PREVALENCE AND DISTRIBUTION OF DENGUE, CHIKUNGUNYA AND ZIKA ARBOVIRUSES AMONG NON-MEASLES FEBRILE RASH CASES IN SELECTED REGIONS OF KENYA: 2008-2014

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Prevalence and Distribution of Dengue, Chikungunya and Zika Arboviruses among Non-Measles Febrile Rash Cases in Selected Regions of Kenya: 2008-2014

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DECLARATION

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

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DEDICATION

This thesis is dedicated to my parents Mr. and Mrs. Kimata, and my siblings Lucille and Joe Kimata, for their continued financial support and encouragement.

I also dedicate it to my late aunt Ruguru Githaiga who would have loved to see her nephews and nieces get the best education and achieve their dreams.

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ABBREVIATIONS AND ACRONYMS

°	Degrees
µl	Microlitre
ATCC	American Type Culture Collection
BSL	Biosafety Level
C	Celsius
CDC	Centers For Disease Control and Prevention
CHIKV	Chikungunya Virus
CI	Confidence Interval
CNS	Central Nervous System
CO_2	Carbon Dioxide
CVR	Centre For Virus Research
DENV	Dengue Virus
DHF	Dengue Haemorraghic Fever
DLSP	Diagnostic and Laboratory System Program
DSS	Dengue Shock Syndrome
ELISA	Enzyme-Linked Immunosorbent Assay
EMEM	Eagles Minimum Essential Medium
EPI	Expanded Programme on Immunization
FBS	Fetal Bovine Serum
FRNT	Focus Reduction Neutralization Test
HRP	Horseradish Peroxidase
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IQR	Interquartile Range
ITNs	Insecticide-Treated Nets
ITROMID	Institute of Tropical Medicine and Infectious Diseases

JKUAT	Jomo Kenyatta University of Agriculture and Technology
KEMRI	Kenya Medical Research Institute
ml	Milliliter
nm	Nanometer
NMFRI	Non-Measles Febrile Rash Illness
OD	Optical Density
ONNV	O'nyong Nyong
PBS	Phosphate Buffered Saline
PFU	Plaque Forming Unit
PRNT	Plaque Reduction Neutralization Test
RNA	Ribonucleic acid
RT-LAMP	Reverse Transcription Loop-Mediated Isothermal Amplification Assay
RT-PCR	Reverse Transcription-Polymerase Chain Reaction
SCDSC	Sub-County Disease Surveillance Coordinator
SFV	Semliki Forest Virus
SINV	Sindbis Virus
SOP	Standard Operating Procedure
TMB	Tetramethylbenzidine
VHF	Viral Hemorrhagic fever
WHO	World Health Organization
WNV	West-Nile Virus
YF	Yellow Fever
YFV	Yellow Fever Virus
ZIKV	Zika Virus

OPERATIONAL TERMS

Co-exposure This is a phenomenon where a patient is bitten by a co-infected mosquito or several bites from infected mosquitoes carrying different viruses.

Prevalence Total number of individuals in a population who have a disease at a specific period.

Plaque Reduction Neutralization Test (PRNT) It is used to measure virus-specific neutralizing antibodies and may be able to determine the cause of the primary infection.

ABSTRACT

In Kenya, 50-60% of suspected measles cases remain undiagnosed as their etiology remains unknown. Clinical symptoms of rash and fever are common manifestations of several infectious disease etiologies, including arboviruses and malaria. Lack of diagnostic capacity has led to the under-reporting of specific causes of febrile rash illness, including arbovirus infections in Kenya. This study aimed to determine the recent and past circulation of arboviral infections amongst suspected cases of measles in Kenya which confirmed negative IgM for measles as well as rubella serological assays. A retrospective study was conducted on 392 randomly selected serum samples collected between 2008 and 2014 from health facilities in Nairobi, North-Eastern and Coastal regions of Kenya. Samples were screened for the presence of IgG and IgM antibodies against dengue, chikungunya, and Zika viruses by Enzyme-Linked Immuno-sorbent Assay (ELISA). Positive samples by ELISA were confirmed by Plaque Reduction Neutralization Test (PRNT) for samples that were positive for either dengue (DEN), Zika (ZIK), or chikungunya (CHIK) viruses. Out of the 392 samples, 5(45.5%) were confirmed to have an active infection of DEN, while 1(0.3%) had an active infection of CHIK and 3 had an active infection of ZIK. Past infection of DEN was confirmed in 17 (56.7%) while 1 (10.0%) sample was positive for CHIKV. Other viruses included were West-Nile (WN), Yellow Fever (YF), Sindbis (SIN), Semliki Forest (SF) and O’nyong Nyong (ONN) viruses. 9(90.0%) samples were confirmed to be ONN, 3 (10.0%) to be WN and 2 (6.7%) to be YF. Dengue, chikungunya and Zika were detected in all years of study 2008-2014 and all study sites except Tana-River and Wajir Counties. The results showed that the prevalence of CHIK and DEN was significantly higher in those aged 14-60 years. The study confirms patients' exposure to these arboviral infections DEN, ZIK and CHIK arboviruses in Nairobi, North-Eastern and Coast Regions. The study recommends that arbovirus diagnosis be included in a comprehensive differential diagnosis for cases presenting with fever and rash from suspected measles cases. Additionally, clinicians should be sensitized to consider testing for arboviruses for those fever and rash cases that are negative for measles and rubella.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Measles and rubella have been the foremost cause of febrile rash illness in children and adults in Kenya (Perry *et al., 2*004; Nandy *et al.*, 2006). Additionally, febrile rash illnesses are common reasons for seeking medical attention in Kenya and Africa (O'Meara *et al.,* 2015). These illnesses are caused by different etiological agents and are among the leading causes of morbidity in Kenya and Africa. According to WHO, (2020) report, measles is one of the leading causes of death in young children in developing countries, particularly in Africa and Asia, despite having a safe and cost-effective vaccine available. Rubella virus is distributed worldwide and is of public health concern due to its teratogenic effects (WHO, 2018). In Kenya, all suspected measles cases are routinely submitted to the measles laboratory for testing at the Kenya Medical Research Institute's (KEMRI)-Centre for Virus Research. Measles negative samples are, in turn, tested for rubella IgM antibodies in support of the national measles/rubella case-based surveillance program. Over the years, it has been observed that approximately 50-60% of these suspected measles cases usually test negative for both rubella and measles by IgM ELISA, and their etiology remains undetermined (EPI, 2015). Febrile rash illness is also a common clinical presentation of arboviruses, and therefore, these arboviral diseases may be present but remain undiagnosed (Ochieng *et al.,* 2015; Kajeguka *et al.,* 2016; Paixao *et al.,* 2018).

Arboviral infections are caused by arboviruses of public importance, which are classified into three main families; *Togaviridae, Flaviviridae,* and *Bunyaviridae*. These viruses cause a range of clinical syndromes in humans ranging from self-limiting febrile illnesses to hemorrhagic fevers (Ochieng *et al.,* 2015). Dengue (DENV) and Zika (ZIKV) belong to the *Flaviviridae* family. In contrast,

Chikungunya (CHIKV) belongs to the *Togaviridae* family. They are among the emerging and re-emerging arboviruses of public health importance regionally and worldwide.

Previous seroprevalence studies have reported exposure of arboviral infection due to DENV and CHIKV infections in Coastal Kenya (Sergon *et al.,* 2008; Sutherland *et al.,* 2011; Ngoi *et al.,* 2016). Kenya is also considered to be endemic for DENV and CHIKV arboviruses and competent vectors to these viruses are present, i.e., *Aedes*, mosquitoes, which have been found in some parts of the country (LaBeaud *et al.,* 2011).

Dengue fever caused by the dengue virus (DENV) is a re-emerging arboviral infection of public health importance (Singal, 2017; Subhadra *et al*., 2021). The infection can vary from a mild febrile illness to severe dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS) (Ross, 2010). Dengue virus is responsible for more than 50 million clinical cases, according to the World Health Organization (WHO, 2009). Dengue fever (DF) is caused by infection with one of the four dengue serotypes (DENV 1 to DENV 4) (WHO, 2020). The primary arthropod vector for dengue is *Aedes aegypti,* commonly found in Africa (Amarasinghe *et al.,* 2011). Dengue cases were reported in early March 2021 with 24 cases testing positive out of 47 (51% positivity rate). In April, another 305 cases tested positive out of 315 (97% positivity rate). Three serotypes of DENV have been detected in Kenya (DENV 1-3) (Konongoi *et al.,* 2016) in Northern and Coastal Kenya. Dengue-4 was detected in 4.5% (10/221) of children with undifferentiated febrile illness (Shah *et al.,* 2020).

Zika virus, which is transmitted mainly by *Aedes* mosquitoes, was first discovered in the Zika forest in Uganda (Gubler *et al.,* 2017). A recent outbreak of ZIKV in the Americas occurred in 2015 (de Oliveira *et al.,* 2017) and several clinical manifestations were described, including microcephaly in children and Guillain Barre

Syndrome in adults (Ladhani *et al.,* 2016). The current level of transmission of ZIKV is unknown in Kenya (Hunsperger *et al.,* 2020). However, the Yellow Fever risk assessment carried out in 2013 (VHF laboratory, 2013) showed the presence of ZIKV antibodies, suggesting it has recently been circulating. Zika virus antibodies were recently detected in Mombasa, Kenya which identified 5 persons sampled in 2013 who were positive for ZIKV IgM and confirmed by $PRNT_{90}$ (Hunsperger *et al.,* 2020). While a study by (Kisuya *et al.,* 2019) about 0.2 % (1/577) as confirmed by PRNT.

Two vectors transmit the chikungunya virus: *Aedes aegypti* and *Aedes albopictus* mosquitoes (WHO, 2020) which are abundant in Kenya. The first outbreak in Kenya occurred in 2004-2005 in Lamu along the Kenyan Coast (Sergon *et al.,* 2008) and later spread to Mombasa (Sang *et al.,* 2008). The most recent outbreak started in 2016 in Mandera County in North-Eastern Kenya (Konongoi *et al.,* 2018), and infection detections continue to date. Chikungunya fever is known to be endemic in the Coast Region and was associated with 12.7% in children (Nyamwaya *et al.,* 2021*)*. In addition, concurrent epidemics of DENV and CHIKV have been reported from Africa (Leroy *et al.,* 2009).

According to (WHO, 2018), when examining measles it is important to include differential diagnosis of other infections such as rubella, scarlet fever, exanthema subitum (roseola), erythema infectious, dengue fever, the early stages of chickenpox, and infectious mononucleosis this is because of fever, rash, and a variety of non-specific symptoms.

Arboviral infections are of major public health concern and cause economic harm because of their high morbidity and mortality rates recorded after outbreaks (Kuniholm *et al.,* 2006). Lack of diagnostics in most health care facilities makes it difficult to estimate the true incidence and burden of arboviruses. Most likely, these arboviral diseases are misdiagnosed as malaria, typhoid, measles, leptospirosis,

dysentery, meningitis and rickettsia (Ochieng *et al.,* 2015). Even though there are reported cases and outbreaks of arboviral infections in parts of Kenya, no study has been conducted to determine their contribution to measles-like illness cases that test negative for measles and rubella virus antibody markers. This study assessed the prevalence and distribution of arboviruses among individuals sampled as suspected measles cases in the measles case-based surveillance in Kenya but tested negative for measles and rubella IgM antibodies. Evidence of other causes of febrile rash illnesses will alert clinicians to consider arboviral infections in their differential diagnostics that may have implications in the management of patients reporting febrile rash illnesses in Kenya. The findings of arboviral infections as causes of febrile rash illnesses will enable policymakers and the national disease control programs to develop algorithms for their management and have a surveillance system for their control.

1.2. Statement of the Problem

About 50-60% of suspected cases test negative for measles and rubella, which poses a public health concern (EPI, 2015). The shared common clinical presentation of arboviruses during the acute phase of the disease poses a challenge during disease diagnosis. Arboviral infections are asymptomatic and mild clinical forms of Zika, chikungunya and dengue may account for a large proportion of all infections. The initial symptoms can be similar, and clinically non-specific, including fever, headache, myalgia, arthralgia, maculopapular rash, retro-orbital pain and lymphadenopathies (Ioos *et al.,* 2014) which may overlap with measles and rubella symptoms.

Dengue has a case-fatality rate of about 5%, and mainly among children and young adults (Beatty *et al.,* 2007). Chikungunya infection is considered to be a relatively benign condition, with many atypical clinical manifestations resulting in significant morbidity and mortality, including Guillain–Barré syndrome (GBS) (Thiberville *et*

al., 2013). Zika virus was associated for the first time with severe neurological complications such as Guillain-Barre syndrome (GBS) in adults, and congenital Zika syndrome (CZS), a spectrum of fetal abnormalities and developmental disorders including microcephaly, when mothers were infected during early pregnancy (Oehler *et al.,* 2014; Cauchemez *et al.*, 2016).

1.3. Justification of the study

Febrile illness with rash is a common presentation of arboviral infections and thus prompted the need to test for arboviruses, which mostly lack effective vaccines (except for YF) and therapeutics and hence require prioritization. In addition, there is no information on other causes of fever and rash in Kenya. The study was carried out in the Northern and Coastal Region because these arboviral infections have been previously detected while Nairobi was used as a comparative site that has never reported any arbovirus outbreaks. This study assessed arboviral infections in non-measles febrile cases because no study has been conducted in Kenya. Therefore, to address this challenge, this study set out to determine the exposure of arboviral infections among suspected measles cases from negative samples collected in the measles case-based surveillance program in Kenya. This is to assess the possible contribution of arbovirus infections to the measles-like illness.

1.4. Research Questions

1. What is the prevalence and co-exposure of DENV, CHIKV and ZIKV arboviruses among non-measles febrile rash cases in Coast, North-Eastern and Nairobi Regions?
2. What are the distribution patterns of infections DENV, CHIKV and ZIKV among non-measles febrile rash cases in Coast, North-Eastern and Nairobi Regions?

3. What is the association of patient demographic characteristics and the PRNT findings among suspected measles cases in Coast, North-Eastern and Nairobi Regions?

1.5. Objectives

1.5.1 General Objective

To determine the prevalence and distribution of DENV, CHIKV and ZIKV arboviruses among non-measles cases in Coast, North-Eastern and Nairobi Regions.

1.5.2 Specific Objectives

1. To determine the prevalence and co-exposure of DENV, CHIKV and ZIKV arboviruses among non-measles cases in Coast, North-Eastern and Nairobi Regions.
2. To determine the distribution patterns of DENV, CHIKV and ZIKV arboviruses among non- measles cases in Coast, North-Eastern and Nairobi Regions.
3. To determine the association of patient demographic characteristics and PRNT findings among suspected measles cases in Coast, North-Eastern and Nairobi Regions.

CHAPTER TWO

LITERATURE REVIEW

2.1. Case-Based Surveillance of Measles in Kenya

Within Kenya, when clinicians suspect measles, they notify the SCDSC (sub-county disease surveillance coordinator), who fills the case-centered form in duplicate and gathers blood sample for lab analysis. The filled form and the specimen are taken to the measles lab of KEMRI, the WHO-official national lab, and case particulars warehoused in the central databank. Suspicion of a measles pandemic arise when there are reports of at least 5 suspected measles cases from a hospital within one month. The outbreak is proven once samples from at least 3 suspected measles cases turn positive from lab analysis. According to WHO (2018), suspected measles case is the incidence of a maculopapular rash and fever with at least one of the signs identified by WHO.

Verified cases of measles are categorized as lab-proven, clinically compatible, or epidemiologically associated. A case is lab-confirmed once ELISA (enzyme-linked immunosorbent assay) detect measles-specific IgM antibody in serum, without measles immunization in one month of prior to collection of specimen. On the other hand, a suspected case is epidemiologically associated with a proven case once the patients have contacted or reside in the same area as an individual with lab-proven measles infection. Moreover, according to Kisangau *et al.* (2018), a suspect measles case is clinically compatible once it meets the medical case description, nonetheless a lab analysis or an epidemiological association is absent.

According to WHO (2018), surveillance indicators are computed as per the WHO standard rules counting: 1) national-level non-measles febrile rash illness (NMFRI)

rate (target of greater or equal to 2 NMFRI cases for every 100,000 population); 2) fraction of sub-counties testifying at least one rash infection case each year (target ≥eighty percent sub-county units); 3) aptness of sample arrival to the lab (described as eighty percent arriving within three days of gathering); and 4) aptness in the results' feedback (described as eighty percent of results returned in one week of the arrival of the sample to the lab.

2.2 Pathogenesis of the Measles virus

Measles is a systemic infection. The primary site of infection is alveolar macrophages or dendritic cells. Two to three days after replication in the lung, the measles virus spreads to regional lymphoid tissues followed by a systemic infection. Following further viral replication in regional and distal reticuloendothelial sites, a second viremia occurs 5 to 7 days after initial infection. During this phase, infected lymphocytes and dendritic cells migrate into the sub epithelial cell layer and transmit measles to epithelial cells. Following amplification in the epithelia, the virus is released into the respiratory tract (CDC, 2021).

2.2.1 Rashes

A majority of communicable illnesses cause the presence of rashes in events of febrile sicknesses. Getting the required information about communicable exanthematous illnesses is indispensable due to varying gravity. For instance, some communicable illnesses are severe, while others are mild and therefore do not necessitate treatment since they are self-managing. Although rashes are an essential part of the diagnosis of certain transmissible infections, rashes are non-specific results that can't be utilized to determine the diagnosis of an illness. Mostly, rashes are an ancillary diagnosis of other infections, counting non-communicable illnesses. Therefore, the identification of a febrile illness that is characterized by a rash requires a comprehensive evaluation of a wide range of conditions. A skin rash is a typical

symptom that may appear during the occurrence of a localized or systemic infection. Hence, a skin rash is a clinically meaningful characteristic diagnosis for some illnesses (Kang *et al.,* 2015).

The classification of febrile rashes depends on several factors that include their distribution, accompanying symptoms and morphology. Some of the various types of rashes include purpuric petechial, pustular, erythema, nodular maculopapular, and generalized diffuse and vesicular rashes. Other classification systems depend on symmetry and distribution, where rashes are classified as either localized or systemic (Fölster-Holst *et al.,* 2009). In most instances, these rashes are neither related to the disease nor the specific diagnosis. Other essential classification systems depend on the causation or the occurrence pattern with non-infectious or infectious rashes and acute or chronic rashes (Kang, 2015).

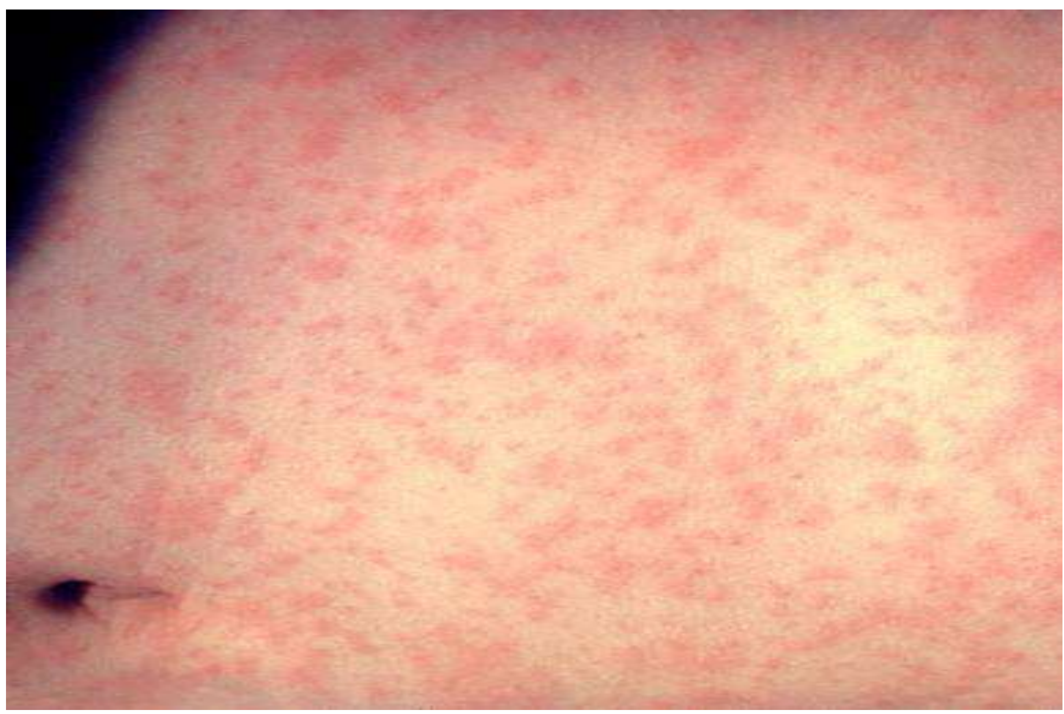
When compared to other virus-related exanthemata, measles virus infection provide a distinctive rash appearance in the skin. For instance, Rubella rash has been defined as macroscopically resembling measles rash, because it causes a pink-reddish "rubelliform" maculopapular rash. Nonetheless, in rubella, virus-related infection occurs deeper inside the dermis, as opposed to a measles skin infection which takes place more superficially within the epidermis and the dermis. According to (Takahashi *et al.,* 1996), keratinocytes infection does not happen in rubella virus infection.

2.2.2 Maculopapular rash

A maculopapular rash is the presence of a raised bump on top of a flat and reddened spot on the skin, as shown in (Figure 2.1). Skin rash is a common characteristic of some illnesses such as measles, rubella and scarlet fever. These are the typical characteristics of maculopapular rashes:

1. Macules: that are flat in appearance

2. Papules: that are small and round bumps
3. Flat and red spots: usually less than half an inch in diameter
4. It can appear anywhere on the body
5. Other symptoms include headaches or fever (Husada *et al.,* 2020).



Source: (Prevention, 2020)

Figure 2.1: Maculopapular rashes (CDC/Public Health Image Library)

Measles rashes: Measles is an infectious disease that is characterized by a skin rash. The skin rash begins from behind the ears and progresses to the face, followed by the neck, torso, and extremities over 2-3 days. When the rash stops evolving, the fever disappears. Then lastly disappearance of the rash may be accompanied by dry desquamation. Also, Koplik spots (enanthem) appear in the mouth either 12 h before or within 24 h of rash appearance (Kang *et al*., 2015).

Rubella rashes: The rash in rubella, like measles which starts from the face to the body. It takes a few hours and occurs faster than measles, and the appearance of the rash is a much lighter color. However, it is not easy to differentiate the rash from measles within 24 h of onset (Kang *et al.,* 2015).

Arboviral rashes: A maculopapular, scarlatiniform or morbilliform rash is observed in the febrile phase of the dengue infection (Azfar *et al.*, 2013).

The most commonly testified manifestation has been the generalized morbilliform maculopapular rash with typically sparing the face and typical islands of intervening skin. (Singal, 2017) argues that the rash develops in three to four days of the inception of fever and is likely to lessen within seven days without some sequel.

The rash linked to zika virus is either papules (morbilliform) or micro papules (scarlatiniform). The rash appears on the face and extends to the limbs and trunk. It might affect soles and palms, which might become swollen. The rash starts to fade in two to three days and goes way completely in seven days. Simultaneously, the eyes turn red and sore and might be sensitive to light (Cordel *et al.,* 2017). Also, small petechiae, red dots, might be seen in the mouth on the palate.

2.3 Arbovirus Infections

Arboviruses are also known as Arthropod-borne viruses. They consist of more than 500 viruses that are found in three families; *Bunyaviridae*, *Togaviridae* (genus *Alphavirus*) and *Flaviviridae*. Sandflies, hematophagous mosquitoes unite these families, and ticks, which are their vectors (Artsob *et al.,* 2017). More than 100 viruses can infect humans, who act as either primary hosts or dead-end hosts. Livestock act as the amplifying or maintenance hosts, which acquire the virus through zoonotic transmission from the dead-end hosts (Gubler, 2001). There is a significant variation in viral epidemiology, ranging from sporadic, low-level transmission to major outbreaks involving millions of cases. Viremic hosts are the

source of blood meal for vectors, after which extrinsic viral incubation occurs, consequently leading to viral amplification within the vector.

The infected vector may transmit the viruses to an uninfected vertebrate host during subsequent blood-feeding, hence sustaining transmission patterns. The development of disease in vertebrate hosts depends on a wide range of factors that include both the host and viral factors. Several permissive conditions are related to active viral transmission, which includes viremic maintenance hosts, as well as climatic conditions that are suitable for the ecological and vector interactions between vectors and the hosts (Gan *et al.,* 2014). According to (Coffey *et al.,* 2013), the viral life cycle is a multistage process that constrains viral evolution and limits viral fitness. Blood supply danger occurs in two instances: during the intrinsic incubation period, a pre-symptomatic, and during any subsequent clinical illness (before viral elimination from the bloodstream). Arboviral diseases have three main acute febrile syndromes across all the infections; acute benign fever (often occurs with arthralgia) in infections with chikungunya, the neuroinvasive disease of the central nervous system (CNS) in West Nile infections and the hemorrhagic fever in infections with dengue. These diseases often occur in tropical areas and subtropical and temperate regions during summer periods because reproduction in arthropod vectors relies on high ambient temperatures. The clinical manifestations of these diseases often overlap with other common acute febrile illnesses, especially during the early phases. Cases may be viremic and asymptomatic (Ludlow *et al.,* 2016).

