SEROPREVALENCE OF CHIKUNGUNYA VIRUS INFECTION AND ITS SOCIODEMOGRAPHIC CHARACTERISTICS IN FEBRILE PATIENTS ATTENDING COAST GENERAL TEACHING AND REFERRAL HOSPITAL KENYA

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Seroprevalence of Chikungunya Virus Infection and Its Sociodemographic Characteristics in Febrile Patients Attending Coast General Teaching and Referral Hospital Kenya

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2022

DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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DEDICATION

I dedicate this thesis to God who has been my strong pillar, my source of strength, knowledge, wisdom and understanding throughout this project and only on his wings have I soared.

Special thanks to the most amazing parents Rachel and Paul who not only nurtured me but also taxed themselves dearly over the years to educate me. Your words of encouragement and push for tenacity still rings in my ears. Thank you for teaching me how to work hard for the things I aspire to achieve. To ‘jojo’and ‘davesy’ you guys are the best cheerleaders’ thank you for supporting me and enduring my absences during the research and thesis preparation.

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TABLE OF CONTENTS

DECLARATION ..... **ii**

DEDICATION ..... **iii**

ACKNOWLEDGEMENT ..... **iv**

TABLE OF CONTENTS ..... **v**

LIST OF TABLES ..... **ix**

LIST OF FIGURES ..... **x**

LIST OF APPENDICES ..... **xi**

LIST OF ABBREVIATIONS AND ACRONYMS ..... **xii**

ABSTRACT ..... **xiii**

CHAPTER ONE **1**

INTRODUCTION **1**

1.1. Background 1

1.2. Statement of the problem 3

1.3. Justification of the study 4

1.4. Research questions 4

1.5. Research Objectives 5

1.5.1. General objective 5

1.5.2. Specific objectives 5

CHAPTER TWO 6

LITERATURE REVIEW 6

2.1. Introduction 6

2.2. CHIKV Virus biology 6

2.3. CHIKV Vectors 7

2.4. CHIKV Transmission cycles (Aetiology). 7

2.5. CHIKV Pathogenesis 9

2.6. Effects of rainfall, temperature and vegetation on survival of the vector 10

2.7. History of outbreaks. 11

2.7.1. Outbreaks in Kenya 11

2.7.2. Worldwide outbreaks 12

2.8. Clinical presentations of CHIKV 14

2.8.1. Acute Phase 14

2.8.2. Post-Acute Phase 14

2.8.3. Chronic Phase 15

2.9. Laboratory diagnosis 15

2.9.1. Viral isolation 16

2.9.2. Molecular Diagnosis of CHIKV. 17

2.9.3. Serological tests 17

2.9.4. Treatment 17

2.9.5. Prevention and Control 18

CHAPTER THREE 19

MATERIALS AND METHODS 19

3.1. Study site 19

3.2 Study population..... 20

3.3. Study design 20

3.3.1. Retrospective survey 20

3.3.2. Inclusion criteria..... 20

3.3.3. Exclusion criteria: 20

3.4. Sample size determination..... 20

3.5. Data collection instrumentation..... 21

3.6. Laboratory analysis for CHIKV. 21

3.6.1. Cell Cultures 21

3.6.2. Growth of Vero Biken Cells 22

3.6.3. Splitting of the Verobiken cells..... 22

3.6.4. Indirect IgG ELISA..... 22

3.6.5. IgM Capture Assay Antigen Preparation. 23

3.6.6. Sandwich IgM ELISA..... 24

3.6.7. Focus Reduction Virus Neutralization Test. 24

3.7. Data analysis..... 25

CHAPTER FOUR **26**

RESULTS **26**

4.1 Demographic characteristics of study participants. 26

4.2 Objective one: Seroprevalence of CHIKV in Febrile patients attending CPGH 27

4.3 Objective two: To Establish socio-demographic correlates of exposure to Chikungunya in febrile patients attending Coast Provincial General Hospital. 29

4.4 Objective Three: Impact of Rainfall and temperature on CHIKV Transmission. 33

CHAPTER FIVE **34**

DISCUSSION **34**

5.1. Discussion 34

5.2. Limitations of the study 38

CHAPTER SIX **39**

CONCLUSIONS AND RECOMMENDATIONS **39**

6.1 Conclusion 39

6.2 Recommendation 39

REFERENCES **41**

APPENDICES **51**

LIST OF TABLES

Table 4.1-1: Demographic characteristics of the study participants. 26

Table 4.3-1: Association between Socio-demographic Characteristics and CHIKV seropositivity 30

Table 4.3-2: Association between Clinical symptoms and CHIKV seropositivity. 31

Table 4.3-3: Chi-square and P-values: for an association between seropositivity and socio-demographic characteristics 32

Table 4.3-4: Chi-square and P-values: for association between seropositivity and Clinical symptoms 32

LIST OF FIGURES

Figure 2.4-1: Life cycle of CHIKV transmission sylvatic cycle on the left and Urban cycle on the right 8

Figure 2.5-1: Pathogenesis in human following Chikungunya virus infection, indicating vireamia and antibodies progression in days (D) and months (M) 10

Figure 2.7-1: Countries with reported cases of CHIKV 13

Figure 2.8-1: Photographs of clinical manifestations in CHIKV patients during an outbreak in Gabon 15

Figure 2.9-1: The Centres for Disease Control and Prevention diagnostic testing algorithm for detection of chikungunya virus (CHIKV) infection. ... 16

Figure 3.1-1: Study site 19

Figure 4.2-1: CHIKV Seroposivity against Age Group 28

Figure 4.4-1: Relationship between Rainfall, Temperature and CHIKV prevalence between January 2014 to December 2015 33

.

LIST OF APPENDICES

Appendix I: **Questionnaire** - A Seroprevalence survey for chikungunya virus exposure in humans at coast provincial general hospital Kenya……..55

Appendix II - Consent Forms for Adults ……...58

Appendix III Results of Seropositivity in different sub-counties …….61

Appendix IV Research Publication ……..64

Appendix V Ethical Clearance ……....74

LIST OF ABBREVIATIONS AND ACRONYMS

Ae.	Aedes
CHIKV	Chikungunya Virus
CI	Confidence Interval
ELISA	Enzyme-Linked Immunosorbent Assay
EMEM	Eagles Minimum Essential Growth Medium
EMEMM	Eagles Minimum Essential Maintenance Medium
FCS	Foetal Calf Serum
FRNT	Focus Reduction Neutralization Test
IgM	Immunoglobulin M
IgG	Immunoglobulin G
KEMRI	Kenya Medical Research Institute
NEAA	Non-Essential Amino Acids
OR	Odds Ratio
P/S	Penicillin /Streptomycin
PBS-Tween 20	Phosphate Buffered Saline
RT-LAMP	Real-Time Loop-Mediated isothermal amplification
RT-PCR	Real-Time Polymerase Chain Reaction

ABSTRACT

Arboviruses including Chikungunya and Dengue are recognized causes of acute febrile illness, in the tropics and have been responsible for outbreaks worldwide. During the past few years, there has been a dramatic resurgence of a well-known arboviral diseases thought to be effectively controlled or unimportant. However, these arbovirus diseases are not usually regarded as a differential diagnosis in patients with fever in Kenyan health institutions because of lack of readily available and affordable diagnostic tests to detect them and a low index of suspicion. Thus, most arboviruses remain undiagnosed and as a result, the frequency of arbovirus disease and the public health threat they pose is greatly underestimated. The nonspecific nature of the clinical signs and symptoms of Chikungunya virus (CHIKV) makes it difficult to differentiate it from illnesses such as malaria, typhoid and other known bacterial infections. Despite this, few surveys have been done to document the magnitude of infection from CHIKV in the Kenyan Coast. This study was done to determine the Seroprevalence of Chikungunya virus in febrile patients attending Coast General Teaching and Referral hospital, to establish socio-demographic correlates of exposure to Chikungunya in febrile patients attending Coast General Teaching and Referral Hospital and lastly to determine the impact of temperature and rainfall in chikungunya transmission

This was a retrospective hospital-based cross-sectional survey conducted at the Coast General Teaching and Referral Hospital in Mombasa Kenya. Samples used were from January 2014 to December 2015. Patients seen at Coast General Teaching and Referral Hospital presenting with febrile symptoms of unknown causes such as malaria, typhoid and other known bacterial infections were eligible. Venous blood that had been collected previously and stored for testing was used. Enzyme-linked Immunosorbent Assays (ELISA) were done to screen for the presence of CHIKV antibodies and confirmed using Focus Reduction Neutralization Test (FRNT). Data analysis was performed using R Studio version 1.2.5033 (2009-2019 R Studio, Inc.) and STATA CORP Version 14.2.

Between January 2014 to December 2015, 476 eligible patients aged between 0 and 81 years were included in this study. Of these, 264 (55.46%) were male, 209 (43.91%) were female while 3 (0.63%) did not indicate their gender. The overall Seroprevalence of CHIKV was 9.7% (46). Age and occupation were significantly associated with seropositivity. CHIKV seropositivity peaked at high temperatures and during low rainfall. This study suggests people in the Kenyan coast are exposed to CHIKV and possibly other arboviruses. Further studies are required to determine the prevalence and distribution of arboviruses in the wider coastal community. There is a need to maintain constant epidemiological and entomological surveillance. Seroprevalence can be predicted using temperature and rainfall patterns.

CHAPTER ONE

INTRODUCTION

1.1. Background

Chikungunya is a mosquito-borne viral disease that is characterized by fever, nausea, rash, vomiting, muscle pain and its hallmark feature being severe arthralgia (Ravi, 2006). It was first described by Marion Robinson and W.H.R. Lumsden in 1955, after an outbreak in 1952 along the border between Mozambique and Tanganyika known as Makonde plateau (Eyase et al., 2020).The virus is a single stranded positive sense RNA Viral genome that is divided into three genotypes, namely; the West African (WA), East/Central/South African (ECSA) and Asian. The ECSA can be further divided into the sub lineage Indian Ocean lineage (IOL). These genotypes are now spread worldwide, with ECSA and Asian genotypes being those predominately found(Sam et al., 2012).

Evidence points that CHIKV could likely have originated From Central/East Africa and subsequently transferred to Asia (Shanmugaraj et al., 2019). The virus circulates continuously in Africa in sylvatic cycle involving wild primates, mosquitoes (Aedes furcifer and Aedes africanus) while in urban areas , the virus is primarily transmitted through the bite of female mosquito vectors, mostly Aedes aegypti and Aedes albopictus (Silva & Dermody, 2017). However, Chikungunya virus has become fully adapted to the urban cycles and no longer require the presence of nonhuman primates and a sylvatic cycle for their maintenance. Thus, the urban transmission cycles of CHIKV, especially in densely inhabited tropical areas, usually result in large outbreaks (de Lima Cavalcanti et al., 2022).

Since the first reports of CHIKV in Africa in 1950s subsequent epidemics of CHIKV have occurred worldwide.CHIKV epidemics display secular, cyclical, and seasonal trends which are characterized by explosive outbreaks interspersed by periods of disappearance ranging from several years to a few decades. Several mechanisms play a role: the human and the mosquito vector susceptibility to the virus; conditions facilitating mosquito breeding (resulting in a high vector density), ability of the vector to efficiently transmit the virus (Elsevier Inc, 2018).

CHIKV has spread drastically over the past 20 years. The virus has been reported to cause many outbreaks worldwide and thus poses a high public health significance. Kenya faced a Chikungunya virus (CHIKV) outbreak in 2004; this was followed by the Indian Ocean islands outbreak in 2005, with more than 260 000 clinical cases (Pialoux et al., 2007). In 2007, CHIKV was exported into Europe, causing an outbreak of Chikungunya fever in Italy (Rezza et al., 2007). This outbreak suggested for the first time the significant potential of the virus ability to move to novel ecological niches through returning travellers who visit countries or areas where Chikungunya is prevalent, and obtain the virus and transmit it when they return to non-prevalent area (Epstein, 2007; Pialoux et al., 2007; Fabrice Simon et al., 2008).

To date, CHIKV continues to be responsible for significant outbreaks worldwide and no specific treatment or vaccines are available to prevent infection. The virus is now fully adapted to an urban transmission Many countries, from different parts of the world, have reported current and/or previous CHIKV transmission cases. Thus, the intensification and expansion of vector-borne diseases are likely to be a significant threat posed by climate change. In fact, although many other complicating factors (like mosquito range limits and viral evolution) exist, climate change will lead to a massive increase in exposure to *Aedes*-borne viruses, as predicted by several modelling studies, in which climate change will result in vector expansion toward temperate zones. This scenario is of major concern since *Aedes*-borne virus expansion into regions that lack previous exposure is a serious risk for public health, given the potential for explosive outbreaks when arboviruses are first introduced into naïve populations. The threat is aggravated by the cocirculation of other arboviruses that cause similar symptoms in infected individuals, resulting in novel challenges for its diagnosis and treatment. Moreover, the introduction of new strains to a susceptible population may result in an explosive number of cases, saturating the already precarious health systems of developing countries. All the above is also aggravated by the lack of proper treatment and effective control measures (i.e., vaccines and vector control).

Although CHIKV mortality rates are low, this virus imposes pronounced morbidity resulting in a substantial impact on the quality of life of infected individuals and significant economic losses, especially in developing countries. , therefore for

effective control, there is a need for documentation of the exact burden of this infection within febrile patients.

This study determined the Seroprevalence of CHIKV in patients at the Kenyan coast, described clinical and the sociodemographic features and clinical manifestations observed in the exposed participants and how temperature and rainfall patterns can be used to predict CHIKV outbreaks.

1.2. Statement of the problem

The impact of arboviral outbreaks and establishing their true burden is still a critical issue but remains a challenging task. Arboviruses are known to cause a broad spectrum of disease ranging from asymptomatic infection to severe disease which may lead to misdiagnosis when the clinical manifestations are shared within co-circulating arboviruses and other bacterial infections such as dysentery, fever, typhoid and pneumonia making it hard to control ,(Fritzell et al., 2018)

Other studies have estimated the burden of arbovirus outbreaks using a range of empirical or extrapolative methods and disease modelling approaches; however, the most reliable data assessment is drawn from Seroprevalence studies which are often lacking as they are expensive and require large workforces and logistical resources (Fritzell et al., 2018).

Even though surveillance systems have been put in place, for example clinicians, laboratory reports and hospitals which are appropriate for prompt detection of outbreaks, they are only designed to estimate the disease burden, therefore, underestimating the total number of cases and because health care seeking varies greatly due to access to care, surveillance data alone is unreliable.

