HUMAN IgG RESPONSE TO NTERM-34KDa SALIVARY PEPTIDE AS A BIOMARKER TO ASSESS TEMPORAL EXPOSURE TO AEDES' BITES AMONG INDIVIDUALS AGED ≥ 6 MONTHS IN LOWER MOSHI-TANZANIA, 2019

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MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES
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DANIEL LASWAI

A Dissertation submitted in Partial Fulfilment of the Requirements for the Degree of Master of Science in Epidemiology and Laboratory Management of the Muhimbili University of Health and Allied Sciences

October, 2021

CERTIFICATION

The undersigned, certify that this research report is the work of the candidate carried out under our direct supervision. The undersigned certifies that they have read and hereby recommend for acceptance by the Muhimbili University of Health and Allied Sciences a dissertation entitled: **HUMAN IgG RESPONSE TO NTERM-34KDa SALIVARY PEPTIDE AS A BIOMARKER TO ASSESS TEMPORAL EXPOSURE TO AEDES' BITES AMONG INDIVIDUALS AGED ≥ 6 MONTHS IN LOWER MOSHI-TANZANIA, 2019** in partial fulfilment of requirements for the Masters of Epidemiology and Laboratory Management.

Dr. Doreen Kamori

(Supervisor)

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Date

_____

Nsiande Lema

(Supervisor)

_____

Date

DECLARATION AND COPYRIGHT

I, **Daniel Laswai** (HD/MUH/T.829/2019) solemnly declare that this research report is my original work and that it has not been presented to any other academic institution for similar or any other degree award, and is not previously or currently under copyright.

Signature …… Date……

ACKNOWLEDGEMENTS

I would like to thank my Almighty God for all His blessings towards this work

The success of this research development has been made possible by the contribution of many people in several different ways. I, therefore, express my sincere gratitude to them all.

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- I also thank my family for their prayers and enormous support.

DEDICATION

I dedicate this research dissertation report to my dear family; Mr. &Mrs. Laswai, my wife, and children.

ABSTRACT

Background: Mosquito-borne diseases inflict significant health, economic and social burden on the populations of endemic areas and are a major cause of important morbidity and mortality. Studies have reported several outbreaks occurring worldwide including Tanzania. The levels of human exposure to vector bites remain an indicator of strengthening vector control measures. Currently, the levels of human exposure to vector bites and their association with climatic changes are mainly evaluated based on both pathogen detection in human populations and entomological methods which have some limitations. Recently, serological *Aedes* salivary biomarkers are most likely reasonable proxies which can offset the limitations. *Aedes* salivary antigen Nterm-34kDa has been dedicated as a useful candidate suitable proxy to assess the level of human temporal exposure to *Aedes* mosquitos' bites ascertained through seroprevalence of IgG. However, these methods have neither been fully evaluated nor attempted to be applied in Tanzania specifically for *Aedes*.

Objective: This study aimed to explored the use of human IgG antibodies to *Aedes* salivary gland protein (Nterm-34kDa) as a biomarker to assess temporal human exposure against *Aedes* bites among individuals aged ≥ 6 months living in Lower Moshi, Northern Tanzania 2019.

Methods: A cross-sectional laboratory-based study which used archived samples collected from individuals aged ≥ 6 months was conducted. All participants of the previous study consented that their samples could be further analysed for research-related studies. A maximum of 500 ul of whole blood samples were collected from each participant in ethylene diamine tetra-acetic acid (EDTA) capillary tubes, samples were stored and transported as per protocols and processed in the laboratory. Sandwich Enzyme-Linked Immunosorbent Assay test (Sero-Well, Sterilin Appleton Woods Limited-France) was used to analyse a total of 713 plasma samples to detect and quantify the presence of immunoglobulin G (IgG) directed against Nterm-34kDa. Rainfall data was provided by the factory's meteorological station (TPC Sugar Factory located at the centre of the villages selected for the study) and recorded in millimetres of rain from January 2019 to January 2020. Data were analysed using Stata Version 14 (Stata Corp, TX, USA) and Graph Pad Prism (San Diego, CA, USA) software. Possible associations

between Nterm-34kDa seroprevalence and participants' characteristics were determined. All statistical tests were regarded as significant at p-values < 0.05.

Results: The seroprevalence of Nterm-34kDa (measured by IgG responses) to salivary peptide were 34.1% (105/308), 45% (91/201), and 26.5% (54/204) during the dry season (time point 1), rainy season (time point 2), and dry season (time point 3), respectively. The Nterm-34kDa seroprevalences were statistically significantly different during time points 2 and 3 (chi^2 =4.1301; *p*=0.042). The levels of IgG increased as rainfall increases, this was observed during the peak of rainfall, suggesting the temporal association between the two variables. The results indicated heterogeneity of the exposure within a population to *Aedes* bites which increases during the rainy season (time point 2, p=0.001, Kruskal Wallis test). Logistic regression analysis showed a significant association between IgG response to salivary peptide and exposure level (risk) according to villages, whereby individuals at Mikocheni village had a lower risk of exposure (OR=0.31; 95% CI=0.1-0.9; p=0.038), independently of either age, sex, and educational level.

Conclusion: These results confirm that human antibody responses to Nterm-34kDa salivary peptide can be isolated from individuals exposed to *Aedes* bites, suggesting that the tool can be used to detect both temporal and real-time human exposure to Aedes's bites. Also, this tool is a sensitive to detect human exposure and is potential in distinguishing seasonal fluctuations in exposure to *Aedes* bites in both high and low transmission settings thus the risk for arboviral transmission. They can also be used as an indicator for evaluating the efficacy of vector control interventions against *Aedes* species.

Keywords: Nterm-34kDa, *Aedes*, Lower Moshi, Tanzania, antibody, temporal, exposure, arboviral

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KEY OF ABBREVIATIONS

ELISA	Enzyme-Linked Immunosorbent Assay
HRP	Horseradish Peroxidase
IgG	Immunoglobulin G
IgM	Immunoglobulin G
MUHAS	Muhimbili University of Health and Allied Sciences
Nterm-34kda	N Terminal -34kilodalton
OD	Optical Density
PBS	Phosphate Buffered Saline
SPSS	Statistical Package for Social Sciences
TMB	Tetramethylbenzidine
TPC	Tanzania Plantation Company
WHO	World Health Organisation

OPERATIONAL DEFINITIONS

Nterm-34kDa- This is an antigenic protein in the sialome of the female of *Aedes aegypti,* which appeared specific to the Aedes genus.

Arboviruses- Are viruses transmitted biologically among vertebrate hosts by hematophagous arthropod vectors. The most well-known include dengue virus, Zika virus, chikungunya virus, yellow fever virus, West Nile virus, and Japanese encephalitis virus.

Biomarker- A substance measured in a biological system as an indicator of exposure, effect, susceptibility, or clinical disease.

Breteau- Defined as the number of mosquito positive containers per 100 houses

Immunoglobin G- Is the most abundant serum immunoglobulins of the immune system. It is secreted by B cells and is found in blood and extracellular fluids. IgG provides protection from infections caused by bacteria, fungi and viruses.

Peptide- This is a short chain of amino acids. The amino acids in a peptide are connected in a sequence by bonds called peptide bonds

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background:

Aedes' mosquitoes are major vectors of re-emerging diseases including arbovirus infections notably Dengue and Chikungunya viruses, which are major public health problems in many tropical regions of the world. The recent (2013) estimate indicates that 390 million dengue infections occur every year, of which 96 million (67–136 million) manifest clinically (with any severity of disease). It is estimated that 4 billion people in 128 countries are at risk of infection with dengue viruses (1). In Sub-Saharan Africa the burden is still high despite the gains achieved following the scaling up of vector control programs, this is due to poor data surveillance, incomplete outbreaks reports, under-reporting to WHO by countries (2,3). Tanzania has experienced with the numbers of dengue outbreak in different years from 2010 up to 2019 which occurred in Dar es Salaam, with the total of 6,873 confirmed cases including 13 deaths (4,5).

To date, no tool can assess the real-time levels of human exposure to vector bites particularly *Aedes* mosquitoes in our local settings in Tanzania. Currently, the level of human exposure is mainly evaluated based on traditional methods; both pathogen detection in human populations and entomological methods which has shown some limitations and challenges (6,7,8,9).

New epidemiologic tools (serological) with new complementary indicators have been used in different settings and has proved and shown to offset the limitation and challenges shown by entomological methods (7).

Therefore, serological analyses of human immunological response towards *Aedes*' salivary antigens (Nterm-34kDa) have been evaluated as proxies for individual exposure to *Aedes* mosquito bites (5). The evaluation of human exposure to *Aedes* bites can be measured by IgG levels response and can be correlated with seasonal fluctuations, such as rainfall, relative humidity, and ambient temperature (7).

One peptide (Nterm-34kDa) protein in *Aedes* aegypti saliva has been recently validated, by several studies, as an appropriate candidate biomarker to assess the levels of temporal human exposure. It has been documented those antibodies against Nterm-34kDa specific

to *Aedes* aegypti are detected in individuals who have been exposed to Aedes bites (7), and levels of IgG against this peptide do correlate well with levels of human exposure to the *Aedes* mosquito (20).

There are about 15 proteins in the sialome of the female of Ae. Aegypti identified, which are potentially antigenic, the putative 34kDa family secreted salivary protein appeared specific to Aedes genus and looked to be an interesting candidate for validation as a biomarker specific to Ae. aegypti bites (21).