2.4 Clinical Manifestations of Arbovirus Infections

Human arboviral infections are usually asymptomatic or present with mild flu-like symptoms. These arboviruses are of clinical importance in humans and veterinary settings due to their association with diseases such as; arthritis, encephalitis, hemorrhagic syndrome, febrile illness, rash, shock, and death (Muyeku, 2011).

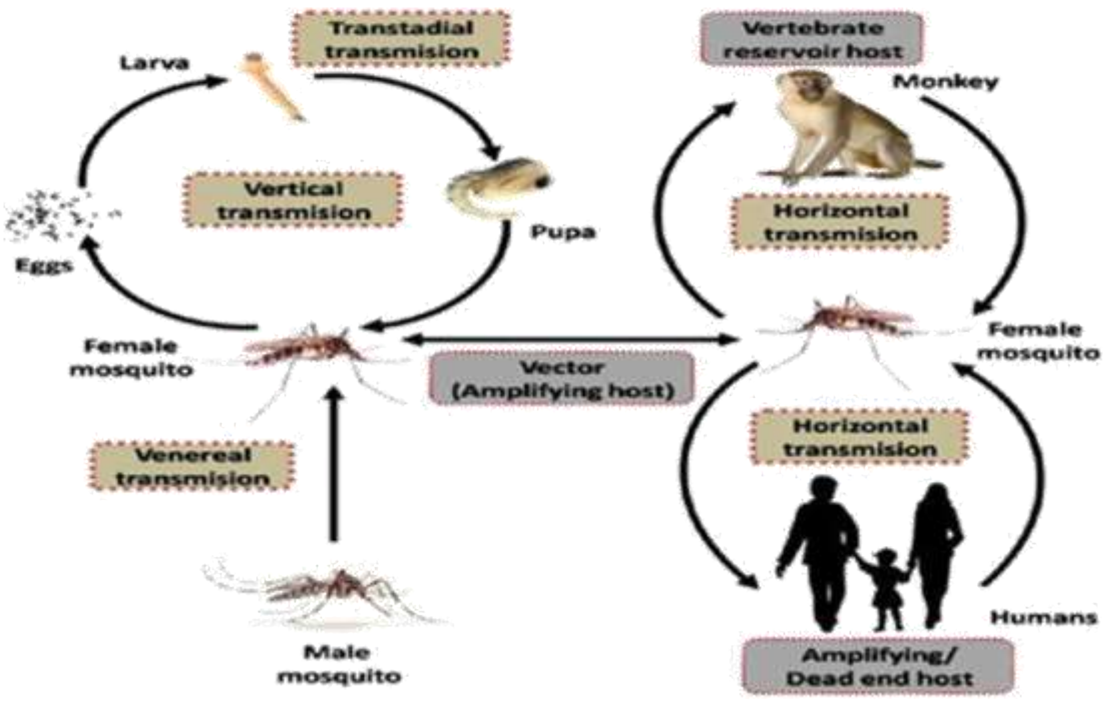
Arboviral infections may be categorized into four units: Acute short benign fevers without or with an exanthema; acute central nervous system infection including mild aseptic meningitis and encephalitis with paralysis, coma, and death; hemorrhagic fever which may be external, internal or extensive, and linked to capillary leakage, jaundice, shock, death, liver and damage; and rash and polyarthritis. According to Alatoom *et al.* (2009), most human illnesses are asymptomatic or might cause nonspecific flulike syndrome and just a tiny fraction of infected individuals advance to frank encephalitis.

2.5 Transmission of Arbovirus Diseases

Transmission of arboviruses occurs between arthropod vectors and vertebrate hosts. In mosquitoes (Figure 2.2), the arboviral infection begins when it feeds on a viremic host. Once the virus enters the mosquito's midgut, replication occurs in midgut epithelial cells, after which it moves to the hemocoel. Later, the virus is disseminated to various secondary organs, including the salivary glands. Subsequently, mosquito bites on different hosts initiate a new transmission cycle. The midgut and salivary glands (anatomical barriers) prevent virus infection and escape. The virus and vector complex interactions determine vector competence, which reflects the ability of the virus to succeed in overcoming different barriers (Kuno *et al.,* 2005). Sylvatic transmission cycles between the invertebrate vector(s) and an enzootic vertebrate reservoir lead to the spread of the virus. The viral level (viremia) in the amplifying vertebrate hosts (non-human primates, birds, and rodents) must be high enough to facilitate infection of the arthropod during the blood meal (Berthet *et al.,* 2014). In some instances, dead-end host transmission may occur during the enzootic cycle. In these hosts, however, viremia is low, which reduces the chances of viral transmission to an arthropod; hence they are not essential in the maintenance of the arbovirus lifecycle. Two cycles maintain arboviruses in nature; these are the invertebrate reservoir cycle and the vertebrate amplification cycle (Pfeffer *et al.,* 2010). Various hematophagous invertebrates act as vectors, but mosquitoes are used as representative

vectors (Karungu *et al.,* 2019). Vertical transmission is common in arboviruses, where an infected female mosquito transmits the virus through transovarial eggs infection (Young *et al.,* 2008). Venereal transmission occurs when infected male mosquitoes transmit the virus to naïve female mosquitoes. Amplification occurs in the vertebrate host after the mosquito transmits the viruses during a blood meal.

The amplifying hosts have high viremias, and this may lead to transmission to other hematophagous mosquitoes, which may be in the same or different species (Vasilakis *et al.,* 2008). Although domestic animals are considered dead-end hosts, epizootic amplification cycles may occur in these animals. Also, in urban settings, some arboviruses such as DENV rely on human amplification. Other factors that significantly affect the lifecycle of arbovirus are the immune status of host populations, geography, and climate. Studies describe two climate-dependent transmission patterns with temperate and cold climates. In temperate climates, viral transmission occurs during the warmer months, which leads to disease absence during the colder months. In cooler climates, virus persistence is usually due to overwintering in the mosquito's eggs (Gubler, 2002) (Figure 2.2).



Source (Gubler, 2002)

Figure 2.2: Transmission of arbovirus infection by mosquitoes to humans

2.6 Epidemiology of Arbovirus Infections

The worldwide distribution of arboviruses represents approximately 30% of all infectious diseases (Jones *et al.,* 2008). The occurrence of arboviral diseases is influenced by a wide range of ecological, socioeconomic and environmental factors, despite the difference in variability in the epidemiology of each virus (Morens *et al.,* 2004). Arboviral disease emergence and re-emergence are significantly affected by human behaviour. For instance, the highly efficient, anthropophilic mosquitoes can easily spread arboviruses during modern travel and trade (Fonseca *et al.,* 2004; Benedict *et al.,* 2007). Other factors that have increased the instances of human

contact with the viruses and their vectors are lumbering and forest-based commercial activities, agricultural development, environmental factors, and population growth. Moreover, poor urbanization and crowded, substandard living conditions associated with the unavailability of enough water and poor waste management ensure that mosquitoes have ideal breeding sites. Climate change also significantly influences the emergence and re-emergence of arboviral diseases (Elliott, 2009). For instance, lengthened warm weather conditions facilitate the existence of vector populations. Additionally, such weathers provide opportunities for vector range, vector composition, vertebrate host, and dynamics changes (Weaver *et al.,* 2010). Increased precipitation cycles, such as those experienced during El Niño/Southern Oscillation (ENSO) phenomenon warm phases, lead to increased outbreaks of arbovirus and the increased abundance of mosquito vectors and their host-seeking activities (Linthicum *et al.,* 1999).

Kenya is known to be endemic for DENV and CHIKV, which continue to be detected in low levels to date. The risk of YF infections spilling into the country is very high and YF antibodies have been detected recently (Chepkorir *et al.,* 2019; Inziani *et al.,* 2020). The other viruses such as ONNV, SINV, SFV, ZIKV and WNV have been detected in previous studies. These viruses are of public health importance in the country. Forty percent (345/868) of the samples tested positive for dengue by either IgM ELISA (Konongoi *et al.,* 2016). 52.9% (100/189) tested positive for CHIKV by either IgM ELISA (Konongoi *et al.,* 2018).

2.6.1 Distribution of Arboviruses

Dengue virus has been detected in Africa, Tropical Asia-Pacific, North-East Australia, South and Central America. ZIKV has been detected in Africa, Asia Pacific, South, Central America and North America. Chikungunya virus has been detected in Africa, the Middle East; Europe; Asia; the Americas; Oceania as shown in Table 2-1 (Braack *et al.,* 2018).

Table 2.1: Distribution of arboviruses, mosquito-host species and amplifying host.

Virus	Acronym	Distribution	Mosquito-host species	Amplifying host
Dengue	DENV1–4	Africa, Tropical/ sub-tropical Asia-Pacific, North-East Australia; South and Central America	*Ae. aegypti*	Humans
Zika	ZIKV	East, West and Southern Africa, Asia Pacific, South and Central America, North America	*Ae. aegypti* *Ae. africanus* *Ae. albopictus*	Several species of African non-human primates
Chikungunya	CHIKV	Africa: Benin, Burundi, Cameroon, Central African Republic, Comoros, Democratic Republic of Congo, Equatorial Guinea, Gabon, Guinea, Kenya, Madagascar, Malawi, Mauritius, Mayotte, Mozambique, Nigeria, Republic of Congo, Reunion, Senegal, Seychelles, Sierra Leone, South Africa, Sudan, Tanzania, Uganda, Zimbabwe; Middle East; Europe; Asia; the Americas; Oceania	*Ae. aegypti,* *Ae. africanus,* *Ae. albopictus,* *Ae. cordellieri,* *Ae. furcifer,* *Ae. fulgens,* *Ae.luteocephalus,* *Ae. neoafricanus,* *Ae. taylori,* *Ae. vittatus*	Non-human primates, humans

2.7 Dengue Virus

Dengue virus denotes a Flavivirus that has a major worldwide burden, since it is present in more than one hundred nations globally and causes about three hundred and ninety million infections annually worldwide. According to WHO (2020), the virus is spread by *Aedes (Stegomyia)* mosquitoes, while non-human apes are the reservoirs. It is a single-stranded RNA virus that is a positive sense. Its genome codes 7 nonstructural and 3 structural proteins. The envelope glycoprotein triggers interactions with cell surface receptors, thus facilitating the virus entry into the cells. It takes roughly four to seven days for the virus to duplicate in the dendritic cells near the bite location and ultimately join the blood after infecting the lymphocytes and macrophages. There exist 4 serotypes of the dengue virus that are antigenically dissimilar and may cause illnesses like DSS, DHF and DF. According to WHO (2020), symptoms of dengue include fever that comes along with two of the following signs throughout the febrile stage: rash, aching around the eyes, swollen glands serious headache, joint and muscle pains, vomiting, and nausea. Also, there is no effective vaccine and treatment for the 4 dengue serotypes, hence it is not possible to manage the transmission of the illness, particularly in pandemic zones (Nedjadi *et al.,* 2015). According to WHO (2017), there are about 5 vaccine candidates under assessment in scientific trials, counting DNA, other live-attenuated subunit and vaccines, along with purified inactivated vaccine candidates. Moreover, they are technological methods like VLP-based and virus-vectored vaccines that are under investigation in pre-clinical assessments.

2.8 Zika Virus

Sporadic cases of the Zika virus among humans have been identified in Sub-Saharan Africa and some parts of Asia (Kindhauser *et al.,* 2016; Petersen *et al.,* 2016). However, in 2007, it was when the clinical importance of the virus was recognized (Duffy *et al.,* 2009). The first viral outbreak among humans occurred in Micronesia at

the Yap Island happened in 2007, but no reports on deaths or hospitalization were made. Various clinical symptoms recorded were arthralgia, headache, malaise, rash, and mild fever (Paixão *et al.,* 2016). The first outbreak of viral infection in American countries was reported in 2015, as the infection rapidly spread through the Caribbean and Central and South America (WHO, 2018). Zika virus is associated with congenital disabilities and congenital diseases, including Guillain-Barre syndrome and microcephaly. Mosquitoes of the *Aedes* genus are the primary Zika virus vectors that are present in various parts of the world (WHO, 2018).

2.9 Chikungunya Virus

Chikungunya virus (CHIKV) causes chikungunya fever that is an acute febrile illness. The virus is an arthropod-borne alphavirus transmitted by mosquitoes of the *Aedes* species (WHO, 2020). Mosquito bites transmit the virus to humans. Its importance as a human pathogen was recognized in the 1950s in Africa, and over the years, numerous cases have been identified in both Asian and African countries (WHO, 2020). Symptoms of CHIKV infection include high fever, rigors, headache, photophobia and a petechial or maculopapular rash (Schwartz *et al., 2*010). The re-emergence of CHIKV occurred in Kenya in 2004, where it spread eastward, leading to millions of cases in the countries within the Indian Ocean (WHO, 2007; Powers *et al.,* 2007; Sergon *et al.,* 2008). The epidemic had a significant impact on health care and public health infrastructure and also resulted in morbidity. An outbreak of CHIKV occurred in Italy in 2007 (Rezza *et al.,* 2007), an epidemic that proved the ability of the virus to move to novel ecological niches, including the Western Hemisphere Europe and Australia. The virus likely originated in Central/East Africa (WHO, 2020). It circulates via the sylvatic cycle between the forest-dwelling mosquitoes of the *Aedes* species and non-human primates in these regions. Although there are sporadic human cases in these areas, large human outbreaks are rare. The cycle is, however, similar to that of dengue viruses in urban areas. Two vectors that transmit the virus in urban areas are *A. aegypti* and *A. albopictus* mosquitoes (Powers

et al., 2007). Several minor periodic outbreaks were recorded through the years, the 2004 outbreak that occurred on the Kenyan coast was the first most massive epidemic (Powers *et al*., 2007; Sergon *et al.,* 2008). The virus spread to other countries along the Indian Ocean, including India and parts of South-east Asia, within four years (Sergon *et al.,* 2007; Sergon *et al.,* 2008). Around 18 countries from various continents including, Europe, Asia, and North America, recorded imported cases of chikungunya fever. Some of the countries developed a local viral autochthonous transmission (Powers *et al.,* 2007; Chretien *et al.,* 2007).

2.10 Seroprevalence of Arboviruses in Africa

In Africa, the first officially recognized dengue outbreaks were reported in Zanzibar in 1823 and 1870. In the early 20th century, outbreaks of dengue fever were reported from South Africa, Egypt, Senegal, and Burkina Faso. By 2020, sporadic cases or outbreaks had been reported in 46 countries in Africa. These countries include Burundi, Comoros, Djibouti, Eritrea, Ethiopia, Kenya, Madagascar, Malawi, Mauritius, Réunion, Rwanda, Seychelles, Somalia, Tanzania, Uganda, Sudan, and South Sudan (East Africa); Benin, Burkina Faso, Cape Verde, Côte D'Ivoire, Gambia, Ghana, Guinea, Guinea-Bissau, Liberia, Mali, Mauritania, Niger, Nigeria, Senegal, Sierra Leone, and Togo (West Africa); Cameroon, Central African Republic, Chad, Congo, the Democratic Republic of the Congo, Equatorial Guinea, and Gabon (Central Africa); Angola, Mozambique, Namibia, Zambia, and Zimbabwe (Southern Africa); and Egypt (North Africa) (Simo *et al.,* 2019). All four DENV serotypes have been reported in Africa. In a recent meta-analysis, the overall seroprevalence of DENV in healthy individuals was 15.6% (95% confidence interval 9.9–22.2%) (Ayukekbong *et al.,* 2017). The seroprevalence of dengue in different regions on the African continent was mostly below 40%, but seroprevalence studies from Mali, Nigeria, Sierra Leone, and Sudan reported regional IgG frequencies of more than 70% (Adam *et al.,* 2021). Evidence indicates that DENV, YFV, ZIKV and CHIKV are endemic in Africa (Amarasinghe, Kuritsk, Letson, & Margolis, 2011).

2.11 Seroprevalence of arboviruses in Kenya

Seroprevalence studies carried out recently in the country have found DENV, CHIKV and ZIKV to be circulating (Njenga *et al.,* 2008, Mease *et al*., 2011; LaBeaud *et al.,* 2015; Ofula *et al.,* 2016; Ochieng *et al.,* 2015; Sergon *et al.,* 2007; Sutherland *et al.,* 2011; Tigoi *et al.,* 2015; Vu *et al.,* 2017; Waitumbi *et al*., 2018; Chepkorir *et al.,* 2019; Kimata *et al.,* 2020; Hunsperger *et al.,* 2020; Inziani *et al.,* 2020). Detection of these viruses is hindered by poor diagnosis due to non-specific clinical presentation and limited surveillance that is being conducted in Africa (Sang *et al.,* 2001). Epidemics of DENV and CHIKV have occurred recently in Kenya (Konongoi *et al.,* 2016; Konongoi *et al.,* 2018; Lutomiah *et al.,* 2016) and continue to circulate in low levels to date.

2.12 Co-Infection of Arboviruses in humans

Several studies have established a relatively high number of patients co-infected with CHIKV/DENV (2-26.3%), with such outbreaks being found in Gabon (Caron *et al.,* 2012), Angola, Gabon, India, Madagascar, Malaysia, Myanmar, Nigeria, Saint Martin, Singapore, Sri Lanka, Tanzania, Thailand and Yemen (Furuya-Kanamori *et al.,* 2016). Moreover, a clinical study conducted in Nicaragua in 2016 identified that about 27% of the patients infected with arbovirus were viremic for multiple agents, including a combination of agents such as ZIKV, DENV and CHIKV (Waggoner *et al.,* 2016) and Colombia (Mercado-Reyes *et al.,* 2019). Cases of co-infection have been reported in several regions throughout the world; hence the phenomenon of arboviral co-circulation and co-infection is becoming common. Regions with reports of these cases include the Pacific (New Caledonia), South America (Colombia and Brazil), the Caribbean (Haiti) and Africa (Nigeria and Angola) (Karpf *et al.,* 1997; Musso *et al.,* 2017; Vogels *et al.,* 2019). These findings, therefore, suggest that in pandemic and endemic regions, co-infections are common. In Kenya, arbovirus

infections are known to co-circulate in the same region, particularly in the Coastal and North-Eastern Region, hence humans have multiple exposures.

2.13 Co-infection of arboviruses in mosquito vectors

A study by Rohani *et al.* (2009) established that *Ae. aegypti* may be stubborn to CHIKV/DENV co-infection. Studies by Vazeille *et al.* (2010) and Nuckols *et al. (2015)* found out the co-infection ability of *Aedes* mosquitoes to be CHIKV/DENV as its ability to expectorate both viruses in their saliva. Out of all the studies conducted concerning DENV/CHIKV co-infection, five studies were performed on entomological surveys to assess the vector(s) associated with co-infection (Lanciotti *et al.*, 2014; Kakumani *et al.*, 2013; Musso *et al.*, 2017; Chan *et al.,* 2012; Weger-Lucarelli *et al.,* 2016). In the South-East Asian region, specifically India in 1964, and Myanmar in 1970-1972, *Ae.aegypti* was the identified primary vector (Kakumani *et al.,* 2013; Weger-Lucarelli *et al*., 2016) causing co-infections.

For the co-infections in the African region, specifically Gabon (2007-2010) and Madgascar (2006) co-infections, *Ae. albopictus* was the primary vector (Chan *et al.*, 2012; Lanciotti *et al.,* 2014; Musso *et al.,* 2017). Mosquitoes which bite patients who are viremic and co-infected have a high risk of acquiring multiple agents. *Ae. aegypti* can, therefore, can be co-infected and therefore co-transmit arboviruses, an aspect that has important implications for viral evolution and epidemiology. There is, however, limited knowledge and an understanding of the issue of the ability of *Aedes* mosquitoes to be co-infected and co-transmit arboviruses.

2.14 Risk Factors and Susceptibility to arboviruses

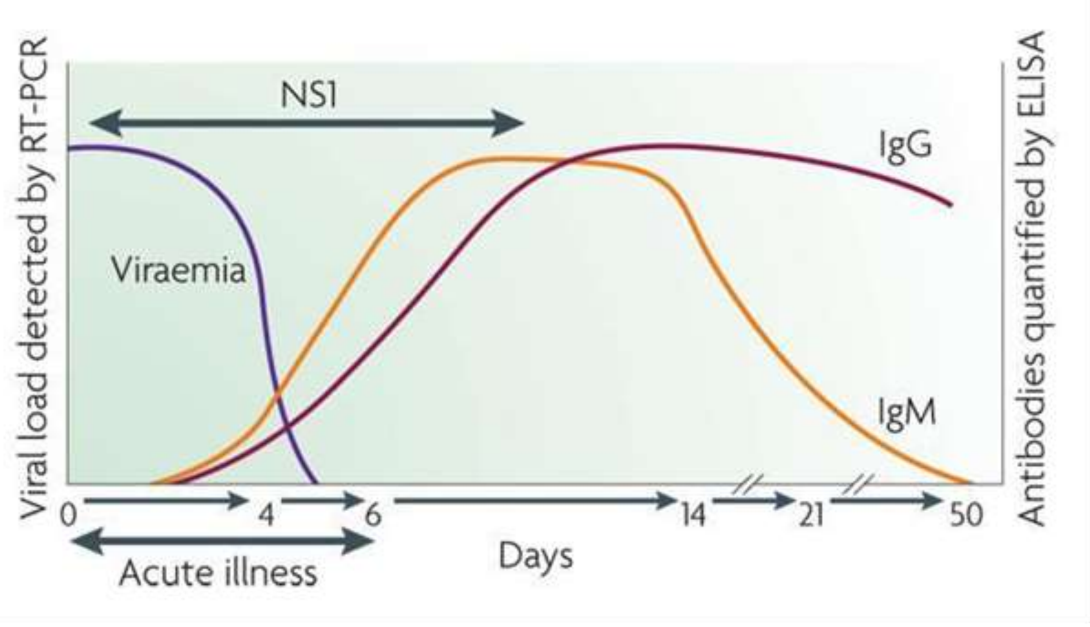
The virus, vector, wild vertebrate host, environmental and humans factors affect risk factors for arboviruses infection. Exposing oneself to mosquito vectors with an arbovirus is one of the risk factors for arboviruses infection. Huang *et al*. (2019) argue that an augmented amount of infected vectors and the accessibility of non-

immune vulnerable human hosts upsurge the possibility of infection. Human undertakings which connect them to forest mosquitoes, for instance, clearing woodlands for farming activities, gathering and hunting, as well as visiting forests surge the possibility of exposure. Also, altering lifestyles renders individuals vulnerable to arthropod biting. For example, urbanization, along with bad drainage and bad waste disposal with open discarded containers and sewers that gather rainwater, building of irrigation dams and schemes, and water storage practices enhance mosquito vector diversity and provide other breeding habitats and renders individuals vulnerable to biting from domesticated *Aedes* mosquitoes which breed within these places. What is more, visiting pandemic zones is a risk factor for vacationers, while augmented air travel results in the conveyance of vulnerable individuals into pandemic zones and viruses and vectors into fresh habitats. Mosquitoes may bite youngsters when herding cattle, playing near forested regions, sleeping without a mosquito net, or fetching firewood. According to Kolawole *et al.* (2018), both sexes and all age groups are at risk of CHIKV infection. The senior along with the immuno-compromised, as a result of organ transplant, HIV/AIDS, individuals on chemotherapy, and diabetics are more vulnerable to clinical illness from infection by arbovirus. Imperceptible infections are prevalent in CHIKV infection, particularly in kids, amongst whom the explicit illness is uncommon. Arthritis happens most commonly amongst adult females in epidemic polyarthritis triggered by the two viruses. Nonetheless, mild infections and asymptomatic illness are prevalent in all the arbovirus infections, and long-term homologous immunity follows recuperation. Thus, as stated by Pierson *et al.* (2020), the most vulnerable age-group in pandemic zone is mostly youngsters who lack immunity to arboviruses.

2.15 Antibody Response to Arbovirus Infection

After arbovirus infection, IgM is seen in the bloodstream in 3-5 days and can persist for 6 months to one year. Lima *et al.* (2012) state that IgM is an indicator of recent

infection, while IgG arises in two weeks and can last for a lifetime, and it indicates past infection with arbovirus.



(Guzman et al., 2010)

Figure 2.3: Antibody profile when infection by Dengue Virus

2.16 Laboratory Diagnosis of Arbovirus Infections

In Kenya, diagnostic analysis for arbovirus illnesses are costly and are not freely obtainable in the medical setting. Nevertheless, certain of the examinations are obtainable in a research context at KEMRI. The examinations are explained below:

2.16.1 Virus isolation

Viral isolation by cell culture is the most definitive test for the detection of viruses in patient samples. This is usually achieved by infection of cells grown in a controlled artificial environment with viral containing samples and observation of morphological changes in the cells. Cells commonly used to isolate arboviruses

include Vero cell lines obtained from the kidney of an African green monkey and C6/36 *Aedes albopictus* larvae cell lines. Virus isolation can also be achieved by inoculating serum samples collected within 24-48 hours of disease onset into susceptible animal models like suckling mice. This method is strictly carried out in a bio-safety level (BSL) 2 or 3 laboratories and the results usually take up to 2 weeks to be released (Artika *et al.,* 2017). Insect cell lines are very sensitive to the dengue virus: the most widely available in the C6-36 cell line derived from *A. albopictus,* CHIKV will produce cytopathic effects in various mammalian cell lines. In vitro cell, culture methods using mosquito cell lines (C6/36) and other mammalian cell lines (BHK-21, HeLa and Vero cells) that produce cytopathic effects have comparable sensitivity to in vivo methods (Fujita *et al.,* 2018).