Chikungunya was not routinely tested in our health facilities, thus remaining undiagnosed, and as such, its prevalence and the public health threat it posed was continually underestimated. Moreover, the disease was also not regarded as a differential diagnosis in patients presenting with fever due to the lack of readily available and affordable diagnostic test to detect them or even a low index of suspicion by clinicians who were not familiar with the clinical presentations of this disease, therefore patients were treated with antimalarial drugs and antibiotics, exposing them to unnecessary side effects; their families and government unnecessary costs and thus

increasing the risk of losing necessary drugs due to development of resistance. Although not a killer disease, the high morbidity rates and incapacitating symptoms it causes among the affected population and could lead to substantial socio-economic impact in affected countries due to absenteeism for example when patients are unable to go to work, school.

1.3. Justification of the study

Fever was one of the most complaint of the patients attending the coast provincial general hospital, quite often the cause of fever was not established; hence there was evidence to suggest CHIKV was the cause of some of these fevers.

Chikungunya is a newly emerging disease, it's relatively uncommon and is not routinely tested and thus goes undocumented and as such its prevalence and the public health threat it poses is greatly underestimated. For effective control, there was a need to document the exact burden of CHIKV among febrile patients in the Kenyan coast.

This study determined the Seroprevalence of CHIKV in patients at the Kenyan coast, described clinical features and the socio demographic features observed in the exposed participants and how temperature and rainfall patterns can be used to predict CHIKV Seropositivity.

The data generated has greatly contributed to the new data on the magnitude of CHIKV exposure. This information forms a basis for stimulating, planning and conducting of further widespread studies and research to determine the role of CHIKV in aetiology of febrile illness in the coastal region and the planning and setting up of public health intervention programs for the prevention of CHIKV. Moreover, this also informs clinicians to consider CHIKV and other arboviruses as a differential diagnosis in febrile patients.

1.4. Research questions

1. What is the prevalence of the Chikungunya virus in coast provincial general hospital?
2. Is there a relationship between the sociodemographic correlates of exposure to chikungunya seropositivity in febrile patients attending Coast General Teaching and Referral Hospital?

3. How do Climatic factors of temperature and rainfall affect Chikungunya transmission?

1.5. Research Objectives

1.5.1. General objective

1. To determine the Seroprevalence of Chikungunya virus in febrile patients attending Coast general teaching and referral hospital

1.5.2. Specific objectives

1. To determine Chikungunya virus prevalence in febrile patients attending Coast general teaching and referral hospital.
2. To determine the relationship of socio-demographic correlates of exposure and clinical manifestations to Chikungunya in febrile patients attending Coast General Teaching and Referral Hospital.
3. To establish the impact of rainfall and temperature the on the transmission of Chikungunya Virus

CHAPTER TWO

LITERATURE REVIEW

2.1. Introduction

Arboviruses are the causative agents of some of the most important emerging infectious diseases and are responsible for posing a high public health significance worldwide (Gubler, 2006). However, these diseases are not usually regarded as a differential diagnosis in patients with fever in Kenyan health institutions because of lack of readily available and affordable diagnostic capacity to properly rule-out malaria as a cause of fever and as well, identify alternative fever-causing pathogens. Patients in the Kenyan coast are therefore treated with antimalarial drugs and antibiotics. Consequently, exposing them to unnecessary side effects, their families and government unnecessary costs and also increasing the chances of losing essential antibiotics due to development of resistance. Thus, most arboviruses remain undiagnosed and as such, the frequency of arbovirus disease and the public health threat they pose is greatly underrated.

Arthropod-borne viruses are known to cause a febrile illness in the tropical and subtropical regions. Their most common presentation being fever. CHIKV is a common cause of fever in some parts of Kenya and therefore it's evident to suggest that CHIKV was the cause of some fevers experienced in the Kenyan Coast.

2.2.CHIKV Virus biology

CHIKV is an alpha virus of the family *Togaviridae*, which comprised of an enveloped, positive single stranded-RNA virus. The virus is divided into three phylogenetic groups namely the West African (WA), East/Central/South African (ECSA), and Asian. The ECSA is divided into the sub lineage Indian Ocean lineage (IOL) (Sam et al., 2012).

Each of these genotypes exhibit different transmission cycles, in Asia transmission appears to be maintained in an urban cycle with *Aedes* sp. Mosquitoes (*Aedes aegypti* and *Aedes albopictus)*, CHIKV transmission in Africa was mostly related to a sylvatic cycle, primarily with *Aedes furcifer* and *Aedes africanus* mosquitoes.

2.3. CHIKV Vectors

Aedes aegypti and *Ae*. *albopictus* are the main vectors of CHIKV.They are highly invasive species and closely associated with the human peridomestic environment (Honório et al., 2018). *Aedes aegypt*i is highly anthropophilic and exhibits endophilic behaviour and is mostly associated with high human density. It uses indoor breeding sites such as water storage vessels, flower vases and concrete tanks. Ae. albopictus shows an eclectic feeding behaviour, preferentially feeding and resting in the peridomicile and is more common in vegetated and urban/urban forest transition habitats, especially where it is sympatric with *Ae. Aegypti.Ae. albopticus* thrives waterfilled breeding sites such as bamboo stumps, cocoa husks, waste containers and vehicle tires.

Aedes species are difficult to control, because they can reproduce in very small amounts of water and their eggs are extremely hardy, hence they can survive drying for more than a year. Distribution of *Ae. aegypti* is limited to hot wet regions and cannot easily spread to other areas (Elsevier Inc, 2018).

2.4. CHIKV Transmission cycles (Aetiology).

The virus is transmitted and maintained in two different cycles namely: sylvatic and urban cycles (See Figure 2.4-1 below). Where the sylvatic cycle refers to an animal to mosquito to a human transmission while urban cycle refers to human to mosquito to human transmission. In Africa, CHIKV is maintained in a sylvatic cycle comprising non-human primates (chimpanzees, monkeys and baboons) and different species of forest-dwelling mosquitoes including *Ae. Africanus, Ae. furcifer-taylori, Ae. dalzieli, Ae. luteocephalus* and *Mansonia, Culex,(Thiboutot et al., 2010)* as shown on Figure 2.4-1, While CHIKV is thought to have evolved from this forest cycle in Central Africa, the virus developed a new urban cycle as it spread through Africa and Asia. Mosquitoes such as *Ae. aegypti* have been implicated as vectors in Africa in urban settings which are densely populated where humans are the major hosts and *Aedes aegypti* as vectors (Diallo et al., 1999). In Asia, the transmission of CHIKV is almost exclusively between *Ae. aegypti* mosquitoes and humans(Myers et al., 1965), and in the Reunion Island, CHIKV was mainly transmitted via *Ae. albopictus*, after a

mutation (E1: A226V), occurred in this CHIKV strain (Tsetsarkin et al., 2007). The mosquito vector implicated in the 2004 epidemics in Kenya and Comoros in 2005 was *Ae aegypti*. In Comoros, larval surveys indicated that at least 11 different species were present on the island (Sang et al., 2008), three of which (*Ae. aegypti, Ae. vittatus* and *Eretmapodites chrysogaster*) had previously been shown to be competent CHIKV vectors (Gilotra & Shah, 1967). Outside epidemics, CHIKV is maintained in a sylvatic cycle involving monkeys, rodents, birds, and other unidentified vertebrates, and forest-dwelling *Aedes* mosquitoes, principally *Ae. furcifer* and *Ae. Africanus (Powers et al., 2000).*

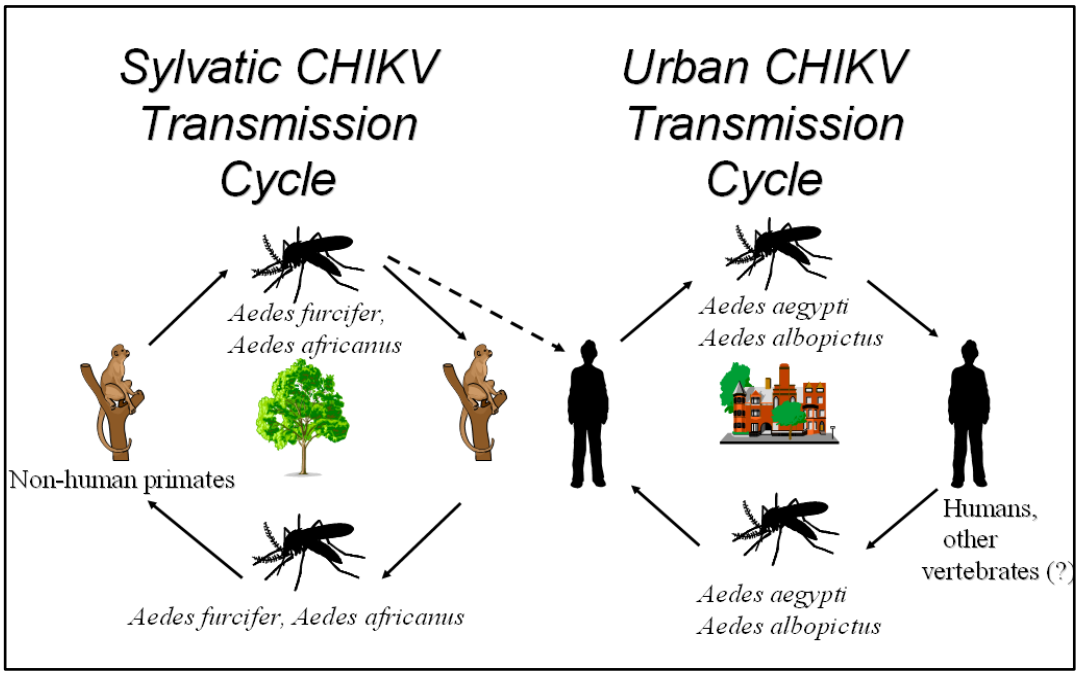

Figure 3.1-1 Life cycle of CHIKV transmission sylvatic cycle on the left and Urban cycle on the right

Source: (Powers, 2010)

The ability of the vectors to transmit the virus is dependent on intrinsic and extrinsic factors which are expressed as vector competence which is the ability of the vector to get infected after ingesting a blood meal with the virus and vectorial capacity refers to the number of infective bites arising from an infected host. Chikv is transmitted when female mosquitoes of the *Aedes aegypti* and *Aedes albopictus* feed on an infected viremic animal. CHIKV then replicates in the midgut and later enters the haemocel

before it is disseminated to the salivary glands where it continues to replicate and remain for the rest of the insect's life. Infected mosquitoes transmit the virus by injecting infectious saliva into a naïve host during a following blood meal hence leading to horizontal transmission. The virus has also been shown to disseminate to mosquito ovaries resulting to the development of infected offspring eggs through vertical transmission, thus allowing viral maintenance during adverse conditions when the vector cannot continue its development cycle. Therefore, the vector serves as a reservoir during inter epidemic periods (Weaver et al., 2020).

After ingesting a blood meal with the virus and egg maturation. The infected female mosquitoes seek for suitable oviposition sites. *Aedes aegypti* lays its eggs in discarded containers or around human homesteads in water storage containers. While *Aedes albopictus* uses both natural and artificial larval habitats.

2.5. CHIKV Pathogenesis

After a subsequent bite by an *Aedes* mosquito, CHIKV replicates in the skin, fibroblasts and disseminates to the liver, joints, muscles, brain and lymphoid tissue. Acute onset of disease is experienced 2–4 days after infection. Symptoms of the infected individuals include fever, headache, a petechial or maculopapular rash and the most distinctive symptom being a severe joint pain that is often incapacitating. Disease onset coincides with rising viral titre, which triggers the activation of an innate immune response, leading to the production of type I interferons (IFNs). Patients successfully clear the virus approximately 1 week after infection, and only at this time is there evidence of CHIKV-specific adaptive immunity (that is, T cell and antibody-mediated responses). (See Figure 2.5-1).

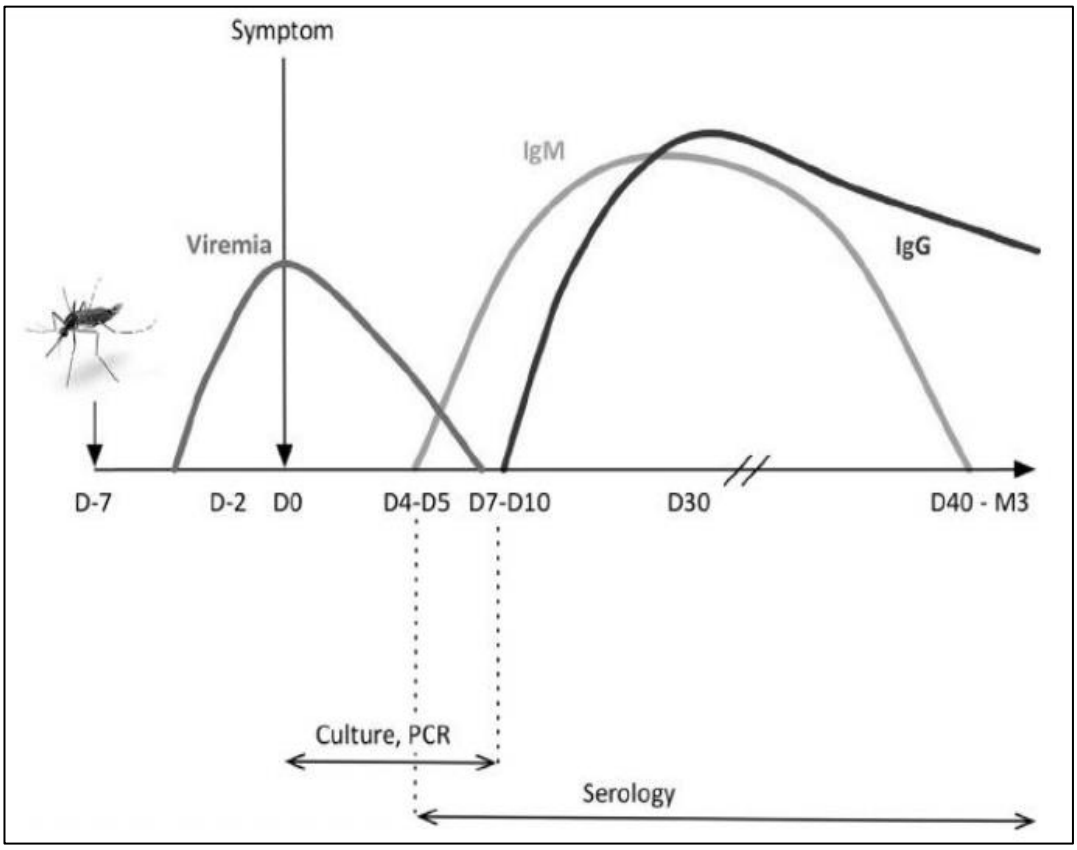

Figure 3.1-1 Pathogenesis in human following Chikungunya virus infection, indicating vireamia and antibodies progression in days (D) and months (M)

Source;(F. Simon et al., 2015)

2.6.Effects of rainfall, temperature and vegetation on survival of the vector

Climate, and particularly temperature and rainfall, have a significant effect on the distribution and abundance of different mosquito species. At higher temperatures, the mosquito life cycle is shorter than at lower temperatures. This in essence determines the geographic distribution of the species. For instance, *Ae. aegypti*, one of major vectors for arboviruses, is more sensitive to low temperature in nature than *Ae. albopictus*, limiting its geographic distribution to tropical and subtropical areas. Fluctuation of temperature has also been found to alter vector competence of disease vectors (Huang et al., 2019).

