Dengue
    a. Ndio
    b. Hapana
2. Chikungunya
    a. Ndio
    b. Hapana

b) Ikiwa ndio umesikia wapi

1. Redio

2. Runinga

3. Huduma za Afya

4. Ndani ya familia yako

5. Magazeti

6. Jirani

7. Sijui

c) Dalili na viashiria

1. Homa

2. Kichwa

3. Maumivu ya viungo

4. Kichefuchefu/kutapika

5. Kuvuja damu

6. Sijui

d) Je ni njia ipi inaweza kuambukiza

1. Dengue

2. Chikungunya

1. Mbu

2. Nzi

3. Hewa

4. Sijui

d) Wakati wa mbu kuuma

1. Mchana

2. Jioni

3. Usiku

4. Asubuhi

5. Sijui

e) Mazingira Anayoishi Mbu

1. Matairi ya gari yaliotumika

2. Maji safi kwenye ndoo/Matenki

4. Uchfu/Maji machafu

5. Sijui

f) Matendo yanayozuia ueneaji wa maradhi

a. Chandarua

Ndio

Hapana

b)Dawa za kuulia mbu

Ndio

Hapana

c)Kuziba madirisha

Ndio

Hapana

d)Mitaro ya maji machifu iko karibu

Ndio

Hapana

e)Vichaka/majani karibu na nyumba yako

Ndio

Hapana

SEHEMU YA 5: HALI YA KIUCHUMI

5.1 **Je, wakazi wa nyumba hii (wote pamoja) wanamiliki chochote kati ya vitu vifuatavyo? (chagua yote yanayohusika)**

Sasa naenda kukuuliza masuali yafuatayo kujua kuhusu hali ya kiuchumi katika familia yako.		
1.	Je kaya ako ina …. Umeme?	1= Ndio 2= Hapana
2.	Je, kaya yako ina ….. Runinga?	1= Ndio 2= Hapana
3.	Je kaya yako ina … Radio	1= Ndio 2= Hapana
4.	Je kaya yako ina…. Pasi?	1= Ndio 2= Hapana
5.	Je,Kuna mtu yeyote kwenye kaya hii ana akaunti ya benki?	1= Ndio 2= Hapana
6.	Je, sakafu imejengwa kwa kutumia vifaa gani vikuu?	1= Udongo/Mchanga/Matope 2= Sementi/Zege 3= other Ingine
7.	Je, kuta za nyumba zimejengwa kwa kutumia vifaa gani kikuu?	Kuta za Asili 1 = Hakuna ukuta 2 = Nyasi 3 = Miwa / Mtende / Mimea / mianzi Kuta za kawaida 4 = miti na Mud 5 = Jiwe na Mud 6 = Wood, Timber Kuta zilizomalizika 7 = Saruji / Zege 8 = Jiwe na Lime / Saruji 9 = Matofali kavu ya jua / Matofali ya Matope 10 = Matofali ya Motoni 11 = Vitalu vya Saruji 12 Nyingine (Taja)
8.	Je , Paa za nyumba hii zimejengwa kwa kutumia kifaa gani kikuu?	1= Mabati

		2= Majani/Nyasi/Majani ya minazi/Udongo 3= Vifaa vngine
9.	Je, ni nyenzo gani kaya hii hutumia kwa ajili ya kupikia?	Umeme 2 = Gesi iliyowekwa kwenye chupa 3 = Parafini / mafuta ya taa 3 = Mkaa 4 = kuni 5 = mabaki ya mazao, majani, nyasi 6 = Chimbuko la wanyama 7 = Hakuna chakula kilichopikwa ndani ya kaya 8 = Nyingine (taja Umeme 2 = Gesi iliyowekwa kwenye chupa 3 = Parafini / mafuta ya taa 3 = Mkaa 4 = kuni 5 = mabaki ya mazao, majani, nyasi 6 = Chimbuko la wanyama 7 = Hakuna chakula kilichopikwa ndani ya kaya 8 = Nyingine (taja
10.	Je, Ni nini chanzo cha mwanga kwenye kaya hii?	1= Umeme 2= Betri/ Tochi au taa ya Sola 3= Other Ingine

RESEARCH OUTPUTS

(i) Publication

Shauri, H. S., Ngadaya, E., Senkoro, M., Buza, J. J., & Mfinanga, S. (2021). Seroprevalence of Dengue and Chikungunya antibodies among blood donors in Dar es Salaam and Zanzibar, Tanzania: A cross-sectional study. *BMC Infectious Diseases*, *21*, 1-6.

(ii) Poster Presentation