Amino-acid sequence of the putative 34kDa family secreted salivary protein of Aedes aegypti (gi: 94468336, NCBI database) is presented and the sequence of the Nterm-34kDa peptide is underlined. The signal peptide (SP) sequence is indicating by a dotted underline (20).

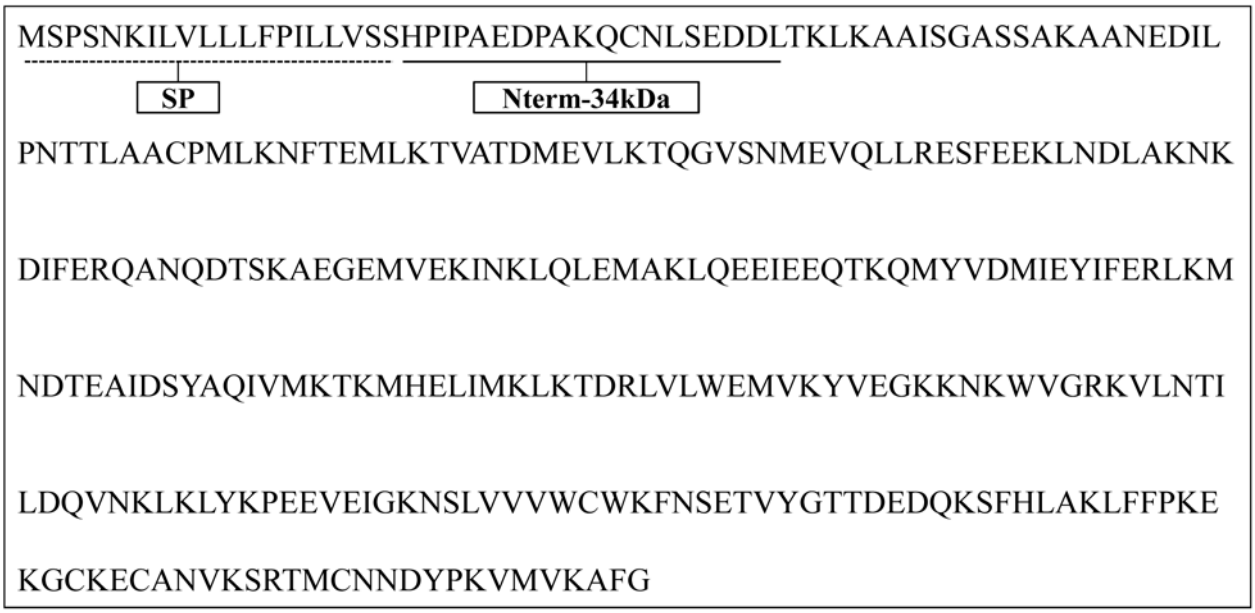

Figure 1: Amino-acid sequence of Nterm-34kDa Peptide.

1.2 Problem statement

Mosquito-borne diseases inflict significant health, economic and social burden on the populations of endemic areas and are a major cause of important morbidity and mortality (1). Studies have reported several outbreaks occurring in Tanzania (22,23-24), and the recent ones in 2018 and 2019 (4,5). The 2014 outbreak resulted in 1018 confirmed cases and 4 deaths, mainly in Dar es Salaam (25), with a few confirmed cases in Kilosa district (5). However, the worst documented dengue outbreak occurred in 2019 in Dar es Salaam and later in Tanga, with 6873 cases and 13 deaths (4,5).

Despite the use of entomological methods to detect the levels of human exposure to mosquito bites and predict the occurrences of outbreaks, still there are limitations and challenges which hindering the fully functioning and utilization of these methods. The salivary proteins (serological biomarkers) such as Nterm-34kDa can be used to offset the limitations shown by the traditional methods as they are cost-effective, simple, rapid, and sensitive, that can measure; the individual exposure to *Aedes* bites, real-time risk of arbovirus transmission in the exposed population, the efficacy of vector control as a necessary step towards outbreaks predictions and improvement of vector control interventions programs and predictors of outbreaks (7). These serological tools have been used in other settings and showed a good performance (19,20,21); however, these methods have not been fully evaluated and have never been attempted to be applied in Tanzania. Therefore, the proposed study was carried out, to evaluate the human IgG response to Nterm-34kDa salivary peptide as a biomarker to assess for human temporal exposure to *Aedes* bites in Lower Moshi-Tanzania, 2019.

1.3 Conceptual framework

Vector density is the key determinant for arbovirus diseases transmission and is largely affected by geographic and climatic factors such as climatic changes, season, and topography. An increase in vector density increases human exposure to vector bites.

During blood feed, the vector (*Aedes*) injects the specific salivary antigen (Nterm-34kDa) into the human body which triggers immunological responses to produce specific antibodies (IgG) against it.

A variation on the levels of IgG to Nterm-34kDa can be compared according to rainfall, age, sex, and village residence

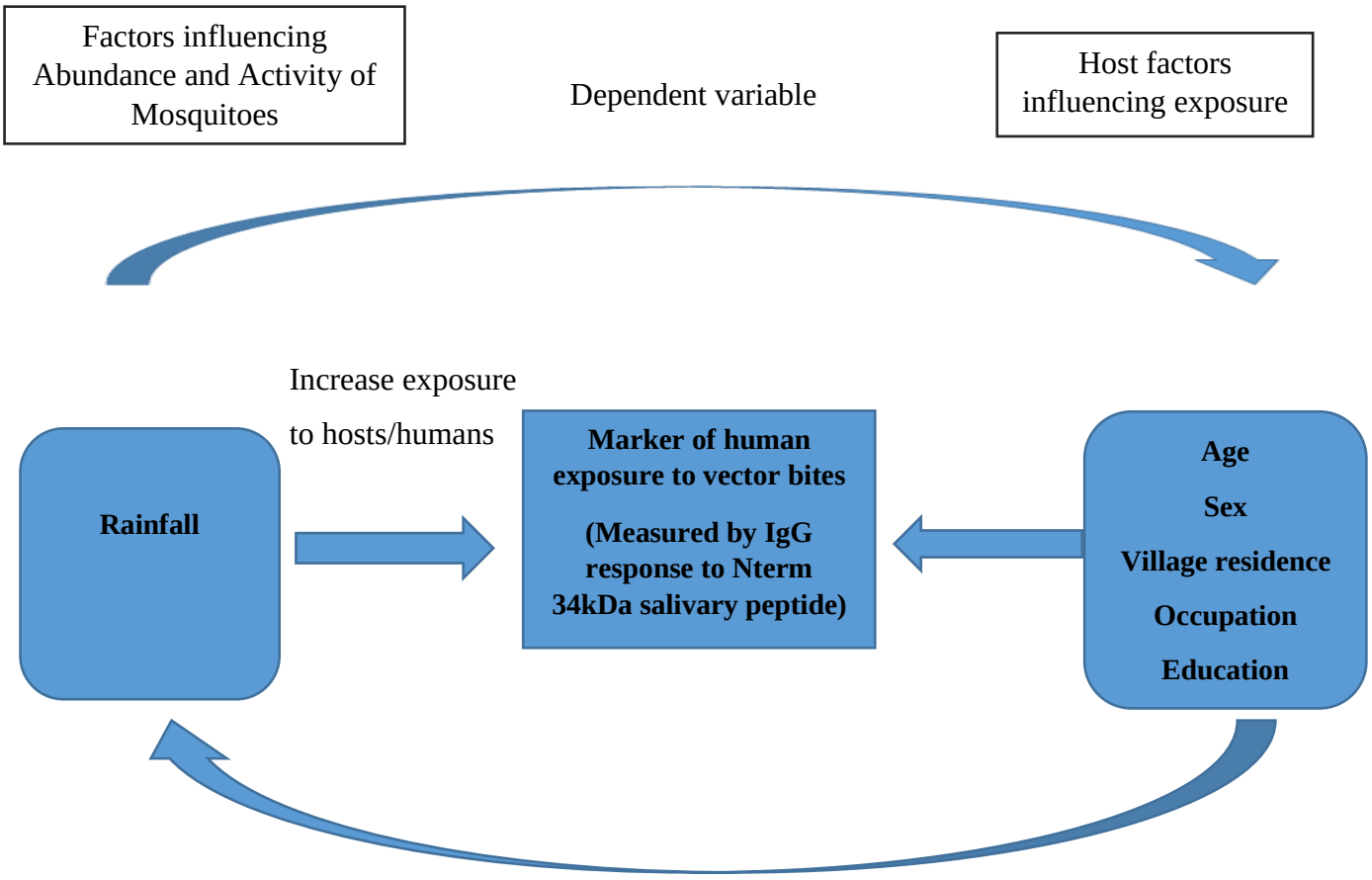

Figure 2: Conceptual frame work

1.4 Rationale

The proposed study aimed to explore the use of human IgG antibodies to *Aedes* salivary gland protein (Nterm-34kDa) as a biomarker to assess temporal human exposure against *Aedes* bites among individuals aged ≥ 6 months living in Northern Tanzania 2019. Lower Moshi, has been selected because it is located in northern Tanzania where there is a high probability of the establishment of the vector-host arbovirus transmission cycle.

The findings from this study will reveal the importance of this serological biomarker as the proxy to assess the heterogeneity of individual exposure to *Aedes* bites. Through examining the changes in IgG Ab response to Nterm-34kDa and the prevalence of immune responders in association with climatic changes, the proper practices on vector control interventions will be implemented, because to date no tool can assess the efficacy of different vector control interventions therefore it is the best way is to incorporate this approach as one among practices in which are in place. Knowing the levels of IgG against Nterm-34kDa, indirectly the abundance of vector density can be detected or evaluated, this can prompt the implementation of a new policies regarding establishment of sensitive surveillance system to monitor the increase of the vector density, hence developing early warning system(s) to the occurrence of risks of transmission and outbreaks. This tool will add to the existing conventional (entomological) tools for the surveillance of arbovirus infections in different settings.