Temperature is an important driver of and limitation on vector transmission since it influences population growth, as well as its dispersion and expansion. *Ae. aegypti* is strongly influenced by climatic factors, with temperatures of 10°C or lower limiting

larval development and adult survival (Slosek, 1986). Optimal temperatures for development, longevity, and fecundity are between 22°C and 32°C (Beserra et al., 2009). Extreme temperatures suppress embryonic development and lead to death within hours of hatching. A temperature of 39°C is referred to as the lethal temperature (Aghdam, H. R. et al., 2009; Mourya et al., 2004). At temperatures favourable to the life cycle, insects not only complete their development but do so more quickly, which may enhance vector competence for arboviruses (Aghdam, H. R. et al., 2009). While temperature governs *Aedes aegypti* reproduction, maturation and mortality rates, rainfall generates the breeding grounds for larvae and pupae. At variance with other mosquito species, *Ae. aegypti's* eggs are laid above the water surface and hatch only when the water level rises and wets them. Increased rainfall enhances the hatching of eggs however low temperatures leads to the subsequent death of the larvae.

The environment can influence distribution, abundance, and even susceptibility of vertebrate hosts. Effects could include migration patterns and reproductive status, and availability of young naïve hosts. One of the most aggressive dispersal of CHIKV in human history was attributed to an abrupt increase of *Ae. aegypti* population followed by emergence of the East-Central-South-African genotype of CHIKV in the coastal region of Kenya (Huang et al., 2019).

2.7. History of outbreaks.

Chikungunya is an emerging arbovirus disease of great public health significance. The virus has been known to cause outbreaks worldwide which have been characterized as dengue-like illnesses with arthralgia and haemorrhagic fevers.

CHIKV was first isolated from the serum of a febrile human in Tanzania in 1953 during a dengue-like illness outbreak (Robinson, 1955; Ross, 1955). Although the initial assessment of the outbreak was thought to be dengue virus, serological and antigenic characterization of the isolates showed that it was an alphavirus closely related to Mayaro and SFV (Casals & Whitman, 1957).

2.7.1. Outbreaks in Kenya

CHIKV has caused many outbreaks, widely distributed around the globe (Eyase et al., 2020). In May 2016, the Kenyan Ministry of Health (KMoH) reported an outbreak of Chikungunya virus (CHIKV) in Mandera County at the border with Somalia. During

this time Outbreaks of CHIKV were occurring in the neighbouring Bula Hawa region, originating from Mogadishu. In Mandera town, 1,792 cases were detected, and an estimated 50% of the health work force was affected by this virus. A cross-border joint response was coordinated between Kenya and Somalia to control the outbreak (Berry et al., 2019). This was the first reported outbreak of CHIKV in Kenya since 2004.

Kenya first faced its largest outbreak in 2004 in Lamu Island, with an estimated 75% of the population infected. The disease also spread to the coastal city of Mombasa, and further to the Comoros and La Réunion islands, causing large outbreaks in 2005–2006 (Sergon et al., 2008) Other outbreaks have been reported to have occurred in the coastal county of Mombasa From mid-December 2017 to mid-May 2018 (Eyase et al., 2020).

CHIKV has been documented to be lowly circulating in few serological studies in Kenya (LaBeaud et al., 2015; Sergon et al., 2008; Tigoi et al., 2015). With an increase of competent vectors seen in the wet and warm season. Mandera County, an area with no previous CHIKV reports had a sporadic outbreak in 2016, thus, the expansion of the vector to new geographical regions. If this trend is not curbed the vectors will spread to more areas affecting naïve hosts, presenting a devastating case of the disease. Despite the evidence of CHIKV outbreaks reported in Kenya and the morbidity associated with them; it has not received sufficient coverage. As it is a newly emerging disease, only a few surveys have been done to document the exact burden of this infection within febrile patients seen in the Kenyan coast.

2.7.2. Worldwide outbreaks

Retrospective case reports suggest that CHIKV outbreaks occurred as early as 1779 but were inaccurately ascribed to dengue virus (Carey, 1971; Halstead, 2015). The virus has been isolated repeatedly from various countries in Africa including the Democratic Republic of Congo, Kenya, Sudan, Malawi, South Africa, Zimbabwe, Senegal, Ivory Coast and Nigeria. Frequent outbreaks were also described in South East Asia Comprising of countries such as India, Malaysia, Indonesia, Cambodia, Vietnam, Myanmar, Pakistan and Thailand (Burt et al., 2017; Nunes et al., 2015; Thiberville et al., 2013). See figure 2.7-1. Earlier before 2004, CHIKV Outbreaks were mostly sporadic. Among those documented were in the Democratic Republic of

Congo, (Pastorino et al., 2004) Malaysia and Indonesia (Laras et al., 2005) Kenya in 2004, Comoros in 2005 (Sergon et al., 2007), Seychelles, Mauritius, Madagascar and Re´union islands during 2005–2006 and in India in 2006/2007 (Ravi, 2006). Significant outbreaks of Chikungunya were first documented in Thailand in the early 1960s as well as India in 1963-1973 (Pialoux et al., 2007). In 2004, an outbreak in Mombasa and the Lamu Island of Kenya resulted in a large-scale outbreak which further spread to Comoros Islands, Reunion Island, and other Islands on the Indian Ocean and eventually to India. The Seroprevalence findings of this study revealed that the outbreak was widespread with attack rates up to 75% (Sergon et al., 2008) and an estimated 266,000 people infected in the Indian Ocean Islands of Reunion and Mauritius (Lam et al., 2001). International air travel also facilitated CHIKV geographical expansion during this epidemic, through viremic travellers importing several CHIKV cases into naive countries, including more temperate regions in Europe and the United States (Gibney et al., 2011; Grandadam et al., 2011; Rezza et al., 2007).

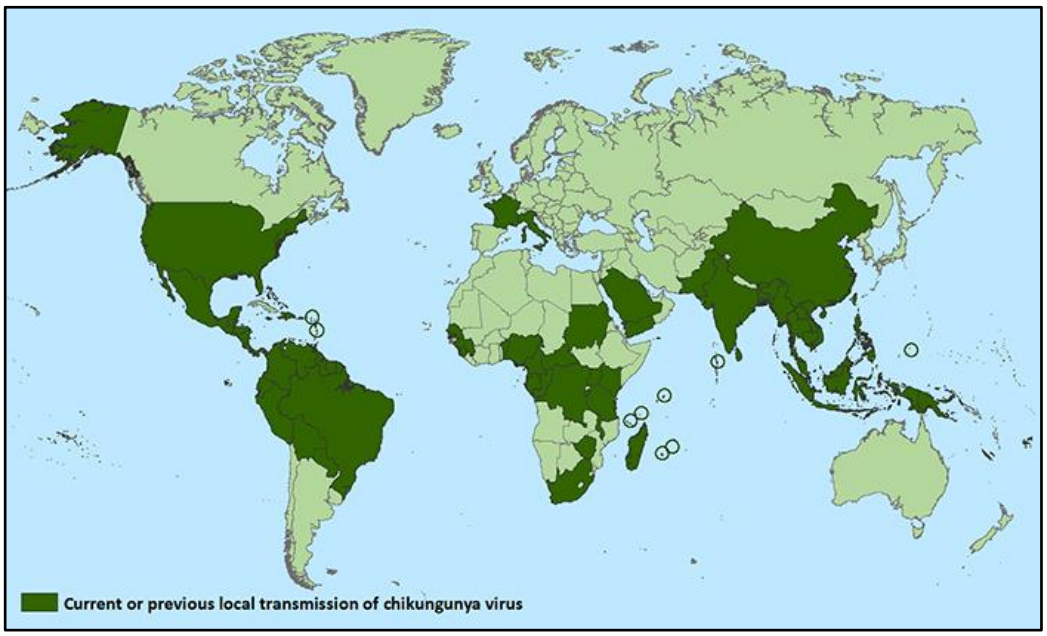

Figure 3.1-1 Countries with reported cases of CHIKV

Source: (CDC, 2016)

CHIKV has been reported to cause several significant outbreaks which were characterized by severe morbidity imposing heavy economic burden and productivity loss (Laras et al., 2005; Pastorino et al., 2004). There were also reports in the 2005–

2006 outbreaks of deaths associated with CHIKV infection in La Reunion Islands (Josseran et al., 2006). These deaths may have occurred under circumstances such as co-infection with other pathogens, infection of individuals with compromised immune systems or the presence of a more virulent variant. For example, the very young and old may be risk groups that fall into the category of individuals with weakened immune systems, particularly those with underlying medical conditions.

2.8.Clinical presentations of CHIKV

Symptomatic CHIKV is characterized into three phases namely: Acute, Post-acute and Chronic Phase (Álvarez-argüelles et al., 2019).

2.8.1. Acute Phase

This takes place during the first three weeks of clinical manifestation. The incubation period lasts up to 3 - 7 days. Clinical manifestation observed includes: Abrupt onset of fever, followed by Malaise.85% of the patients are symptomatic (Weaver et al., 2020) the phase lasts 7-10 days.

Poly arthralgia manifests after the onset of Fever affecting multiple joints such as wrists, ankles, hands resulting in intense pain that is debilitating leading to immobilization. Other symptoms observed include a maculopapular rash in some patients. (See figure 2.8-1) Patients who are old and those with underlying conditions are likely to develop severe complications leading to death.

Patients in this phase may recover fully while some may suffer persistent joint pain or even have a relapse of signs and symptoms lasting for months or years.

2.8.2. Post-Acute Phase

Patients may remain asymptomatic 2-3 weeks after onset of disease. Generally, most patients

Show improvements in their clinical condition and relapses occur after a brief “healing” period. Studies have shown that, on average, the symptoms persist in 50–90% of patients after the second or third week, and the percentage of patients with persistent polyarthralgia after the acute phase of CHIKV infection is more frequent in those older than 40 years and women (Sissoko et al., 2009).

Clinical manifestations observed in this phase include; stiffness, oedematous polyarthritis of the fingers, toes, morning joint pain and severe tenosynovitis. A0 non-specific clinical manifestation that is not associated with CHIKV could occur; this includes Alopecia, depression, anxiety and chronic fatigue.

2.8.3. Chronic Phase

This phase is characterized by chronic pain, mental health issues, stiffness, arthritis, pain accompanied by oedema or morning joint pain.

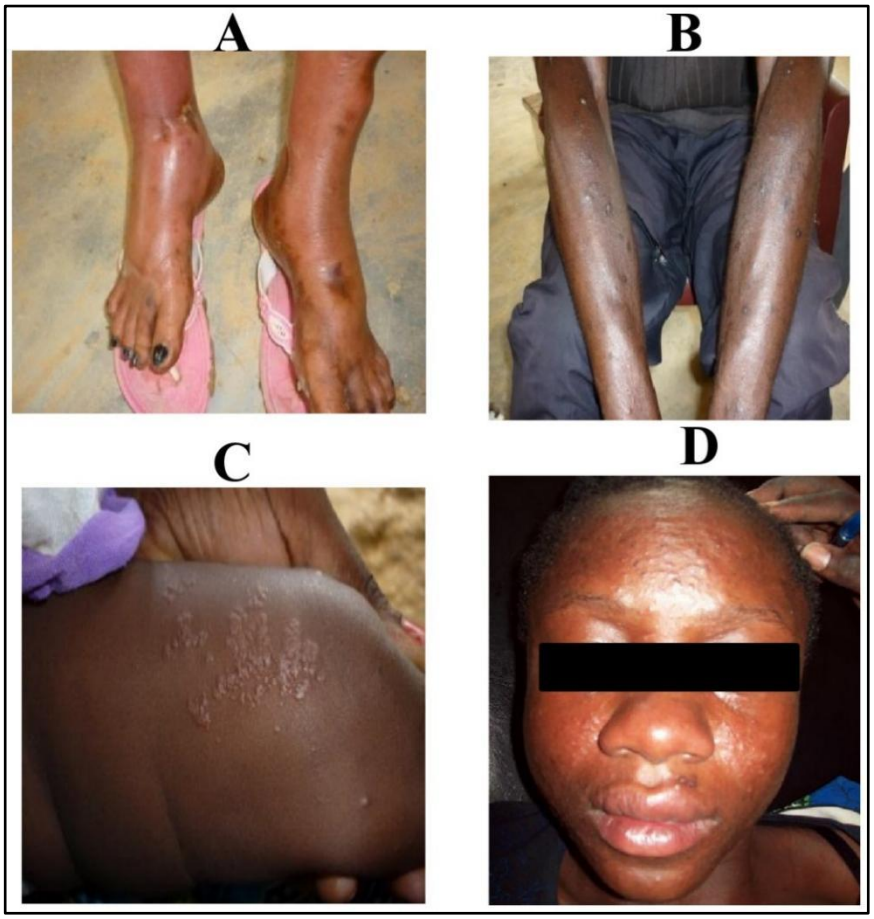

Figure 3.1-1 Photographs of clinical manifestations in CHIKV patients during an outbreak in Gabon

Source; (Nkoghe et al., 2012)

2.9.Laboratory diagnosis

CHIKV diagnosis is carried out by detecting Viral RNA and the identification of specific immune response elicited. Laboratory analysis of CHIKV is dependent on the quality of the sample and when it was collected during the disease (Dash et al., 2011). Reason being, CHIKV replicates rapidly in the host leading to high titres of Viral RNA

which can be detected by RT-PCR at <6 days after onset of clinical illness (Álvarez-argüelles et al., 2019; Johnson et al., 2016). While antibodies like IgM are detected in serum 5-7 days after onset of illness. (See Figure 2.9-1.)

Samples used during virological diagnosis include serum, plasma, whole blood, urine, cerebrospinal fluid etc. while for serological diagnosis serum samples are obtained by using a serum separator.