2.16.2 Nucleic Acid Amplification Test

Tests such as reverse transcription-polymerase chain reaction (RT- PCR) and reverse transcription loop-mediated isothermal amplification assay (RT-LAMP) are available to detect arboviral nucleic acids in serum samples obtained from patients in acute phases (Kutsuna *et al.,* 2020; Stanley *et al.,* 2021). RT-PCR results are usually available within 1 or 2 days, while the RT-LAMP can yield results within a few hours (Silva *et al., 2*019).

Severe infection may be identified molecularly through detection of virus-related RNA through RT-PCR (reverse transcription-polymerase chain reaction) or through antigen examinations. A nucleic acid amplification test measures and amplifies the genetic material of arbovirus to identify the existence of the virus. This test may identify an existing infection with the virus, before antibodies to the virus can be detected, nonetheless, there ought to be a particular amount of virus existing in the specimen to identify it.

2.16.3 Serological diagnosis

Serological approaches, for instance ELISA (enzyme-linked immunosorbent assays), can confirm the existence of a past or recent infection by detecting IgG and IgM antibodies. According to Tarde*i et al.* (2000), the IgM antibodies can be detected 7 days after infection and are peak at two to four weeks after the inception of infection. These antibodies stay noticeable for around three months. IgG antibody amounts take a longer time to increase than IgM, nonetheless they remain in the body for several years. The existence of IgG indicates a previous infection, while IgM indicate a recent infection.

Commonly used tests are the IgM capture enzyme-linked immunosorbent assay (MAC-ELISA) which can be bought commercially or use in house kits eg CDC ZIKA MAC-ELISA which are developed in the laboratory and the indirect IgG Enzyme-Linked Immuno-sorbent Assay. Results of MAC-ELISA are ready in 2-3 days. Cross-reactivity between antibodies against other viruses in the same family of *Alphaviridae* and *Flaviviridae* is a common problem with ELISA tests. For instance, a cross-reaction between CHIKV, ONNV, SFV or SINV may occur when testing for an *Alphavirus*. A virus confirmatory test can be performed using PRNT, focus reduction neutralization tests (FRNT), while a specific virus type can be confirmed by PCR (Muyeku, 2011). Tests for measuring neutralizing antibodies are particularly useful in confirming both the diagnosis and for determining the serotype of an isolate (Guzman *et al.*, 2010).

Plaque reduction neutralization tests (PRNT) can measure virus-specific neutralizing antibodies and may be able to determine the cause of the primary infection with high specificity and clarify cross-reacting results; however, PRNT is expensive and very labour intensive (CDC, 2016).

2.17 Prevention of Arbovirus Infections

According to WHO (2016), the inhibition of flaviviruses and alphaviruses is focused on path control and guarding people from mosquito bites by destroying breeding sites, insecticide-treated nets, insecticide sprays, as well as insect repellants to prevent insects from entering the house. Failloux *et al.* (2017) note that regular surveillance of vertebrate hosts and susceptible vectors is employed to identify arbovirus activity promptly, so that precautionary measures could be introduced before outbreaks take place. Virus-like particle vaccine, subunit vaccine, inactivated vaccine, live-attenuated vaccine, chimeric vaccine, nucleic acid vaccine, and recombinant virus-vectored vaccine have been established; CHIKV commercial vaccine is expected to be developed soon (Edelman *et al.,* 2000; Wang *et al.,* 2011). Vaccines for the other arboviruses such as DENV are currently under clinical trials.

2.18 Treatment and Management of Arbovirus infections

According to Goh *et al.* (2020), there is no cure for arbovirus infections. However, their management mostly supports treatment of symptoms like paracetamol for fever and painkillers for arthralgia. Also, bed rest is highly recommended. Moreover, intravenous fluid replacement is crucial treatment for hemorrhagic fever. Nevertheless, in case of severe hemorrhagic fever, a blood transfusion might be necessary. Arboviral infections generally are self-limiting and there are no specific treatments available. Supportive care, including fluid resuscitation, and analgesia (if needed for joint, muscle, or bone pain) are the mainstays of management.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study Sites

The archived sera were collected from health facilities in Nairobi, Coast and North-Eastern Regions (Figure 3.1) in Kenya.

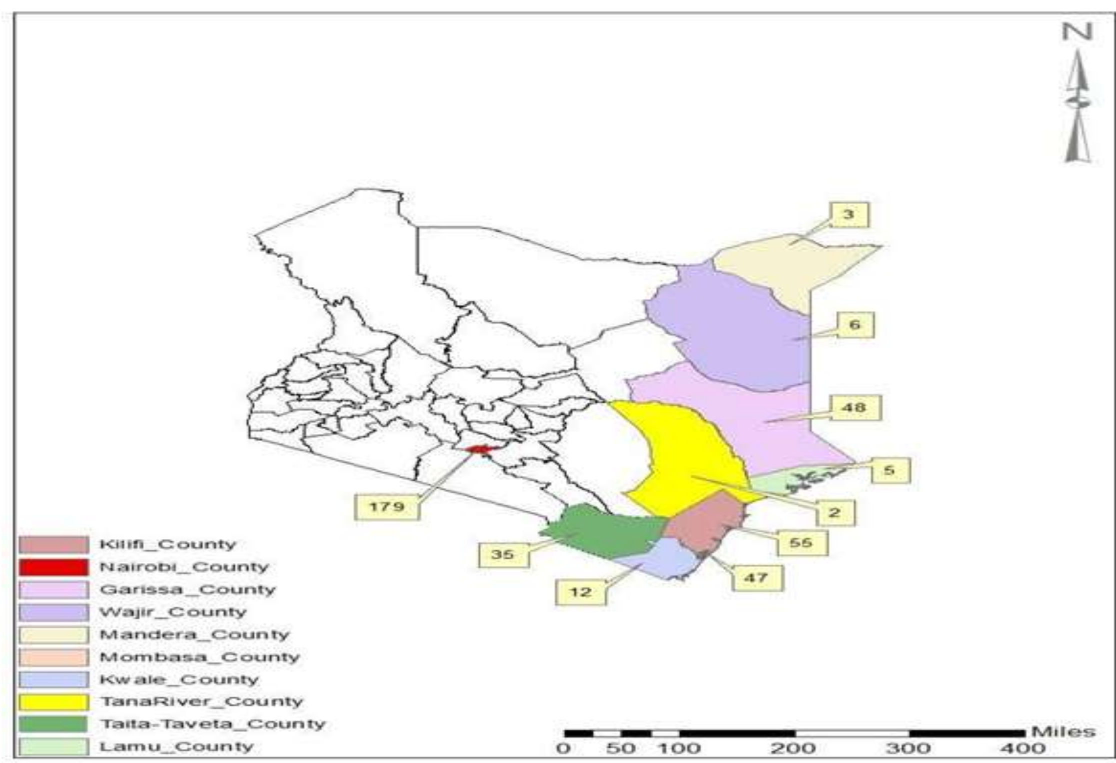


Figure 3.1: Map of Kenya showing the study sites and total numbers of samples used in that particular site: Nairobi Region, Coast Region (Mombasa, Kwale, Kilifi, Tana-River, Taita-Taveta and Lamu) and North-Eastern (Wajir, Mandera and Garissa).

3.2.1 Nairobi County

Nairobi County is the capital city of Kenya. It is situated in the south-central part of the country that lies (01° 17´ 36 ° 48´E). It is located at an altitude of 1661 m above sea level with an average monthly temperature range between 22° and 28° (Agha *et al.*, 2017). Nairobi County has not reported any arbovirus outbreak. Still, cases are detected randomly e.g dengue and chikungunya, in patients with travel history from endemic areas (Konongoi *et al.,* 2016) but were included in this study as a comparative site major travel hub with a high risk of virus introduction and transmission.

3.2.2 North-Eastern region

This region comprises 3 counties; Garissa, Wajir and Mandera. This region experiences low rainfall, has high temperatures, proximity to the lakes and rivers, has forested areas and wildlife. These environmental factors promote the transmission of arboviral infections by mosquitoes. Also, the region borders countries like Somalia and Ethiopia, which have experienced various arboviral outbreaks. During rainy seasons, these areas experience flooding providing good breeding grounds for the vectors (Arum *et al.,* 2015).

3.2.3 Coastal region

The region comprises six counties namely; Mombasa, Kwale, Kilifi, Tana River, Taita-Taveta and Lamu. Being a holiday destination, there is a constant influx of travelers from all over the world. It has environmental factors that favour transmission of arboviral infections such as low rainfall, high temperatures, closeness to the ocean and rivers, forests, presence of wildlife and birds, putting the region at risk of infection (Lutomiah *et al.,* 2016; Nyamwaya *et al.,* 2016; Agha *et al.,* 2017). Some areas like Tana River County experiences occasional flooding, which provides breeding grounds for the vectors.

3.2 Study Design

This was a retrospective study that utilized archived human sera collected from January 2008 to December 2014.

3.3 Study Samples

This study utilized archive samples from measles case-based surveillance collected from North-Eastern, Coast and Nairobi Regions. The Measles surveillance in Kenya follows the World Health Organization guidelines, in which a suspected measles case is defined by a patient having fever and one of the following symptoms: rash, cough, coryza, or conjunctivitis. The samples were stored in the World Health Organization /Ministry of Health Regional Reference Laboratory at the Kenya Medical Research Institute -Expanded Programme on Immunization (EPI) laboratory at 20ºC and the volume was 200µl.

3.4 Sample Size Determination

The minimum sample size required for this study was calculated using the formula developed by Cochran (Cochran, 1963). The prevalence used to calculate the sample size was 51%; this was based on a serosurvey study by (Labeaud *et al.,* 2013). The sample size was estimated using DENV's prevalence of 51% as previously described by (Naing *et al.*, 2006). The sample size was thus determined as

$$N= Z^2 P (1-p)/d^2$$

N =sample size

Z = Standard normal deviation at the required confidence interval (1.96)

p = proportion in the target population estimated to have measured character (q = 1 – p)

d = level of statistical significance at 95% confidence level = 0.05

$$= 1.96^2 \times 0.51\ (1\text{-}0.51)/0.05^2\text{=}385$$

385 was the minimum number of samples to be used in this study, to which 7 samples were added in case there was not enough serum in some of the vials, making a total of 392 samples.

3.5 Sampling Criteria

A simple random sampling procedure was used. The investigator used the database to identify the negative samples to both measles and rubella and captured their district of residence (regions). The total number of negative samples for both measles and rubella IgM antibodies was calculated. The percentage of negative samples in each year was calculated by dividing the total number of negative samples in that year by the total number of negative samples multiplied by 100. The sample size of 385 was distributed proportionately according to year and sites.

The required number of negative samples in each region was randomly selected from the total number of negative samples in that region in that year. Random numbers were generated using a computer and using the sample interval that determined every k^{th} sample to be selected from a list of samples received from (2008-2014) from the three regions.

3.6 Laboratory Procedures

3.6.1 Enzyme-Linked Immunosorbent assay (ELISA)

The samples were heated-inactivated at 56°C then screened for Dengue Virus (DENV), Chikungunya Virus (CHIKV). This was done using commercial kits, namely: DENV IgG (Novatec GMBH), DENV IgM (Novatec GMBH), CHIKV IgM µ-capture (Novatec GMBH), CHIKV IgG capture ELISA kit (Novatec GMBH) (Appendix 1) as per the manufacturer´s instructions. Briefly, CHIKV/DENV IgG

microplates were coated with anti-human Ig antibodies. The diluted samples, positive and negative controls were added and incubated at 37 ºC for 1 hour. After 1 hour, wells were washed to remove all unbound antibodies, the antigen was added and plates were incubated at room temperature. Next, the plates were washed, the detector antibody was added into all the wells and the plates were incubated at room temperature. After washing, horseradish peroxidase (HRP) labelled streptavidin was added to bind to the captured specific immune complex, washed and the immune complexes were visualized by adding Tetramethylbenzidine (TMB) substrate which gave a blue colour. Stop solution was added to stop the reaction, which produced a yellow colour. The absorbance was measured at 450 nm using ELISA plate reader ELx800 (Biotek).

Serologically, CHIKV/DENV IgM was detected by ELISA. Briefly, the microplates were coated with anti-human IgM antibodies to bind to the corresponding antibodies of the diluted samples. All the other steps were followed as described for CHIKV/DENV IgG and the absorbance was measured at 450 nm using ELISA plate reader ELx800 (Biotek).

For ZIKV, a laboratory-developed test (CDC Zika MAC ELISA) was developed. Briefly, a 96 well immunological plate was coated with diluted goat anti-human IgM (1:2000) in coating buffer (CDC-Fort Collins Arbovirus Disease Branch) followed by overnight incubation at 4° C. The following day, the coating buffer was discarded. The wells were then coated with blocking buffer (5% Skimmed milk, 0.5% Tween 20 and Phosphate Buffered Saline (PBS) for 30 minutes. This was followed by washing the plates 5 times. The patients' serum, positive control and negative control were diluted (1:400) and added into their respective wells, then incubated at 37°C for 1 hour. After incubation, the plates were washed 5 times. ZIKV antigen and normal antigen were diluted (1:2000) and added into their respective wells. The plates were then incubated at 4°C overnight. After the incubation and washing step, diluted conjugate (DB621-HRP) in blocking buffer (1:4000) was added to all the wells,

followed by another wash 5X and incubation. Tetramethyl benzidine (TMB) substrate was added to all the wells in the dark and then added stop solution. The optical density (OD) values were read using an ELx800 absorbance reader at 450nm (CDC, 2018; Basile *et al.,* 2018) (Appendix 1).

3.6.2 Virus propagation, Confirmation and Quantification

Dengue, ZIKV, CHIKV and other arboviruses were propagated in Vero cell lines and C6/36 cell lines as previously described (Igarashi, 2008).

3.6.2.1 Virus propagation

Virus isolates were propagated in the laboratory in Vero cells and C6/36 cells cultured in MEM Minimum Essential Medium (MEM) (Gibco) and Dulbecco's Modified Eagle Medium (DMEM) (Gibco) supplemented with 10% Fetal bovine serum (FBS) (Gibco), 2 mM L-glutamine, 1X penicillin/ streptomycin respectively. 200µl of the virus isolate was inoculated onto an 80% confluent monolayer in a 25cm^2 cell culture flask (Corning), incubated for 1 hr at 37°C and 5ml of maintenance media with 2% FBS was added. The flask was observed daily for cytopathic effects (CPE) and infected culture supernatant (ICF) was harvested when 70%-80% CPE was observed. The flasks were frozen at -80 °C overnight to lyse cells and release bound viruses from the cells. The ICF was clarified by low speed centrifugation at 2800 revolutions per minute (rpm) for 10 minutes to separate the virus from cellular debris. The supernatant was harvested and stored in 1ml aliquots at -80 °C (Igarashi, 2008) (Appendix 2).

3.6.2.2 Virus confirmation by Polymerase Chain Reaction

a) RNA extraction

The viral RNA was purified from the cell culture supernatant using the QIAamp Viral RNA Mini kit spin protocol (QIAGEN-USA) as per the manufacturer's instructions. Briefly, 140 µl of the culture supernatant was added to 560 µl of lysis buffer containing carrier RNA, incubated for 10 minutes at room temperature and 560 µl of absolute ethanol added to the mixture. 630 µl of the resultant solution was applied to a QIAamp mini-column in 2 ml collection tubes and spun at 7500 rpm for 1 minute. This step was repeated twice with the remaining solution after which 500 µl of wash buffer was applied to the column, centrifuged at 13700 rpm for 3 minutes and the filtrate discarded. 60 µl of the elution buffer was added then be added to the spin column and the latter spun at 7500 rpm for 1 minute to elute the purified viral RNA.

b) Complementary deoxyribonucleic nucleic acid (cDNA) synthesis from viral RNA

In a 200 µl PCR tube, 10 µl of the extracted sample RNA was mixed with 2 µl of 50 ng/µl random hexamer primer and 1 µl of 10 mM deoxy nucleotide solution (dNTPs), and incubated in a thermocycler for 5 minutes at 65°C and immediately chilled for 1 minute at 4°C. The following components were then added to the PCR tube: 4 µl of 5X First Strand Buffer (Invitrogen), 1 µl of 0.1 M DTT, 1 µl of RNAse OUT (40 U/µl) and 1 µl of Superscript III Reverse transcriptase (200 U/µl). The mixture was then incubated in a thermocycler for 5 minutes at 25°C, 50 minutes at 50°C and 15 minutes at 70°C. A total of 20 µl cDNA was obtained (Konongoi *et al.,* 2018).

c) PCR amplification

The PCR amplification of targeted viral sequences in the cDNA was performed in a 25-µL reaction containing: 12.5 µl of Amplitaq Gold 360 PCR master mix (Applied

Biosystems USA), 0.5µl of 50 picomoles each of forward and reverse primer, 2 µl of the cDNA and 9.5 µl of DEPC treated water. Samples were first tested using alphavirus family primers (VIR2052F 5'-TGG CGC TAT GAT GAA ATC TGG AAT GTT-3)' and (VIR2052R 5'-TAC GAT GTT GTC GTC GCC GAT GAA-3') and flavivirus family primers (FU1 5'- TAC AAC ATG ATG GGA AAG AGA GAG AA-3' and CFD2 5'- GTG TCC CAG CCG GCG GTG TCA TCA GC-3') (Konongoi *et al.,* 2018). In all the PCR reactions and a negative control was included. Electrophoresis of the amplified DNA products was done on a 2% agarose gel in 1% Tris-borate EDTA buffer stained with ethidium bromide. An ultra-violet (UV) trans illuminator was used to visualize the PCR product bands and images recorded using a gel photo imaging system (Sang *et al.,* 2003) (Appendix 3).

Table 3.1: Primers used in this study

Virus	Primer	Primer sequence	Cycling conditions			
Alphavirus	Vir2052F	5′-TGG CGC TAT GAT GAA ATC TGG AAT GTT-3′	95°C (10 minutes)	95°C (30 seconds) 49°C (30 seconds)	72°C (10 minutes)	4°C (hold)
	Vir2052R	5′-TAC GAT GTT GTC GTC GCC GAT GAA-3′		72°C (30 seconds) for 35 cycles		
Flaviirus	Fu1	5′-TAC AAC ATG ATG GGA AAG AGA GAG AA-3′	95°C (10 minutes)	95°C (30 seconds) 55°C (30 seconds) 68°C (45 seconds) for 35 cycles	72°C (7 minutes)	4°C (hold)
	CDF2	5′-GTG TCC CAG CCG GCG GTG TCA TCA GC-3′				

3.6.3 Plaque assay

Quantification of the viruses: CHIKV, DENV, ZIKV and other targeted arbovirues was done as previously described by (Russell *et al.,* 1967; Kaur *et al.,* 2016). These

viruses were characterized previously and stored in the laboratory. Vero cell lines derived from the African green monkey kidney were used in the study. Serial dilutions of the virus inoculum were performed by adding 50µl of the virus inoculum to the plate containing 450µl of maintenance media. Serial dilutions of 1:10 for each virus were performed by transferring 50µl to the consecutive Eppendorf tubes. 100µl of the appropriate virus dilution was used to infect 70% confluent Vero cells on the plates. The plates (Corning) were incubated for 1hr at 37°C in 5% CO_2 to allow adsorption. 2.5% methylcellulose was then used as an overlay medium by adding 2 milliliters into each well (12 well) and incubated at 37°C in 5% CO_2 and observed for plaque formation. After plaques were formed, the overlay media was discarded, and the plaques were fixed on the appropriate days with 3% formaldehyde and left overnight. The formalin was gently poured out, and 2ml of (0.5%) crystal violet was added to stain the plaques and washed to remove excess stain. The plates were left to dry. The virus titre was estimated as plaque-forming units per ml (pfu/ml) (Appendix 4).

$$Plaque\ forming\ units\ per\ ml\ \left({}^{pfu}/_{ml}\right) = \frac{Number\ of\ plaques}{Dilution\ factor\ x\ volume}$$

3.6.4 Plaque reduction neutralization test (PRNT)

Plaque Reduction Neutralization Tests were conducted to confirm the ELISA positive results (WHO, 2007; Roehrig *et al.,* 2008) for CHIKV, DENV and ZIKV and rule out cross-reactivity with viruses in the same family. The PRNT panel selected for detection of alphaviruses for CHIKV ELISA positive included Semliki Forest Virus (SFV) (mosquito isolate), Sindbis (SINV) (mosquito isolate), O 'nyong nyong (ONNV) (SG 650) and CHIKV (Lamu 33 isolate) viruses (Kimata *et al.,* 2020). The panel for flaviviruses for DENV and ZIKV positive included DENV (008/01/2012 isolate), ZIKV (MR766), YFV (mouse brain inoculation) and WNV (mosquito isolate

from Kenya) (Chepkorir *et al.,* 2019) were obtained from the KEMRI Viral Haemorraghic Fever (VHF) laboratory. The viruses used in this study were selected because they are known to be circulating in parts of the country the samples were collected from. Each virus was diluted to a standard concentration to produce approximately 20-50 plaques/well in 24-well tissue culture plates (Corning) containing a monolayer of Vero (African green monkey cells ATCC). Each serum sample was serially diluted from 1:20 to 1:320 using maintenance media in sterile Eppendorf tubes. A constant volume of the diluted virus with the required concentration was added into each 1.5 ml tube (Eppendorf) containing the diluted serum from 1:20 to 1:320 and incubated for 1 hour at 37°C. The virus-serum mixture (30µl of the diluted virus with 30µl of the diluted serum) was then transferred to 24-well plates (Corning) with confluent Vero cell monolayers (African green monkey cells ATCC) and incubated at 37° C, 5% CO_2 for 1 hour for adsorption process, after which an over-lay of 2.5% methylcellulose (Sigma) was added. After 3-14 days, depending on the virus tested, the plates were fixed with 3% formalin (Sigma) and stained with 0.5% crystal violet (Sigma) prepared in absolute ethanol (Sigma). For etiological diagnosis, the $PRNT_{90}$ antibody titer for each virus was required to be four-fold greater than the other viruses in the same family to arrive at an etiological diagnosis (Tigoi *et al.,* 2015; Chepkorir *et al.,* 2019) (Appendix 4).

3.7 Data Management

Data collected was entered in a database that included: Laboratory identification number, country, district of residence, and date of specimen collection, date specimen sent to the laboratory, gender and age. Microsoft Excel was used for data entry and storage and analyzed using Statistical Package for Social Sciences (SPSS). Only authorized personnel were able to access the data. Data files were stored in a password-protected computer. Data was also be backed up online using the Dropbox online software from Dropbox Inc, USA.

3.8 Data Analysis

Data was stored in a password-protected database linked to laboratory results; the data were analyzed using SPSS version 22. The main outcomes of interest were IgM and IgG antibodies against DENV, CHIKV and ZIKV and neutralization tests for other arboviruses, ONNV, SFV, SINV, YFV and WNV. The IgM and IgG status was dichotomized as either positive or negative based on the values of optical density (OD). The frequency of the viruses by ELISA and PRNT was obtained and determined the prevalence. Data on counts/proportions were compared using Chi-Square. The association of patient demographic characteristics and with PRNT findings was calculated using Chi-square. The factors considered include the age of the patients and gender. All tests were performed at a 5% level of significance.

3.9 Ethical Considerations

Approvals to carry out this study were obtained from Kenya Medical Research Institute's Scientific Ethics Review Unit (SERU no. 3122) (Appendix 5 and 6). Permission to use the World Health Organization/Ministry of Health measles surveillance samples were obtained. The analysis was conducted on a sub-sample of stored serum samples from survey participants. All specimens that were used were not linked to personal identifiers.

CHAPTER FOUR

RESULTS

4.1 Demographic Characteristics of Study Participants in Coast, North-Eastern and Nairobi Regions from 2008-2014

In total, 392 human archived serum samples were collected from patients presenting with febrile rash illness at both Public and Private Health Facilities in Nairobi (n=179), North-Eastern (n=59) and Coast (n=156). The regions were later divided into counties which includes Nairobi (n=179), Mombasa (n=47), Kwale (n=12), Kilifi (n=55), Lamu (n=5), Taita-Taveta (n=35), Tana River (n=2), Wajir (n=6), Garissa (n=48) and Mandera (n=3). The age range of participants was 5months-60 years. The median age was 4.7 years and Interquartile Range (IQR) was 2.8-6.7 years). The ages were further classified into four categories less than 5 years (52.8%), 5-9 years (34.4%), 10-14 years (6.4%) and 14-60 years (6.4%).