1.5 Research question

What is the response of human IgG to Nterm-34kDa salivary peptide as a biomarker for assessing temporal human exposure to *Aedes* bites among individuals aged ≥ 6 months in Lower Moshi-Tanzania-2019?

1.6 specific research questions

1. What is the temporal and spatial variation in Nterm-34kDa Aedes salivary peptide seroprevalence among individuals aged ≥ 6 months in Lower Moshi 2019?
2. What is the temporal variation of rainfall patterns and Nterm-34kDa seroprevalence in Lower Moshi 2019?

3. What is the association between social-demographic characteristics and exposure to Aedes mosquito bites among individuals aged ≥ 6 months in Lower Moshi 2019?

1.7 Objectives

1.7.1 Broad Objective

To evaluate the performance of salivary peptide (Nterm-34kDa) IgG response as a serological biomarker to assess temporal exposure to Aedes bites among individuals aged ≥ 6 months in Lower Moshi-Tanzania-2019.

1.7.2 Specific Objectives

1. To determine temporal and spatial variation in Nterm-34kDa Aedes salivary peptide seroprevalence among individuals aged ≥ 6 months in Lower Moshi 2019
2. To determine the temporal variation of rainfall patterns and Nterm-34kDa seroprevalence in Lower Moshi 2019
3. To determine the association between social-demographic characteristics and exposure to Aedes mosquito bites among individuals aged ≥ 6 months in Lower Moshi 2019

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Description of Nterm-34KDa salivary peptide, host immune response and limitations

This is among 15 proteins in the sialome of the female of Ae. Aegypti identified, which are potentially antigenic, the putative 34kDa family secreted salivary protein appeared specific to *Aedes* genus (21) . During blood feed the vector (*Aedes*) injects the cocktail of salivary proteins including specific salivary antigen (Nterm-34kDa) into human body which trigger human body to produce of specific antibodies (IgG) against it, that can be used to evaluate exposure to disease vectors (43,44). Evolution of knowledge in past fifteen years regarding approach towards understanding the composition and complexity of vectors salivary glands extracts made the way towards the development of novel complementary serological tools that can directly assess the temporal human exposure to vectors bites by measuring human-vector contacts ascertained by the human antibody response (33). Previous studies indicated IgG immune response to Nterm-34kDa salivary peptide can assess relative human temporal exposure to Aedes bites since it is not expected to last for more than 15–30 days (28), therefore may be very useful, especially in both settings with lower and higher mosquito density, providing a better view of human exposure to *Aedes* vectors and disease risk, and eventually increasing the sensitivity and/or specificity of the immunoassays (46). However, These SGE has shown some limitations as antigen for immunoassays, such as it is difficult to obtain the enough quantity of saliva as it is laborious and time-consuming. Moreover, specificity may be compromised due to different in saliva composition which varies between the species and due to technical variations in the procedure of saliva collection or SGE preparation. Furthermore, there is high chance of cross-reactivity between the species genus and genus level. However, there is large group of vector species which family- and genus-specific salivary proteins, that highlighting the importance of these biomarkers as potential and reliable tools to evaluate the human exposure to vector bites within a population (45).

2.2 Performance, challenges and limitations of traditional methods and serological tools towards assessing temporal human exposure against vector bites

Currently, the level of human exposure is mainly evaluated based on traditional methods; both pathogen detection in human populations and entomological methods (4), such as identification of breeding sites, the capture of mosquitoes by trapping, aspirators, indoor spraying, and human landing catches and pupal monitoring (5). Some evaluation indices such as Breteau, adult productivity, house, and adult density have also been used (6). These entomological methods showed some limitations and challenges such as, identification of larval habitats which are labour-time consuming, very fastidious and there are ethical concerns when it comes to human landing catches (4). In addition, they are difficult to apply when there is a change in environmental conditions, they cannot give the vectors distribution densities as they cannot be applied in areas with low Aedes mosquitoes' population (7). Furthermore, these methods cannot be applied to gauge the heterogeneity of individual exposure and attractiveness to mosquitoes (5), moreover, the tools are poorly predictors of diseases transmission and risk of outbreaks (4).

New epidemiologic tools (serological) with new complementary indicators which are cost-effective, simple, rapid, and sensitive should be developed, that can measure; the individual temporal exposure to *Aedes* bites, real-time risk of arbovirus transmission in the exposed population, the efficacy of vector control as a necessary step towards outbreaks predictions and improvement of vector control interventions programs (18). There is a correlation between human exposure to vector bites *(Aedes)* with an increase in vector abundance, whereby if there are favourable climatic conditions also the vector density increases, this suggesting that human antibodies response can be used to evaluate the abundance and fluctuations of vector population that can bite human population (6).

During blood feeding, vectors inject salivary proteins, some of these salivary proteins which are functional and necessary for blood uptake and immunogenic characteristics that enable initiation of a specific immune response to the host immune cells to produce antibodies against each specific immunogenic salivary protein (10).

Studies have shown that various salivary proteins from vectors can be used as an indicator to evaluate the level of exposure against arthropod vectors bite such as triatoma, Phlebotomus, ticks, glossina, and anopheles (11,12,13,14,15). The study done among children sensitive to mosquito bites supported the role of mosquito saliva-specific IgE

antibodies in the pathogenesis of mosquito bites (16,17). It has been shown that the levels of IgG against gSG6-P1 correlates well with levels of exposure to Anopheles mosquito (18), and are always detected in malaria-infected individuals (19). There is a correlation between human exposure to vector bites *(Aedes)* with an increase in vector density which is highly affected by the changes in climatic variables such as climatic changes, season, and topography (20).

2.3 Temporal and spatial variation in Nterm-34kDa Aedes salivary peptide seroprevalence

Human contacts with *Aedes* mosquito trigger the production of IgG specific to Nterm-34kDa, which varies temporally and spatially across settings (26). Since, the level of exposure of the human population against vector bite is mainly evaluated by traditional entomo-parasitological methods which are not ideal under certain conditions (7), The measure of human antibody response to *Aedes* salivary proteins (biomarkers) which are serological tools represents a novel approach since it can overcome all the limitations of traditional methods (20). knowing the temporal seroprevalence of IgG against Nterm-34kDa and its spatial variation within a geographical area, this biomarker can be useful for surveillance of arboviruses and establishment of vector control programs (7). Since it can explore the association between levels of human exposure to *Aedes* mosquito, it can be able to discriminate exposed individuals to Aedes vector bites from those who are not exposed, and can classify individuals according to their level of exposure to *Aedes* vectors, and usable at a population level (26). Therefore, there is a need to prompt an extensive surveillance system of these vectors which are surviving and adapt easily in different geographical areas which were not previously found. For these reasons, there is a great need for continuous entomologic and serological arboviral surveillance to gather new data about the circulation (emergence and re-emergence) of arthropod-borne viruses globally. Seroprevalence of IgG appears to be an important indicator during assessing and evaluating; the actual human and level of individual exposure to vector bites, reflected by human antibodies levels response to vector salivary proteins (27). However, the IgG immune response to Nterm-34kDa salivary peptide represents a relevant temporal biomarker to assess recent relative exposure of humans to *Aedes* bites since it is not expected to last for more than 15–30 days (28). The Nterm-34kDa salivary protein is

antigenic and specific to *Aedes* genus whereby during biting, the female *Aedes* mosquito injects this antigen which results in the production of human antibodies (IgG) by the immunological response (29).

The measure of human antibody (Ab) response to vector salivary proteins represents a novel approach, the antibodies produced can be serologically detected and quantified where the quantities reflect the level of exposure to vector bites (7). Individuals exposed to Aedes bites could develop IgG response to Nterm-34kDa salivary peptide (20). A study done among people living in an urban area in Bolivia showed relatively low levels of IgG against Aedes aegypti (30), compared to Reunionian individuals who were much exposed, with an overall of 88.2% represented specific IgG responses which were higher than the cut-off (7).

The antibody (IgG) response against Aedes saliva appears to increases significantly and appeared to be specific to the mosquito genus among French soldiers and it has been demonstrated that IgM and IgG responses to the whole saliva could be a promising indicator of Ae. aegypti exposure in temporarily exposed populations regarding Aedes species (21).

A large dengue epidemic that occurred in Santa Cruz in 2007 showed the seroprevalence (52.7%) of IgG against Aedes aegytpti (26). It has also been documented that the IgG levels vary with age, where it is highest in infants and decreases progressively with age (31). The seroprevalence of the IgG levels also increases as the exposure to Aedes bites increases (68.8% and 72%) among immune responders during exposure 1 and 2 respectively compared to other groups (32), this was higher compared to another recent study which reported only 19% of immune responders for Aedes aegypti (33). Therefore, this study will help to determine the seroprevalence of IgG response to Nterm-34kDa Aedes salivary peptide in Lower Moshi-2019.

2.4 Temporal variation of rainfall pattern and Nterm-34kDa seroprevalence

An alarming increase of mosquito-borne arboviruses worldwide has been largely attributed to the effects of the global climatic changes (5). The seasonality of Aedes abundance and human exposure to bites is highly correlated with changes in climatic variables, such that when there are favourable climatic conditions the vector density

increases, this suggesting that human antibodies response can be used to evaluate the abundance and fluctuations of vector population that can bite human population (37).