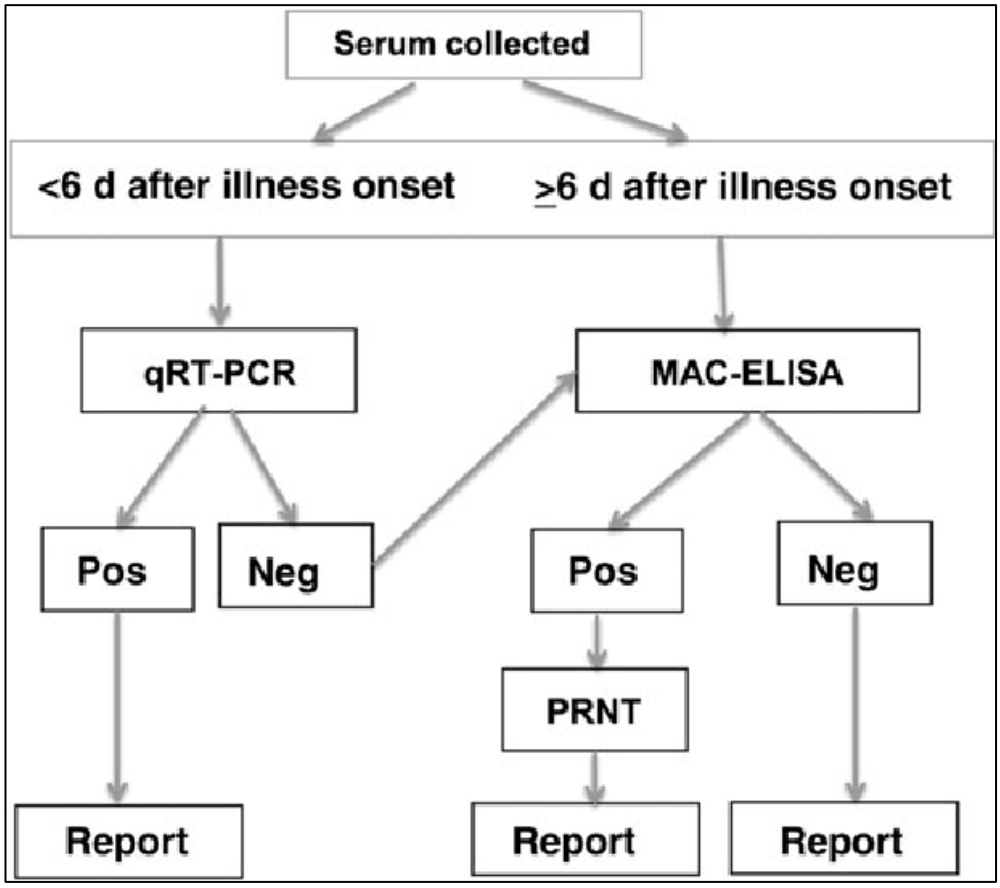

Figure 3.1-1 The Centres for Disease Control and Prevention diagnostic testing algorithm for detection of chikungunya virus (CHIKV) infection.

Source: (Johnson et al., 2016)

2.9.1. Viral isolation

This method is considered as the gold standard but it's rarely used since it is time-consuming and the degree of success is dependent on several complicating factors such as time of specimen collection, maintenance of cold chain, processing and storage of samples

This is performed on field-collected mosquitoes or acute serum specimens. Serum obtained from whole blood collected during the first week of illness is inoculated into a susceptible cell line. CHIKV will produce typical cytopathic effects (CPE) within three days after inoculation in a variety of cell lines, including Vero, BHK-21, and HeLa cells. This test is performed in T-25 flasks. Virus isolation is confirmed either by, using CHIKV-specific antiserum, by RT-PCR of the culture supernatant or by immunofluorescence assay (IFA)

2.9.2. Molecular Diagnosis of CHIKV.

A reverse transcription-polymerase chain reaction is used to detect the viral nucleic acid in a patient's acute phase serum. Real-time, closed system assays should be utilized, due to their increased sensitivity and lower risk of contamination.

Real-time loop-mediated isothermal amplification (RT-LAMP) assay has been shown to be a helpful molecular tool for rapid diagnosis (Álvarez-argüelles et al., 2019)

2.9.3. Serological tests

Enzyme-Linked Immunosorbent Assay is used to detect CHIKV IgM and IgG antibodies.

Following a CHIKV infection, IgM appears in the blood in the first week after the onset of infection and may persist for several months to 2 years. While IgG antibodies become detectable 4-10days in serum and may persist for years conferring lifelong immunity. Improving the test design has contributed to fewer chances of antibody cross-reactivity than in the past. Plaque Reduction Neutralisation Test and Focus Reduction Neutralisation Test are used as a confirmatory test.

2.9.4. Treatment

Despite, the high public health threat posed by CHIKV, the disease lacks a definitive treatment. CHIKV is managed by giving ibuprofen, naproxen, or another non-steroidal anti-inflammatory agent (NSAID) to relieve arthralgia (Kaur et al., 2013). Antipyretics like paracetamol or acetaminophen for fever and any other necessary symptomatic treatment. For patients with severe joint pains that are not relieved by NSAID, narcotics (e.g., morphine) or short-term corticosteroids can be used. Patients are advised to drink plenty of fluids to replenish fluid lost from sweating, vomiting, and

other insensible losses. Bed rest is highly recommended**.** A blood transfusion may be required by patients suffering from haemorrhagic fever.

2.9.5. Prevention and Control

CHIKV transmission can be prevented in two ways**;**

1. Personal protection from mosquito bites.
2. Public health measures that aim in reducing the vector population

Personal protection involves wearing clothes that minimize skin exposure and also reducing time spent outdoors, repellents can also be applied on exposed skin or clothing while adhering to the product label. Other ways to prevent mosquito bites is to ensure that the rooms have air conditioning, window screens, using mosquito coils as well as sleeping during the day under a mosquito net to prevent mosquito bites.

Public health measures involve vector control by eliminating larval habitats by removing tins tyres, discarded containers and stagnant water around the houses which act as mosquito breeding sites. Other measures include covering domestic water storage containers. Aerial spraying can also be carried out to reduce the number of adult mosquitoes.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study site

This study was carried out at the Coast General Teaching and Referral hospital formerly known as (Coast provincial General Hospital), the facility is located in Mombasa county the area covers 2,755km^{2}and lies between latitudes 3° 56' and 4° 10' south of the equator and the longitudes 39° 46' east. The county has a tropical climate characterized by a mean annual rainfall of about 1196mm. The driest month being February with 15mm of rainfall and most precipitation falling in May; 302mm. The annual maximum temperature ranges from (20 -33^0 C). Its mean relative humidity for an average year is recorded as 77.6% *(Kenya Meteorological Department, 2012).* The total population of the study area is 939370 as per the 2009 census (Kenya National Bureau of Statistics, 2009) with a human population density of 4,292 per km^2 with 37.6 % of this population living below the poverty line. Adjacent to the study area is the Kenya Tanzanian borders that serves traders from Tanzania and neighbouring Uganda. (See figure 3.1-1).

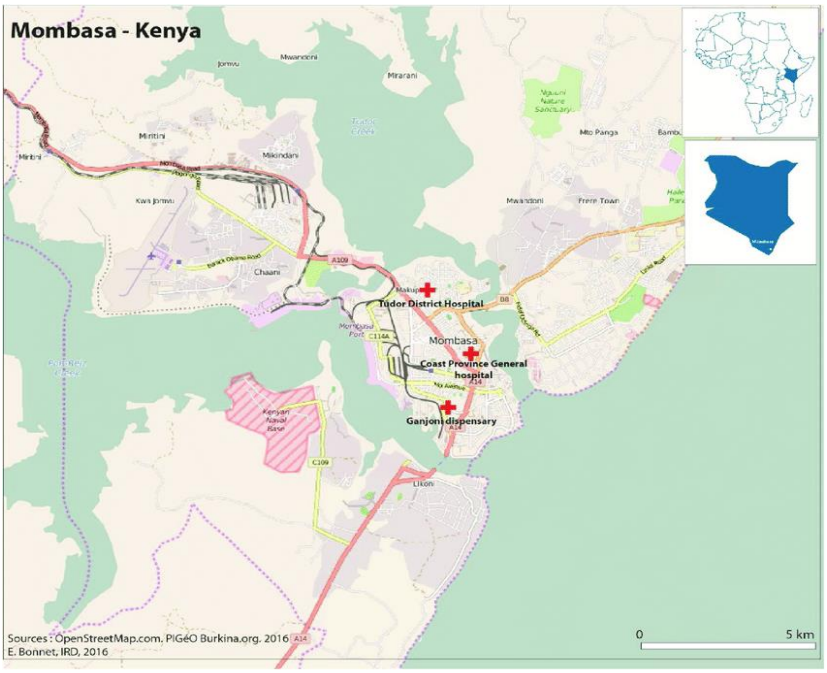

Figure 3.1-1 Study site

***Source**:(Lim et al., 2020)*

3.2 Study population

The study comprised of both rural and urban patients who sought treatment at the Coast provincial general hospital.

3.3. Study design

3.3.1. Retrospective survey

This was a retrospective hospital-based cross-sectional study that was done with samples which had been collected between the year 2014 and 2015. Sections of the population that attended the hospital and had given consent were tested and the data obtained used to determine the Seroprevalence. (See informed consent on page 48 Appendix II.)

3.3.2. Inclusion criteria.

1. This study included patients with a fever that was not due to malaria, typhoid or any other known cause such as Pneumonia.
2. This study included all age brackets.
3. The study included patients that were willing to give consent.

3.3.3. Exclusion criteria:

1. The study excluded patients that suffered from a fever of known cause.
2. Those unable or unwilling to give consent were also excluded
3. Those not febrile were also not included in the study.

3.4. Sample size determination.

We presume that the people attending the Coast Provincial General Hospital during the study period represent the total population. Therefore, the sample size was determined by (Banoo et al., 2007) as follows:

$$N = \frac{Z^2_{1-\alpha/2}\, P\,(1-P)}{d^2}$$

Where:

N = Minimum sample size required.

Z= standard normal distribution at 5% significance level=1.96

P= the prevalence of CHIKV recorded in patients in coastal Kenya in 2013 =22%

D= degree of precision = 5%

So Therefore n = $\frac{(1.96)2 \times 0.22(1-0.22)}{(0.05)2}$

$$n = \frac{3.8416 \times 0.22\ (0.78)}{0.0025}$$

n= 263.687424 rounded off to 264 samples

The population of samples to be tested were determined by; (100/50) n

= (100/50)264.

=2*264

=528 samples.

The sample size was adjusted to 476 samples.

The initial sample size was 264 samples .It was increased by a factor of 2 in order to increase the study's statistical power and accommodate all the study objectives ,In order to give a clear picture of the Kenyan coast ,the sample size was also added to counter any missing data and non-responses from the study participants .Oversampling by 10 % to 20% of the computed sample size is required depending on how much the researcher would anticipate discrepancies (Naing et al., 2006). Only 476 samples were tested in this study due lab viable.

3.5.Data collection instrumentation.

Data were obtained from the previous collected close-ended questionnaires administered by KEMRI under the SATREP Project on arboviruses.

The designed questionnaire is annexed in appendix one.

3.6. Laboratory analysis for CHIKV.

3.6.1. Cell Cultures

Vero cells were used for this work. These are mammalian cells derived from an adult African green monkey (kidney cell line, ATCC CCL81)). They have adherent growth properties and epithelial morphology and thus, have been known to support arbovirus growth. These cells were used since most arboviruses grow well in them and are also

suitable for performing Focus Reduction Neutralization Assays, in addition to preparing assay antigen for ELISAs.

3.6.2. Growth of Vero Biken Cells

The Vero cells were propagated in 15 seal-cap tissue culture flasks containing Growth Medium (Eagles Minimum Essential Growth Medium (EMEM) and maintained in Maintenance Medium (Eagles Minimum Essential Maintenance Medium (EMEMM). The Growth Medium was supplemented with 10% heat-inactivated filtered Fetal Calf Serum (FCS) whereas the Maintenance Medium was supplemented with 2% heat-inactivated filtered Fetal Calf Serum (FCS). Both medias were supplemented with Non-essential amino acids (NEAA): Glutamine (Gln), antibiotics: Penicillin/Streptomycin (P/S) and sodium bicarbonate (NaHCO3). The flasks were incubated in a humidified 37°C incubator with 5% CO_2. Cells were monitored daily and media was changed after every 3-4 days..

3.6.3. Splitting of the Verobiken cells

Once a >90% confluent monolayer was achieved after propagation, the cells were split inside a laminar airflow which had been decontaminated using 70% ethanol. Old-growth medium was discarded and cells rinsed once with 3ml of 0.05% trypsin-EDTA and incubated at 37^0C for 5 minutes. The flask was rocked gently to dislodge the cell monolayer. The cell suspension was then transferred to a 15ml conical tube and centrifuged at 1000rpm for 4min. The resultant supernatant was discarded and cell pellet re-suspended in 10ml of growth media and transferred cells to new tissue culture flasks at a ratio of 1:5. The Vero cells were split after every 48-72 hours to prevent overgrowth and cell death (Ammerman et al., 2009).

3.6.4. Indirect IgG ELISA.

The positive control was derived after a serial dilution (initial 1:1000 dilution, then two twofold serial dilutions up to 2-12 times dilution) of the febrile patients' sera while the negative control (1:1000 dilution) was set after drawing a scatter plot for the OD's of samples at 492 nm. Ninety-six well flat-bottomed microtitre plates/Falcon Immuno plates (Beckton Dickinson) were coated with purified CHIKV in coating buffer and

then incubated at 37 °C for 1 h or at 4 °C overnight and then blocked with Blockace at room temperature for 1 h. The microtitre plates were then washed three times using PBS-Tween 20. Excess washing buffer was retrieved after washing by firmly tapping the microtitre plates on absorbent paper. Patient sample serum, 100µl, of 1:1000 dilutions in 10 % Blockace in PBS-Tween 20 was added to each well in duplicate and incubated at 37 °C for 1 h. Thus, allowing the human sera to react with the antigen-coated wells. The plates were washed as described above. A hundred µl of 1:5,000-diluted horseradish peroxidase-conjugated goat anti-human IgG (American Qualex, California) was added to each well and the plates incubated at 37 °C for 1 h. The plates were washed as described above. A hundred µl of o-phenylenediamine (5 mg o-phenylenediamine dihydrochloride and 0.03 % hydrogen peroxide in 10 ml of 0.05 M citrate-phosphate buffer at pH 5.0) was added to each well and subsequent incubation in the dark at room temperature for 10-30 min. The reaction was stopped by adding 100 µl of 1N H2SO4 to each well. The OD at 492 nm for each well was measured using Multiscan EX reader (Thermo-Lab systems) using Ascent Software Version 2.6 (Thermo Scientific) or equivalent for data acquisition. Each sample was tested in duplicate and hence the mean OD for each sample was calculated. ELISA titres were calculated from standardized reciprocal dilution values by using Thermo-Labsystem's Ascent photo spectrometric data analysis software, version 2.6. A sample titer equivalent to three standard deviations from the baseline was considered to be positive.