Out of the 392 samples, 158 (40%) were females and 234 (60%) males. The year 2008 had 72 (18.4%) samples, 2009 had 87 samples (22.2%), 2010 had 41 samples (10.5%), 2011 had 74 samples (18.0%), 2012 had 31 samples (7.9%), 2013 had 6 samples (1.5%) and 2014 had 81 samples (20.7%) (Table 4.1).

Table 4.1: Demographic characteristics of the study participants in Coast, North-Eastern and Nairobi Regions from 2008-2014

Region		Coast						Nairobi	North-Eastern			
County		Mombasa	Kilifi	Kwale	Lamu	Taita Taveta	Tana River-	Nairobi	Mandera	Garissa	Wajir	Total
n		47	55	12	5	35	2	179	3	48	6	392
	<5	28 (13.5)	26 (12.6)	8 (3.9)	3 (1.4)	22 (0.5)	1 (0.5)	91 (44.0)	1 (0.5)	24(11.6)	3 (1.4)	207(52.8)
Age	5-9	11 (8.1)	18 (13.3)	4 (3.0)	2 (1.5)	12(8.9)	1 (0.7)	69 (51.1)	0	17 (12.6)	1 (0.7)	135(34.4)
	10-14	4(16.0)	4 (16.0)	0	0	1 (4.0)	0	11 (44.0)	1(4.0)	5(20.0)	0	25 (6.4)
	14-60	4 (16.0)	7 (28.0)	0	0	1 (4.0)	0	8 (32.0)	1 (4.0)	2(8.0)	2 (8.0)	25 (6.4)
Gender	Female	20 (12.7)	28 (17.7)	4 (2.5)	3 (1.9)	13 (8.2)	0	73 (46.2)	1 (0.6)	15(9.5)	1 (0.6)	158 (40.3)
	Male	27 (11.5)	27 (11.5)	8 (3.4)	2 (0.9)	22 (9.4)	2(0.9)	106 (45.3)	2 (0.9)	33 (14.1)	5 (2.1)	234(59.7)
Year	2008	2 (2.8)	10 (13.9)	0	0	13(18.1)	0	43 (59.7)	0	4 (5.6)	0	72(18.4)
	2009	8 (9.2)	14 (16.1)	1 (1.1)	0	0	0	54 (62.1)	0	10 (11.5)	0	87(22.2)
	2010	2 (4.9)	3 (7.3)	2 (4.9)	0	11(26.8)	1 (2.4)	16 (39.0)	0	5 (12.2)	1 (2.4)	41(10.5)
	2011	7 (9.5)	6 (8.1)	1 (1.4)	2 (2.7)	5 (6.8)	0	41 (55.4)	3 (4.1)	7 (9.5)	2 (2.7)	74 (18.9)
	2012	3 (9.7)	6 (19.4)	2 (6.5)	0	3 (9.7)	0	12 (38.7)	0	2 (6.5)	3 (9.7)	31 (7.9)
	2013	2 (33.3)	0	0	0	0	0	2 (33.3)	0	2 (33.3)	0	6 (1.5)
	2014	23 (28.4)	16 (19.8)	6 (7.4)	3 (3.7)	3 (3.7	1 (1.2)	11 (13.6)	0	18 (22.2)	0	81 (20.7)

4.2 Prevalence of DENV, CHIKV and ZIKV arboviruses in Coast, North-Eastern and Nairobi Regions from 2008-2014.

a) Active infection of DENV, CHIKV and ZIKV

Out of the 392 samples, 5 (45.5%) were DENV IgM positive and confirmed by PRNT to be DENV while 6 samples (54.5%) were confirmed to be ZIKV by PRNT. One sample (0.3%) was CHIKV IgM positive was confirmed by PRNT. Three samples were ZIKV IgM positive were confirmed by PRNT to be 1 (33.3%) ZIKV, 1(33.3%) WNV and 1 (33.3%) YFV as shown in (Table 4.2).

Table 4.2: Prevalence of Active infection DENV, CHIKV and ZIKV arboviruses in Coast, North-Eastern and Nairobi Regions from 2008-2014.

Variable	Frequency (%)
DENV IgM + PRNT positive	
Positive	11 (2.8)
Negative	381 (97.2)
PRNT positive (n=11)	
DENV	5 (45.5)
ZIKV	6 (54.5)
CHIKV IgM + PRNT	
Positive	1 (0.3)
Negative	391 (99.7)
PRNT positive (n=1)	
CHIKV	1 (100.0)
ZIKV IgM + PRNT	
Positive	3 (0.8)
Negative	389 (99.2)
PRNT positive (n=3)	
ZIKV	1 (33.3)
WNV	1 (33.3)
YFV	1 (33.3)

b) Past infection of DENV, CHIKV and ZIKV arboviruses

Out of the 392 samples, 30 samples were confirmed by DENV IgG and PRNT; 17 (56.7%) was confirmed to be DENV, 8 (26.7%) to be ZIKV, 3 (10.0%) to be WNV and 2 (6.7%) to be YFV. Out of the 10 samples that were CHIKV IgG and PRNT positive; 1 (10.0%) was positive for CHIKV while 9(90.0%) were confirmed to be ONNV. None of the samples neutralized with SFV or SINV as shown in (Table 4.3).

Table 4.3: Prevalence of past infection DENV, CHIKV and ZIKV arboviruses in Coast, North-Eastern and Nairobi Regions from 2008-2014.

Variable	Frequency (%)
DENV IgG+PRNT	
Positive	30 (7.7)
Negative	362 (92.3)
PRNT positive (n=10)	
DENV	17 (56.7)
ZIKV	8 (26.7)
WNV	3 (10.0)
YFV	2 (6.7)
CHIKV IgG+ PRNT	
Positive	10 (2.6)
Negative	382 (97.4)
PRNT positive (n=10)	
CHIKV	1 (10.0)
ONNV	9 (90.0)

c) Co- exposure

Co-exposure with more than one virus was observed in this study; 4 participants had been exposed to alphaviruses (ONNV) and flaviviruses (WNV and ZIKV).

4.3 Virus propagation, confirmation and quantification

a) Viral titres used in the study

Alphaviruses:

i. CHIKV $\frac{28}{0.1x\ 10^{6}}$

$=2.8\ x10^{8}$ pfu/ml

i) ONNV $\frac{100}{0.1x10^{6}}$

$= 1.0\ x\ 10^{9}$ pfu/ml

iii) SINV $\frac{28}{0.1x\ 10^{5}}$

$=2.8\ x\ 10^{7}$ pfu/ml

iv) SFV $\frac{68}{0.1\ x10^{4}}$

$=6.8x\ 10^{8}$ pfu/ml

Flaviviruses:

i) DENV-2 $\frac{36}{0.1x\,10^4}$

$= 3.6 x 10^6$ pfu/ml

ii) WNV $\frac{17}{0.1x10^4}$

$=1.7 x 10^6$ pfu/ml

iii) ZIKV $\frac{35}{0.1x\,10^3}$

$= 3.5 x 10^5$ pfu/ml

iv) YFV $\frac{67}{0.1x\,10^4}$

$= 6.7 x 10^6$ pfu/ml

4.4 Distribution patterns of DENV, CHIKV and ZIKV arboviruses among non-measles cases in Coast, North-Eastern and Nairobi Regions from 2008-2014.

In 2008, Dengue IgM was confirmed in 3(4.2) samples while Dengue IgG was confirmed in 7(9.7) samples. In 2009, Dengue IgM was confirmed in 1 (1.1), Dengue

IgG was confirmed in 9(10.3) while Chikungunya IgG was confirmed in 4 (4.6). In 2010, Dengue IgM was confirmed in 1(2.4) sample, Dengue IgG was confirmed in 1 (2.4%) sample while chikungunya IgG was confirmed in 1(2.4) sample. In 2011, Zika IgM was confirmed 2(2.7) samples, chikungunya IgG was confirmed in 1(1.4) while Dengue IgG was confirmed in 2(2.7) samples. In 2012, Chikungunya IgM was confirmed in 1(3.2) sample, Dengue IgM was confirmed in 1 (3.2) sample, Zika IgM was confirmed in 1(3.2) sample, Chikungunya IgG was confirmed in 2 (6.5) samples while Dengue was confirmed in 3(9.7) samples. In 2013, Dengue IgM was confirmed in 1(16.7) and Dengue IgG was confirmed in 1(16.7). In 2014, Dengue IgM was confirmed in 4(4.9), Chikungunya IgG was confirmed in 2(2.5) samples while Dengue IgG was confirmed 7(8.6). There was no significance difference in the virus tested in the various years.

In Garissa, Dengue IgM was confirmed in 1(2.1) sample, Chikungunya IgG was confirmed in 4(8.3) samples while Dengue IgG was confirmed in 7 (14.6). In Kilifi, Dengue IgM was confirmed in 2(3.6) samples, Chikungunya IgG was confirmed in 1 (1.8) sample and Dengue IgG was confirmed in 4(7.3) samples. In Kwale, Dengue IgM was confirmed in 1(8.3) sample and Dengue IgG were confirmed in 1(8.3) sample. In Lamu, Chikungunya IgM was confirmed in 1(20) sample. In Mandera, no virus was detected. In Mombasa, Dengue IgM was detected in 4(8.5) samples, Chikungunya IgG was confirmed in 2(4.3) samples and Dengue IgG was confirmed in 6(12.8) samples. In Nairobi, Chikungunya IgM was confirmed in 1(0.6) sample, Dengue IgM was confirmed in 3(1.7) samples, Zika IgM was confirmed in 2(1.1) samples, chikungunya IgG was confirmed in 2(1.1) samples while dengue IgG was confirmed in 11 (6.1)

In Taita Taveta, Zika IgM was confirmed in 1(2.9) sample and Dengue IgG was confirmed in 1(2.9) sample. No virus was detected in Tana-River and Wajir counties as shown in (Table 4.4).

Table 4.4: Distribution patterns of DENV, CHIKV and ZIKV arboviruses among non- measles cases in Coast, North-Eastern and Nairobi Regions from 2008-2014.

Variable	CHIKV IgM+PRNT	DENV IgM+PRNT	ZIKV IgM+PRNT	CHIKV IgG+PRNT	DENV IgG+PRNT
Year					
2008	0	3 (4.2)	0	0	7 (9.7)
2009	0	1 (1.1)	0	4 (4.6)	9 (10.3)
2010	0	1 (2.4)	0	1 (2.4)	1 (2.4)
2011	0	0	2 (2.7)	1 (1.4)	2 (2.7)
2012	1 (3.2)	1 (3.2)	1 (3.2)	2 (6.5)	3 (9.7)
2013	0	1 (16.7)	0	0	1 (16.7)
2014	0	4 (4.9)	0	2 (2.5)	7 (8.6)
P value	0.094	0.109	0.121	0.352	0.243
County					
Garissa	0	1 (2.1)	0	4 (8.3)	7 (14.6)
Kilifi	0	2 (3.6)	0	1 (1.8)	4 (7.3)
Kwale	0	1 (8.3)	0	0	1 (8.3)
Lamu	0	0	0	1 (20.0)	0
Mandera	0	0	0	0	0
Mombasa	0	4 (8.5)	0	2 (4.3)	6 (12.8)
Nairobi	1 (0.6)	3 (1.7)	2 (1.1)	2 (1.1)	11 (6.1)
Taita taveta	0	0	1 (2.9)	0	1 (2.9)
Tana-River	0	0	0	0	0
Wajir	0	0	0	0	0
P value	1.000	0.258	0.666	0.077	0.541

4.5 Association of patient demographic characteristics and the PRNT findings among suspected measles cases in Coast, North-Eastern and Nairobi Regions from 2008-2014

Association of patient demographic characteristic and with PRNT findings were calculated using chi-square. The results showed that the prevalence of CHIKV and DENV was significantly higher in those aged 14-60 years. The prevalence of CHIKV and DENV was 8 and 32% resctively (p=0.001). There was no significant difference by gender and days of onset of symptoms and sample collection among all the selected viruses tested as shown in (Table 4.5).

Table 4.5: Association of patient demographic characteristics and the PRNT findings among suspected measles cases in Coast, North-Eastern and Nairobi Regions from 2008-2014

Variable	**CHIKV IgM+PRNT**	**DENV IgM+PRNT**	**ZIKV IgM+PRNT**	**CHIKV IgG+PRNT**	**DENV IgG+PRNT**
Age in years					
<5	0	6 (2.9)	2 (1.0)	0	13 (6.3)
5-9	1 (0.7)	2 (1.5)	1 (0.7)	7 (5.2)	7 (5.2)
10-14	0	0	0	1 (4.0)	2 (8.0)
14-60	0	3 (12.0)	0	2 (8.0)	8 (32.0)
P value	0.472	0.067	1.000	**0.001**	**0.001**
Gender					
Female	1 (0.6)	4 (2.5)	1 (0.6)	4 (2.5)	12 (7.6)
Male	0	7 (3.0)	2 (0.9)	6 (2.6)	18 (7.7)
P value	0.403	1.000	1.000	1.000	0.972
Days from onset to collection					
0-5 days	1 (0.3)	10 (2.8)	2 (0.6)	8 (2.3)	26 (7.4)
>5 days	0	1 (2.6)	1 (2.6)	2 (5.1)	4 (10.3)
P value	1.000	1.000	0.270	0.261	0.522

CHAPTER FIVE

DISCUSSION

5.1. Seroprevalence of DENV, CHIKV and ZIKV arboviruses (2008-2014).

This study found the presence of IgG suggesting past infection to arboviruses and the presence of IgM suggests a current/recent arbovirus infection. Neutralizing antibodies to DENV, WNV, ZIKV, YFV, CHIKV and ONNV hence confirming them as active and past infection to these arboviruses. These arboviral infections have been previously reported in North-Eastern and Coast regions (Mease *et al.,* 2011; Tigoi *et al.,* 2015; LaBeaud *et al.,* 2015; Konongoi *et al.,* 2016; Konongoi *et al.,* 2018), but none have been reported in Nairobi County. The results indicate the potential of these arboviral infections to cause measles-like illnesses, thus pointing out the necessity of differential diagnosis in febrile cases that test negative for measles/rubella.

Measles is a major childhood problem that causes significant illness, disability and 1 million deaths annually in developing countries (Onoja *et al., 2*019). In Kenya, the measles case definition recommended by both WHO is acute illness lasting at least 3 days, with maculopapular rash, fever (≥ 38.3°C), and one or more of cough, coryza, or conjunctivitis (WHO, 2015). However, no data exists on the reliability or validity of this case definition in areas where arboviruses are endemic. Therefore, febrile rash illnesses that are clinically diagnosed as measles but test negative for measles should be investigated for other infections, including arboviruses. The results of this study have shown that arboviruses can present as measles-like illnesses. Thus clinicians should not solely rely on the measles case definition to identify cases, especially in areas where arboviruses are endemic.

This finding of arboviral infections on samples of suspected measles cases carried out to determine the etiologies of fever and maculopapular rash agrees with other studies that

have yielded results indicating that some of these etiological agents were overlooked or left out such as first, in Mozambique by (Antonio *et al.,* 2019), where CHIKV IgG and IgM were detected among measles-negative samples. Secondly, in Brazil by (De Moraes *et al.,* 2011), where dengue virus was detected in 22 cases. 2 of these 22 cases met Brazil's clinical case definition for rubella, which would have been reported to be rubella. Still, these 2 cases were diagnosed as dengue infection. Third, in Rio de Janeiro, Brazil, a study by (Oliveira *et al.,* 2001) showed that dengue fever, rubella, parvovirus B19, measles and human herpesvirus 6 were among the causes of rash between 1994 and 1998. Possibly, the main contributor to these occurrences could be the fact that arboviruses are overlooked because of the unavailability of resources in terms of equipment for diagnostics assays and specialized laboratories.

5.2 Cross-reactivity of arboviruses

Cross-reactivity was observed in samples that had tested positive for anti-CHIKV IgG on ELISA neutralizing with ONNV on PRNT assay. This finding is consistent with other studies reported in Kenya and Cameroon, where cross-reactivity was observed between CHIKV and ONNV (Kuniholm *et al.,* 2006; LaBeaud *et al.,* 2015; Tigoi *et al.,* 2015). This could be due to both viruses may have been co-circulating in the region and the continued evolution of ONNV has made it antigenically more similar to CHIKV than previously reported (Chanas *et al.,* 1979).

Cross-reactivity was observed in samples that had tested positive for anti-DENV IgG and anti-ZIKV IgM neutralizing with WNV and YFV on PRNT assay. This finding agrees with the findings of (Montoya *et al.,* 2018; Maeki *et al.,* 2019), where these flaviviruses were found to be cross-reacting this is because these viruses are antigenically related.

b) Co-exposure status to arboviruses

Co-exposure with more than one virus was observed in this study; 4 participants had been exposed to alphaviruses (ONNV) and flaviviruses (WNV and ZIKV). This could be due to a situation where the patient was bitten by a co-infected mosquito or several bites from infected mosquitoes carrying different viruses (Adesina *et al.,* 1991; Olaleye *et al.,* 1990). This phenomenon was also observed in (Baba *et al.,* 2013; Tigoi *et al.,* 2015), where samples had more than one virus; this could be due to multiple exposures to these arboviral infections. This is possible because these viruses co-circulate in the same areas. The lack of sensitive diagnostic methods contributes to the underestimation of these arboviruses

5.3 Distribution patterns of arboviruses in the selected counties (2008-2014)

In this study, CHIKV IgM antibodies were confirmed in a sample collected from a child aged between 5-9 years who reported to a health facility with febrile rash illness. This finding suggests the circulation of CHIKV virus in Mombasa County, Coastal region, as early as 2014. The Coastal region experienced the largest outbreak of CHIKV in Lamu County in 2004 (Sergon *et al.,* 2008). Therefore, this indicates continued circulation of CHIKV outside of the outbreaks and may also suggest it had been circulating at low levels even before the reported outbreak, which occurred in Mombasa in 2018 (WHO, 2018). This is likely to happen because only a few cases present with a maculopapular rash, and they easily go undetected. The Coastal region also experienced a large dengue outbreak in 2013 (Ellis *et al.,* 2015), 2013-2014 (Lutomiah *et al.,* 2016), which could have led to underreporting of CHIKV because of its similar clinical symptoms (WHO, 2009). The Coastal region has competent vectors for CHIKV, as shown by (Mbaika *et al.,* 2016; Agha *et al.,* 2017; Mulwa *et al.,* 2018), and this is attributed to the exacerbation of the intensity of the CHIKV transmission and outbreaks**.**

CHIKV neutralizing antibodies were also detected in a sample collected from a 5-year-old in Nairobi County in the year 2009, indicative of past exposure to the virus. Nairobi County has never experienced any arboviral outbreak when compared with Mombasa County. This finding of CHIKV in negative measles cases indicates how such co-circulation of viruses with similar clinical presentation could complicate measles diagnosis (Kimata *et al.,* 2020).

O'nyong-nyong virus neutralizing antibodies were detected in Lamu, Taita-Taveta, Kilifi, Mombasa, Garissa and Nairobi, indicating past exposure of the virus in all three regions from samples collected between 2009 and 2014. A large number of samples neutralizing with ONNV were observed in the Coastal region, a finding consistent with the findings from a previous study by (LaBeaud *et al.,* 2015) that found ONNV exposure to be common in Coastal Kenya between the years 2014 to 2015. The last documented ONNV outbreak in Kenya occurred in 1961; that outbreak spread to neighbouring countries, eventually affecting more than 2 million individuals but without any recorded fatalities (Rezza *et al.,* 2017). Neutralizing antibodies against SINV or SFV in the measles negative samples were not detected in this study, but this doesn't rule out the possibility of these viruses circulating among undiagnosed measles cases (Kimata *et al.,* 2020). This is because these viruses have been detected in serological surveys carried out in Kenya (Tigoi *et al.,* 2015; Ofula *et al.,* 2016) and also during vector surveillance carried out by (Ochieng *et al.,* 2013). ONNV was detected in 90% of the samples; a recent study by (LaBeaud *et al.,* 2015) had a prevalence of 56% in Coastal Kenya, which is lower than the findings of this study. This could be due to the abundance of the *Anopheles* mosquitoes in that region that transmits the virus.

DENV neutralizing antibodies were detected in Garissa, Kilifi, Kwale, Mombasa and Nairobi, indicating the presence of past and recent infection of the virus in 2008, 2009, 2011, 2012, 2013 and 2014. Three of the counties are from the Coastal Region, which is likely because this area is endemic for DENV and the presence of competent vectors,

which then exacerbates the situation (Chepkorir *et al.,* 2014). The high percentage could be attributed to the two DENV outbreaks that occurred in Kenya during the study period. In 2011/2012, in Mandera, North-Eastern Kenya and 2013/2014 in Mombasa, Coast Region (Lutomiah *et al*., 2016; Konongoi *et al*., 2016). In an another study carried out in Kenya among children all the 4 dengue serotypes were detected (Shah *et al.,* 2020).

Zika virus seropositivity was detected in Garissa, Kilifi, Mombasa, and Nairobi from 2008 to 2014. This finding of ZIKV in Mombasa agrees with a study by (Hunsperger *et al.,* 2020), where ZIKV IgM antibodies were detected in Mombasa Kenya from samples collected in 2013, indicating it's been circulating in the country. This virus shares the same vector with DENV, which are abundant in Coastal Kenya (Lutomiah *et al.,* 2013). There is a possibility that the virus spread into Kenya due to the presence of the mosquito vector, increased foreign travel and commerce, and a growing human population. This finding of ZIKV antibodies in Kenya agrees with other studies carried out in different parts of the country that ZIKV continues to circulate at low levels (Chepkorir *et al.,* 2019; Kisuya *et al.,* 2019; Gobillot *et al.,* 2020).

On the other hand, WNV seropositivity was detected in Garissa and Nairobi in the year 2008, 2009, 2011 and 2014. Garissa is found in Northern Kenya. WNV is known to be transmitted by

Culex mosquitoes (WHO, 2017). It has been isolated from mosquitoes (Crabtree *et al.,* 2009; LaBeaud *et al.,* 2011; Ochieng *et al.,* 2013) and ticks in North-Eastern Kenya (Lwande *et al.,* 2013). Therefore, this indicates it's been circulating at low levels. In a study by Nyamwaya *et al.* (2016), WNV was detected in wild birds in Tana River and Garissa Counties, Kenya. Recent serosurveys carried out have also indicated the presence of WNV antibodies in humans in the country (Tigoi *et al.,* 2015; Chepkorir *et al.,* 2019; Inziani *et al.,* 2020). There is no evidence of any arbovirus being detected in Nairobi previously.

Seropositivity to YFV was found in three samples, one each from Garissa, Taita Taveta and Nairobi Counties in the years 2008, 2009 and 2011. In Kenya, the first and last documented outbreak of YFV occurred in 1992-1993 (Sanders *et al.,* 1998), but outbreaks were reported in the neighbouring countries such as Ethiopia in 1960-1962, 2013 (Alhakimi *et al.,* 2015; Ellis *et al.,* 2008; Lilay *et al.,* 2017 (Sudan in 2003-2005) (Gould *et al.,* 2008), South Sudan in 2012 (Alhakimi *et al.,* 2015) and Uganda in 2010-2011, 2016 (Henderson *et al.,* 1968; Onyango *et al.,* 2004). After the largest YF outbreak in Ethiopia occurred in 1960-1962, YFV antibodies were reported in Northern Kenya (Henderson *et al.,* 1968), which confirmed the extent of exposure. However, our findings could possibly be due to vaccination, though there was no data on the patient's vaccination history. West-Nile Virus and Yellow Fever Virus antibodies were detected in the samples collected. These viruses were recently detected in serosurveys carried out in various parts of the county (Chepkorir *et al.,* 2019; Inziani *et al.,* 2020).

Nairobi County was chosen as a comparative site that has not experienced any arboviral outbreak. However, it recorded (10.6%) of arboviral prevalence by ELISA. This could be attributed to being a major travel hub where travelers come in from different continents as well as domestic tourists, but the travel history of the patients was not captured. This finding has public health implications because surveillance is mainly done in areas where outbreaks have occurred.

5.4 Association of patient demographic characteristics and PRNT findings

Association of age, gender and days from onset of the symptoms and sample collection and PRNT findings show that the prevalence of CHIKV and DENV was significantly higher in those aged 14-60 years. This observation could be attributed to the fact that this age group is active and mostly stays outdoors, where they come in contact with mosquitoes that transmit the viruses.

5.5 Limitations

This study is considered a success, but at the same time acknowledges existence limitations. First, over 90% of the study participants are children, given that measles and rubella surveillance is focused on children. Second, the travel history of the patients was not captured during sample collection.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

1. It was confirmed that patients' had been exposed to arboviral infections DENV, ZIKV and CHIKV in Nairobi, North-Eastern and Coast Regions. These infections are often diagnosed as measles cases; hence misdiagnosis of these arboviral infections occurs, posing a public health concern. Findings of these arboviral infections outside of the reported outbreaks indicate the continuous circulation of these viruses at low levels.
2. The presence of co-exposure indicates co-circulation of these arboviral infections in the same area, emphasizes the need to develop diagnostic kits to test these infections, especially in outbreak-prone areas.
3. Dengue, ZIKV and CHIKV were detected in all the three regions: Nairobi, North-Eastern and Coastal Region from 2008-2014.
4. This study determined that age as a risk factor for dengue and chikungunya viruses. This is an important finding in designing disease control strategies and awareness creation

6.2 Recommendations

1. The findings of this study indicate that clinical diagnosis is not sufficient in detection of febrile rash cases and laboratory confirmation is essential to determine other causes of febrile rash illnesses in Kenya. Arboviruses are overlooked because diagnostic assays are not typically available at the county and sub-county levels. The tests are often performed in very few specialized laboratories at the national level. A further reason is the lack of ELISA kits for other arboviruses, making it difficult to detect these viruses, causing illness.