Development and survival rates of Aedes mosquito depend on temperature, that low or high affect mortality of Aedes, also it affects the rate of development (life cycle of Aedes). Both temperature and humidity have effects on *Aedes* activity and therefore host finding, while rainfall affects breeding sites; all of these directly affect their abundancy and indirectly have effects on human exposure to bites and arboviruses diseases transmission (39).

The evaluation of human exposure to *Aedes* bites measured by IgG levels response can be correlated with rainfall, relative humidity, and ambient temperature (26). The levels of IgG response to *Aedes* salivary peptide antigen were compared according to different seasons (Time points).

A higher correlation of IgG levels was observed to increase from dry to the rainy season (28.78 to 98.98%) respectively, also the IgG levels varied according to the season and were associated with the intensity of rainfall (20).

A correlation was found among the population exposed to Aedes whereby the IgG response increases as the rainy increases (34), similar findings were observed in different sites in different mosquito genus whereby the IgG response, density of adult mosquito, and exposure rate increases during rainy intensity (15).

Therefore, these findings demonstrated that there is an association between climatic changes and IgG Abs response to Nterm-34kDa and can reveal the real-time human exposure to Aedes bite(20).

Temporal variation in Nterm-34kDa seroprevalence and rainfall were compared between the peak of the dry and rainy seasons (according to time points).

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study Design.

A cross-sectional laboratory-based study design which used archived samples was conducted. These samples were obtained from a survey conducted for seven months among adults and children aged ≥ 6 months in Lower Moshi, Tanzania in 2019. The survey had three time points in which samples were collected; including during a dry season in March (time point 1) as a baseline, followed by two follow-up time points, whereby time point 2 was conducted during the rainy season in June, and the last time point 3 during the subsequent dry season in September 2019.

3.2 Study Area.

The study was conducted in lower Moshi. Lower Moshi (3021'S, 37020'E), is located 10 kilometres from Moshi municipality, and about 800 meters above sea level, south of Mount Kilimanjaro in the northern part of Tanzania. Most of the population in the area is engaged in agricultural activities with irrigated rice and sugarcane cultivation as main crops. The non-irrigated crops include maize, beans, and banana. Two rivers, namely Njoro and Rau provide the water for irrigation. Livestock in this area is mainly cattle, goats, sheep, and poultry. Lower Moshi has been selected because it is located in northern Tanzania where there is a high probability of the establishment of the vector-host arbovirus transmission cycle, basing on risk factors such as proximity to large wetlands (rice fields, rivers, swamps, and dams), livestock farms, reports of febrile illness patients through previous studies, interface areas for domestic-wild animals and vector abundance, as well as suitable climate which offers conducive breeding habitat for mosquito vectors. Also, it is among the endemic areas for mosquito-transmitted diseases like arboviruses (Chikungunya) (42). Therefore, the estimation of the level of human–mosquito contact is an important indicator to measure the risk of transmission of such diseases.

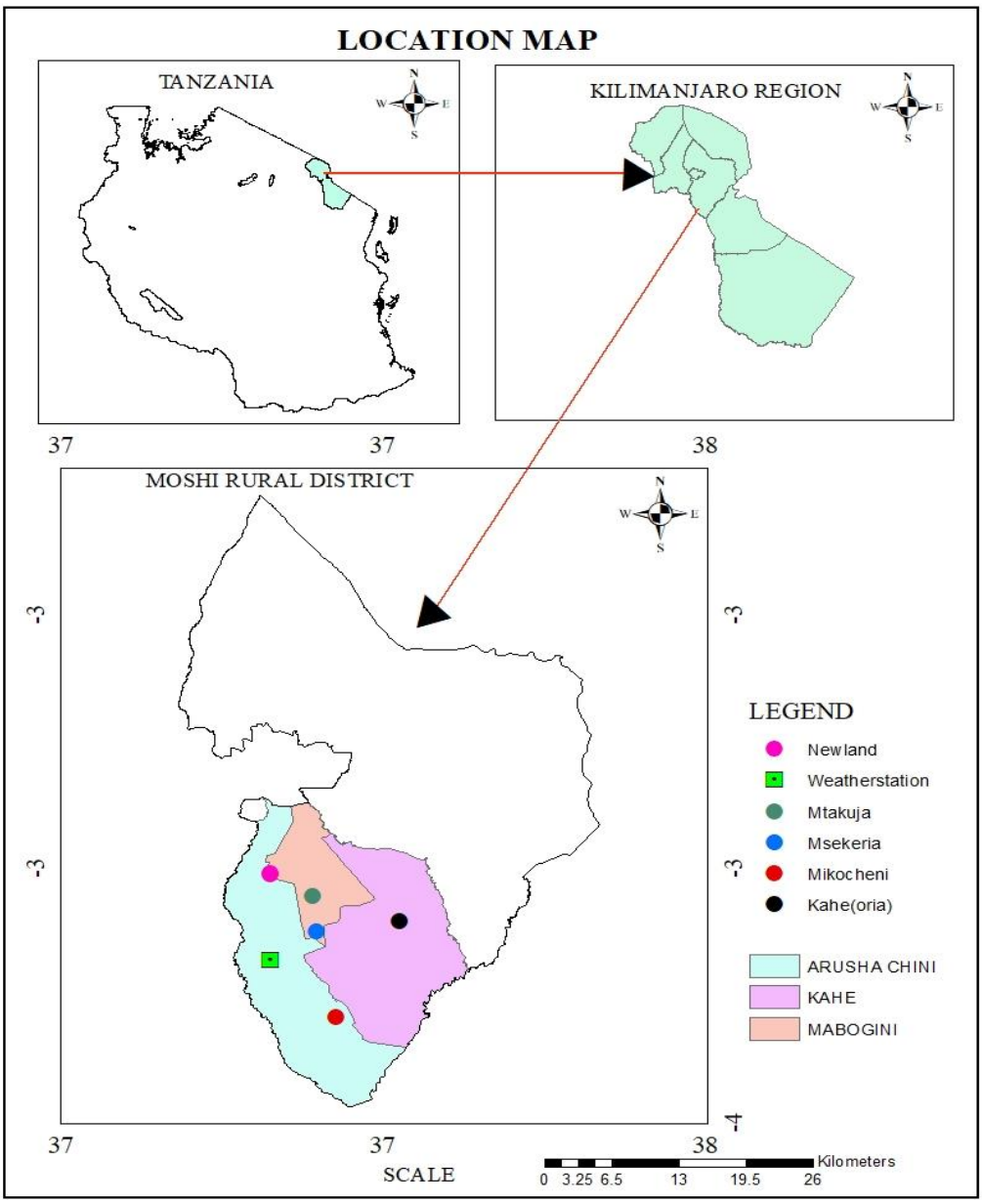

Figure 3: A map showing the study site.

3.3 Study population and Sampling strategy

This study utilized archived blood plasma samples that were collected in dry and rainy seasons from individuals aged ≥ 6 months.

Briefly individuals aged ≥ 6 months were recruited in the previously three cross sectional surveys conducted for a period of seven months among adults and children in lower Moshi, Tanzania in 2019. The study population were stratified by age whereby the age strata was from six months to five years, six years to fifteen years and sixteen years and

above. Within the age strata, participants were further stratified by sex (men and women). To ensure comparison between the three age strata, equal numbers of participants were recruited in each stratum (41). In total 713 blood samples were collected whereby, 308 study participants were enrolled during the baseline survey (Time point 1) whose blood samples were collected. Of them, 201 were followed in the second survey (Time point 2) and collected blood samples, and 204 in the third survey and samples were collected. The previous study aimed to explore the use of human antibodies against gambiae salivary gland protein 6 peptide 1 (gSG6-P1) as a biomarker of Anopheles exposure and assessed temporal exposure to mosquito bites in populations living in Lower Moshi, Northern Tanzania. (41)

3.4 Sample size estimation.

In this study, a total of 713 cryo-preserved plasma samples collected from individuals enrolled in the previous surveys (three-time points studies) were analysed.

Absent participants during the follow-up studies had either travelled or absent for other community and employment activities resulted into the decrease of expected samples (924 samples) that were supposed to be collected and analysed.

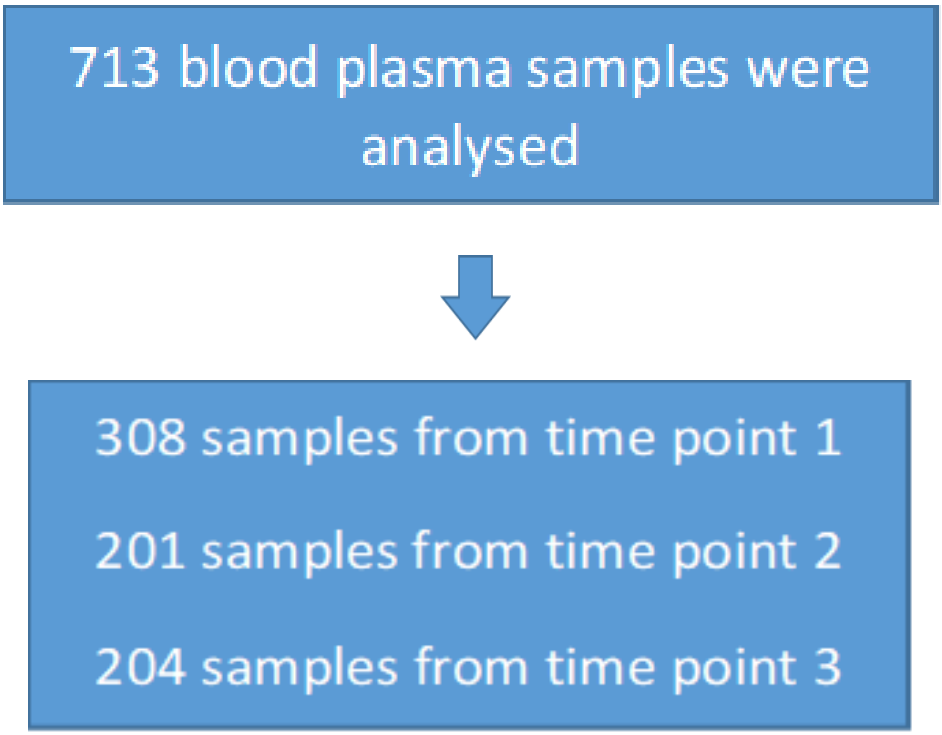

Figure 4: A flow chart to show distribution of samples used for analysis at time point 1, 2 and 3.