3.6.5. IgM Capture Assay Antigen Preparation.

The Vero cell line was grown 175cm^2 flasks (NUNC®) until 80% confluence was achieved. The growth medium (MEM 10% FCS) was then removed and 1 ml of seed virus stock in 4ml maintenance media (MEM 2% FCS) inoculated in each flask. The virus was allowed to adsorb by incubating for 2 hours (with tilting after every 20 min). Maintenance media was added and flasks incubated at 37°C for 2-4 days. The resulting Inoculated Culture Fluid (ICF) was then collected in 50ml centrifuge tubes, spun at 1800 × g at 4°C for 10 minutes. The ICF was later titrated using antigen detection ELISA.

3.6.6. Sandwich IgM ELISA.

Ninety-six well Falcon Immuno plates (Becton Dickinson) was coated with 100 µl of a 1:500 dilution of goat anti-human IgM (BioSource International) in coating buffer and the coated plates incubated at 37 °C for 1 h or at 4 °C overnight. A hundred µl of Blockace was then added in each well except for the blank wells at room temperature for 1 hour. Plates were washed as described in the indirect IgG ELISA method above. To each well, 100 µl of patient serum sample diluted 1:100 using PBS-Tween 20 with sodium azide as assay diluent was added to the Falcon immunoplate (Becton Dickinson), then incubated at 37 °C for 1 h. Plates were washed as described above. The CHIKV infected culture fluid was diluted to 100EIA/100 µl in 10 % Blockace in PBS-Tween 20 assay diluent and the plate incubated at 37 °C for 1 h. Plates were washed as described above. A hundred µl, 1: 100,000 dilution of rabbit anti-CHIKV polyclonal antibodies was added and the plate incubated at 37 °C for 1 h. Plates were washed as described above. A hundred microliters diluted 1:5,000 times of horseradish peroxidase-conjugated goat anti-rabbit IgG (American Qualex, California) was added and the plate incubated at 37 °C for 1 h. Plates were washed as described above. A hundred µl of the substrate, o-phenylenediamine (5 mg o-phenylenediamine dihydrochloride and 0.03 % hydrogen peroxide in 10 ml of 0.05 mM citrate-phosphate buffer at pH 5.0), was added to each well and subsequent incubation in the dark at room temperature for 10-30 min. The reaction was then stopped by adding 100 µl of 1N H_2SO_4 to each well. OD at 492 nm for each well was be measured using Multiscan EX reader (Thermo-Lab systems) using Ascent Software Version 2.6 (Thermo Scientific) or equivalent. Each sample was tested in duplicate and hence the mean OD for each sample was calculated. On each test plate, 1:100 dilutions of the negative-control and positive-control serum samples were run simultaneously. A P/N (positive control (or sample) OD492/negative control OD492) ratio $\geq$2.0 will be considered positive.

3.6.7. Focus Reduction Virus Neutralization Test.

Focus reduction neutralization test (FRNT) was used to determine the presence of arbovirus specific neutralizing antibodies in test sera already screened as positive by ELISA. Briefly, Vero cells at a concentration of 1.5 x 10 cells/ml were seeded into 96-

well plates (Nunc) at a volume of 2 ml/well. Cells were cultured in Growth Medium for 1 day at 37 ºC, 5% CO_2. Sera were diluted with Maintenance Medium (EMEM, 2% FCS, P/S supplemented) in 1:20, 1:40 and 1:80 dilutions and then mixed with an equal volume of standard virus solution (120 FFU/0.1 ml). The virus-serum mixture was then incubated for 1 hour at 37 ºC and then overnight at 4 ºC. The next morning 100 µl/well of the mixture was added to Vero cells in duplicate wells and allowed to adsorb by spreading inoculums every 30 minutes for 1.5-2 hours in the incubator. 4 ml of overlay medium (EMEM, 1% FCS, 1.25% Methylcellulose, P/S supplemented) was added into each well, and the plates incubated at 37 ºC, 5% CO_2 for 4-8 days. After the final day, 2 ml of 3.7% formaldehyde solution was poured over the overlay medium and incubated for 3 hours at room temperature, and the wells then rinsed with water. Staining solution (1% NP - 40 in 1 x PBS) was used to stain each for 30 minutes at room temperature, after which the dye was discarded, plates rinsed with 1 x PBS and blocked using 100 ul block ace for 30 min. 100 ul of the conjugate (500 x goat anti-rabbit IgG, American Qualex) was added and incubated for 1 hr at. Substrate buffer (10 mg/ml DAB) was added and the plates incubated at room temperature for 5 to 10 min. Plates were washed once with 1 x PBS (-) and dried. Foci were counted for each set of duplicate wells and the percentage reduction calculated by comparing with the control virus (100% foci formation). More than 90% focus reduction was regarded as positive.

3.7. Data analysis.

Data collected from the questionnaires were entered and analyzed using Microsoft Office-Excel 2016 (Microsoft, California, USA) and RStudio version 1.2.5033 (2009-2019 RStudio, Inc.). Descriptive data are presented in tabular forms. Associations between binary or

Categorical variables were investigated using chi-square tests. Association of chikungunya seropositivity and symptoms was analyzed by logistic regression. Odds ratios (OR) were computed at 95% confidence intervals (CI) to determine the strength of association between seropositivity and variables. All tests were statistically significant at $P \leq 0.05$.

CHAPTER FOUR

RESULTS

4.1 Demographic characteristics of study participants.

A total of 476 patients were recruited into the study between January 2014 and December 2015. All 476 had a fever of unknown cause. Of these, 264 (55.46%) were male, 209 (43.91%) were female while 3 (0.63%) did not indicate their gender. Of the 476, some participants had been vaccinated against yellow fever.

Table 4.1-1 Demographic characteristics of the study participants.

Variable	**N=476**	**Proportion % (100%)**
Gender		
Female	209	43.91%
Male	264	55.46%
Gender not Indicated	3	0.63%
Age groups		
0-19	129	27.10%
20-29	130	27.31%
30-39	114	23.95%
40-49	36	7.56%
50-59	27	5.67%
60-69	22	4.62%
70-79	2	0.42%
80-89	3	0.63%
Age not indicated	13	2.73%
Yellow fever Vaccination status		
Complete	85	17.9%
Incomplete	27	5.7%
Not vaccinated	360	75.6%
Vaccination status not indicated	4	0.8%
Marital status		

Married	223	51.89%
Single	199	46.22%
Widowed	4	0.84%
Marital status not Indicated	5	1.05%
Sub-county		
Changamwe	30	6.30%
Kisauni	142	29.83%
Mvita	67	14.08%
Nyali	43	9.03%
Likoni	23	4.83 %

Demographic information for the 476 study Participants included in this study is shown in Table 4.1-1. The participants were clustered into 10-year age groups. The participants were drawn from both sexes and their ages were between 0-89 years. This dataset had as many males (264, 55.46 %) as it had females (209, 43.91%), 3 (0.63%) did not indicate their gender.

112(23.6%) of these participants had received yellow fever vaccination, 85 (17.9%) had completed the vaccination 27(5.7%) had incomplete vaccination 360(75.6%) had not been vaccinated, 4 (0.8%) did not indicate their vaccination status. About 142 (29.83%) of the study participants were residing in Kisauni sub-county. For the sub-counties not listed in Table 4.1-1 see appendix III.

4.2 Objective one: Seroprevalence of CHIKV in Febrile patients attending CPGH

Sera from the 476 study participants were analysed for CHIKV antibodies using an indirect IgG ELISA, Sandwich IgM ELISA and were later confirmed using Focus Reduction Neutralisation Test to determine the overall Seroprevalence. By ELISA, 74 (15.5%) participants tested IgG seropositive, while 9 (1.9%) participants tested IgM positive. All the 83 seropositive samples were further subjected to FRNT; only 46 (9.7%) of these had neutralizing antibodies against CHIKV. The prevalence rates for the different tests are shown in Table 4.2-1.

Table 4.2-1 CHIKV seropositivity using different tests

CHIKV seropositivity Test	Samples tested	Positive
IgM ELISA	476	9 (1.9%)
IgG ELISA	476	74(15.5%)
FRNT	83	46 (9.7%)

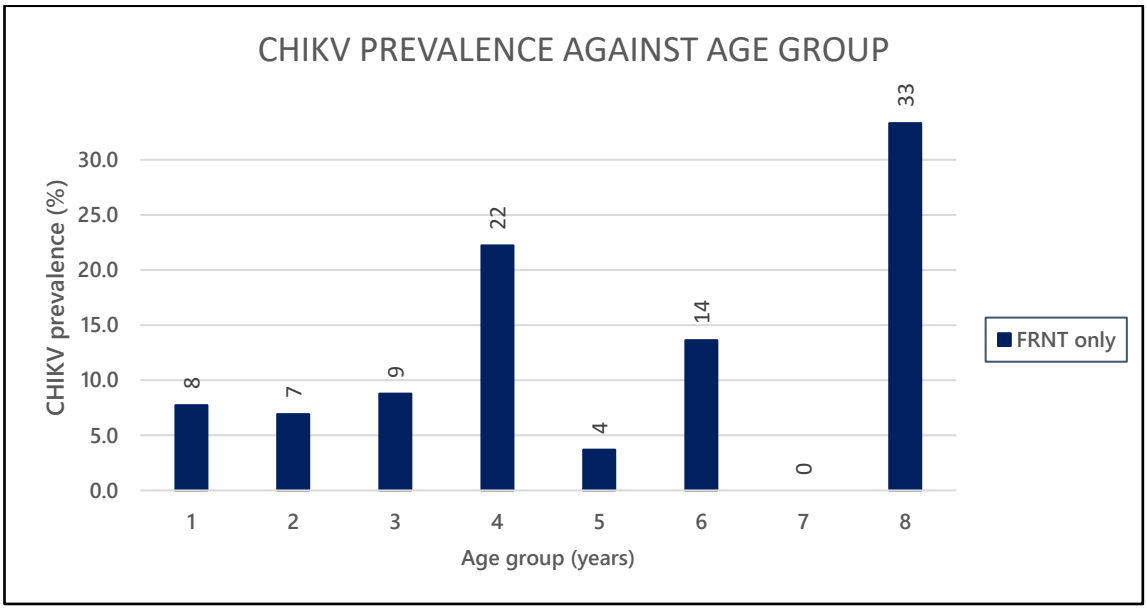

Figure 4.2-1 CHIKV Seroposivity against Age Group

Seroprevalence among those aged 80-89 years was 33% followed by 40-49 (See figure 4.2-1). Of the 209 females that were tested, only 22(10.5%) tested positive for CHIKV while those that tested positive in the 264 men tested were 24 (9.1%). Under marital status category, those that were married registered a high prevalence of 52.2% followed by singles at 45.6%. Study participants residing at the Kisauni sub-county had a higher Seroprevalence 28.26% as compared to the rest sub-counties.

4.3 Objective two: To Establish socio-demographic correlates of exposure to Chikungunya in febrile patients attending Coast Provincial General Hospital.

To determine whether the sociodemographic characteristic and clinical symptoms were associated with CHIKV, logistic regression was carried out. Those that had CHIKV neutralising antibodies were compared to that were seronegative. Gender, marital status, vaccination status, occupation, Village and sub-county were not associated with CHIKV seropositivity. Those aged 40-49 had a higher odd that was statistically significant OR 3.4 95Cl (1.20 to 9.42) P=0.0183* this is shown in Table 4.3-1.

Table 4.3-1 Association between Socio-demographic Characteristics and CHIKV seropositivity

Characteristic	Seronegativee	Seropositive	Univariate Analysis		
			Odds ratio	95% CI	P-value
Gender					
Female	187 (89.5%)	22 (10.5%)	Reference		
Male	240 (90.9)	24 (9.1%)	0.85	0.46 to 1.57	0.601
Gender not indicated	3(100%)	0			
Age group					
0-19	119	10	Reference		
20-29	121	9	0.89	0.34 to 2.27	0.7982
30-39	104	10	1.14	0.45 to 2.89	0.7729
40-49	28	8	3.4	1.20 to 9.42	0.0183*
50-59	26	1	0.46	0.02 to 2.55	0.4655
60-69	19	3	1.88	0.40 to 6.82	0.3697
70-79	2		0	-	0.9899
80-89	2	1	5.95	0.26 to 67.65	0.1597
Age not indicated	9	4			
Marital status					
Married	223	24	Reference		
Single	199	21	0.98	0.53 to 1.82	0.95
Widowed	4		0	_____	0.985
Gender not indicated	4	1			
Vaccination status					
Incomplete			Reference		
Yes				0.11 to 2.58	0.365824
No				0.31 to 4.10	0.927926
Occupation			----------	----------	0.2232
Village			----------	----------	0.9802
Sub-county			----------	----------	0.7524

Among the clinical symptoms analysed, none of them was associated with CHIKV seropositivity. Those with eye infection (OR 1.17, 95Cl 0.27 to 3.54, P=0.8), Jaundice (OR 1.01, 95Cl 0.40 to 2.23) had an increased chance of CHIKV seropositivity however, it was not statistically significant as shown in Table 4.3-2.

Table 4.3-2 Association between Clinical symptoms and CHIKV seropositivity

			Univariate Analysis		
Clinical symptoms	**Seronegative**	**Seropositive**	**Odds ratio**	**95% CI**	**P-value**
Rash					
No	329	39(11.85%)	**Reference**		
Yes	99	7(7.07%)	0.6	0.24 to 1.30	0.225
Not indicated	2				
Eye Infection					
No	404	43(10.64%)	Reference		
Yes	24	3(12.5%)	1.17	0.27 to 3.54	0.8
Not indicated	2				
Swollen Joints					
No	84	8(9.52%)	Reference		
Yes	2		0.0	-----	0.993
Not indicated	344	38(11.05%)			
Chills					
No	65	7(10.76%)	Reference		
Yes	18	1(5.56%)	0.52	0.03 to 3.17	0.548
Not indicated	347	38(10.95%)			
Jaundice					
No	361	39(10.80%)	Reference		
Yes	64	7(10.94%)	1.01	0.40 to 2.23	0.977
Not indicated	5				
Headache					
No	170	20(11.76%)	Reference		
Yes	254	26(10.24%)	0.87	0.47 to 1.62	0.657
Not indicated	6				

Chi-squares were calculated to establish the sociodemographic and clinical symptoms that correlated to CHIKV seropositivity and their P-values reported. Gender, Age

group, Marital status, Vaccination status and Sub-county were not associated with CHIKV. However, the occupation was significantly associated with CHIKV (Chisq statistic 104.32 P=0.000344) as shown in Table 4.3-3

Table 4.3-3 Chi-square and P-values: for an association between seropositivity and socio-demographic characteristics

	Chi-sq. Statistic	**P-Value**
Gender	0.13468	0.7136
Age group	12.4	0.08816
Marital status	0.4301	0.8065
Vaccination status	1.768	0.4131
Occupation	104.32	0.000344*
Sub-county	30.615	0.4857

As for the clinical symptoms shown in Table 4.3-4, none of them was statically significantly. Hence there was no association to CHIKV.