Awareness creation among the clinicians should be done on these arboviral infections that cause measles-like illnesses.

2. Control measures and active surveillance should be put in place to detect these arboviruses on time and emphasizes should on developing diagnostic kits to test these infections, especially in outbreak-prone areas.
3. Inclusion of arboviruses in the differential diagnosis of the non-measles febrile rash illnesses is a necessity. Further research should be done by testing for additional pathogens that should also be considered in measles/rubella control. A similar periodic reassessment be conducted to gauge the contribution of arboviruses to febrile rash illness in Kenya.
4. Infection regulation measures that target the crucial risk factors ought to be introduced to lessen the disease burden within these regions. Also, the community must be enlightened on the risks factors linked to these diseases and potential control and prevention measures ought to be applied to stop the spread. These strategies must be site and virus specific in order to be effective.

REFERENCES

Adam, A., & Jassoy, C. (2021). Epidemiology and Laboratory Diagnostics of Dengue, Yellow Fever, Zika, and Chikungunya Virus Infections in Africa. *Pathogens (Basel, Switzerland), 10*(10), 1324.

Adesina, O. A., & Odelola, H. A. (1991). Ecological distribution of Chikungunya haemagglutination inhibition antibodies in human and domestic animals in Nigeria. *Trop Geogr Med, 43*(3), 271-275.

Agha, S. B., Chepkorir, E., Mulwa, F., Tigoi, C., Arum, S., Guarido, M. M., Ambala, P Chelangat, B., Lutomiah, J., Tchouassi, D P.,Turell, M J., Sang, R. (2017). Vector competence of populations of *Aedes* aegypti from three distinct cities in Kenya for chikungunya virus. *PLoS Negl Trop Dis, 11*(8), e0005860.

Agha, S. B., Tchouassi, D. P., Bastos, A. D. S., & Sang, R. (2017). Assessment of risk of dengue and yellow fever virus transmission in three major Kenyan cities based on Stegomyia indices. *PLoS Negl Trop Dis, 11*(8), e0005858.

Alhakimi, H. A., Mohamed, O. G., Khogaly, H. S. E., Arafa, K. A. O., & Ahmed, W. A. (2015). Epidemiological, Clinical and Entomological Characteristics of Yellow Fever Outbreak in Darfur2012. *AIMS public health, 2*(1), 132-141.

Alatoom, A., & Payne, D. (2009). An Overview of Arboviruses and Bunyaviruses. *Laboratory Medicine, 40*(4), 237-240.

Amarasinghe, A., Kuritsk, J. N., Letson, G. W., & Margolis, H. S. (2011). Dengue virus infection in Africa. *Emerg Infect Dis, 17*(8), 1349-1354.

Anis, E., Grotto, I., Mendelson, E., Bin, H., Orshan, L., Gandacu, D., Warshavsky, B., Shinar, E., Slater, P. E., & Lev, B. (2014). West Nile fever in Israel: the reemergence of an endemic disease. *TheJournalofinfection*, *68*(2),170–175.

Antonio, V. S., Amade, N. A., Muianga, A. F., Ali, S., Monteiro, V., Mula, F., Chelene, I., Oludele, J., Chongo, I., José, A., Augusto, O., Gudo, E. S. (2019). Retrospective investigation of antibodies against chikungunya virus (CHIKV) in serum from febrile patients in Mozambique, 2009-2015: Implications for its prevention and control. *PLoS One, 14*(3), e0213941.

Artika, I. M., & Ma'roef, C. N. (2017). Laboratory biosafety for handling emerging viruses. *Asian Pacific Journal of Tropical Biomedicine, 7*(5), 483-491.

Artsob, H., Lindsay, R., & Drebot, M. (2017). Arboviruses. In S. R. Quah (Ed.), *International Encyclopedia of Public Health (Second Edition)* (pp. 154-160). Oxford: Academic Press.

Arum, S. O., Weldon, C. W., Orindi, B., Landmann, T., Tchouassi, D. P., Affognon, H. D., & Sang, R. (2015). Distribution and diversity of the vectors of Rift Valley fever along the livestock movement routes in the northeastern and coastal regions of Kenya. *Parasit Vectors, 8*, 294.

Ayukekbong, J. A., Oyero, O. G., Nnukwu, S. E., Mesumbe, H. N., & Fobisong, C. N. (2017). Value of routine dengue diagnosis in endemic countries. *World J Virol, 6*(1), 9-16.

Azfar, N. A., Malik, L. M., Jamil, A., Jahangir, M., Nuzhat, Tirmizi, Majid, A., Ashraf, M. Malik, M. (2013). *Cutaneous manifestations in patients of dengue fever*.

Basile, A. J., Goodman, C., Horiuchi, K., Sloan, A., Johnson, B. W., Kosoy, O., Laven, J. Panella, A. J., Sheets, I., Medina, F., Mendoza, E. J., Epperson, M., Maniatis,

P., Semenova, V., Steward-Clark, E., Wong, E., Biggerstaff, B. J., Lanciotti, R., Drebot, M., Safronetz, D., Schiffer, J. (2018). Multi-laboratory comparison of three commercially available Zika IgM enzyme-linked immunosorbent assays. *J Virol Methods, 260*, 26-33.

Baba, M., Logue, C. H., Oderinde, B., Abdulmaleek, H., Williams, J., Lewis, J., Laws, T. R., Hewson, R., Marcello, A., D. A. (2013). Evidence of arbovirus co-infection in suspected febrile malaria and typhoid patients in Nigeria. *J Infect Dev Ctries, 7*(1), 51-59.

Barrett, A. D. T., & Higgs, S. (2007). Yellow Fever: A Disease that Has Yet to be Conquered. *AnnualRevieof Entomology, 52*(1), 209-229.

Benedict, M. Q., Levine, R. S., Hawley, W. A., & Lounibos, L. P. (2007). Spread of the tiger: global risk of invasion by the mosquito *Aedes* albopictus. *Vector Borne Zoonotic Dis, 7*(1), 76-85.

Beatty, M., Letson, G., Edgil, D., & Margolis, H. (2007). Estimating the total world population at risk for locally acquired dengue infection. Abstract presented at the 56th Annual Meeting of the American Society of Tropical Medicine and Hygiene. *Am J Trop Med Hyg, 77*, 170-257.

Berthet, N., Nakouné, E., Kamgang, B., Selekon, B., Descorps-Declère, S., Gessain, A.,Manuguerra, J. C., Kazanji, M. (2014). Molecular characterization of three Zika flaviviruses obtained from sylvatic mosquitoes in the Central African Republic. *Vector Borne Zoonotic Dis, 14*(12), 862-865.

Braack, L., Gouveia de Almeida, A. P., Cornel, A. J., Swanepoel, R., & de Jager, C. (2018). Mosquito-borne arboviruses of African origin: review of key viruses and vectors. *Parasites & vectors, 11*(1), 29-29

Burt, F. J., Grobbelaar, A. A., Leman, P. A., Anthony, F. S., Gibson, G. V. F., & Swanepoel, R. (2002). Phylogenetic relationships of southern African West Nile virus isolates. *Emerg Infect Dis, 8*(8), 820-826. doi:10.3201/eid0808.020027.

Caron, M., Paupy, C., Grard, G., Becquart, P., Mombo, I., Nso, B. B., Kassa Kassa, F., Nkoghe, D., Leroy, E. M. (2012). Recent introduction and rapid dissemination of Chikungunya virus and Dengue virus serotype 2 associated with human and mosquito coinfections in Gabon, central Africa. *Clin Infect Dis, 55*(6), e45-53.

Crabtree, M., Sang, R., Lutomiah, J., Richardson, J., & Miller, B. (2009). Arbovirus surveillance of mosquitoes collected at sites of active Rift Valley fever virus transmission: Kenya, 2006-2007. *J Med Entomol, 46*(4), 961-964.

Cauchemez, S., Besnard, M., Bompard, P., Dub, T., Guillemette-Artur, P., Eyrolle-Guignot, D., Salje, H., Van Kerkhove, M. D., Abadie, V. Garel, C., Fontanet, A., Mallet, H. P. (2016). Association between Zika virus and microcephaly in French Polynesia, 2013-15: a retrospective study. *Lancet, 387*(10033), 2125-2132.

Chretien, J. P., Anyamba, A., Bedno, S. A., Breiman, R. F., Sang, R., Sergon, K., Powers, A. M. Onyango, C. O., Small, J. Tucker, C. J., Linthicum, K. J. (2007). Drought-associated chikungunya emergence along coastal East Africa. *Am J Trop Med Hyg, 76*(3), 405-407.

Centres of Disease Control (2021). Measles.Retrieved from https://www.cdc.gov/vaccines/pubs/pinkbook/downloads/meas.pdf

CDC (2018). Zika MAC-ELISA. Retrieved from https://www.cdc.gov/zika/pdfs/zika-mac-elisa-instructions-for-use.pdf

Chan, M., & Johansson, M. A. (2012). The Incubation Periods of Dengue Viruses. *PLoS One, 7*(11), e50972..

Chepkorir, E., Lutomiah, J., Mutisya, J., Mulwa, F., Limbaso, K., Orindi, B., Ng'ang'a, Z.,Sang, R. (2014). Vector competence of *Aedes* aegypti populations from Kilifi and Nairobi for dengue 2 virus and the influence of temperature. *Parasit Vectors, 7*, 435.

Chepkorir, E., Tchouassi, D. P., Konongoi, S. L., Lutomiah, J., Tigoi, C., Irura, Z., Eyase, F., Venter, M., Sang, R. (2019). Serological evidence of Flavivirus circulation in human populations in Northern Kenya: an assessment of disease risk 2016–2017. *Virology Journal, 16*(1), 65.

Cochran, W. G. (1963). Sampling Techniques.

Collins, N. D., & Barrett, A. D. T. (2017). Live Attenuated Yellow Fever 17D Vaccine: A Legacy Vaccine Still Controlling Outbreaks In Modern Day. *Current infectious disease reports, 19*(3), 14-14.

Cordel, N., Birembaux, X., Chaumont, H., Delion, F., Chosidow, O., Tressières, B., & Herrmann Storck, C. (2017). Main Characteristics of Zika Virus Exanthema in Guadeloupe. *JAMA Dermatology, 153*(4), 326-328.

Crabtree, M., Sang, R., Lutomiah, J., Richardson, J., & Miller, B. (2009). Arbovirus surveillance of mosquitoes collected at sites of active Rift Valley fever virus transmission: Kenya, 2006-2007. *J Med Entomol, 46*(4), 961-964.

Danis, K., Papa, A., Theocharopoulos, G., Dougas, G., Athanasiou, M., Detsis, M., Baka, A., Lytras, T., Mellou, K., Bonovas, S., Panagiotopoulos, T. (2011). Outbreak of West Nile virus infection in Greece, 2010. *Emerg Infect Dis, 17*(10), 1868-1872.

Davis, L. E., DeBiasi, R., Goade, D. E., Haaland, K. Y., Harrington, J. A., Harnar, J. B., Pergam, S. A., King, M. K., DeMasters, B. K., Tyler, K. L. (2006). West Nile virus neuroinvasive disease. *Ann Neurol, 60*(3), 286-300.

De Moraes, J. C., Toscano, C. M., de Barros, E. N., Kemp, B., Lievano, F., Jacobson, S., Afonso, A.M. S., Strebel, P. M., Cairns, K. L. (2011). Etiologies of rash and fever illnesses in Campinas, Brazil. *J Infect Dis, 204 Suppl 2*, S627-636.

de Oliveira, W. K., de França, G. V. A., Carmo, E. H., Duncan, B. B., de Souza Kuchenbecker, R., & Schmidt, M. I. (2017). Infection-related microcephaly after the 2015 and 2016 Zika virus outbreaks in Brazil: a surveillance-based analysis. *The Lancet, 390*(10097), 861-870.

Duffy, M. R., Chen, T.-H., Hancock, W. T., Powers, A. M., Kool, J. L., Lanciotti, R. S.,Pretrick,M., Marfel, M., Holzbauer, S., Dubray, C., Guillaumot, L., Griggs, A., Bel, M., Lambert, A. J., Laven, J., Kosoy, O., Panella, A., Biggerstaff, B. J., Fischer, M., Hayes, E. B. (2009). Zika Virus Outbreak on Yap Island, Federated States of Micronesia. *New England Journal of Medicine, 360*(24), 2536-2543.

Elliott, R. M. (2009). Bunyaviruses and climate change. *Clinical Microbiology and Infection, 15*(6), 510-517.

Ellis, B. R., & Barrett, A. D. T. (2008). The enigma of yellow fever in East Africa. *Rev Med Virol, 18*(5), 331-346. doi:10.1002/rmv.584

Ellis, E. M., Neatherlin, J. C., Delorey, M., Ochieng, M., Mohamed, A. H., Mogeni, D. O., Hunsperger, E., Patta, S., Gikunju, S., Waiboic, L., Fields, B., Ofula, V., Konongoi, S.L, Torres-Velasquez, B., Marano, N., Sang, R., Margolis, H.S., Montgomery, J. M., Tomashek, K. M. (2015). A household serosurvey to

estimate the magnitude of a dengue outbreak in Mombasa, Kenya, 2013. *PLoS Negl Trop Dis, 9*(4), e0003733.

Expanded Programme on Immunization (EPI), (2015). [Measles laboratory data].

Failloux, A.-B., Bouattour, A., Faraj, C., Gunay, F., Haddad, N., Harrat, Z., Jancheska, E., Kanani, K.,Kenawy, M. A., Kota, M., Pajovic, I., Paronyan, L., Petric, D., Sarih, M., Sawalha, S., Shaibi, T. Sherifi, K., Sulesco, T., Velo, E., Gaayeb, L.,Victoir, K., Robert, V. (2017). Surveillance of Arthropod-Borne Viruses and Their Vectors in the Mediterranean and Black Sea Regions Within the MediLabSecure Network. *Current tropical medicine reports, 4*(1), 27-39.

Fujita, R., Kato, F., Kobayashi, D., Murota, K., Takasaki, T., Tajima, S., Lim, C., Saijo, M.,Isawa, H., Sawabe, K. (2018). Persistent viruses in mosquito cultured cell line suppress the multiplication of flaviviruses. *Heliyon, 4*(8), e00736. doi:

Fölster-Holst, R., & Kreth, H. W. (2009). Viral exanthems in childhood – infectious (direct) exanthems. Part 1: Classic exanthems. *JDDG: Journal der Deutschen Dermatologischen Gesellschaft, 7*(4), 309-316.

Fonseca, D. M., Keyghobadi, N., Malcolm, C. A., Mehmet, C., Schaffner, F., Mogi, M., Fleischer, R.C., Wilkerson, R. C (2004). Emerging vectors in the Culex pipiens complex. *Science, 303*(5663), 1535-1538

Furuya-Kanamori, L., Liang, S., Milinovich, G., Soares Magalhaes, R. J., Clements, A. C. A., Hu, W., Brasil, P., Frentiu, F. D.. Dunning, R., Yakob, L. (2016). Co-distribution and co-infection of chikungunya and dengue viruses. *BMC Infect Dis, 16*(1), 84.

Gan, V. C. H., & Leo, Y. S. (2014). Current epidemiology and clinical practice in arboviral infections - implications on blood supply in South-East Asia. *ISBT science series, 9*(1), 262-267.

Gobillot, T. A., Kikawa, C., Lehman, D. A., Kinuthia, J., Drake, A. L., Jaoko, W., Mandaliya, K., John-Stewart, G., McClelland, R. S., Overbaugh, J. (2020). Zika Virus Circulates at Low Levels in Western and Coastal Kenya. *J Infect Dis, 222*(5), 847-852

Goh, V. S. L., Mok, C.-K., & Chu, J. J. H. (2020). Antiviral Natural Products for Arbovirus Infections. *Molecules (Basel, Switzerland), 25*(12), 2796.

Gould, E. A., & Higgs, S. (2009). Impact of climate change and other factors on emerging arbovirus diseases. *Trans R Soc Trop Med Hyg, 103*(2), 109-121.

Gould, L. H., Osman, M. S., Farnon, E. C., Griffith, K. S., Godsey, M. S., Karch, S., Mulenda, B. El Kholy, A., Grandesso, F., de Radiguès, X., Brair, M. E., Briand, S., El Tayeb el, S. M., Hayes, E. B., Zeller, H., Perea, W. (2008). An outbreak of yellow fever with concurrent chikungunya virus transmission in South Kordofan, Sudan, 2005. *Trans R Soc Trop Med Hyg, 102*(12), 1247-1254.

Gubler, D. J. (2001). Human arbovirus infections worldwide. *Ann N Y Acad Sci, 951*, 13-24

Gubler, D. J. (2002). The global emergence/resurgence of arboviral diseases as public health problems. *Arch Med Res, 33*(4), 330-342.

Gubler, D. J., Vasilakis, N., & Musso, D. (2017). History and Emergence of Zika Virus. *J Infect Dis, 216*(suppl_10), S860-S867.

Guzman, M. G., Halstead, S. B., Artsob, H., Buchy, P., Farrar, J., Gubler, D. J., Hunsperger, E., Kroeger, A., Margolis, H, S., Martínez, E., Nathan, M. B. Pelegrino, Jose Luis Simmons, Cameron Yoksan, Sutee Peeling, R. W. (2010). Dengue: a continuing global threat. *Nature Reviews Microbiology, 8*(12), S7-S16.

Hayes, E. B., Komar, N., Nasci, R. S., Montgomery, S. P., O'Leary, D. R., & Campbell, G. L. (2005). Epidemiology and transmission dynamics of West Nile virus disease. *Emerg Infect Dis, 11*(8), 1167-1173.

Heinisch, O. (1965). Cochran, W. G.: Sampling Techniques, 2. Aufl. John Wiley and Sons, New York, London 1963. Preis s. *Biometrische Zeitschrift, 7*(3), 203-203.

Huang, Y.-J. S., Higgs, S., & Vanlandingham, D. L. (2019). Arbovirus-Mosquito Vector-Host Interactions and the Impact on Transmission and Disease Pathogenesis of Arboviruses. *Frontiers in microbiology, 10*, 22-22.

Hunsperger, E., Odhiambo, D., Makio, A., Alando, M., Ochieng, M., Omballa, V., Munyua Peninah., Bigogo Godrey., Njenga Kariuki., Widdowson, Marc.-Alain. (2020). Zika Virus Detection with 2013 SerosurveyMombasa, Kenya. *Emerging Infectious Disease journal, 26*(7), 1603.

Husada, D., Kusdwijono, Puspitasari, D., Kartina, L., Basuki, P. S., & Ismoedijanto. (2020). An evaluation of the clinical features of measles virus infection for diagnosis in children within a limited resources setting. *BMC Pediatrics, 2*1), 5-5.

Igarashi, A. (2008). Production of Type 2 Dengue (D2) Monoclonal Antibody and Cell Culture Derived D2 Antigen for Use in Dengue IgM-Capture ELISA.

Inziani, M., Adungo, F., Awando, J., Kihoro, R., Inoue, S., Morita, K., Obimbo, E., Onyango, F., Mwau, M. (2020). Seroprevalence of yellow fever, dengue, West Nile and chikungunya viruses in children in Teso South Sub-County, Western Kenya. *International Journal of Infectious Diseases, 91*, 104-110.

Ioos, S., Mallet, H. P., Leparc Goffart, I., Gauthier, V., Cardoso, T., & Herida, M. (2014). Current Zika virus epidemiology and recent epidemics. *Med Mal Infect, 44*(7), 302-307. doi:10.1016/j.medmal.2014.04.008

Iwamoto, M., Jernigan, D. B., Guasch, A., Trepka, M. J., Blackmore, C. G., Hellinger,W.C.,.Petersen, L. R. (2003). Transmission of West Nile Virus from an Organ Donor to Four Transplant Recipients. *New England Journal of Medicine, 348*(22), 2196-2203.

Jones, K. E., Patel, N. G., Levy, M. A., Storeygard, A., Balk, D., Gittleman, J. L., & Daszak, P. (2008). Global trends in emerging infectious diseases. *Nature, 451*(7181), 990-993.

Kajeguka, D. C., Kaaya, R. D., Mwakalinga, S., Ndossi, R., Ndaro, A., Chilongola, J. O., Mosha, Franklin W., Schiøler, Karin L., Kavishe, Reginald A., Alifrangis, M. (2016). Prevalence of dengue and chikungunya virus infections in north-eastern Tanzania: a cross sectional study among participants presenting with malaria-like symptoms. *BMC Infect Dis, 16*, 183-183. doi:10.1186/s12879-016-1511-5

Kaur, P., Lee, R. C., & Chu, J. J. (2016). Infectious Viral Quantification of Chikungunya Virus-Virus Plaque Assay. *Methods Mol Biol, 1426*, 93-103. doi:10.1007/978-1-4939-3618-2_9

Kakumani, P. K., Ponia, S. S., S, R. K., Sood, V., Chinnappan, M., Banerjea, A. C., Medigeshi, G. R., Malhotra, P., Mukherjee, S. K., Bhatnagar, R. K. (2013). Role of RNA interference (RNAi) in dengue virus replication and identification of NS4B as an RNAi suppressor. *J Virol, 87*(16), 8870-8883.

Kang, J. H. (2015). Febrile Illness with Skin Rashes. *Infection & chemotherapy, 47*(3), 155-166.

Kariuki Njenga, M., Nderitu, L., Ledermann, J. P., Ndirangu, A., Logue, C. H., Kelly, C. H. L., Sang, R., Sergon, K., Breiman, R .Powers, A. M. (2008). Tracking epidemic Chikungunya virus into the Indian Ocean from East Africa. *J Gen Virol, 89*(Pt 11), 2754-2760

Karungu, S., Atoni, E., Ogalo, J., Mwaliko, C., Agwanda, B., Yuan, Z., & Hu, X. (2019). Mosquitoes of Etiological Concern in Kenya and Possible Control Strategies. *Insects, 10*(6).

Karpf, A. R., Lenches, E., Strauss, E. G., Strauss, J. H., & Brown, D. T. (1997). Superinfection exclusion of alphaviruses in three mosquito cell lines persistently infected with Sindbis virus. *Journal of Virology, 71*(9), 7119-7123.

Kindhauser, M. K., Allen, T., Frank, V., Santhana, R. S., & Dye, C. (2016). Zika: the origin and spread of mosquito-borne virus. *Bulletin of the World Health Organization, 94*(9), 675-686.

Kisangau, E., Sergon, K., Ibrahim, Y., Yonga, F., Langat, D., Nzunza, R., Borus, Peter Galgalo, Tura Lowther, S. (2018). Progress towards elimination of measles in Kenya, 2003-2016. *Pan African Medical Journal, 31*.

Kisuya, B., Masika, M. M., Bahizire, E., & Oyugi, J. O. (2019). Seroprevalence of Zika virus in selected regions in Kenya. *Trans R Soc Trop Med Hyg, 113*(12), 735-739.

Kiwanuka, N., Sanders, E. J., Rwaguma, E. B., Kawamata, J., Ssengooba, F. P., Najjemba, R., . . .Campbell, G.L (1999). O'nyong-nyong fever in south-central Uganda, 1996-1997: clinical features and validation of a clinical case definition for surveillance purposes. *Clin Infect Dis, 29*(5), 1243-1250

Kolawole, O. M., Seriki, A. A., Irekeola, A. A., & Ogah, J. I. (2018). The Neglect and Fast Spread of Some Arboviruses: A Note for Healthcare Providers in Nigeria. *Diseases (Basel, Switzerland), 6*(4), 99.

Konongoi, L., Ofula, V., Nyunja, A., Owaka, S., Koka, H., Makio, A., Koskei, E., Eyase, F.,Langat, D., Schoepp, R. J., Rossi, C. A., Njeru, I., Colden, R., Sang, R. (2016). Detection of dengue virus serotypes 1, 2 and 3 in selected regions of Kenya: 2011-2014. *Virol J, 13*(1), 182.

Konongoi, S. L., Nyunja, A., Ofula, V., Owaka, S., Koka, H., Koskei, E., Eyase, F.,Langat, D., Mancuso, J., Lutomiah, J., Sang, R. (2018). Human and entomologic investigations of chikungunya outbreak in Mandera, Northeastern Kenya, 2016. *PLoS One, 13*(10), e0205058.

Kuniholm, M. H., Wolfe, N. D., Huang, C. Y., Mpoudi-Ngole, E., Tamoufe, U., LeBreton, M., Burke, D.S., Gubler, D. J. (2006). Seroprevalence and distribution of Flaviviridae, Togaviridae, and Bunyaviridae arboviral infections in rural Cameroonian adults. *Am J Trop Med Hyg, 74*(6), 1078-1083.