3.5 Inclusion and Exclusion Criteria.

3.5.1 Inclusion criteria.

All the archived blood plasma samples collected from individuals aged ≥ 6 months who were enrolled at three different time points from the previous survey.

3.5.2 Exclusion criteria.

None of the samples were excluded which were collected from individuals aged ≥ 6 months enrolled at three different time points from the previous survey.

3.6 Variables.

3.6.1 Dependent variable.

The dependent variable was the level of exposure to Aedes bites (as measured by IgG response to specific Nterm-34kDa salivary peptide).

3.6.2 Independent variables.

Independent variables were age, sex, rainfall, educational level and village residence.

3.6.3 Data collection methods for independent variables

Information on these variables (age, sex, rainfall, educational level and village residence) were extracted from the structured data base of the previously conducted survey.

Age, Sex, Educational level and Village residence were collected through structured questionnaire while Rainfall data was provided by TPC Sugar Factory located at the centre of the villages selected for the study. Daily rainfall data issued was recorded in millimetres of rain at the factory's meteorological station from January 2019 to January 2020.

3.7 Laboratory Analysis.

A maximum of 500 ul of whole blood samples were collected from each participant in ethylene diamine tetra-acetic acid (EDTA) capillary tubes, samples were stored in cool boxes contained ice packs, transported and processed in the laboratory, were by blood samples were centrifuged to obtain plasma sample and stored in cryo-tubes.

A total of 713 plasma samples were analysed by a Sandwich Enzyme-linked immunosorbent assay (Sero-Well, Sterilin Appleton Woods Limited) - for evaluation and quantification of human IgG levels. The ELISA had a sensitivity of >90% and specificity of 93%. There is a possibility of cross reactivity due to presence of common antigenic determinants, also freeze-thaw cycles of the specimen may affect the absorbance values.

Elisa optimization was performed before the analysis of the studied samples, and this was done by using control samples (positive and negative), the known positive samples from individuals who have been exposed to *Aedes* bites, and the negative control samples from neonates that are not been exposed to *Aedes* bites, this was to ensure the testing of the developed ELISA procedures, reagents used and equipment (ELISA reader) if are in good working condition to give the quality and reliable results (data).

Under this section I participated on sample collection, packaging and transportation during the previous conducted study survey, and also, I performed laboratory work on sample analysis.

Table 1: Laboratory reagent preparation and procedures.

Reagent's preparations	**Procedures**
N-Term 34kDa aliquots preparation The Nterm-34 kDa salivary peptide has been selected as previously described (18) and synthesized and purified (>95%) by Genepep SA (St-Jean de Vedas, France). The peptides were shipped in lyophilized form and then suspended milliQ water and stored in aliquots at -20°C	Coating
Washing buffer	Blocking
Blocking buffer	Incubation with the plasma sample
Stopping buffer	Capture, secondary Abs and substrate
	Detection

3.8 Evaluation of human IgG antibody levels.

ELISA technique was performed as previously described (18). Briefly, the peptide (20µg/mL in 100 µl of Phosphate Buffer Saline, PBS) was coated for 150 minutes at 37°C into Maxisorp plates (Nunc, Roskilde, Denmark).

Plates were blocked by Protein-Free Blocking-Buffer (Pierce, Thermo Scientific, France). Each eluate was incubated in triplicate at 4°C overnight at 1/20 dilution in PBS-Tween 1%

Mouse biotinylated Ab to human IgG (BD Biosciences, San Diego, CA) was incubated at 1/1000 dilution in PBS-Tween 1% and peroxidase-conjugated streptavidin (GE Healthcare, Orsay, France) was added (1/1000 dilution in PBS-Tween 1%).

Colorimetric development was carried out using 2, 2'-azino-bis (3-ethylbenzthiazoline 6-sulfonic acid) diammonium (ABTS; Thermo Scientific, France), and absorbance (OD) was measured at 405 nm.

3.9 Interpretation.

Individual results were expressed as the ΔOD value calculated according to the formula ΔOD = ODx − ODn, where ODx represents the mean of individual OD values in the two wells containing antigen and ODn (the OD value) in a well without antigen.

A subject was considered as an "immune responder" if ΔOD was higher than the cut-off (Cut-off = mean (ΔOD unexposed) + 2SD).

3.10 Data analysis plan.

Data were checked for correctness and completeness, then entered in statistical software, counterchecked, cleaned, and analysed. Statistical Package for Social Sciences (SPSS) version 20 (IBM Corp, Chicago, IL) was used for entry and analysis. Data were summarized in tables and presented in figures.

Data were analysed using Stata Version 14 (Stata Corp, TX, USA) and Graph Pad Prism (San Diego, CA, USA) software. Kruskal-Wallis test was used for comparison between more than two groups. Chi^2 test was used to compare temporal variation in exposure and IgG Abs positives response to Nterm-34kDa for bivariate analysis. The logistic regression analysis was performed to determine the associations between Nterm-34kDa seroprevalence and participants' characteristics, however subjecting the variable to multivariate analysis was to assess the association between the probability of being an immune responder and *Aedes* exposure level independent of potential confounders. The

proportion was used to show the level of exposure to Aedes' bites. All statistical tests were regarded as significant at p-value < 0.05.

Under this section I performed data extraction, cleaning and analysis, and finally preparing the results.

3.11 Utilization and Dissemination of Information

The community will be informed on the results obtained from this study through the means of presentations in symposia, workshops, and conferences. Additionally, the dissertation report will be kept at the MUHAS repository and findings will be published in international peer-reviewed journals, contributing information to the wider scientific and health provider community. Findings will also be presented to TFELTP and MoHCDGEC.

3.12 Ethical Issues.

Ethical clearance to conduct the study was obtained from MUHAS Research and Ethics Committee. An approval to conduct the study using the archived sample was sought from the Principal Investigator of the previously conducted study. Regarding the previous study, written informed consent and/or ascent was obtained from the adult participants while children's guardians or parents were asked to give the consent. Approval to conduct the surveys was provided by Kilimanjaro Christian Medical University College (KCMUCo) Research Ethics and Review Committee (CRERC). Permission to carry out the study in Lower Moshi was sought from the District Executive Director (DED) of Moshi District Council and local government leaders of Lower Moshi (41). All participants of the previous study consented that their samples could be further analysed for research-related studies.

3.13 Expected outcome.

A proxy for exposure to Aedes has been evaluated as a tool to assess temporal exposure to mosquito bites and a tool to estimate Aedes mosquito density. The impact of vector density dynamics on the risk of arbovirus diseases transmission has been assessed too. Also, the Nterm-34kDa antigen can be validated now, for evaluating the efficacy of vector control interventions implemented within the long or short term.

3.14 Feasibility.

This study was supported financially by the Field Epidemiology and Laboratory Training program and the principal investigator (PI) of the previously conducted longitudinal time points study in 2019.

CHAPTER FOUR

4.0 RESULTS

4.1 Socio-demographic characteristics of the study participants

In total, 713 plasma samples were analysed, these samples were collected during time point 1 (308), time point 2 (201), and time point 3 (204**).** The plasma samples collected from children aged between 6 and 15 years represented the largest age group, whereby 77 (25.0%) samples were collected in time point 1, 50 (24.9%), time point 2, and 64 (31.4%) time point 3 during the previous survey. Almost 70.0% of participants in each time points were females. Less than 40% of participants in all three-time points had primary education. **(Table 2)**

Table 2: Characteristics of the studied population

Variable	Time point 1 N=308 n (%)	Time point 2 N=201 n (%)	Time point 3 N=204 n (%)
Village			
Oria	52 (16.88)	45 (21.39)	40 (19.61)
Mtakuja	40 (12.99)	35 (17.41)	34 (16.67)
Newland	49 (15.91)	28 (13.93)	25 (12.25)
Mikocheni	84 (27.27)	44 (21.89)	54 (26.47)
Mserekia	83 (26.95)	51 (25.37)	51 (25.00)
Age-groups (years)			
0-5	49 (15.91)	32 (15.92)	32 (15.69)
6-15	77 (25.00)	50 (24.88)	64 (31.37)
16-30	37 (12.01)	18 (8.96)	16 (7.84)
31-45	50 (16.23)	31 (15.42)	28 (13.73)
46-65	19 (19.84)	43 (21.39)	41 (20.10)
66+	35 (11.36)	27 (13.43)	23 (11.27)
Sex			
Female	215 (69.81)	136 (67.66)	140 (68.63)
Male	93 (30.19)	65 (32.34)	64 (31.37)
Education Level			
No formal education	56 (18.18)	35 (17.41)	31 (15.20)
Children ages <5 years	37 (12.01)	25 (12.44)	26 (12.75)
Pupils at primary school	76 (24.68)	52 (25.87)	64 (31.37)
Had Primary education	119 (38.64)	79 (39.30)	74 (36.27)
Have secondary education and above	20 (6.49)	10 (4.98)	9 (4.41)