Table 4.3-4 Chi-square and P-values: for association between seropositivity and Clinical symptoms

Variable	**Chisq. Statistic**	**P-Value**
Rash	1.077	0.2994
Eye Infection	3.04E-29	1
Swollen Joints	3.84E-29	1
Chills	0.024069	0.8767
Jaundice	4.55E-31	1
Headache	0.081816	0.7749

4.4 Objective Three: Impact of Rainfall and temperature on CHIKV Transmission.

Data on rainfall and temperature for the study area was obtained from the Kenya meteorological department.

Seropositivity was lowest in the months when precipitation was highest, and vice versa, While

Temperature was positively associated with CHIKV seropositivity i.e. seropositivity was highest when temperatures are highest, and vice versa However the correlations were not significant at 0.05 significance level**.** This information is presented in Figure 4.4-1.

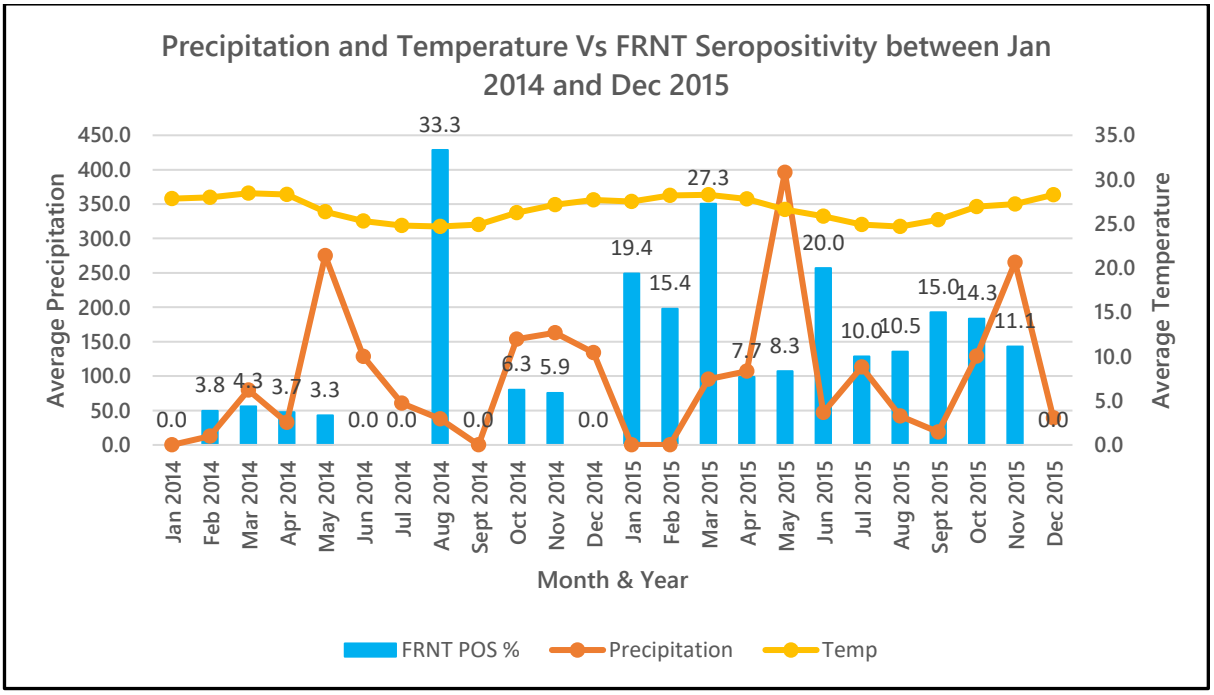

Figure 4.4-1 Relationship between Rainfall, Temperature and CHIKV prevalence between January 2014 to December 2015

CHAPTER FIVE

DISCUSSION

5.1.Discussion

Diagnosis of febrile diseases in our health facilities is a challenge because of lack of resources. Thus most febrile illnesses are treated as bacterial infections due to lack of diagnostic capacity to properly rule-out cause of fever and as well, identify alternative fever-causing pathogens (D'Acremont et al., 2010). This, in turn, leads to a lot of pressure to essential drugs, increasing the risk of losing necessary drugs due to development of resistance. Chikungunya and dengue have similar clinical presentations therefore it's difficult to distinguish the two diseases clinically (Mardekian & Roberts, 2015). Most clinical Chikungunya and Dengue cases are self-limiting. Therefore, early diagnosis and especially patients with dengue is very crucial for patient management in case of severe symptoms if done on time.

Previous studies conducted on CHIKV occurred during outbreaks and were focused on febrile patients (Anjichi, V. K. et al., 2016). The prevalence reported was 23.2%. The point prevalence of 9.7% reported in our study was lower.

Age and gender are important factors for certain specific behaviours that cause higher exposure to the *Aedes aegypti* bites. This behaviour may include staying outdoors during daytime when the vectors are active and without the use of any personal protection measures, such practices and lifestyles of the participants may make them vulnerable to CHIKV infection. In this study, all age groups had been affected suggesting that there was no background immunity for CHIKV infection. Those in the age group 80-89 years' registered a high CHIKV Seroprevalence and a higher likelihood of getting CHIKV (OR =5.95 95Cl 0.26 to 67.65 P= 0.1597) although it was not statistically significant. This finding is consistent with the results of previous studies in Lamu, Nigeria and Malaysia, where the infection was found to be more prevalent in elderly people (Azami et al., 2013; Omatola et al., 2020; Sergon et al., 2008). Host factors affect the clinical presentation of CHIKV infections. It has been observed that young children present with arthralgia less frequently than older children and adults. Patients older than 65 years of age may present a more complicated clinical picture during acute chikungunya, with more frequent manifestations other than arthralgia,

including high rates of neurological complaints (Natrajan et al., 2019). This could be a result of anatomical and functional changes, degree of exposure, and impaired immune functions in elderly people *(Ang et al., 2017; Omatola et al., 2020)*. Additionally, elderly people maintain sedentary lifestyles as they stay for long periods in unscreened places, hence are exposed to *Aedes aegypti*. Despite those in the age group 80-89 having higher seropositivity, age group 40-49 years who were significantly associated with CHIKV infection (OR=3.4 95Cl 1.20 to 9.42 P=0.0183) this is can be attributed to the increased susceptibility to the movement of older people outdoors when the vector activity is at its peak (daytime) and the variations in peoples' immune response that may make them susceptible to CHIKV infection.

Findings of this study indicated that males had higher seropositivity, unlike females, as shown in Table 4.3-1. This is in agreement with previous studies that found males to be prone to infection as documented by (Azami et al., 2013 ; Sissoko et al., 2008) which was attributed to different cultural habits and behaviours that predisposed men to the vector. The lower rate of transmission in females may be due to the effect of herd immunity whereas high rates of CHIKV transmission in males might be due to their adventurous nature; they hunt and fend for the family, irrespective of the place and time. They enter forested areas, swamps, and may not protect themselves from mosquito bites.

Under the Marital status category, the results indicated that those that were married had higher seropositivity as compared to those single and divorced. This has been corroborated by a previous study by (Omatola et al., 2020) that found married people had a seropositivity of 45.5% divorced 43.8% and Singles 19%. CHIKV is spread through human to mosquito to human cycle. Thus, people infected with the virus may be infective by other mosquitoes that bite them after an infectious mosquito bite. Infected persons may remain infective to *Aedes aegypti* for as long as 7 days after onset of illness. Asymptomatic people may still transmit the virus on to a mosquito that bites them. Households with several members are likely to be more seropositive. For instance, if one of them is asymptomatic the other person can horizontally be infected after a blood meal by *Aedes aegypti*.

Using the Univariate analysis occupation was found insignificant but when using CHI square test, it was found to be statistically significant.

Among the clinical manifestations analysed the commonest symptoms were eye infection 12.5%, jaundice 10.94% and Headache 10.24%. While, rash, chills and muscle ache were less prominent features. Although none of the clinical manifestations was significantly associated with CHIKV infection. Those with eye infection (OR 1.17, 95Cl 0.27 to 3.54, P=0.8), and jaundice (OR 1.01, 95Cl 0.40 to 2.23) had a higher chance of getting CHIKV. CHIKV has been shown to occasionally to infect the eyes and cause severe systemic diseases such as Renal, liver and respiratory failure (Pellot et al., 2012). Some studies suggest that CHIKV infects fibroblast in the scleral connective tissues found in the stroma of smooth muscles of ciliary bodies, iris and other fibres of the ocular muscles where it replicates actively producing infectious particles which damage the ocular tissues (Moizéis et al., 2018).

Chikungunya virus has become fully adapted to the urban cycles and no longer requires the presence of nonhuman primates and a sylvatic cycle for their maintenance thus, the urban transmission cycles of CHIKV especially in densely inhabited tropical areas, usually result in large outbreaks where a sustained low level of virus circulation is enough to maintain these virus to population (de Lima Cavalcanti et al., 2022). In this study, the village and sub-county were not statistically significant. However, some of the sub-counties registered a high number of seropositive patients included Kisauni, Changamwe, Jomvu, Mvita, Rabai; with most of these sub-counties being from Mombasa county. Mombasa County has 6 sub-counties namely Mvita, Nyali, Changamwe, Jomvu, Kisauni, and Likoni. Mvita and Changamwe have high population densities that is mainly attributed to the proximity of vital infrastructures such as electricity, roads, water and employment opportunities provided by the Export Processing Zones, and facilities such as airports and ports. Kisauni Sub-county has the highest population representing 20.66 per cent of the County's population, and this is largely attributed to low-cost housing and ease of access to most parts of the county from the sub-county, furthermore, the largest slums such as Mshomoroni, Junda, Kisumu ndogo are located there (County Government of Mombasa, 2018). Jomvu sub-county has the lowest population and this is attributed to fewer settlements and poor

infrastructure in the sub-county compared to the other sub-counties. Most of the economic activities of the regions are farming, fishing, tourism, industrial activities and mining. Mining and sand harvesting contribute to environmental degradation by leaving behind sites that are not rehabilitated which collect rainwater and act as breeding sites for vectors. As the population continues to grow more pressure is exerted on the available water resources because of the increase in demand for water. This means that pollution of available water resources is also high because of waste being produced with inadequate waste management strategies. Despite the increase in human traffic, sewage management systems have not been upgraded to cope with the rising demand. Unpleasant odours at the market are common due to uncollected rotting garbage and poor sanitation hence sanitation is an issue of major concern (Un-habitat/ECA/ Unep/ Unesco, 2005) and as such this enhances ample breeding sites for mosquitoes, therefore, increased contact between human and vectors in this region.

The main contributor to environmental degradation in the region is solid waste such as plastic bags, bottles, cans, garden and kitchen waste, vegetable waste and oil waste, logging (charcoal burning), bush fire (burning vegetation by farmers), overgrazing, dumping of solid waste by the hotels next to the ocean.

Temperature is an important driver of and limitation on vector transmission as it influences its population growth, as well as its dispersion and expansion. *Ae. aegypti* is strongly influenced by climatic factors, with temperatures of 10°C or lower limiting larval development and adult survival (Slosek, 1986). Optimal temperatures for development, longevity, and fecundity are between 22°C and 32°C (Beserra et al., 2009). Extreme temperatures suppress embryonic development and lead to death within hours of hatching 39°C is referred to as the lethal temperature (Couret et al., 2014; Mourya et al., 2004). At temperatures favourable to the life cycle, insects not only complete their development but do so more quickly, which may enhance vector competence for arboviruses (Couret et al., 2014). While temperature governs *Aedes aegypti* reproduction, maturation and mortality rates (Zequi & Lopes, 1993), rainfall generates the breeding grounds for larvae and pupae. Whenever rainfall increased CHIKV seropositivity decreased as shown in Figure 4.4-1. *Ae. Aegypti's* eggs are laid above the water surface and hatch only when the water level rises and wets them.

Increased rainfall forces the hatching of eggs and as the temperatures are low and high surface runoff this leads to the subsequent death of the larvae hence low transmission.

The Kenyan coast has favourable temperatures that are conducive for the vector proliferation with low rainfall providing breeding sites; therefore, high rainfall causes surfaces runoff washing off the laid eggs and such the vector capacity is reduced, besides, most people are found outdoors when the climate is favourable and hence they are more exposed to the vector; thus increased seropositivity.

5.2.Limitations of the study

Having been a sub-study we used bio banked sera that had been collected and used for an ongoing parent study that aimed to determine the Seroprevalence of DENV at the Kenyan coast. Therefore, our samples had been repeatedly frozen and thawed, and this might have resulted in false-negative IgG and IgM capture ELISA results. Due to the limitations of the antigen of interest, Focus Reduction Neutralisation Test was not carried out on the samples that tested negative for IgM and IgG ELISA. Due to the nature of the samples used molecular and Virus isolation tests were not carried out.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

1. The Point prevalence of chikungunya fever infection in febrile patients attending coast provincial general hospital Kenya was 9.7%. This suggests that the people in the Kenyan coast are exposed to CHIKV and possibly other arboviruses**.**
2. The disease is endemic due to the favourable climate that supports Vector proliferation and there is a need to maintain a high Epidemiological and entomological surveillance.
3. Rainfall and temperature can be used to predict CHIKV outbreaks.

6.2 Recommendation

Out of the 476 cases tested for the fevers of unknown origin only 46 cases tested positive for CHIKV, there is a high likelihood that the virus may have mutated or the participants might have had some other infection leading to fever; therefore;

1. More studies should be carried out to check for Coinfection within arboviruses.
2. Reporting of fever cases should be closely monitored as this will aid in the early identification of cases thus enabling control measures that will contain transmission.
3. Periodic surveys should also be carried out to determine CHIKV prevalence to prevent outbreaks before they occur.
4. Tests such as specific IgM, Viral isolation and molecular virus detection should be used as they will aid in determining the real burden of disease caused by CHIKV and its role in causing fever. Specific findings from such studies when conducted on a regular periodic basis could supplement surveillance to provide insights on CHIKV circulation in at-risk population and also inform clinicians and the community of the possible differential diagnosis in febrile illness thus saving them the costs incurred by treating febrile illness such as CHIKV with antimalarial drugs or antibiotics.

5. This study found that most people infected were adults and was attributed to their increased movements outdoors. There is, therefore, need to educate the masses about CHIKV prevention which can be prevented in two ways; Personal protection from mosquito Bites and Public health measures that aim in reducing the vector population Personal protection involves wearing clothes that minimize skin exposure and also reducing time outdoors. Repellents can also be applied on exposed skin or clothing adhering to the product label. Other ways to prevent mosquito bites ensure that the rooms have air conditioning, window screens, using mosquito coils as well as sleeping during the day under a mosquito net to prevent mosquito bites.