Kuno, G., & Chang, G.-J. J. (2005). Biological transmission of arboviruses: reexamination of and new insights into components, mechanisms, and unique

traits as well as their evolutionary trends. *Clinical microbiology reviews, 18*(4), 608-637.

Kutsuna, S., Saito, S., & Ohmagari, N. (2020). Simultaneous diagnosis of dengue virus, Chikungunya virus, and Zika virus infection using a new point-of-care testing (POCT) system based on the loop-mediated isothermal amplification (LAMP) method. *J Infect Chemother, 26*(12), 1249-1253.

Labeaud, A., Banda, T., Teng, C., Heimbaugh, C., Muchiri, E., Mungai, P., Mutuku, F.,Bustinduy, A., Gildengorin, G., Long, K., Harris, E., Weaver, Scott., Heise, M., Powers, A.,Kitron, U., King, C. (2013). *Dengue and Chikungunya Seroprevalence in Rural Coastal Kenya*.

LaBeaud, A. D., Sutherland, L. J., Muiruri, S., Muchiri, E. M., Gray, L. R., Zimmerman, P. A., Hise, A. G., King, C.H (2011). Arbovirus prevalence in mosquitoes, Kenya. *Emerg Infect Dis, 17*(2), 233-241.

LaBeaud, A. D., Banda, T., Brichard, J., Muchiri, E. M., Mungai, P. L., Mutuku, F. M., Borland, E., Gildengorin, G., Pfeil, Sarah., Teng, C. Y., Long, K., Heise, M., Powers, A. M., Kitron, U., King, C. H. (2015). High rates of o'nyong nyong and Chikungunya virus transmission in coastal Kenya. *PLoS Negl Trop Dis, 9*(2), e0003436.

LaBeaud, A. D., Sutherland, L. J., Muiruri, S., Muchiri, E. M., Gray, L. R., Zimmerman, P. A., Hise, A. G., King, C.H (2011). Arbovirus prevalence in mosquitoes, Kenya. *Emerg Infect Dis, 17*(2), 233-241.

Ladhani, S. N., Connor, C., Kirkbride, H., Brooks, T., & Morgan, D. (2016). Outbreak of Zika virus disease in the Americas and the association with microcephaly,

congenital malformations and Guillain–Barré syndrome. *Archives of Disease in Childhood, 101*(7), 600

Laine, M., Luukkainen, R., & Toivanen, A. (2004). Sindbis viruses and other alphaviruses as cause of human arthritic disease. *Journal of Internal Medicine, 256*(6), 457-471

Lanciotti, R. S., & Valadere, A. M. (2014). Transcontinental movement of Asian genotype chikungunya virus. *Emerg Infect Dis, 20*(8), 1400-1402.

Leroy, E. M., Nkoghe, D., Ollomo, B., Nze-Nkogue, C., Becquart, P., Grard, G., Pourrut, X.,Charrel, R., Moureau, G., Ndjoyi-Mbiguino, A., De Lamballerie, X. (2009). Concurrent Chikungunya and Dengue Virus Infections during Simultaneous Outbreaks, Gabon, 2007. *Emerg Infect Dis, 15*(4), 591-593.

Lilay, A., Asamene, N., Bekele, A., Mengesha, M., Wendabeku, M., Tareke, I., Girmay, A., Wuletaw, Y., Adossa, A., Ba, Y., Sall, A., Jima, D., Mengesha, D. (2017). Reemergence of yellow fever in Ethiopia after 50 years, 2013: epidemiological and entomological investigations. *BMC Infect Dis, 17*(1), 343-343.

Lima, J. R. C., Rouquayrol, M. Z., Callado, M. R. M., Guedes, M. I. F., & Pessoa, C. (2012). Interpretation of the presence of IgM and IgG antibodies in a rapid test for dengue: analysis of dengue antibody prevalence in Fortaleza City in the 20th year of the epidemic. *Revista da Sociedade Brasileira de Medicina Tropical, 45*, 163-167.

Linthicum, K. J., Anyamba, A., Tucker, C. J., Kelley, P. W., Myers, M. F., & Peters, C. J. (1999). Climate and satellite indicators to forecast Rift Valley fever epidemics in Kenya. *Science, 285*(5426), 397-400.

Lutomiah, J., Barrera, R., Makio, A., Mutisya, J., Koka, H., Owaka, S., Koskei, Edith., Nyunja, Albert., Eyase, Fredrick.,Coldren, Rodney., Sang, R. (2016). Dengue Outbreak in Mombasa City, Kenya, 2013–2014: Entomologic Investigations. *PLoS Negl Trop Dis, 10*(10).

Lutomiah, J., Bast, J., Clark, J., Richardson, J., Yalwala, S., Oullo, D., Mutisya, J., Mulwa, F., Musila, L., Khamadi, S., Schnabel, D., Wurapa, E., Sang, R. (2013). Abundance, diversity, and distribution of mosquito vectors in selected ecological regions of Kenya: public health implications. *J Vector Ecol, 38*(1), 134-142.

Ludlow, M., Kortekaas, J., Herden, C., Hoffmann, B., Tappe, D., Trebst, C., Griffin, D. E.,Brindle, H. E., Solomon, T., Brown, A. S.,van Riel, D., Wolthers, K. C., Pajkrt, D., Wohlsein, P. Martina, B. E. E., Baumgärtner, W., Verjans, G. M., Osterhaus, A. D. M. E. (2016). Neurotropic virus infections as the cause of immediate and delayed neuropathology. *Acta Neuropathologica, 131*(2), 159-184.

Lwande, O. W., Lutomiah, J., Obanda, V., Gakuya, F., Mutisya, J., Mulwa, F., Michuki, G. Chepkorir, E., Fischer, A., Venter, M., Sang, R. (2013). Isolation of tick and mosquito-borne arboviruses from ticks sampled from livestock and wild animal hosts in Ijara District, Kenya. *Vector Borne Zoonotic Dis, 13*(9), 637-642.

Mackenzie, J. S., Gubler, D. J., & Petersen, L. R. (2004). Emerging flaviviruses: the spread and resurgence of Japanese encephalitis, West Nile and dengue viruses. *Nat Med, 10*(12 Suppl), S98-109.

Maeki, T., Tajima, S., Ikeda, M., Kato, F., Taniguchi, S., Nakayama, E., Takasaki, T.., Lim, C. K. Saijo, M. (2019). Analysis of cross-reactivity between flaviviruses with sera of patients with Japanese encephalitis showed the importance of

neutralization tests for the diagnosis of Japanese encephalitis. *Journal of Infection and Chemotherapy, 25*(10), 786-790.

Malkinson, M., & Banet, C. (2002). The role of birds in the ecology of West Nile virus in Europe and Africa. *Curr Top Microbiol Immunol, 267*, 309-322.

Mary Muyeku. (2011). Seroprevalence of Chikungunya,Yellow Fever and West Nile viruses in children at the Alupe District Hospital in Western Kenya. (M.Med), University of Nairobi, University of Nairobi, Kenya. Retrieved from http://erepository.uonbi.ac.ke/handle/11295/3785?show=full

Mathiot, C. C., Grimaud, G., Garry, P., Bouquety, J. C., Mada, A., Daguisy, A. M., & Georges, A. J. (1990). An Outbreak of Human Semliki Forest Virus Infections in Central African Republic. *The American journal of tropical medicine and hygiene, 42*(4), 386-393.

Mathiot, C. C., Grimaud, G., Garry, P., Bouquety, J. C., Mada, A., Daguisy, A. M., & Georges, A. J. (1990). An outbreak of human Semliki Forest virus infections in Central African Republic. *Am J Trop Med Hyg, 42*(4), 386-393.

Mbaika, S., Lutomiah, J., Chepkorir, E., Mulwa, F., Khayeka-Wandabwa, C., Tigoi, C., Oyoo-Okoth, E., Mutisya, J., Ng'ang'a, Z.,Sang, R. (2016). Vector competence of *Aedes* aegypti in transmitting Chikungunya virus: effects and implications of extrinsic incubation temperature on dissemination and infection rates. *Virol J, 13*, 114.

McIntosh, B. M., Worth, C. B., & Kokernot, R. H. (1961). Isolation of Semliki Forest virus from *Aedes* (Aedimorphus) argenteopunctatus (Theobald) collected in Portuguese East Africa. *Trans R Soc Trop Med Hyg, 55*, 192-198.

Mease, L. E., Coldren, R. L., Musila, L. A., Prosser, T., Ogolla, F., Ofula, V. O., Schoepp, Randal J., Rossi, Cindy A., Adungo, N. (2011). Seroprevalence and distribution of arboviral infections among rural Kenyan adults: a cross-sectional study. *Virol J, 8*, 371.

Mercado-Reyes, M., Acosta-Reyes, J., Navarro-Lechuga, E., Corchuelo, S., Rico, A., Parra, E., Tolosa, N. Pardo, L., González, M., Martìn-Rodriguez-Hernández, J., Karime-Osorio, L., Ospina-Martinez, M., Rodriguez-Perea, H., Del Rio-Pertuz, G., Viasus, D. (2019). Dengue, chikungunya and zika virus coinfection: results of the national surveillance during the zika epidemic in Colombia. *Epidemiol Infect, 147*.

Montoya, M., Collins, M., Dejnirattisai, W., Katzelnick, L. C., Puerta-Guardo, H., Jadi, R., Schildhauer, S., Supasa, P., Vasanawathana, S., Malasit, P., Mongkolsapaya, J., de Silva, A.D., Tissera, H., Balmaseda, A., Screaton, G., de Silva, A. M., Harris, E. (2018). Longitudinal Analysis of Antibody Cross-neutralization Following Zika Virus and Dengue Virus Infection in Asia and the Americas. *J Infect Dis, 218*(4), 536-545.

Morens, D. M., Folkers, G. K., & Fauci, A. S. (2004). The challenge of emerging and re-emerging infectious diseases. *Nature, 430*(6996), 242-249.

Morrill, J. C., Johnson, B. K., Hyams, C., Okoth, F., Tukei, P. M., Mugambi, M., & Woody, J. (1991). Serological evidence of arboviral infections among humans of coastal Kenya. *J Trop Med Hyg, 94*(3), 166-168.

Mulwa, F., Lutomiah, J., Chepkorir, E., Okello, S., Eyase, F., Tigoi, C., Kahato, M., Sang, R. (2018). Vector competence of *Aedes* bromeliae and *Aedes* vitattus mosquito populations from Kenya for chikungunya virus. *PLoS Negl Trop Dis, 12*(10), e0006746.

Murray, K. O., Garcia, M. N., Yan, C., & Gorchakov, R. (2013). Persistence of detectable immunoglobulin M antibodies up to 8 years after infection with West Nile virus. *The American journal of tropical medicine and hygiene, 89*(5), 996-1000.

Musa, A. A., Muturi, M. W., Musyoki, A. M., Ouso, D. O., Oundo, J. W., Makhulu, E. E., Wambua, L.,Villinger, J., Jeneby, M. M. (2020). Arboviruses and Blood Meal Sources in Zoophilic Mosquitoes at Human-Wildlife Interfaces in Kenya. *Vector Borne Zoonotic Dis, 20*(6), 444-453.

Musso, D., Rouault, E., Teissier, A., Lanteri, M. C., Zisou, K., Broult, J., Grange, E. Nhan, T. X., Aubry, M. (2017). Molecular detection of Zika virus in blood and RNA load determination during the French Polynesian outbreak. *J Med Virol, 89*(9), 1505-1510.

Naing L, W. T., Rusli BN. (2006). Practical issues in calculating the sample size for prevalence studies.Archives of Orofacial Sciences(1),914.doi:http://citeseerx.ist.psu.edu/viewdoc/download?

Nandy, R., Handzel, T., Zaneidou, M., Biey, J., Coddy, R. Z., Perry, R., Strebel, P., Cairns, L. (2006). Case-fatality rate during a measles outbreak in eastern Niger in 2003. *Clin Infect Dis, 42*(3), 322-328

Nedjadi, T., El-Kafrawy, S., Sohrab, S. S., Desprès, P., Damanhouri, G., & Azhar, E. (2015). Tackling dengue fever: Current status and challenges. *Virology Journal, 12*, 212-212.

Ngoi, C. N., Price, M. A., Fields, B., Bonventure, J., Ochieng, C., Mwashigadi, G., Hassan, Amin S., Thiong'o, Alexander N., Micheni, Murugi., Mugo, Peter., Graham, Susan., Sanders, E. J. (2016). Dengue and Chikungunya Virus

Infections among Young Febrile Adults Evaluated for Acute HIV-1 Infection in Coastal Kenya. *PLoS One, 11*(12), e0167508.

Nuckols, J. T., Huang, Y. J. S., Higgs, S., Miller, A. L., Pyles, R. B., Spratt, H. M., Horne, K. M., Vanlandingham, D. L. (2015). Evaluation of Simultaneous Transmission of Chikungunya Virus and Dengue Virus Type 2 in Infected *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae). *J Med Entomol, 52*(3), 447-451

Nyamwaya, D., Wang'ondu, V., Amimo, J., Michuki, G., Ogugo, M., Ontiri, E., Sang, R., Lindahl, J., Grace, D., Bett, B. (2016). Detection of West Nile virus in wild birds in Tana River and Garissa Counties, Kenya. *BMC Infect Dis, 16*(1), 696.

O'Meara, W. P., Mott, J. A., Laktabai, J., Wamburu, K., Fields, B., Armstrong, J., Taylor, S. M., MacIntyre, C., Sen, R., Menya, D., Pan, W., Nicholson, B. P., Woods, C. W.,

Holland, T. L. (2015). Etiology of pediatric fever in western Kenya: a case-control study of falciparum malaria, respiratory viruses, and streptococcal pharyngitis. *Am J Trop Med Hyg, 92*(5), 1030-1037

Ochieng, C., Ahenda, P., Vittor, A. Y., Nyoka, R., Gikunju, S., Wachira, C., Waiboci, Lilian., Umuro, Mamo., Kim, Andrea A., Nderitu, Leonard., Juma, Bonventure., Montgomery, Joel M. Breiman, Robert F. Fields, B. (2015). Seroprevalence of Infections with Dengue, Rift Valley Fever and Chikungunya Viruses in Kenya, 2007. *PLoS One, 10*(7), e0132645.

Ochieng, C., Lutomiah, J., Makio, A., Koka, H., Chepkorir, E., Yalwala, S., Mutisya, James. Musila, Lillian., Khamadi, Samoel., Richardson, Jason., Bast, Joshua., Schnabel, David., Wurapa, Eyako Sang, R. (2013). Mosquito-borne arbovirus

surveillance at selected sites in diverse ecological zones of Kenya; 2007 - 2012. *Virology Journal, 10*, 140-140.

Ofula, V. O., Oundo, J., Irura, Z., Chepkorir, E., Tigoi, C., Ongus, J., Coldren, R., Sang, R., Schoepp, R., Rossi, C. (2016). Evidence of presence of antibodies against selected arboviruses in Ijara and Marigat Districts, Kenya. *International Journal of Infectious Diseases, 45*, 188-189.

Oehler, E., Watrin, L., Larre, P., Leparc-Goffart, I., Lastere, S., Valour, F., Baudouin, L., Mallet, H.,Musso, D., Ghawche, F. (2014). Zika virus infection complicated by Guillain-Barre syndrome--case report, French Polynesia, December 2013. *Euro Surveill, 19*(9).

Olaleye, O. D., Omilabu, S. A., Ilomechina, E. N., & Fagbami, A. H. (1990). A survey for haemagglutination-inhibiting antibody to West Nile virus in human and animal sera in Nigeria. *Comp Immunol Microbiol Infect Dis, 13*(1), 35-39.

Oliveira, S. A., Siqueira, M. M., Camacho, L. A., Nogueira, R. M., Spinetti, C. C., Cubel Garcia, R. C., Knowles, W., Brown, D. W. (2001). The aetiology of maculopapular rash diseases in Niterói, State of Rio de Janeiro, Brazil: implications for measles surveillance. *Epidemiology and infection, 127*(3), 509-

Onoja, A., & Ajagbe, O. (2019). Measles in Developing Countries.

Paixão, E. S., Barreto, F., Teixeira, M. d. G., Costa, M. d. C. N., & Rodrigues, L. C. (2016). History, Epidemiology, and Clinical Manifestations of Zika: A Systematic Review. *American journal of public health, 106*(4), 606-612.

Paixao, E. S., Teixeira, M. G., & Rodrigues, L. C. (2018). Zika, chikungunya and dengue: the causes and threats of new and re-emerging arboviral diseases. *BMJ Glob Health, 3*(Suppl 1), e000530

Patrican, L. A., & DeFoliart, G. R. (1987). *Aedes* triseriatus and La Crosse virus: similar venereal infection rates in females given the first bloodmeal immediately before mating or several days after mating. *Am J Trop Med Hyg, 36*(3), 648-652.

Perry, R. T., & Halsey, N. A. (2004). The Clinical Significance of Measles: A Review. *J Infect Dis, 189*(Supplement_1), S4-S16

Petersen, L. R., Jamieson, D. J., Powers, A. M., & Honein, M. A. (2016). Zika Virus. *N Engl J Med, 374*(16), 1552-1563.

Pfeffer, M., & Dobler, G. (2010). Emergence of zoonotic arboviruses by animal trade and migration. *Parasites & Vectors, 3*(1), 35

Pierson, T. C., & Diamond, M. S. (2020). The continued threat of emerging flaviviruses. *Nature Microbiology, 5*(6), 796-812

Powers, A. M., & Logue, C. H. (2007). Changing patterns of chikungunya virus: re-emergence of a zoonotic arbovirus. *J Gen Virol, 88*(Pt 9), 2363-2377.

Reisen, W., & Brault, A. C. (2007). West Nile virus in North America: perspectives on epidemiology and intervention. *Pest Manag Sci, 63*(7), 641-646.

Rezza, G., Chen, R., & Weaver, S. C. (2017). O'nyong-nyong fever: a neglected mosquito-borne viral disease. *Pathogens and global health, 111*(6), 271-275.

Rohani, A., Potiwat, R., Zamree, I., & Lee, H. L. (2009). Refractoriness of *Aedes aegypti* (Linnaeus) to dual infection with dengue and chikungunya virus. *Southeast Asian J Trop Med Public Health, 40*(3), 443-448.

Ross, T. M. (2010). Dengue virus. *Clinics in laboratory medicine, 30*(1), 149-160.

Russell, P. K., & Nisalak, A. (1967). Dengue virus identification by the plaque reduction neutralization test. *J Immunol, 99*(2), 291-296.

Roehrig, J. T., Hombach, J., & Barrett, A. D. (2008). Guidelines for Plaque-Reduction Neutralization Testing of Human Antibodies to Dengue Viruses. *Viral Immunol, 21*(2), 123-132.

Rwaguma, E. B., Lutwama, J. J., Sempala, S. D., Kiwanuka, N., Kamugisha, J., Okware, S., Bagambisa, G., Lanciotti, R., Roehrig, J. T., Gubler, D. J. (1997). Emergence of epidemic O'nyong-nyong fever in southwestern Uganda, after an absence of 35 years. *Emerg Infect Dis, 3*(1), 77-77.

Shan, C., Xie, X., Ren, P., Loeffelholz, M. J., Yang, Y., Furuya, A., Dupuis, A. P., 2nd.,Kramer, L. D.,Wong, S. J., Shi, P. Y. (2017). A Rapid Zika Diagnostic Assay to Measure Neutralizing Antibodies in Patients. *EBioMedicine, 17*, 157-162

Sampathkumar, P. (2003). West Nile Virus: Epidemiology, Clinical Presentation, Diagnosis, and Prevention. *Mayo Clinic Proceedings, 78*(9), 1137-1144.

Sanders, E. J., Marfin, A. A., Tukei, P. M., Kuria, G., Ademba, G., Agata, N. N., Ouma, J. O., Cropp, C.B., Karabatsos, N., Reiter, P., Moore, P. S. Gubler, D. J. (1998). First recorded outbreak of yellow fever in Kenya, 1992-1993. I. Epidemiologic investigations. *Am J Trop Med Hyg, 59*(4), 644-649.

Sanders, E. J., Rwaguma, E. B., Kawamata, J., Kiwanuka, N., Lutwama, J. J., Ssengooba, F. P., Lamunu, M., Najjemba, R., Were, W. A., Bagambisa, G., Campbell, G. L. (1999). O'nyong-nyong fever in south-central Uganda, 1996-1997: description of the epidemic and results of a household-based seroprevalence survey. *J Infect Dis, 180*(5), 1436-1443

Sang, R., Arum, S., Chepkorir, E., Mosomtai, G., Tigoi, C., Sigei, F., Lwande, Olivia Wesula., Landmann, Tobias., Affognon, Hippolyte., Ahlm, Clas., Evander, M. (2017). Distribution and abundance of key vectors of Rift Valley fever and other arboviruses in two ecologically distinct counties in Kenya. *PLoS Negl Trop Dis, 11*(2), e0005341

Sang, R. C., Ahmed, O., Faye, O., Kelly, C. L., Yahaya, A. A., Mmadi, I., Toilibou, A., Sergon, K. Brown, J., Agata, N., Yakouide, A., Ball, M. D., Breiman, R. F., Miller, B. R., Powers, A. M. (2008). Entomologic investigations of a chikungunya virus epidemic in the Union of the Comoros, 2005. *Am J Trop Med Hyg, 78*(1), 77-82.

Sang, R. C., & Dunster, L. M. (2001). The growing threat of arbovirus transmission and outbreaks in Kenya: a review. *East Afr Med J, 78*(12), 655-661.

Sang, R. C., Gichogo, A., Gachoya, J., Dunster, M. D., Ofula, V., Hunt, A. R., . . . Dunster, L. M. (2003). Isolation of a new flavivirus related to cell fusing agent virus (CFAV) from field-collected flood-water Aedes mosquitoes sampled from a dambo in central Kenya. *Arch Virol, 148*(6), 1085-1093

Schwartz, O., & Albert, M. L. (2010). Biology and pathogenesis of chikungunya virus. *Nature Reviews Microbiology, 8*(7), 491-500

Schwameis, M., Buchtele, N., Wadowski, P. P., Schoergenhofer, C., & Jilma, B. (2016). Chikungunya vaccines in development. *Hum Vaccin Immunother, 12*(3), 716-731.

Sejvar, J. J., Haddad, M. B., Tierney, B. C., Campbell, G. L., Marfin, A. A., Van Gerpen, J. A., Fleischauer, A., Leis, A. A., Stokic, D. S. Petersen, L. R. (2003).

Neurologic manifestations and outcome of West Nile virus infection. *Jama, 290*(4), 511-515.

Sergon, K., Njuguna, C., Kalani, R., Ofula, V., Onyango, C., Konongoi, L. S., Bedno, S.., Burke, H.,Dumilla, A. M.,Konde, J., Njenga, M. K., Sang, R., Breiman, R. F. (2008). Seroprevalence of Chikungunya virus (CHIKV) infection on Lamu Island, Kenya, October 2004. *Am J Trop Med Hyg, 78*(2), 333-337.

Silva, S. J. R. d., Pardee, K., & Pena, L. (2019). Loop-Mediated Isothermal Amplification (LAMP) for the Diagnosis of Zika Virus: A Review. *Viruses, 12*(1), 19

Smithburn, K. C., Hughes, T. P., Burke, A. W., & Paul, J. H. (1940). A Neurotropic Virus Isolated from the Blood of a Native of Uganda. *American Journal of Tropical Medicine, 20*, 471-472.

Stanley, J., Chongkolwatana, V., Duong, P. T., Kitpoka, P., Stramer, S. L., Dung, N. T. T., Grimm, K. E., Pojanasingchod, A., Suksomboonvong, P., Galel, S. A. (2021). Detection of dengue, chikungunya, and Zika RNA in blood donors from Southeast Asia. *Transfusion, 61*(1), 134-143.

Solomon, T., & Vaughn, D. W. (2002). Pathogenesis and clinical features of Japanese encephalitis and West Nile virus infections. *Curr Top Microbiol Immunol, 267*, 171-194.

Subhadra, S., Sabat, J., Dwibedi, B., Panda, S., Mandal, M. C., Rath, S., Ho, L., Palo, S.,Pati, S.,Turuk, J. (2021). Prevalence and trend of emerging and re-emerging arboviral infections in the state of Odisha. *VirusDisease, 32*(3), 504-510.

Sutherland, L. J., Cash, A. A., Huang, Y. J., Sang, R. C., Malhotra, I., Moormann, A. M., King, C. L. Weaver, S. C.., King, C. H., LaBeaud, A. D. (2011). Serologic

evidence of arboviral infections among humans in Kenya. *Am J Trop Med Hyg, 85*(1), 158-161.

Tardei, G., Ruta, S., Chitu, V., Rossi, C., Tsai, T. F., & Cernescu, C. (2000). Evaluation of immunoglobulin M (IgM) and IgG enzyme immunoassays in serologic diagnosis of West Nile Virus infection. *Journal of Clinical Microbiology, 38*(6), 2232-2239.