4.2 To determine temporal and spatial variation in Nterm-34kDa Aedes salivary peptide seroprevalence.

4.2.1 IgG response to salivary Nterm-34kDa peptide in the studied population

The IgG response to salivary Nterm-34kDa peptide in the studied population based on samples collected at three-time points was analysed. For each time point, the seroprevalence was different, the specific IgG level was more pronounced during time point 2 compared to time point 1 and 3; hereby IgG response to salivary Nterm-34kDa peptide was (105/308) 34.1% during time point 1 (dry season), (91/201) 45.3% during time point 2 (rainy season) and (54/204) 26.5% during time point 3 (another dry season) (Fig 3). IgG responses to salivary Nterm-34kDa peptide were presented and compared to the total monthly rainfall recorded in the same studied area. Also, the seroprevalence varied seasonally from the start to the end of the study, whereby lowest IgG levels were observed during the first dry season (January to March), thereafter increased during the rainy season (April to June), followed by a decrease during the second dry season (July to September). Altogether, these results suggest that human IgG antibodies to Nterm-34kDa peptide could be developed whenever there is human exposure to *Aedes* bites and it can differ within the same population based on the season of exposure.

4.2.2 Temporal and spatial variation in Nterm-34kDa seroprevalence according to time points and villages.

The Nterm-34kDa seroprevalence was 34.1% (105/308) during time point 1 that was conducted in all villages during the dry season. The seroprevalence increased to 45.3% (91/201) at time point 2 conducted at the end of the rainy season (chi^2 =0.1605; p=0.689) and then followed by a significant decline to 26.5% (54/204) at time point 3 conducted during the subsequent dry season (chi^2 =4.1301; p=0.042) (**Fig 3**).

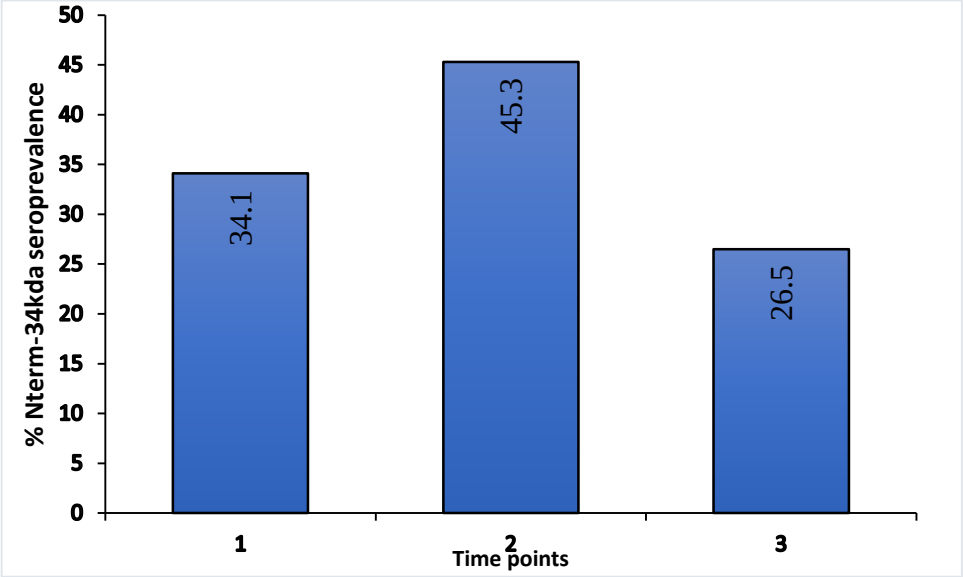

Figure 2: Nterm-34kDa seroprevalence measured during the dry season (time point 1) in March, rainy season (time point 2) in June, and another dry season (time point 3) in September 2019.

Also, it was observed that there was a temporal variation in Nterm-34kDa seroprevalence at time points 1, 2, and 3 to each village included in the study, whereby the seroprevalence pattern was almost similar for all villages except for Mserekia village. There was no statistical significance in seroprevalence variation by the village of residence at all time points during the study (**Fig 4).**

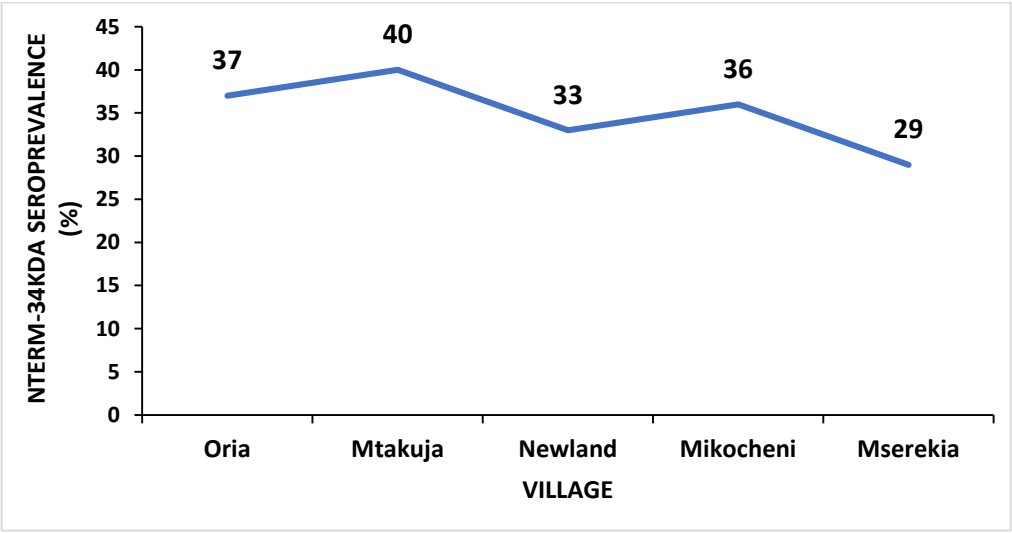

Figure 3: Temporal variation of Nterm34-kDa seroprevalence for the five villages included in the study during the three-time points.

4.2.3 Individual IgG response to Nterm-34kDa

The IgG response to Nterm-34kDa salivary peptide within a population was different between individuals across the time points during the study. High inter-individual heterogeneity in specific IgG Ab levels was observed during time point 2 (rainfall season). Also, the seroprevalence of individual exposure levels was significantly increased during the rainy season, p=0.001(Kruskal Wallis test) (Fig 5).

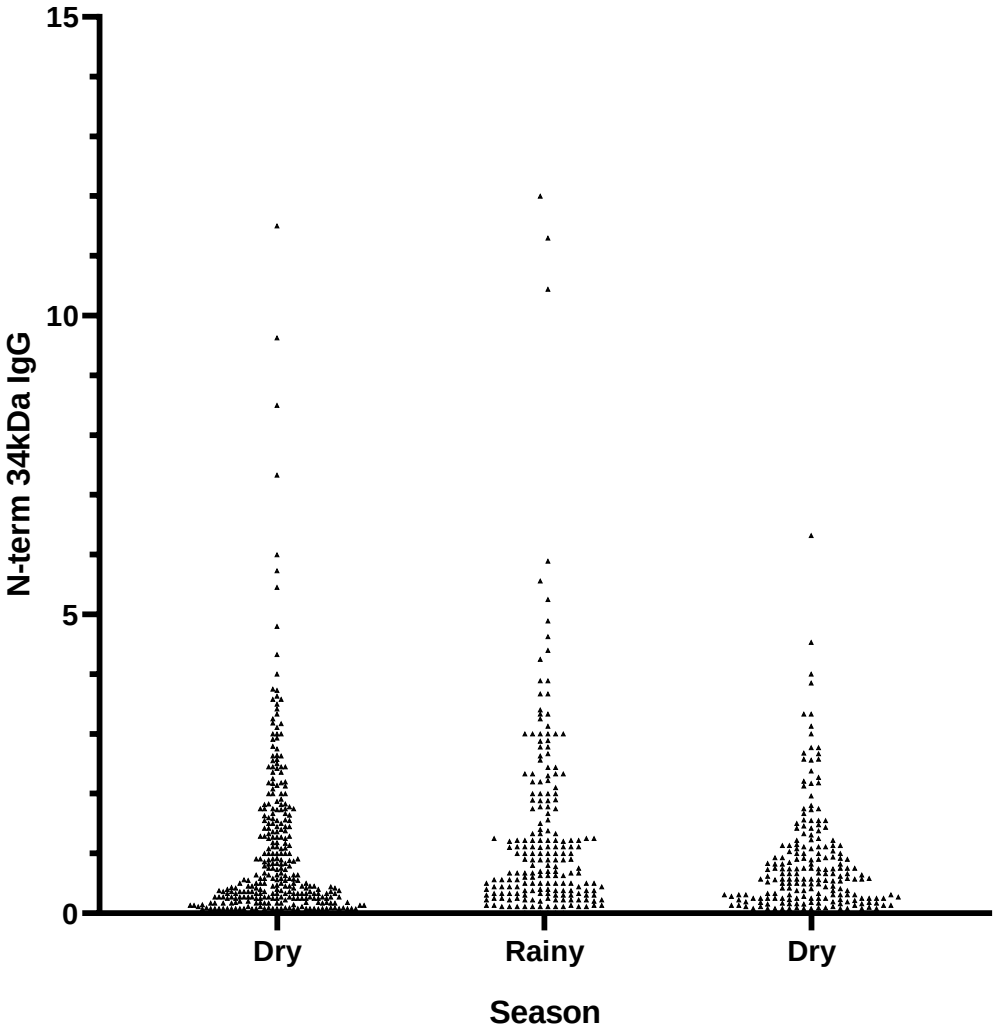

Figure 4: Evolution of individual IgG response to Nterm-34kDa (ΔOD) between dry and rainy season across the time points. (Black points indicate individual IgG response).