Public health measures involve vector control by eliminating larval habitats by removing tins tyres, discarded containers and stagnant water around the houses which act as mosquito breeding sites. Other measures include covering domestic water storage containers. Aerial spraying can also be carried out to reduce the number of adult mosquitoes.

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APPENDICES

APPENDIX I: QUESTIONNAIRE

A SEROPREVALENCE SURVEY FOR CHIKUNGUNYA VIRUS EXPOSURE IN HUMANS AT COAST PROVINCIAL GENERAL HOSPITAL KENYA.

1. Date of hospital visit …….

2. Study Number ……..…

3. Village ……

4. Sub-location …….

5. Location …….

6. Division ……

7. District …….

8. Province ……

9 Gender:

Male ( ) Female( )

Age (years) ( ) Date of birth ( )

Marital status: Single ( ) Married ( ) divorced ( )

10. Have you ever been vaccinated for Chikungunya fever?

Yes ( ) No ( )

If Yes, when?

Year ( ) Date ( ) Age ( )

11.Presenting Complaints:

A)Fever:

Yes ( ) No ( )

Duration of symptoms…….

b) Rash:

Yes ( )No ( )

Duration of symptoms…….

c)Eye infection:

Yes ( ) No ( )

Duration of symptoms……..

d)Jaundice:

Yes ( ) No ( )

Duration of symptoms……..

e)Headache:

Yes ( ) No ( )

Duration of symptoms……..

f)Symptoms suggestive of meningitis-meningoencephalitis

Yes ( ) No ( )

Duration of symptoms……..

g)Bleeding diathesis:

Yes ( ) No ( )

Duration of symptoms……..

List any other symptoms

…… ……

Any other remarks:

……
……
……

Name of person completing the form: ……...
……..

Signature …….. Date …….. Time ……

APPENDIX II: CONSENT FORMS FOR ADULTS

Informed Consent for Adults aged 18 and above, in English.

My name is……and I work in this health facility. We think that Chikungunya fever is an important cause of febrile illness in this area. To be sure about the exact magnitude of the problem, we are conducting a study to determine the prevalence of Chikungunya fever. The information we gather is useful to the government and other policymakers who may consider preventative programs in this community or other communities in the future. We will summarize our findings from this study and disseminate it to various stakeholders including the Ministry of Health, KEPI, KEMRI, and others. The KEMRI's ethical review committee, who are responsible for conducting such reviews at the national level, has approved this study.

Research Procedures: You are here to have a little blood drawn from you because your clinician has recommended some tests. If you agree to be a participant in this study, we will ask you a few questions regarding where you reside and your vaccination status. Then we will take 2 extra millilitres of blood during the procedure. We will use sterile and disposable instruments that are clean and safe. The extra blood taken from you will be transported to the KEMRI laboratories in Nairobi for analysis of Chikungunya Fever Status. The tests we conduct may also identify other infections related to Chikungunya fever if these are present. To ensure complete confidentiality of the test results, no names will be attached to the blood samples, but an identification number assigned to you will be used to label the sample.

Risk/benefits: During this procedure, there will be no long-lasting effect. However, you may feel a brief moment of pain or fear. You will not be given any monetary benefits; neither will you incur any costs. The study will benefit your community since by helping us and the government to understand the problems your community is facing as a result of Chikungunya fever, we will be able to recommend and design appropriate interventions to minimize the impact of this infectious disease.

Participant's Rights: Your participation in this study is voluntary and if you decline to participate, you will not be denied any services that are normally available to you.

Confidentiality: We will make every effort to protect your identity. You will not be identified in any report or publication of this study or its results.

Contact Information: If you have questions now or in the future regarding your rights or this study, you may ask any of the field officers involved in this study or contact Dr Allan ole Kwallah (Principal Researcher) of KEMRI at 02027222541 ext. 2256/2290.

Consent for the individual for blood sample:

May I now ask if you would like to participate in the study?

Ridhaa ya watu waliozidi umri wa miaka 18, kwa Kiswahili

Jina langu ni…….... Mimi ni mfanyakazi wa kituo hiki cha afya. Tunafanya utafiti juu ya ugonjwa uitwao Chikungunya fever ambao tunafikiri ni mojawapo ya magonjwa yanayoambukiza jamii hii. Ili tujua umuhimu wa Chikungunya fever huku, tumeamua kufanya utafiti. Matokeo ya utafiti huu yatasambazwa kwa serikali na wadau kama Wizara ya Afya, KEPI, na KEMRI na wengineo, ambao watatengeneza miradi mbalimbali itakayolenga kupunguza maambukizi na kuboresha huduma za walioathirika katika jamii hii, na nyingine hapo baadaye. Utafiti huu umepitishwa na kuruhusiwa na kamati inayohusika na utoaji wa vibali vya utafiti ya KEMRI, yaani ERC.

Utaratibu:

Daktari wako ameshakueleza kwamba anahitaji upimwe damu ili ajue kama una magonjwa fulani. Ikiwa utakubali kujihusisha na utafiti huu, kwanza tutakuuliza maswali machache juu ya umri wako, unapoishi, na kama umeshapewa chanjo ya Chikungunya fever. Baadaye, tutatoa kiasi cha 2 mls Zaidi cha damu, zoezi ambalo litachukua muda mfupi tu. Tutatumia vifaa visafi na salama ambavyo vitafunguliwa mbele ya macho yako, na vitatumika kwako tu na kutupwa mara tu baadaye. Baadaye, damu tutakayotoa kwa huu utafiti itapelekwa maabara kwa upimaji wa chikungunya fever na virusi vingine jinsia. Ili kuhakikisha usiri wa jina lako katika utafiti huu, jina na maelezo yako hayataandikwa kwenye sampuli ya damu, bali sampuli itatambulishwa na namba tu. Ni utaratibu wa utafiti huu kuwa watafiti hawatakujulisha majibu yako.

Faida/Mapungufu:

Utasikia maumivu kidogo ama woga wakati unachomwa sindano, lakini hutapata maumivu ya muda mrefu. Hutapata malipo yoyote ya kifedha, na pia hutatumia pesa zako mwenyewe katika utafiti huu. Utapewa neti ya kuzuia kuumwa na mbu. Utafiti huu utasaidia jamii yako kwa sababu tukifahamu matatizo ya jamii hii, tutaweza kushauri na kutengeneza miradi mbalimbali ya kupunguza athari za Chikungunya fever.

Haki za mshirika:

Ushiriki wako katika utafiti huu ni wa hiari kabisa. Ukikataa kushiriki, hutanyimwa huduma zinazotolewa kwa kawaida.

Usiri/Utunzaji wa taarifa:

Katika utafiti huu, tutahakikisha kuwa maelezo yako, jina lako ni siri kabisa. Jina lako halitaandikwa au kuhusishwa kwa hii fomu, sampuli, na popote ndani ya ripoti nzima tutakayotoa baadaye.

Mawasiliano

Iwapo utakuwa na swali kuhusiana na haki zako ama utafiti huu, unaruhusiwa kuwasiliana na afisa yeyote wa utahini, au Mkuu wa utafiti huu, Dr. Allan ole kwallah wa KEMRI, Nambari ya simu 0202722541 ext. 2256/2290

Ridhaa ya kutolewa damu:

Napenda kukuuliza ridhaa yako ya ushiriki wako katika zoezi la utoaji damu

Nimeelewa maelezo ya hapo juu yanayohusu utafiti huu, na ninakubali kushiriki katika zoezi hili. Naelewa kuwa ushiriki wangu ni wa hiari, na pia kama sitakubaliana muda wowote naruhusiwa kujitoa katika zoezi hili. Natoa ridhaa damu yangu itumike katika upimaji wa Chikungunya fever.

Sahihi/dole gumba……

Tarehe…….

APPENDIX III; Results of Seropositivity in different sub-counties

RESubcounty Vs FRNT

Count of FRNT_Status	**Column Labels**		
Row Labels	**NEG**	**POS**	**Grand Total**
bura	3		3
changamwe	23	7	30
galole	4	1	5
ganze	11	2	13
garsen	18	1	19
Hindi	1		1
Jomvu	10	3	13
kaloleni	8	1	9
Kilifi		1	1
kilifi north	2	1	3
kilifi south	25	1	26
KINANGO	2	1	3
Kisauni	129	13	142
Lamu east	1		1
Likoni	22	1	23
Lunga Lunga	3		3
Magarini	2		2
Malindi	1		1
matuga	21	1	22
mkunumbi	1		1
msambweni	14	1	15
mvita	61	6	67
mwatate	9	1	10
N/A	1	1	2

nyali	40	3	43
Rabai	2		2
SHELA	1		1
Taita taveta	6		6
tana delta	1		1
Voi	6		6
Wundanyi	2		2
Grand Total	**430**	**46**	**476**

APPENDIX IV; Research Publication

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Seroprevalence of Chikungunya Virus in Febrile Patients in the Kenyan Coast

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Abstract: *Fever is one of the main complaints in patients seen at the Coast Provincial General Hospital in Mombasa, Kenya. There is anecdotal evidence to suggest that Chikungunya virus is a cause of some of those fevers, but published data is scanty. This was a hospital-based cross-sectional study conducted in patients presenting with fevers of unknown origins. We tested for CHIKV antibodies using Enzyme-linked Immunosorbent Assay (ELISA) and confirmed using Focus Reduction Neutralization Test (FRNT). Prevalence rates were determined using proportions and rates. The odds ratio was used to measure the association between CHIKV seropositivity and associated risk factors. Of the 488 eligible participants who were recruited for this study, 269 (54.7%) were males, 213 (46.3%) were female while 6 (1.2%) did not indicate their gender. A total of 90 (18.4%) participants had been vaccinated against yellow fever. The overall seroprevalence of CHIKV was 9.84% (48). Age, gender, yellow fever vaccination status, sub-county, occupation and clinical manifestations were not associated with seropositivity. At high temperatures, CHIKV seropositivity peaked to 11.11% in 2014 and in 20.75% in 2015. Chikungunya is an important cause of fever in the Kenyan coast with a seroprevalence of 9.84%. There is a need to maintain constant epidemiological and entomological surveillance. Seroprevalence can be predicted using temperature and rainfall patterns.*

Date of Submission: 20-05-2019 Date of acceptance: 05-06-2019

I. Introduction

Chikungunya, a mosquito-borne viral disease, is characterized by fever, nausea, rash, vomiting, and arthralgia and muscle pain[1]. The virus has been reported to cause many outbreaks worldwide and thus poses a high public health significance. Kenya faced a Chikungunya virus (CHIKV) outbreak in 2004; this was followed by the Indian Ocean islands outbreak in 2005, with more than 260 000 clinical cases [2]. In 2007, CHIKV was exported into Europe, causing an outbreak of Chikungunya fever in Italy [3]. This outbreak suggested for the first time the significant potential of the virus to move to novel ecological niches through returning travellers [2, 4-6].

Although not a killer disease, high morbidity rates and prolonged polyarthritis lead to considerable disability in a proportion of the affected population and can cause a substantial socioeconomic impact in affected countries.

Even though CHIKV causes fever and nonspecific clinical manifestations similar to malaria and other bacterial infections, it is not routinely tested at the health facilities and therefore goes undiagnosed and as such its prevalence has continuously been underestimated. The misdiagnosis of CHIKV coupled with lack of effective diagnostic tools in areas where CHIKV is prevalent makes this disease a potential burden to these regions and at the increased potential for global spread. As it is a new and emerging disease it has not received sufficient coverage. For effective control, there is a need for documentation of the exact burden of this infection within febrile patients.

In this study, we describe the seroprevalence and establish sociodemographic correlates of exposure to Chikungunya in febrile patients attending Coast Provincial General Hospital.

II. Material And Methods

This was a cross-sectional survey conducted at the Coast Provincial General Hospital (CPGH) in Mombasa Kenya. CPGH is a major referral hospital located along the Indian Ocean shoreline in Kenya. It serves the region that lies between the Somali Border to the northeast and the Tanzania border in the southwest. The catchment is comprised of both urban and rural population.

Study setting and Sample collection

The study was conducted between January 2014 and December 2015. Patients seen at

CPGH presenting with febrile symptoms of unknown causes such as malaria, typhoid and other known bacterial infections were eligible. Venous blood was collected from consenting individuals and sera separated and stored for testing.

Laboratory procedures

Indirect IgM Indirect IgGELISA

Both indirect IgM and IgG ELISA screening tests were performed according to well-described methods [7]. For IgM ELISA each sample was tested in duplicate, and the OD for each well was measured at 492 nm using Multiscan EX reader (Thermo-Lab systems) using Ascent Software Version 2.6 (Thermo Scientific). The mean OD was calculated. On each test plate, 1:100 dilutions of the negative-control and positive-control serum samples were run simultaneously. A P/N (positive control (or sample) OD/negative control OD) ratio equal to or greater than 2.0 at 492 nm was considered positive. For the IgG ELISAOD was read the same way**.** ELISA titres were calculated from standardized reciprocal dilution values. A sample titre 1≥30000 was considered positive.

Focus Reduction Neutralization Tests (FRNT)

Focus reduction neutralization test (FRNT) was used to determine the presence of arbovirus specific neutralizing antibodies in test sera already screened as positive by ELISA. Briefly, Vero cells at a concentration

1.5 x 10 cells/ml were seeded into 96-well plates (Nunc) at a volume of 2 ml/well. Cells were cultured in

Growth Medium for 1 day at 37 ºC, 5% CO_2. Sera were diluted with Maintenance Medium (EMEM, 2% FCS, P/S supplemented) in 1:20, 1:40 and 1:80 dilutions and then mixed with an equal volume of standard virus solution (120 FFU/0.1 ml). The virus-serum mixture was then incubated for 1 hour at 37 ºC and then overnight at 4 ºC. The next morning 100 µl/well of the mixture was added to Vero cells in duplicate wells, and allowed to adsorb by spreading inoculums every 30 minutes for 1.5-2 hours in the incubator. 4 ml of overlay medium (EMEM, 1% FCS, 1.25% Methylcellulose, P/S supplemented) was added into each well, and the plates incubated at 37 ºC,

5% CO_2 for 4-8 days. After the final day, 2 ml of 3.7% formaldehyde solution was poured over the overlay medium and incubated for 3 hours at room temperature, and the wells then rinsed with water. Staining solution (1% NP - 40 in 1 x PBS) was used to stain each for 30 minutes at room temperature, after which the dye was discarded, plates rinsed with 1 x PBS and blocked using 100 ul block ace for 30 min. 100 ul of the conjugate (500 x goat anti-rabbit IgG, American Qualex) was added and incubated for 1 hr at. Substrate buffer (10 mg/ml DAB) was added and the plates incubated at room

temperature for 5 to 10 min. Plates were washed once with 1 x PBS (-) and dried. Foci were counted for each set of duplicate wells and the percentage reduction calculated by comparing with the control virus (100% foci formation). More than 90% focus reduction was regarded as positive.