Takahashi, H., Umino, Y., Sato, T. A., Kohama, T., Ikeda, Y., Iijima, M., & Fujisawa, R. (1996). Detection and Comparison of Viral Antigens in Measles and Rubella Rashes. *Clinical Infectious Diseases, 22*(1), 36-39.

Thiberville, S.-D., Boisson, V., Gaudart, J., Simon, F., Flahault, A., & de Lamballerie, X. (2013). Chikungunya Fever: A Clinical and Virological Investigation of Outpatients on Reunion Island, South-West Indian Ocean. *PLoS Negl Trop Dis, 7*(1), e2004.

Tigoi, C., Lwande, O., Orindi, B., Irura, Z., Ongus, J., & Sang, R. (2015). Seroepidemiology of Selected Arboviruses in Febrile Patients Visiting Selected Health Facilities in the Lake/River Basin Areas of Lake Baringo, Lake Naivasha, and Tana River, Kenya. *Vector Borne Zoonotic Dis, 15*, 124-132.

Valentine, M., Murdock, C., & Kelly, P. (2019). Sylvatic cycles of arboviruses in non-human primates. *Parasites & Vectors, 12*.

Vasilakis, N., & Weaver, S. C. (2008). Chapter 1 The History and Evolution of Human Dengue Emergence *Advances in Virus Research* (Vol. 72, pp. 1-76): Academic Press.

Vazeille, M., Mousson, L., Martin, E., & Failloux, A.-B. (2010). Orally Co-Infected *Aedes* albopictus from La Reunion Island, Indian Ocean, Can Deliver Both

Dengue and Chikungunya Infectious Viral Particles in Their Saliva. *PLoS Negl Trop Dis, 4*(6), e706.

Viral Haemorraghic Fevers Laboratory. (2013). YF risk assessment study in 2013.

Vogels, C. B. F., Rückert, C., Cavany, S. M., Perkins, T. A., Ebel, G. D., & Grubaugh, N. D. (2019). Arbovirus coinfection and co-transmission: A neglected public health concern? *PLoS biology, 17*(1), e3000130-e3000130.

Vu, D. M., Mutai, N., Heath, C. J., Vulule, J. M., Mutuku, F. M., Ndenga, B. A., & LaBeaud, A. D. (2017). Unrecognized Dengue Virus Infections in Children, Western Kenya, 2014-2015. *Emerg Infect Dis, 23*(11), 1915-1917.

Waggoner, J. J., Gresh, L., Vargas, M. J., Ballesteros, G., Tellez, Y., Soda, K. J.,… & Pinsky, B. A. (2016). Viremia and Clinical Presentation in Nicaraguan Patients Infected With Zika Virus, Chikungunya Virus, and Dengue Virus. *Clin Infect Dis, 63*(12), 1584-1590.

Waitumbi, J., Buddhdev, N., Awinda, G., Kanyara, L., Irura, Z., Ofula, V., Konongoi, L., Sang, R. (2018). Zika Virus Screening in the Kenyan Olympic Team Attending the 2016 Olympic Games in Brazil. *Open Journal of Preventive Medicine, 08*, 183-187. doi:10.4236/ojpm.2018.86017

Wamala, J. F., Malimbo, M., Okot, C. L., Atai-Omoruto, A. D., Tenywa, E., Miller, J. R. …& Mbonye, A. K. (2012). Epidemiological and laboratory characterization of a yellow fever outbreak in northern Uganda, October 2010-January 2011. *Int J Infect Dis, 16*(7), e536-542.

Weaver, S. C., & Reisen, W. K. (2010). Present and future arboviral threats. *Antiviral Res, 85*(2), 328-345

Weger-Lucarelli, J., Duggal, N. K., Bullard-Feibelman, K., Veselinovic, M., Romo, H., Nguyen, C., Rückert, C. ., Brault, A. C., Bowen, R. A., Stenglein, M., Geiss, B. J., Ebel, G. D. (2016). Development and Characterization of Recombinant Virus Generated from a New World Zika Virus Infectious Clone. *Journal of Virology, 91*(1), e01765-01716

Weger-Lucarelli, J., Rückert, C., Chotiwan, N., Nguyen, C., Garcia Luna, S. M., Fauver, J. R., Foy, B.D., Perera R., Black W C., Kading R.C., Ebel, G. D. (2016). Vector Competence of American Mosquitoes for Three Strains of Zika Virus. *PLoS Negl Trop Dis, 10*(10), e0005101.

World Health Organization (2007). Guidelines for plaque reduction neutralization testing of human antibodies to dengue viruses.

World Health Organization (2009). Dengue. http://www.who.int/tdr/publications/ documents/ dengue-diagnosis.pdf

World Health Organization (2009). Dengue guidelines for diagnosis, treatment, prevention and control: new edition. Geneva: World Health Organization.

World Health Organization. (April 2015).WHO African Regional measles and rubella surveillance guidelines Retrieved from https://www.afro.who.int/publications/ who-african-regional-measles-and-rubella-surveillance-guidelines-0

World Health Organization (2016). A toolkit for integrated vector management in sub-Saharan Africa. . Retrived from https://apps.who.int/iris/bitstream/handle/10665/ 250267/9789241549653-eng.pdf

World Health Organization (5th December 2017). Immunization, Vaccines and Biologicals-Dengue vaccinere search. Retrieved from https://www.who.int/immunization/ research/development/dengue_vaccins/en/

World Health Organization (2018).Chikungunya – Mombasa, Kenya.Retrievedfrom https://www.who.int/csr/don/27-february-2018-chikungunya-kenya/en/

World Health Organization (2018). Rubella: Retrieved from https://www.who.int/immunization/monitoring_surveillance /burden/vpd/WHO_Surveillance Vaccine Preventable_20_Rubella_R2.pdf?ua=1

World Health Organization (2020). Chikungunya Virus. Retrieved from https://www.who.int/news-room/fact-sheets/detail/chikungunya

World Health Organization (2020). Dengue and severe dengue. Retrieved from Dengue and severe dengue

World Health Organization (2020). Dengue and severe dengue. Retrieved from https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue

World Health Organization (2020). Measles. Retrievedfromhttps://www.who.nt/immnization/diseases/measles/en /

World Health Organization (2018). Zikavirus. Retrieved from https://www.who.int/newsroom/factssheets/detail/zikavirus

World Health Organization (2015). Regional Office for Africa. African Regional guidelines for measles and rubella surveillance-Revised April 2015.

Young, D. S., Kramer, L. D., Maffei, J. G., Dusek, R. J., Backenson, P. B., Mores, C. N., Bernard,KA, Ebel, G. D. (2008). Molecular epidemiology of eastern equine encephalitis virus, New York. *Emerg Infect Dis, 14*(3), 454-460.

Yvonne Kimata, P. B., Rosemary Nzunza, Victor Ofula, Edith Chepkorir, Rebecca Waihenya, and Rosemary Sang. (2020). Serological Evidence of Chikungunya

Virus Infection among Suspected Measles Cases in Selected Regions of Kenya: 2008–2014. *Vector-Borne and Zoonotic Diseases, 0*(0), null.

APPENDICES

Appendix I: ELISA Laboratory procedures

1. ZIKV IgM ELISA

According to the in-house kit, the CDC Zika MAC-ELISA is intended for the qualitative detection of Zika virus IgM antibodies in human sera or cerebrospinal fluid (CSF).

Materials

1. Zika Vero E6 Tissue Culture Antigen (CDC catalog #AV0002 or AV0003): Antigens produced from different Zika strains; only one is required for the assay. Lyophilized Zika antigen (inactivated) prepared for use in Zika MAC-ELISA.
2. Flavivirus IgM positive control (CDC catalog #AV0004): Chimeric monoclonal antibody specific for Flavivirus; lyophilized.
3. Detecting antibody conjugate: Horseradish peroxidase conjugated monoclonal antibody 6B6C-1. Available from: Hennessy Research, catalog #DC153-100 or
4. Goat anti-human IgM (Kirkegaard and Perry Laboratories, catalog #01-10-03)
5. Deionized water
6. Hydrochloric acid (to adjust pH of coating buffer)
7. Sodium carbonate (Na_2CO_3); (Sigma)
8. Sodium bicarbonate ($NaHCO_3$); (Sigma.)
9. Phosphate buffered saline (PBS); (Sigma)
10. Tween 20 (available from multiple commercial sources, e.g. Sigma
11. Nonfat dry milk (available from multiple commercial sources, e.g. Sigma,
12. Sulfuric acid (H_2SO_4); (Sigma)
13. Immulon II HB flat-bottomed 96 well plates, Dynatech Technologies catalog #3455 (available from multiple commercial sources, e.g. Sigma)

14. Enhanced K-Blue TMB substrate (3, 3’, 5, 5' tetramethylbenzidine base; Neogen Corp, catalog # 308175
15. Normal human sera—tested negative for Zika virus antibodies

Formulations

1. Coating buffer: Carbonate/bicarbonate buffer, pH 9.6 Na_2CO_3 + 2.93 g $NaHCO_3$ diluted in 1L water.

1. Wash buffer: Phosphate buffered saline (PBS); 0.05% Tween 20, pH 7.2. 2.59g PBS is available in powdered form
2. Blocking buffer: PBS/ 5% milk/ 0.5% Tween 20
3. Stop solution: 1 N H_2SO_4
4. Detecting antibody conjugate: Conjugate can be diluted up to 1:4000 in blocking buffer
5. Flavivirus IgM positive control: Flavivirus IgM positive control diluted up to 1:1000 in wash buffer
6. Zika viral antigen:
    - Zika Vero E6 Antigen: diluted up to 1:2000 in wash buffer
7. Normal antigen:
    - Normal Vero E6 Antigen: diluted up to 1:2000 in wash buffer
    - Goat anti-human IgM: diluted 1:2000 in coating buffer
8. Patient serum: diluted 1:400 in wash buffer
9. Negative control: Normal human sera diluted to 1:1000

Procedure

1. Plates were sensitized with Goat anti-human IgM in coating buffer (1:2000). The 75µl was added to all the wells then incubated overnight at 4°C.
2. The following day the plates were dumped off the coating antibody, then blocking of the plates was done by adding the 200µl of blocking buffer per well followed by incubation for 30 minutes.

3. The plates were washed 5 times with 300µl of the washer buffer using an automatic plate washer.
4. Serum samples were diluted in the ratio of 1:400 with the wash buffer and thoroughly mixed with a vortex. Positive and negative controls are diluted 1:1000 in wash buffer.
5. The serum samples (50µl) and controls were dispensed into the respective wells, covered with a foil supplied with the kit, and incubated for 1 hour at 37 °C.
6. After incubation, each well was washed five times with 300µl of the washing buffer using an automatic washer.
7. Zika and Normal Vero E6 antigen diluted in wash buffer 1:2000 then added into the respective wells followed by overnight incubation at 4°C.
8. Washing was done 5 times again with 300µl of washing solution using an automatic washer.
9. 50µl of the conjugate (horseradish peroxidase-conjugated monoclonal antibody), diluted in blocking buffer, was added in all the wells then incubated at 37°C for 1 hour.
10. Washing was done 5 times twice, and 75 µl TMB solution was added to all wells. Incubation followed by for 10 minutes in the dark
11. After incubation, 50µl Stop Solution was added to all wells.
12. The plates were read using a 450nm filter.

Interpretation of results

Before the results can be calculated for each clinical specimen, the test must be determined to be valid. For a test to be valid, the following ratio must be greater than or equal to 2.0. This is the P/N of positive control.

Mean OD of the test specimen reacted with Zika viral antigen (P)

Mean OD of the normal human serum reacted with Zika viral antigen (N)

This is the P/N of the test specimen

All test specimen P/N values greater than or equal to 3.0 should be reported as presumptive IgM-positive. P/N values that lie between 2.0 and 3.0 should be considered equivocal. All specimens for whom Specimen P/N is < 2 report as negative.

2. CHIKV IgM µ-capture ELISA

This was a qualitative immunoenzymatic determination of IgM-class antibodies against Chikungunya using ELISA as per the Manufacturer‘s instructions

Procedure

1. Serum samples were diluted in the ratio of 1:100 with the sample diluent supplied by the manufacture and thoroughly mixed with a vortex. Positive and negative controls were ready to use and hence will not be diluted.
2. The serum samples (50µl) and controls were dispensed into the respective wells, covered with a foil, and incubated for 1 hour at 37 °C.
3. After incubation, each well was washed three times with 300µl of washing solution. This was followed by dispensing 50 µl Chikungunya antigen Solution 1 into all wells except for the blank well and covered with foil, then incubated for 30 minutes at room temperature.
4. Washing step was repeated, followed by adding 50 µl Chikungunya Solution 2 (biotinylated Chikungunya antibody, stabilizers, preservatives, and an inert blue dye) into all wells except for the blank well. The plate was then covered with the foil and incubated for 30 min at room temperature.
5. The washing step was repeated again. Then, 50 µl Streptavidin peroxidase conjugate was added into all wells except for the blank and covered with a foil and incubated for 30 min at room temperature.

6. Washing was repeated again and 100 µl TMB solution was added into all wells. Incubation followed by for exactly 15 min in the dark.
7. After incubation, 100µl Stop Solution (0.2 M sulphuric acid solution) was added into all wells in the same order and at the same rate as for the TMB substrate.
8. The absorbance of the specimens was measured at 450nm within 30 min after the addition of the Stop Solution.

CHIKV IgG Capture ELISA

A qualitative immunoenzymatic determination of IgG-class antibodies to Chikungunya was performed based on the manufactures instructions (NovaTec Immundiagnostica).

Procedure

1. All samples were diluted in the ratio of 1:100 using the sample diluent and 50µl dispensed into the microtiter strip wells pre-coated with anti-human IgG to bind corresponding antibodies of the specimen. Also, 50µl of positive and negative controls and substrate blank were also dispensed to the marked wells. The wells were then covered using a foil supplied in the kit and incubated for 1-hour ± 5 min at 37±1°C.
2. After washing the wells three times with 300µl of washing solution to remove all unbound sample and control material, 50µl Chikungunya antigen solution 1 was added except for the blank well. The plate was then covered with foil and incubated for 30 minutes at room temperature.
3. After a further washing step as two above, 50 µl biotinylated Chikungunya antibody solution 2 was pipetted into the wells except for the blank well. The plate was covered with foil and incubated for 30 minutes at room temperature.
4. After washing again, 50µl Streptavidin peroxidase conjugate was dispensed into all wells except for the blank to bind to the captured Chikungunya-specific

immune complex. The plate was covered with foil and incubated for 30 minutes at room temperature without exposing it to direct sunlight.

5. This immune complex was visualized by adding 100 µl TMB solution into all wells giving a blue reaction product. The plate was incubated for exactly 15 minutes in the dark.
6. This was followed by adding 100µl Sulphuric acid (Stop Solution) to stop the reaction. Finally, the absorbance was read at 450 nm using an ELISA microwell plate reader, ELx808 IU (Biotek, USA) ELx808 IU (Biotek, USA).

Interpretation of Results for both CHIKV IgM and IgG ELISA

The cut-off was the mean absorbance value of the Cut-off control determinations. Samples were considered positive if the absorbance value was higher than 10% over the cut-off. Samples found with an absorbance value of 10% above or below the cut-off were not considered. Samples were considered negative if the absorbance value is lower than 10% below the cut-off.

3. DENV IgM ELISA

This was a qualitative immunoenzymatic determination of IgM-class antibodies against Dengue using ELISA as per the Manufacturer‘s instructions.

Procedure

1. Serum samples were diluted in the ratio of 1:100 with the Sample diluent supplied by the manufacture and thoroughly mixed with a vortex. Positive and negative controls were ready to use.
2. The serum samples (100µl) and controls were dispensed into the respective wells, covered with a foil and incubated for 1 hour at 37 °C.

3. After incubation, each well was washed three times with 300µl of washing solution, and this was followed by dispensing 100µl of Dengue IgM conjugate then covered with a foil.
4. Incubation was done for 30 minutes at room temperature away from direct sunlight. After incubation, each well was washed three times with 300µl of washing solution.
5. 100 µl TMB solution added into all wells. Incubation for exactly 15 min in the dark was done.
6. After incubation, 100µl Stop Solution (0.2 M sulphuric acid solution) was added into all wells in the same order and at the same rate as for the TMB substrate.
7. The absorbance of the specimen was measured at 450nm within 30 min after the addition of the Stop Solution

DENV IgG ELISA

A qualitative immunoenzymatic determination of IgG-class antibodies to Dengue was performed based on the manufactures instructions (NovaTec Immundiagnostica).

Procedure

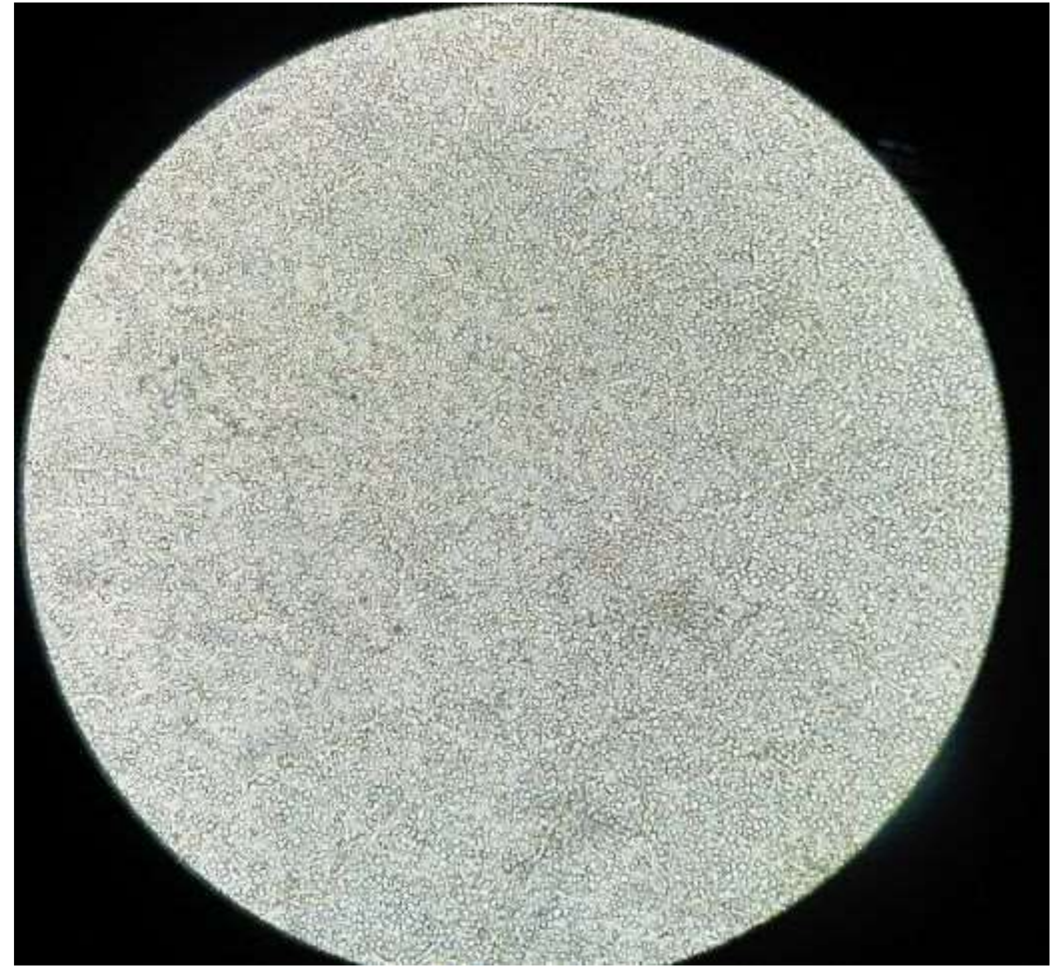
1. Serum samples were diluted in the ratio of 1:100 with the sample diluent supplied by the manufacture and thoroughly mixed with a vortex. Positive and negative controls were ready to use.
2. The serum samples (100µl) and controls were dispensed into the respective wells, covered with a foil, and incubated for 1 hour at 37 °C.
3. After incubation, each well was washed three times with 300µl of washing solution, and this was followed by dispensing 100µl of Dengue IgG conjugate then covered with a foil.
4. Incubation was done for 30 minutes at room temperature away from direct sunlight. After incubation, each well was washed three times with 300µl of washing solution.

5. 100 µl TMB solution added into all wells. Incubation for exactly 15 minutes in the dark was done.
6. After incubation, 100µl Stop Solution (0.2 M sulphuric acid solution) was added into all wells in the same order and at the same rate as for the TMB substrate.
7. The absorbance of the specimen was measured at 450/620nm within 30 min after the addition of the Stop Solution

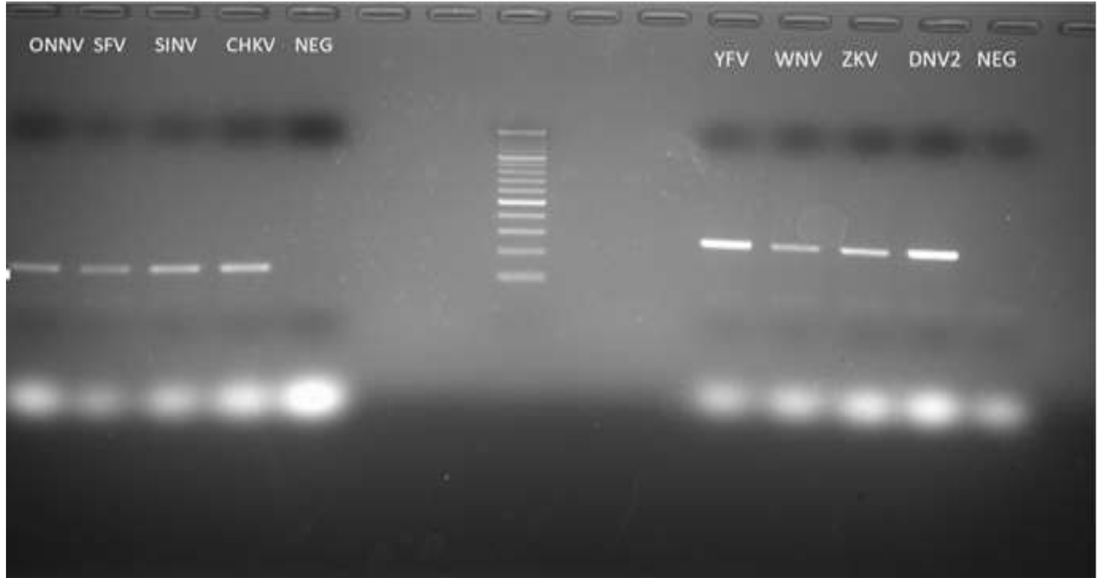
Interpretation of Results for both DENV IgM and IgG ELISA

The cut-off was the mean absorbance value of the Cut-off control determinations. Samples were considered positive if the absorbance value was higher than 10% over the cut-off. Samples found with an absorbance value of 10% above or below the cut-off were not considered. Samples were considered negative if the absorbance value is lower than 10% below the cut-off.

Appendix II: Cytopathic effects of dengue virus in Vero cells indicated by rounding up of cells



Appendix III: A gel picture showing the viruses used to rule out cross-reactivity were confirmed by PCR



Appendix IV: A plate showing plaques (titre) of chikungunya virus on monolayers of (African green monkey cells ATCC)

Appendix V: Kenya Medical Research Institute Scientific and Ethical Review Unit Approval

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200 NAIROBI - Kenya
Tel: (254) (020) 2722541, 254 (020) 2713349, 0722-205901, 0733-400003 Fax (254) (020) 2720030
Email: director@kemri.org info@kemri.org Website: www.kemri.org

KEMRI/RES/7/3/1 July 28, 2016

TO: YVONNE KIMATA,
PRINCIPAL INVESTIGATOR

THROUGH: DR. ROSEMARY SANG,
ACTING DIRECTOR, CVR,
NAIROBI

FOR DIRECTOR
CENTRE FOR VIRUS RESEARCH
P.O. BOX 54828
NAIROBI
2/8/2016

Dear Madam,

RE: **SERU PROTOCOL NO. 3122 (*REQUEST FOR 1ST AMENDMENT*): SEROPREVALENCE AND DISTRIBUTION OF DENGUE, CHIKUNGUNYA AND ZIKA ARBOVIRUSES AMONG NON-MEASLES FEBRILE RASH CASES IN SELECTED AREAS IN KENYA *(VERSION 1.2 DATED 04/04/2016)***

This is to inform you that at the 253rd Committee B Meeting of the KEMRI Scientific and Ethics Review Unit (SERU) held on **20th July, 2016** the request for amendment for the above referenced research proposal was discussed.

The Committee acknowledges receipt of the following documents:

1. 5 copies of the cover letters
2. 5 copies of the amendment submission form
3. 5 copies of the protocol
4. 5 copies of CVs of non-KEMRI co-investigators
5. 5 copies of ethics certificates

The Committee noted the following amendment:

1. Addition of Zika Virus to the protocol to be analyzed with the dengue and chikungunya arboviruses.

The Committee concluded that the amendment is justified and will not alter the risk/benefit status of the study and is therefore **granted approval** for implementation.

You are also required to submit any further requests for changes to the approved protocol to the SERU prior to initiation.