4.3 Temporal variation of rainfall pattern and Nterm-34kDa seroprevalence.

The rainfalls were more intense from April until June then followed by three months of drought from June to September, 2019. The curve of rainfall was closely associated with the increase of the percentage of N-term 34kDa seropositive which were ascertained by an increase of specific IgG Abs level response. The monthly rainfall pattern for lower Moshi in the year 2019 is shown in **Fig 6.**

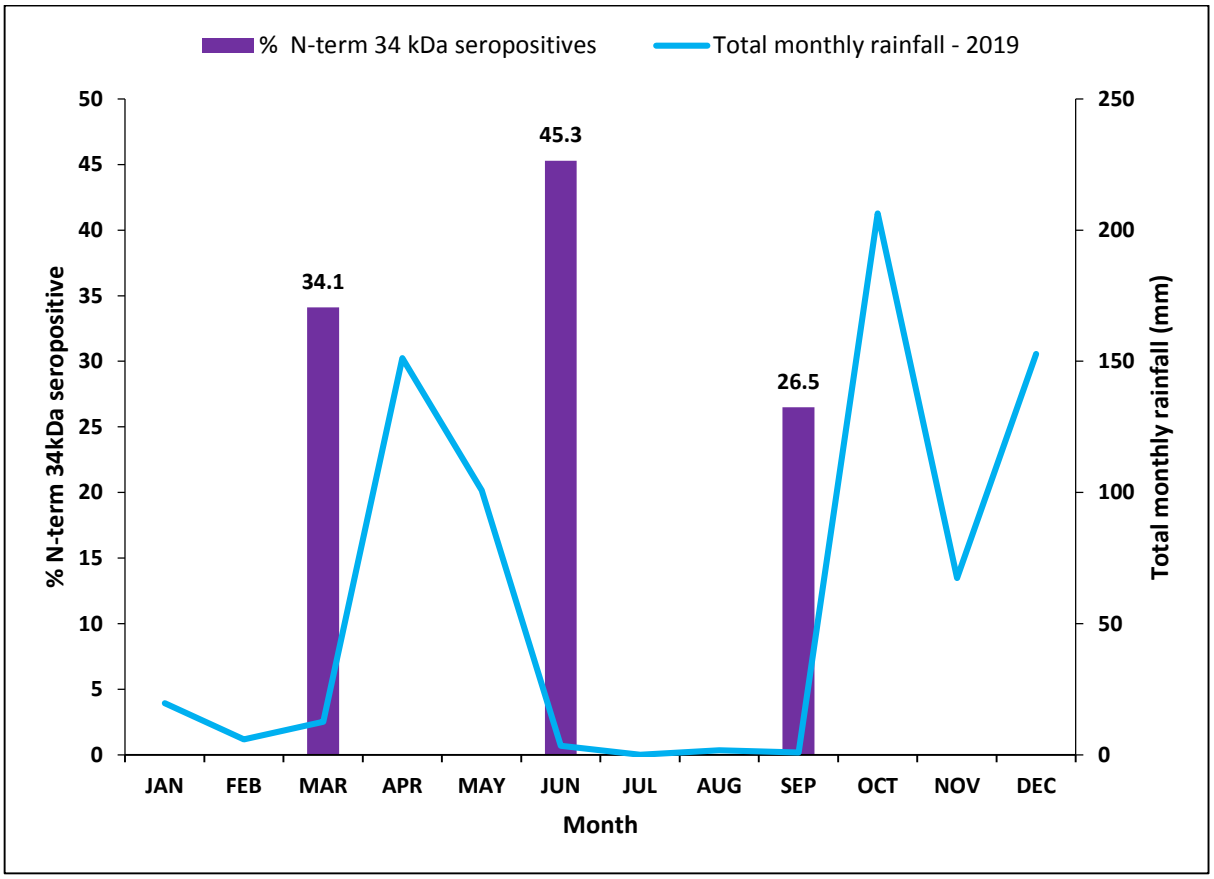

Figure 5: Total monthly rainfall and % N-term 34kDa seropositive.

4.4 Factors associated with risk for exposure to *Aedes* bites using anti-Nterm-34kDa IgG levels as a proxy for exposure.

The associations between anti-Nterm-34kDa antibody seropositivity, the village of residence, and demographic characteristics (age, sex, education level) were explored by logistic regression (Table 3). In the univariate analysis, the risk of exposure to *Aedes* bites was significantly lower among residents of Mikocheni village (OR=0.31; 95% CI=0.1-0.9; p=0.038) as compared to Newland residents during the dry season (time point 3). The risk of exposure to *Ades* bites was not of statistical significance by villages of residence during the dry (time point 1) and rainy season (time point 2). During all three time points, the risk of exposure to *Aedes* bites had no significant associations with age, sex, and education level. However, in the multivariate logistic regression analysis; only participants living in Mikocheni village still had a significantly lower risk of exposure to

Aedes bites as compared to the rest of the villages (AOR=0.31; CI=0.1-0.9; *p*=0.038), while exposure to *Aedes* mosquito bites was not significantly associated with any of the other variables.

Table 3: Associations between socio-demographic characteristics of studied population and exposure to Aedes mosquito bites

Variable	Timepoint 1(n=308)				Timepoint 2 (n=201)				Timepoint 3(n=204)			
	COR (95% CI)	*p*-value	AOR (95% CI)	*p*-value	COR (95% CI)	*p*-value	AOR (95% CI)	*p*-value	COR (95% CI)	*p*-value	AOR (95% CI)	*p*-value
Village												
Oria	1.87 (0.5-2.7)	0.682			2.61 (1.0-6.9)	0.055	2.61 (1.0-6.9)	0.055	0.67 (0.2-2.0)	0.471	0.67 (0.2-2.0)	0.471
Mtakuja	1.38 (0.6-3.3)	0.473			0.91 (0.3-2.5)	0.862	0.91 (0.3-2.5)	0.862	1.10 (0.4-3.2)	0.861	1.10 (0.4-3.2)	0.861
Newland	1				1		1		1		1	
Mikocheni	1.15 (0.5-2.4)	0.720			0.88 (0.3-2.3)	0.803	0.88 (0.3-2.3)	0.803	0.31 (0.1-0.9)	0.038	0.31 (0.1-0.9)	0.038
Mserekia	0.84 (0.3-0.8)	0.652			1.37 (0.5-3.5)	0.506	1.37 (0.5-3.5)	0.506	0.61 (0.2-1.7)	0.345	0.61 (0.2-1.7)	0.345
Age												
0-15	1		1		1				1			
16-45	1.54 (0.9-2.7)	0.140	3.03 (0.6-14.9)	0.174	0.85 (0.4-0.8)	0.660			0.93 (0.4-2.2)	0.873		
46+	1.34 (0.7-2.4)	0.312	2.81 (0.6-13.6)	0.198	1.15 (0.8-2.9)	0.211			1.55 (0.8-3.1)	0.482		
Sex												
Female	1				1				1			
Male	0.95 (0.6-1.6)	0.854			0.27 (0.7-2.3)	0.436			1.27 (0.7-2.5)	0.482		
Education Level												
No formal education	1		1		1				1			
Pupils at primary school	1.30 (0.6-2.7)	0.492	3.32 (0.7-16.6)	0.145	0.68 (0.3-1.6)	0.393			0.73 (0.3-2.0)	0.546		
Primary education and above	1.59 (0.8-3.1)	0.178	1.43 (0.7-2.9)	0.318	1.06 (0.5-2.3)	0.882			1.10 (0.4-2.8)	0.839		

COR- crude odds ratio, AOR- adjusted odds ratio, CI- confidence interval

CHAPTER FIVE

5.0 DISCUSSION

This study evaluated human antibody responses to the *Aedes* Salivary gland protein Nterm-34kDa peptide as a serological biomarker for temporal human exposure against *Aedes'* bites. The present study reported results of a large-scale epidemiologic study of antibody response to *Aedes* saliva in Tanzania.

5.1 To determine temporal and spatial variation in Nterm-34kDa Aedes salivary peptide seroprevalence

Our results showed that individuals exposed to *Aedes* bites could develop IgG response to Nterm-34kDa salivary peptide. The serological responses as shown by IgG response to *Aedes* were detected in many people across all the villages included in the study during all three-time points. In this study, the IgG responses to the Nterm-34kDa peptide was detected in 34.1%, 45.3%, and 26.5% individuals during time points 1, 2, and 3 respectively. In contrast, a low proportional (19%) of immune responders for both A*e.aegypti* and A*e.alboictus* salivary gland extracts (SGE) has been reported previously in Reunion Island (33). This discrepancy could be because the antigenic sequence found in 34kDa putative protein is probably detected in low levels as compared to other specific antibodies in serum. Moreover, high specific IgG responses (88.2%) have also been detected among Reunionian individuals (7). This high seroprevalence was probably due to low cross-reactivity between two species using a complementary approach.