Data Analysis

Data were analysed using STATA (Stata Corp Version 14.2). Descriptive statistics were computed for both quantitative and categorical variables. Exact 95% binomial confidence intervals (CIs) were calculated for seroprevalences. The chi-square test or Fisher's exact test, where appropriate, were used to test for associations between binary or categorical variables. We used generalized linear models assuming a binomial distribution to calculate crude and adjusted odds ratios (OR) using univariate analysis. Statistical significance was set at $p<0.05$.

III. Result

A total of 488 patients aged between 0 and 81 years were recruited for the study and blood samples drawn successfully. Of these, 269 (54.7%) were male, 213 (46.3%) were female while 6 (1.2%) did not indicate their gender. A total of 90 (18.4%) participants had been vaccinated against yellow fever.

By ELISA, 76 (15.67%) participants tested IgG seropositive, while 10 (2.06%) participants tested IgM positive. All the 86 seropositive samples were further subjected to FRNT; 48 (9.84%, 95% CI 9.2-11.11%) of these had neutralizing antibodies against CHIKV.

The seroprevalence in those aged 36-81 years was 12.84%, while in those aged 7-18 years it was 11.29%. The odds of seropositivity in those aged 36-81 years were 2.062 (95% CI 0.58 -7.3) when age group 0- 6 years was used as the baseline. The seroprevalence in females was 10.8% while in males it was 8.99%. The odds of being seropositive in females were 1.226 (95% CI 0.67–2.24) when the odds in males were used as the baseline. Amongst occupations, the group “others” had a seroprevalence of 13.98%, while in fishermen the seroprevalence was 11.11%. For the occupation status “others” the odds were 2.16 (95% CI 1.15-4.06).

Amongst clinical manifestations, 11.11% of those with eye infection were seropositive, with the odds of seropositivity for being 1.226 (95% CI 0.67-2.24). Seroprevalence in people residing in Changamwe was 16.13%, with the odds of CHIKV seropositivity being 1.877 (95% CI 0.68 -5.16). These data are presented in Table 1.

CHIKV seropositivity peaked at 11.11% in 2014 when the temperature and rainfall were 26℃ and 100mm respectively. It peaked at 20.75% in 2015 when the temperatures and rainfall were 26℃ and 47 mm respectively. Seropositivity declined to zero when temperatures were above 26.8 ℃ and rainfall was above 150 mm (Fig. 1). The odds of seropositivity were 2.78 (95% Cl 0.28-27.21) when the temperatures were above ≥ 26.8℃ and 1.36 (95% Cl 0.14-13.54) when rainfall was low (Fig. 1).

Fig. 1: Relationship between rainfall, temperature and CHIKV prevalence between January 2014 and December 2015.

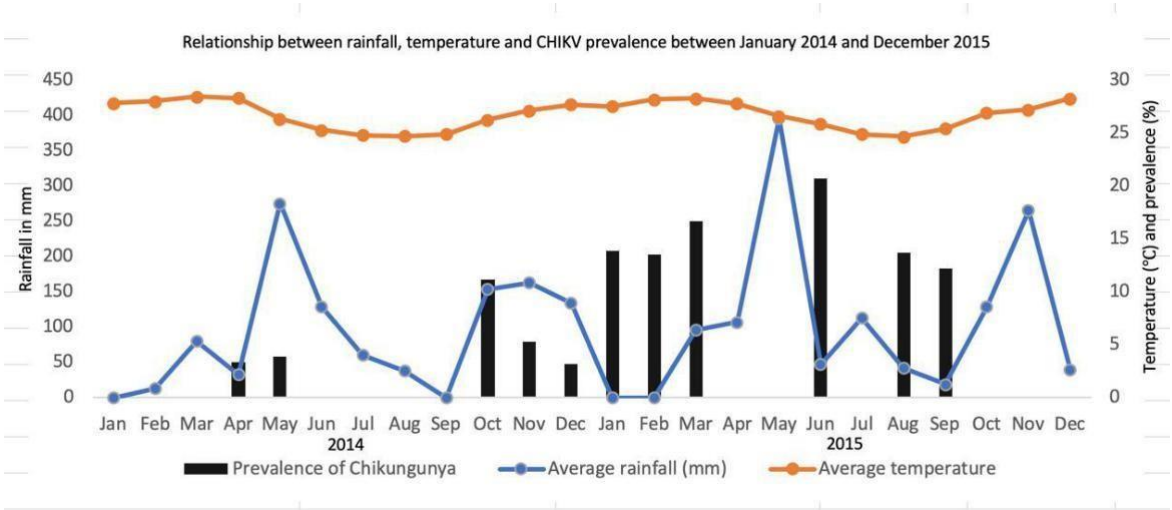

Table no 1: Seroprevalence (%) and odds ratios for the presence of CHIKV-neutralizing antibodies.

Characteristic	Number (%)	Seropositive (%)	Seronegative (%)	OR	95 CI	*P value*
Overall	488 (100)	48 (9.8)	440 (90.6)			
Age groups						
0-6	45 (9.2)	3 (6.67)	42 (93.33)	Ref	-	-
7-18 years	62 (12.7)	7 (11.29)	55 (88.71)	1.78	0.43-7.39	0.42
18-35	217 (44.5)	14 (6.45)	203 (93.55)	0.97	0.27-3.52	0.96
36-81	148 (30.3)	19 (12.84)	129 (87.16)	2.06	0.58-7.37	0.26
Adults and children						
Children under 18 years	107 (21.9)	10 (9.35)	97 (90.65)	Ref	-	
Adults	365 (74.79)	33 (9.04)	332 (90.96)	0.96	0.46-2.03	0.92
Gender						
Male	269 (55.1)	25 (9.3)	244 (50)	Ref	-	-
Female	213 (43.6)	23 (10.8)	190 (38.9)	1.23	0.670-2.24	0.51
Vaccination status						
Incomplete	27 (5.5)	3 (11.11)	24 (88.89)	Ref	-	-
Complete	90 (18.4)	6 (6.67)	84 (93.33)	0.57	0.13-2.48	0.45
Not vaccinated	364 (74.6)	39 (10.71)	325 (89.29)	0.96	0.28-3.34	0.95
Occupation						
Driver	29 (5.9)	3 (10.34)	26 (89.66)	Ref	-	-
Student	128 (26.2)	8 (6.25)	120 (93.75)	0.58	0.14-2.34	0.44
Fisherman	9 (1.8)	1 (11.11)	8 (88.89)	1.08	0.10-12.31	0.95
Farmer	45 (9.2)	2 (4.44)	43 (95.56)	0.40	0.1-2.64	0.33
Teacher	35 (7.2)	3 (8.57)	32 (91.43)	0.81	0.15-4.43	0.81

Others	186 (38.1)	26 (13.98)	160 (86.02)	1.40	0.39-5.1	0.61
Clinical manifestations						
Swollen joints	2 (0.4)	0 (0)	2 (100)	Ref		
Chills	19 (3.9)	1 (5.26)	18 (94.74)	0.45	0.05-3.92	0.46
Jaundice	73 (16.0)	7 (9.59)	66 (90.41)	0.95	0.41-2.20	0.90
Headache	288 (59.0)	27 (9.38)	261 (90.63)	0.88	0.48-1.62	0.68
Rash	106 (21.95)	7 (6.60)	99 (93.4)	0.58	0.25-1.33	0.19
eye infection	27 (5.5)	3 (11.11)	24 (88.89)	1.14	0.33-3.95	0.83
Sub counties						
Changamwe	31 (6.4)	5 (16.13)	26 (83.87)	Ref	-	-
Likoni	23 (4.7)	3 (13.0)	20 (86.96)	0.78	0.16-3.72	0.75
Kaloleni	9 (1.8)	1 (11.1)	8 (88.89)	0.65	0.06-6.63	0.71
Kisauni	143 (29.3)	12 (8.39)	131 (91.61)	0.48	0.15-1.48	0.19
Mvita	68 (13.9)	7 (10.29)	61 (89.71)	0.60	0.17-2.08	0.41
Nyali	43 (8.8)	3 (6.98)	40 (93.02)	0.39	0.08-1.82	0.21
Others	166 (34.0)	16 (9.64)	150 (90.36)	0.46	0.15-1.42	0.17
Rainfall						
Low rainfall	488 (100)	38 (7.79)	450 (92.21)	Ref		
High rainfall	488 (100)	10 (2.05)	478 (97.95)	0.73	0.07-7.28	0.79
Temperature						
<26.8	488 (100)	16 (3.28)	472 (96.72)	Ref		
≥26.8	488 (100)	32 (6.56)	456 (93.44)	2.78	0.28-27.21	0.36

*****Occupation group **'others'** comprises all occupations other than Student, Fisherman, Farmer, Driver and Teacher. They include Craftsman, Cleaner, Vendors, Business people, and Mechanics.

*****Sub counties group **'others'** comprises all other than sub-counties Changamwe, Likoni, Kaloleni, Kisauni, Mvita and Nyali. They include Mwatate, Kinango, Ganze, Garsen and Msambweni.

IV. Discussion

Previous studies conducted on CHIKV occurred during outbreaks and were focused on febrile patients

[8]. The prevalence reported was 23.2%. The point prevalence of 9.83% determined in our study was lower.

Women had a higher prevalence and a higher likelihood of having CHIKV infection than men. This is consistent with previous reports from the Comoros and Reunion islands [9, 10] which indicate that women are more prone to CHIKV infection. The higher prevalence in women may relate to gender differences in exposure to infection due to community-specific habits, customs and behaviour; also, most of the women and the elderly people spend most of their time home during the day, while men are at home less than half of the time. As the mosquitoes that transmit

Chikungunya do not travel far and often stay within the same households for days, women and the elderly are at high risk of contracting the virus.

Those in the 36-81 years age group had a higher CHIKV seroprevalence although it was not statistically significant. We attribute this increased susceptibility to the movement of older people outdoors when the vector activity is at its peak (daytime) and the variations in peoples immune response[11].

Whenever temperature and rainfall increased CHIKV seropositivity decreased. Temperature is an important driver of and limitation on vector transmission since it influences it population growth, as well as its dispersion and expansion. Ae. aegypti is strongly influenced by climatic factors, with temperatures of 10°C or lower limiting larval development and adult survival [12]. Optimal temperatures for development, longevity, and fecundity are between 22°C and 32°C [13]. Extreme temperatures suppress embryonic development and lead to death within hours of hatching [14]. 39°C is referred to as the lethal temperature [15-17]. At temperatures favourable to the life cycle, insects not only complete their development but do so more quickly, which may enhance vector competence for arboviruses [17]. While temperature governs Aedes aegypti reproduction, maturation and mortality rates [18], rainfall generates the breeding grounds for larvae and pupae. At variance with other mosquito species, Ae. Aegypti‟s eggs are laid above the water surface and hatch only when the water level rises and wets them. Increased rainfall forces the hatching of eggs and as the temperatures are low this leads to the subsequent death of the larvae.

The most important finding of this study is that CHIKV seroprevalence can be predicted using temperature and rainfall patterns. This in turn can be used to anticipate outbreaks and can be used to develop be spoke interventions.

V. Conclusion and Recommendations

Since the seroprevalence of CHIKV is quite high in febrile patients, there is a need to maintain high levels of epidemiological and entomological surveillance. Fever should be closely monitored as this will aid in the early identification of cases thus enabling control measures that will contain transmission. Findings from such serological studies, when conducted on a regular periodic basis, could supplement surveillance to provide insights on CHIKV circulation in the at-risk population.

VI. Limitations Of Study

Having been a sub-study we used bio banked sera that had been collected and used for an ongoing parent study that aimed to determine the seroprevalence of DENV at the Kenyan coast. Therefore, our samples had been repeatedly frozen and thawed, and this might have resulted in false-negative IgG and IgM capture ELISA results.

Acknowledgements

The project was nested within a JICA-JST-SATREPS project based at KEMRI. We appreciate technical support provided by the Production Department, KEMRI Nairobi and Centre for Infectious and Parasitic Control Research (CIPDCR) Infectious Disease Research Laboratory (IDRL) in Alupe, Kenya. JICA- JST-SATREPS, who funded this study, had no role in the study design, in the collection, analysis and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication.

VII. Ethical Statement

Approval for the study was obtained from the ethical review committee at KEMRI (KEMRI/SERU/2979). Informed consent was sought from all participating children and adults by the phlebotomist. The study was conducted according to the principles of the Helsinki Declaration. In the case of children, consent was sought from their parents or guardians.

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APPENDIX IV; Ethical clearance

Elizabeth Njeri Ng'ang'a. "Seroprevalence of Chikungunya Virus in Febrile Patients in the Kenyan Coast." IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 14.3 (2019): 40-44.

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KEMRI/RES/7/3/1 **August 02, 2016**

TO: **NG'ANG'A NJERI ELIZABETH,**
PRINCIPAL INVESTIGATOR

THROUGH: **MR. TOM MOKAYA,**
ACTING DIRECTOR, CIPDCR,
BUSIA

Dear Madam,

RE: PROTOCOL NO. SSC 2979 (*RESUBMISSION4 OF INITIAL SUBMISSION*): SEROPREVALENCE OF CHIKUNGUNYA IN FEBRILE PATIENTS ATTENDING COAST PROVINCIAL GENERAL HOSPITAL KENYA

Reference is made to your letter dated 19th July, 2016. The KEMRI/Scientific and Ethics Review Unit (SERU) acknowledges receipt of the revised study documents on the 1st August, 2016.

This is to inform you that the Committee notes that the issues raised during the 240th meeting of the KEMRI/Ethics Review Committee (ERC) held on 16th June, 2015 have been adequately addressed.

Consequently, the study is granted approval for implementation effective this day, **2nd August, 2016** for a period of one year. Please note that authorization to conduct this study will automatically expire on **August 01, 2017.** If you plan to continue data collection or analysis beyond this date, please submit an application for continuation approval to SERU by **June 20, 2017**.

You are required to submit any proposed changes to this study to SERU for review and the changes should not be initiated until written approval from SERU is received. Please note that any unanticipated problems resulting from the implementation of this study should be brought to the attention of SERU and you should advise SERU when the study is completed or discontinued.

You may embark on the study.

Yours faithfully,

FOR:

DR. EVANS AMUKOYE,
ACTING HEAD,
KEMRI/SCIENTIFIC AND ETHICS REVIEW UNIT