Previous evidence indicates that IgG response to this biomarker is not expected to last for more than 15-30 days, therefore together with entomological tools, the biomarker can be the better tool to assess temporal exposure of humans to *Aedes* bites (28). Recently, Drame and colleagues have shown the potential of An. Gambiae saliva for use as an immunological exposure marker to assess the risk of malaria transmission and the efficiency of antivectorial strategies in malaria-endemic areas (15). The study done in lower Moshi revealed that there were temporal and spatial variations in Anti-gSG6-P1 IgG in exposure to *Anopheles* mosquito bites, and it can be used as a potential tool in detecting and distinguishing temporal and spatial variations (41). Several studies showed the existence of this species-specific protein as one of the quantitative biomarkers for

human exposure in all settings (32,36), recently it has been shown that this biomarker can be used against *Aedes*(5). Also, two previous studies have validated and recommended that IgG antibodies to Nterm-34kDa peptide can be used as a pertinent candidate biomarker of *Aedes* bite in African and Asian individuals (20)**.** In Tanzania various arboviral vector control programs have been established and still number of outbreaks has been occurring (4,5), the use of this biomarker can be integrated with the existing national traditional tools parallel with vector control practices and policy so as to enhance the maximum establishment of vector control programs in the country.

In the present study, the spatial variation in Nterm-34kDa seroprevalence according to villages was observed. However, there was no statistically significant difference in the variation of exposure by the village during time points 1 and 3, perhaps because all villages are located within more or less similar altitudinal ranges. In contrast, this pattern was observed in Mserekia village which showed the lowest prevalence during our study period and thus, presumably reflecting that a low number of individuals are directly exposed to *Aedes*. A significant variation of IgG Ab levels was observed between villages for both years as previously reported by Elanga Ndille et al in seven villages of Southern Benin (West Africa) (20). In addition, in this study, the specific IgG levels showed considerable inter-individual variations. This also has been reported by previous studies that the inter-individual heterogeneity in specific IgG level was observed whatever the studied months (7,20). These results suggests that IgG response to Nterm-34kDa salivary peptide may represent a reliable biomarker to detect variation in human exposure to Ae. Aegypti mosquito bites. This tool (s) can be deployed in areas where there is a low-level transmission risks since few people can be tested and show inter-individual variation within the same geographical location and observe for the immunological response which ascertained by measuring the antibodies levels.

5.2 Temporal variation of rainfall pattern and Nterm-34kDa seroprevalence

In this study there were temporal variations of Nterm34-kDa seroprevalence according to three-time points across the survey, it was observed to be higher during time point 2 which also corresponded with the increase of rainfall. These findings are also supported by other studies which showed similar findings of which Nterm34-kDa seroprevalence were varied significantly with seasons and was higher during intense rainfall seasons

(20,32,30,38). This is probably due to the fact that during the end of intense rainfall, the mosquito breeding sites increases, together with favourable temperature, the mosquito abundance,
and outdoor activities also do increases. This could also be probably due to the production of mosquitoes in containers filled by people during scant rain season as observed in other previous studies (39). It is well known that greater proliferation of Ae. aegypti adult mosquito occurs during rainfall, especially in the African rural context (40). The previously developed mathematical model which was applied to field data showed that rainfall triggered the dynamics of Aedes mosquito aggressiveness (39). Generally, these results revealed that there is a temporal association between rainfall pattern and the level of IgG responses to Nterm-34kDa salivary peptide which can also reflect the real intensity of human exposure to *Aedes* bites. By knowing this temporal variation of IgG response based on season and climatic factors, best practices on the proper vector control programs control, monitoring, and evaluation can be implemented in a proper time.

5.3 Factors associated with risk for exposure to *Aedes* bites using anti-Nterm-34kDa IgG levels as a proxy for exposure.

In the objective to highlight the potential association of anti-Nterm-34kDa IgG levels as a proxy for exposure with risk factors to *Aedes* bites. Multivariate analysis showed no influence of sex, age, and educational level in the level of IgG antibody response to Nterm-34kDa. Age was not a confounding factor for the association between IgG response to Nterm-34kDa and the level of human exposure to mosquito bites (32). Therefore, this indicates that this biomarker is non-specific to the sex and age of individuals. Further studies are therefore needed to cement whether the antibody response to saliva is age-dependent. The lower seroprevalence of Nterm-34kDa among residents from Mserekia village than other villages while the risk of exposure to *Aedes* bites was significantly lower among residents from Mikocheni village and was not associated with age, sex, and level of education. The reason for this variation could be the dryland environment found in the Mikocheni village which does not much support the abundance and mosquito activities hence contributing to the low risk of human-mosquito contacts.

5.4 Study limitation

The present study did not explore serological tools and entomological tools at the same time so as to have fully comparison regarding the use within a population in different settings.

5.5 Study mitigation

Due to financial constrains it was not possible to parallelly explore both tools at the same time.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

Our results showed that individuals exposed to *Aedes* bites could develop IgG response to Nterm-34kDa salivary peptide. The seroprevalence differed among individuals, this suggesting that the tool can eventually distinguish the level of temporal human exposure to *Aedes* vector bite within a population into both high and lower vector transmission settings where the traditional tools can't be fully applied.

This biomarker is a promising tool to assess the risk for transmission and may be used to guide vector control interventions and surveillance programs together with entomological tools, to assess the efficiency of anti-mosquito strategies, to estimate exposure levels, and to identify new infestation areas.

6.2 RECOMMENDATIONS.

Further validation studies with large sample size are needed to be conducted, that will take into consideration evaluating the serological tools and entomological indicators at the same time to have a full comparison and generalizability of the study results. This tool will add into existing conventional (entomological) tools for the surveillance of arbovirus infections in different settings such as low arboviral transmission settings.

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APPENDICE

Appendix 1: Proposed NTERM-34kDa Elisa Protocol

Materials and reagents

- 96 wells flat bottomed Maxisorp ELISA plates
- Non powdered gloves
- 10 MLS falcon tubes
- 50mls falcon tubes
- Falcon tube racks
- Pipettes (Single channeled, 100uL multi-channelled)
- 1ml Tips
- 100uL Tips
- ELISA reader
- Incubator
- 20ug Nterm-34kDa peptide antigen
- Phosphate Buffered Saline (PBS)
- 1:20 Test sera
- Bovine Serum Albumin (BSA)
- 1: 25000 Horseradish peroxidase goat antihuman IgG antibody
- 1% Tween 20
- Tetra-Methyl Benzene (TMB)
- Phosphate Citrate buffer (PH 4)
- 0.003% Hydrogen Peroxide
- 1M sulphuric acid
- Distilled deionized water

Reagent's preparations

1. Nterm-34kDa Peptide aliquots preparation
    - Suspend 10mg Nterm-34kDa peptide in 500mls distilled deionized water
    - Make 10mls aliquots of the antigen (enough for a single ELISA run)
    - Freeze the antigen at -20 degrees centigrade until use

2. **Washing Buffer**
    - 0.5 ml Tween 20
    - 1000 ml PBS Buffer
    - Store at 4 °C

3. **Blocking Buffer**
    - 100 ml Washing Buffer
    - Ready to use casein blocker solution
    - Store at 4 °C

4. **Stop Buffer**
    - Ready to use SDS solution

PROCEDURE

Coating

1. Dilute the antigen with PBS-Tween 1% and coat appropriate wells of ELISA plate with the antigen by adding 100 µl of the diluted antigen solution.

2. Cover the plate with an adhesive plastic and incubate for 2 ½ hours at 37 degrees.

3. Wash the plate with 200 µl of washing buffer three times.

Blocking

4. Add 200 µl of 1% BSA to block the non-specific binding sites in the coated wells and control well

5. Cover the plate with an adhesive plastic and incubate at 37 °C for 1 hour

6. Wash the plate with 200 µl of washing buffer three times.

Incubation

7. Dilute the HRP-conjugated antibody with Blocking Buffer and add 100 µl of the diluted antibody to each well of the plate.

8. Cover the plate with an adhesive plastic and incubate at 37 °C for 1 hour 30 minutes.

9. Wash the plate with 200 µl of washing Buffer five times.

Detection

10. Add 100 µl of TMB Reagent per well with a multichannel pipette.

11. After sufficient color development, add 100 µl of 1M sulphuric acid to the wells.

Note: 10~15 minutes is enough for color development.

12. Read the absorbance of each well using 405 nm

Appendix II: Ethical clearance letter

UNITED REPUBLIC OF TANZANIA
MINISTRY OF EDUCATION, SCIENCE AND TECHNOLOGY
MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES
OFFICE OF THE DIRECTOR – POSTGRADUATE STUDIES

In reply quote;

Ref. No. GA.97/261/03 17th November, 2021

Daniel Laswai,
MSc. Epidemiology and Laboratory Management,
School of Public Health and Social Sciences,
MUHAS.

Re: SUBMISSION OF ERROR FREE DISSERTATION

Please refer to the heading above.

We acknowledge receipt of your loose bound error free dissertation title **"Human IgG Response to Interm-34KDa Salivary Peptide As a Biomarker o Assess Temporal Exposure to Aedes Bites Among Individuals Aged > 6 Months I Lower Moshi-Tanzania, 2019."**

By this letter you are advised to proceed with the binding process and submit two hard bound copies in blue color and CD as per regulations.

In addition, kindly submit the report and it's dataset or transcripts for qualitative research, to the research and data management system available through **https://rdms.muhas.ac.tz/demo_1/dashboard-one.php.** The link is also shared with you through your SARIS account. Use your registration number as username and password is 1234 to log in and change your password.

Dr. Emmanuel Balandya
DIRECTOR OF POSTGRADUATE STUDIES

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