Yours faithfully,

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

01 AUG 2016

In Search of Better Health

Appendix VI: Kenya Medical Research Institute Scientific and Ethical Review Unit Approval letter 2

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840 - 00200 NAIROBI - Kenya
Tel: (254) (020) 2722541, 254 (020) 2713349, 0722-205901, 0733-400003 Fax (254) (020) 2720030
Email: director@kemri.org info@kemri.org Website:www.kemri.org

KEMRI/RES/7/3/1 **September 8, 2015**

TO: **YVONNE KIMATA,**
PRINCIPAL INVESTIGATOR

THROUGH: **THE DIRECTOR, CVR,**
NAIROBI

FOR DIRECTOR
CENTRE FOR VIRUS RESEARCH
P.O. BOX 54628
NAIROBI.

Dear Madam,

RE: KEMRI/SERU/CVR/002/3122 (*RESUBMISSION 1: INITIAL SUBMISSION*): SEROPREVALENCE AND DISTRIBUTION OF DENGUE AND CHIKUNGUNYA ARBOVIRUS AMONG NON-MEASLES FEBRILE RASH CASES IN SELECTED AREAS IN KENYA *(VERSION 1.1 DATED AUGUST 31 2015)*

Reference is made to your letter dated 31st August, 2015. The ERC Secretariat acknowledges receipt of the revised proposal on 2nd September, 2015.

This is to inform you that the KEMRI Scientific and Ethics Review Unit (SERU) reviewed the documents and is satisfied that the issues raised at the 242nd meeting of the KEMRI ERC held on 18th August 2015 have been adequately addressed.

The study is granted approval for implementation effective **8th September 2015**. Please note that authorization to conduct this study will automatically expire on **7th September 2016.** If you plan to continue with data collection or analysis beyond this date, please submit an application for continuing approval to the ERC Secretariat by **27th July, 2016**.

Any unanticipated problems resulting from the implementation of this protocol should be brought to the attention of SERU. You are also required to submit any proposed changes to this protocol to SERU prior to initiation and advise the SERU when the study is completed or discontinued.
You may embark on the study.

Yours faithfully,

PROF. ELIZABETH BUKUSI,
ACTING HEAD,
KEMRI/SCIENTIFIC AND ETHICS REVIEW UNIT

In Search of Better Health

Appendix VII: WHO Approval Letter

23 July 2015

TO WHOM IT MAY CONCERN

RE: USE OF MEASLES SURVEILLANCE SAMPLES FOR STUDY ON DENGUE AND CHIKUNGUNYA

The WHO measles laboratory network supports countries in confirmation of measles and rubella from suspected cases of measles detected through case-based surveillance. The KEMRI measles laboratory is classified as a national reference laboratory within the network.

Samples held in network laboratories are for diagnostic use to support Public Health, and as part of differential diagnoses, anti-rubella IgM testing is routinely undertaken within the laboratories on all samples negative for measles. As a large proportion of samples turn out negative for either measles or rubella, laboratories are encouraged to develop capacity for differential diagnostics for other viral infections that cause rash illness used in the standard case definition for measles surveillance.

We have been approached by Ms Yvonne Mukami Kimata, an MSc student, seeking concurrence to use measles diagnostic samples within the KEMRI network laboratory to test for dengue and chikukungunya viruses as differential diagnoses for measles, and which will contribute to her thesis work.

We have no objection to use of the above samples archived in the measles laboratory repository.

Yours faithfully,

Dr Peter Borus

Scientific Officer

Appendix VIII: Board of Postgraduate Studies Proposal Approval Letter

JOMO KENYATTA UNIVERSITY
OF
AGRICULTURE AND TECHNOLOGY
DIRECTOR, BOARD OF POSTGRADUATE STUDIES

P.O. BOX 62000
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REF: JKU/2/11/TM303-2124/2014 20th June 2016

KIMATA YVONNE MUKAMI
C/o S
JKUAT

Dear Ms. ~~Too~~ Mukami,

RE: APPROVAL OF RESEARCH PROPOSAL AND SUPERVISORS

Kindly note that your MSc. research proposal entitled: **"SEROPREVALENCE AND DISTRIBUTION OF DENGUE AND CHIKUNGUNYA ARBOVIRUSES AMONG NON-MEASLES FERBRILE RASH CASES IN SELECTED AREAS IN KENYA** "has been approved. The following are your approved supervisors:-

1. Dr. Rosemary Sang
2. Dr. Peter Borus
3. Prof. Rebecca Waihenya

Yours sincerely,

PROF. MATHEW KINYANJUI
DIRECTOR, BOARD OF POSTGRADUATE STUDIES

Copy to: Dean, SOBMS

JKUAT is ISO 9001:2008 and 14001:2004 Certified
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Appendix IX: Publication

VECTOR-BORNE AND ZOONOTIC DISEASES
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Serological Evidence of Chikungunya Virus Infection Among Suspected Measles Cases in Selected Regions of Kenya: 2008–2014

Yvonne Kimata,[1] Peter Borus,[2] Rosemary Nzunza,[2] Victor Ofula,[2] Edith Chepkorir,[3] Rebecca Waihenya,[1] and Rosemary Sang[2]

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Abstract

Chikungunya virus (family *Togavirdae* and genus *Alphavirus*) is an emerging and reemerging virus of public health importance both regionally and globally. In Kenya, about 50–60% of the suspected measles cases remain undiagnosed once measles and rubella is ruled out by immunoglobulin M (IgM) ELISA thus prompted the need to do differential diagnosis on the measles/rubella negative samples. Nothing is known about the role played by chikungunya infection among these suspected measles cases. Febrile rash illness is a common clinical presentation of arboviruses, including chikungunya. In this study, we conducted a serosurvey to explore the possible role of chikungunya infections among suspected measles cases in Kenya that had tested negative for measles and rubella. Sera were tested by commercially available ELISA for the presence of IgG and IgM antibodies against the chikungunya virus. All positive samples for chikungunya by ELISA were confirmed by plaque reduction neutralization test (PRNT), and to rule out cross-reactivity with other alphaviruses a panel of viruses was used, namely o' nyong' nyong, Semliki Forest, and Sindbis viruses. Of the 392 serum samples screened, 0.3% ($n=1$) tested positive for IgM antibodies, while 4.6% ($n=18$) tested positive for IgG antibodies against the chikungunya virus. PRNT results indicated 2 (11%) chikungunya positives and 7 (38.9%) o' nyong' nyong positives. We recommend awareness among health care providers and improved surveillance for these arboviruses by both serology and molecular testing. Testing for other pathogens should also be done to improve disease detection and diagnosis.

Keywords: febrile, rash, illlness

Introduction

Measles infection is one of the causes of high morbidity and mortality among children in developing countries (Perry et al. 2004, Nandy et al. 2006). Clinical symptoms caused by measles infection include cough, coryza, and conjunctivitis, followed by a maculopapular rash and high fever (39–40°C) (WHO 2015). In Kenya, all suspected measles cases, are routinely submitted to the measles laboratory at the Kenya Medical Research Institute's (KEMRI), Centre for Virus Research (CVR). At the CVR, the samples are tested for measles. If negative, cases are tested for rubella IgM antibodies in support of the national measles/rubella case-based surveillance program. Over the years, it has been observed that ~50–60% of these suspected measles cases usually test negative for both rubella and measles using enzyme-linked immunosorbent assay (ELISA).

Chikungunya (CHIKV) and other arboviruses such as o' nyong' nyong (ONNV), Sindbis (SINV), and Semliki Forest virus (SFV) are among arboviruses causing emerging and reemerging infections of public health importance regionally and globally (Paixao et al. 2018). It has been shown that CHIKV is endemic in Coastal and Northern parts of Kenya (Sergon et al. 2004, Konongoi et al. 2018). It has caused frequent outbreaks in the country (WHO 2016, 2018, Konongoi et al. 2018). Even though CHIKV, ONNV, SFV, and SINV are known to be circulating in Kenya (Morrill et al. 1991, Sergon et al. 2008, Mease et al. 2011, Sutherland et al. 2011, LaBeaud et al. 2015, Ochieng et al. 2015, Tigoi et al. 2015,

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Grossi-Soyster et al. 2017), no study has been conducted to test these arboviruses on non-measles cases that present as febrile rash illness. In addition, the disease burden of these arboviral diseases in Kenya is not well defined. To address these gaps, this study set out to determine the exposure of chikungunya virus among suspected measles cases collected in the measles case-based surveillance program in Kenya. This was to assess the possible contribution of chikungunya infections to cause measles-like illness.

Materials and Methods

Study design

We conducted a laboratory-based, retrospective study using 392 randomly selected archived human sera. These samples were collected between January 2008 and December 2014 from health facilities performing measles case-based surveillance in 10 counties from 3 regions in Kenya. These include Nairobi County, Coastal region (Lamu, Taita-Taveta, Mombasa, Tana River, Kwale, and Kilifi), and North-Eastern region (Mandera, Wajir, and Garissa) as shown in (Fig. 1). These counties were preselected because of past or recent reports of CHIKV outbreaks (Coastal and North Eastern regions) or because they are a major travel hub with a high risk of introduction and transmission (Nairobi County).

The 392 archived serum samples used in this study were selected based on probability proportional to size from the total number of samples collected from each of the three regions: Nairobi $n = 179$ (46%), North-Eastern $n = 57$ (14%) and Coast $n = 156$ (40%).

This study was approved by Kenya Medical Research Institute's Scientific and Ethics Review Unit (SERU no.3122).

Serum specimens' collection, processing, storage, and transportation

Samples were collected from all counties in Kenya. Measles surveillance in Kenya follows World Health Organization guidelines, in which a suspected measles case is defined by a patient having fever and one of the following symptoms: rash, cough, coryza, or conjunctivitis (WHO 2015). Five milliliters whole blood was collected from all

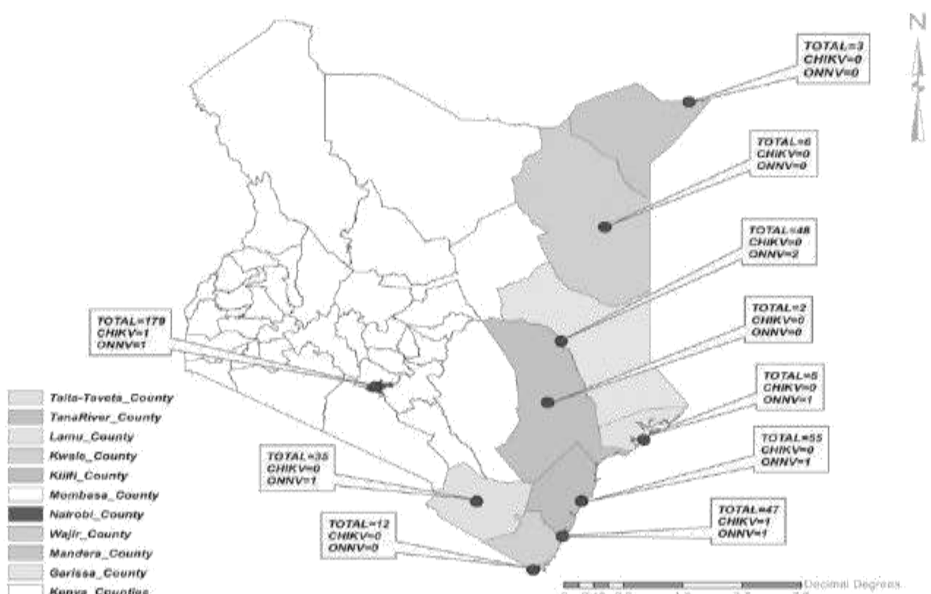

FIG. 1. Map of Kenya showing the study sites and total numbers of samples used per site: Nairobi Region, Coast Region (Mombasa, Kwale, Kilifi, Tana-River, Taita-Taveta, and Lamu), and North-Eastern Region (Wajir, Mandera, and Garissa) and number of CHIKV and ONNV positives in each county.

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suspected cases, and serum was separated at the various health facilities then shipped to KEMRI's reference laboratory for testing by ELISA method. In addition, from each suspected patient, a case investigation form was filled and the information recorded included; region, health facility, gender, age, date of birth, residence, date of onset of rash, presence of fever, type of rash, measles vaccination history, status of the patient, tracer information, date of specimen collection, and type of specimen. The form was sent together with the serum to the laboratory. The serum was stored in a biobank at −20°C.

Laboratory testing

Enzyme-linked immunosorbent assay. CHIKV IgM and IgG antibodies were measured to assess serological evidence of recent exposure (IgM) and prior exposure (IgG) to CHIKV.

CHIKV IgG and IgM ELISA. All the 392 serum samples were heat-inactivated at 56°C for 30 min before testing. We screened the specimens for anti-CHIKV antibodies using commercial IgG and IgM capture ELISA kits (Novatec Immunodiagnostica GmbhH) according to the manufacturer's instructions. Briefly, CHIKV IgG microplates were coated with anti-human IgG antibodies. The diluted samples were added and incubated at 37°C for 1 h. After 1 h, the wells were washed to remove all unbound antibodies, the antigen was added, and the plates incubated at room temperature for 30 min. Next, the plates were washed, and the detector antibody was added into all the wells. The plates were incubated at room temperature for 30 min. After washing, the horseradish peroxidase-labeled streptavidin was added to bind to the captured specific immune complex during incubation at room temperature for 30 min and washed. The immune complexes were visualized by adding Tetramethylbenzidine (TMB) substrate, which gave a blue color. Stop solution (0.2 M sulfuric acid solution) was added and incubated for 15 min to stop the reaction that produced a yellow color. The absorbance was measured at 450 nm using ELISA plate reader ELx800 (Biotek).

CHIKV IgM antibodies were detected using microplates that were coated with anti-human IgM antibodies to bind to the corresponding antibodies of the diluted samples. All the other steps were followed as described for CHIKV IgG and the absorbance was measured at 450 nm using ELISA plate reader ELx800 (Biotek).

Plaque reduction neutralization test. Plaque reduction neutralization test (PRNT) assays were undertaken to confirm the CHIKV IgG and IgM ELISA positive results and to rule out cross-reactivity with other closely related alphaviruses. All the alphavirus isolates used in this study were obtained from KEMRI Viral Haemorrhagic Fever (VHF) laboratory; including CHIKV Lamu 33 (Gen Bank accession no. HQ456255), (Kariuki Njenga et al. 2008), SFV ATH00510 (Gen Bank accession no. KF283988), (Lwande et al. 2013), SINV NVS/305 (Gen bank accession no KY616988), (Sigei et al. 2018), and ONNV SG 650 (Gen bank accession no AF079456), (Lanciotti et al. 1998). In brief, all the viruses were pretitred using plaque assay, then diluted in maintenance media (Minimum Essential medium Sigma with Earle's salts with reduced $NaHCO_3$(Sigma), supplemented with 2% heat-inactivated fetal bovine serum (FBS) (Gibco), 2% L-glutamine (Sigma), and 2% penicillin/amphotericin B (Sigma) to a standard concentration that produced ∼20–50 plaques per 100 μL. Each serum sample was serially diluted from 1:20 to 1:320 using maintenance media in sterile Eppendorf tubes. A constant volume of the diluted virus with the required concentration was added into each 1.5 mL tube (Eppendorf) containing the diluted serum from 1:20 to 1:320 and incubated for 1 h at 37°C. The virus-serum mixture (30 μL of the diluted virus with 30 μL of the diluted serum) was then transferred to 24-well plates (Corning) with confluent Vero cell monolayers (African green monkey cells ATCC) and incubated at 37°C, 5% CO_2 for 1 h for adsorption process, after which an overlay of 2.5% methylcellulose (Sigma) was added. After 3–5 days, depending on the virus tested, the plates were fixed with 3% formalin (Sigma) and stained with 0.5% crystal violet (Sigma) prepared in absolute ethanol (Sigma). For etiological diagnosis, the $PRNT_{90}$ antibody titer for each virus was required to be four-fold greater than the other viruses in the same family to arrive at an etiological diagnosis (Tigoi et al. 2015, Chepkorir et al. 2019). A sample was considered indeterminate if it neutralized two viruses and did not meet the four-fold greater threshold.

Data analysis

Data analysis was performed using SPSS version 22. The IgM and IgG titers for CHIKV were dichotomized as either positive or negative based on optical density (OD) values. PRNT results were stratified by the age of the patients, gender and county of occurrence, and by region (North-Eastern, Coast, and Nairobi). Association of seropositivity to CHIKV and ONNV with age, gender, and site of collection was tested using chi-square. The proportion of positive sample for each virus was determined.

Results

Patient demographic characteristics

A total of 392 archived human serum samples were analyzed (Nairobi (n = 179) (46%), North-Eastern (n = 57) (14%), and Coast (n = 156) (40%)), the median age of the suspected measles cases was 4.7 years and interquartile range (IQR) was 2.8–6.7 years. Of the 392 samples, 52.8% were <5 years, 34.4% were 5–9 years, 6.4% were 10–14 years, and 6.4% were >14 years of age. One hundred and fifty-eight (40%) were female and 234 (60%) were male (Table 1).

Serological detection of ONNV and CHIKV

Out of the 392 samples tested, 1 (0.3%) sample tested positive for both anti-CHIKV IgM/IgG and 18 (4.6%) tested positive for anti-CHIKV IgG. Out of the 18 samples that were positive by ELISA, 9 (50%) were positive by PRNT. Only 2 (11%) had neutralizing antibodies against CHIKV while 7 (39%) for ONNV (Fig. 1). The other 9 samples (50%) had neutralizing antibodies for both CHIKV and ONNV, which did not meet the four-fold greater threshold hence considered indeterminate (Table 2). None of the samples had neutralizing antibodies against SINV and SFV. CHIKV was detected from a sample collected in Mombasa County in 2014 while the other one was collected from Nairobi County

Table 1. Patient Demographic Characteristics Collected from Coast, North-Eastern, and Nairobi Regions in Kenya (2008–2014)

		Coast n=*156 (40%)*						*Nairobi* n=*179 (46%)*	*North-Eastern* n=*57 (14%)*		
	Total (%)	*Mombasa*	*Kilifi*	*Kwale*	*Lamu*	*Taita Taveta*	*Tana-River*	*Nairobi*	*Garissa*	*Wajir*	*Mandera*
N	*392*	*47*	*55*	*12*	*5*	*35*	*2*	*179*	*48*	*6*	*3*
Age (years)											
<5	207 (52.8)	28 (14)	26 (13)	8 (4)	3 (1)	22 (11)	1 (1)	91 (44)	24 (12)	3 (1)	1 (0)
5–9	135 (34.4)	11 (8)	18 (13)	4 (3)	2 (2)	12 (9)	1 (1)	69 (51)	17 (12)	1 (1)	0
10–14	25 (6.4)	4 (16)	4 (16)	0	0	0	0	11 (44)	5 (20)	0	1 (4)
>14	25 (6.4)	4 (16)	7 (28)	0	0	1 (4)	0	8 (32)	2 (8)	2 (8)	1 (4)
Gender											
Female	158 (40)	20 (13)	28 (17)	4 (3)	3 (2)	13 (8)	0	73 (46)	15 (9)	1 (1)	1 (1)
Male	234 (60)	27 (12)	27 (12)	8 (3)	2 (1)	22 (9)	2 (1)	106 (45)	33 (14)	5 (2)	2 (1)

in 2009. ONNV was detected in the years 2009 (3/7), 2010 (2/7), 2011 (1/7) and 2014 (1/7). The counties in which ONNV was detected were Nairobi (1/7), Garissa (2/7), Mombasa (1/7), Kilifi (1/7), Taita-Taveta (1/7), and Lamu (1/7) (Table 2).

There was no significant difference in seropositivity of CHIKV and ONNV with age, gender, and site of collection (Table 3).

Discussion

This study found two cases of chikungunya infection among samples that were negative for acute measles in selected areas in Kenya during the 2008–2014 period. This finding of CHIKV in negative measles cases is an indication of how such co-circulation of viruses with similar clinical presentation could complicate measles diagnosis. The low prevalence rate of CHIKV observed in this study could be attributed to the early timing of sample collection. It is known that CHIKV IgM antibodies are highly detectable 4–20 days after the onset of illness (Natrajan et al. 2019) and may persist for about 2 months. Therefore, samples collected during the first week after onset of illness should have preferably been tested by both serological and virological methods (RT-PCR). This was not done due to resource limitation.

In this study, CHIKV IgM antibodies were detected in a sample collected from a 6 year old who reported to a health facility with febrile rash illness. This finding suggests the circulation of CHIKV virus in Mombasa County, Coastal region, as early as 2014. The Coastal region experienced the largest outbreak of CHIKV in Lamu County in 2004 (Sergon et al. 2008). Therefore, this indicates continued circulation of CHIKV outside of the outbreaks and may also suggest it had been circulating at low level even before the reported

Table 2. Chikungunya and O'nyong-Nyong Virus Plaque Reduction Neutralization Test Positivity by Endpoint Titers for Archived Serum Samples from Coast, North-Eastern, and Nairobi Regions in Kenya (2008–2014)

Demographic characteristics					$PRNT_{90}$ *titers*				
Sample ID	*Collection year*	*County*	*Age*	*Gender*	*CHIKV*	*ONNV*	*SINV*	*SFV*	*Inferred infection*
KEM-14-0509	2014	Lamu	6	M	1:40	1:160	—	—	ONNV
KEM-14-0910	2014	Mombasa	6	F	1:160	1:20	—	—	CHIKV
KEM-14-1015	2014	Kilifi	2	M	1:20	1:20	—	—	IND
KEM-10-0591	2010	Taita-Taveta	4	F	1:20	1:80	—	—	ONNV
KEM-10-0317	2010	Kilifi	6	F	1:20	1:160	—	—	ONNV
KEM-10-0683	2010	Taita-Taveta	3	M	1:20	1:40	—	—	IND
KEM-11-0510	2011	Mombasa	6	M	1:20	1:80	—	—	ONNV
KEM-12-0947	2012	Kilifi	20	F	1:80	1:40	—	—	IND
KEM-09-0948	2009	Garissa	9	F	1:40	1:160	—	—	ONNV
KEM-14-0581	2014	Garissa	1	M	1:20	1:40	—	—	IND
KEM-09-1318	2009	Garissa	5	M	1:20	1:80	—	—	ONNV
KEM-09-1319	2009	Garissa	7	M	1:80	1:40	—	—	IND
KEM-10-1117	2010	Garissa	13	M	1:40	1:40	—	—	IND
KEM-09-0390	2009	Nairobi	1	M	1:40	1:20			IND
KEM-14-0889	2014	Nairobi	3	M	1:20	1:40	—	—	IND
KEM-09-0529	2009	Nairobi	5	M	1:80	1:20	—	—	CHIKV
KEM-09-0545	2009	Nairobi	36	M	1:20	1:80	—	—	ONNV
KEM-12-0282	2012	Nairobi	9	F	1:20	1:40	—	—	UND

PRNT, plaque reduction neutralization test; M, male; F, female; CHIKV, chikungunya virus; ONNV, o 'nyong' nyong virus; SINV, sindbis virus; SFV, semliki forest virus; (—), titer <1:20 and IND-indeterminate.

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TABLE 3. ASSOCIATION BETWEEN SEROPOSITIVITY OF CHIKUNGUNYA AND O 'NYONG' NYONG VIRUSES WITH AGE, GENDER, AND SITE

	CHIKV	p *value*	*ONNV*	p *value*
Age				
<5	0	0.281	1 (14.3)	0.121
5–9	2 (100.0)		5 (71.4)	
10–14	0		0	
>14	0		1 (14.3)	
Gender				
Female	1 (50.0)	1.000	3 (42.9)	1.000
Male	1 (50.0)		4 (57.1)	
County				
Garissa	0	0.974	2 (28.6)	0.197
Kilifi	0		1 (14.3)	
Kwale	0		0	
Lamu	0		1 (14.3)	
Mandera	0		0	
Mombasa	1 (50.0)		1 (14.3)	
Nairobi	1 (50.0)		1 (14.3)	
Taita Taveta	0		1 (14.3)	
Tana-River	0		0	
Wajir	0		0	

outbreak, which occurred in Mombasa in 2018 (WHO 2018). This is likely to happen because only a few cases present with a maculopapular rash, and they easily go undetected.

The Coastal region also experienced a large dengue outbreak in 2013 (Ellis et al. 2015), 2013–2014 (Lutomiah et al. 2016), which could have led to underreporting of CHIKV because of its similar clinical symptoms (WHO 2009). The Coastal region has competent vectors for CHIKV as shown by Mulwa et al. (2018), Agha et al. (2017), and Mbaika et al. (2016), and this is attributed to the exacerbation of the intensity of the CHIKV transmission and outbreaks.

CHIKV neutralizing antibodies were also detected in a sample collected from a 5 year old in Nairobi County in the year 2009, indicative of past exposure to the virus. Nairobi County has never experienced any arboviral outbreak, when compared with Mombasa County.

O'nyong-nyong virus neutralizing antibodies were detected in Lamu, Taita-Taveta, Kilifi, Mombasa, Garissa, and Nairobi, indicating past exposure to the virus in all the three regions from samples collected between 2009 and 2014. A large number of samples neutralized with ONNV were observed in Coastal region, a finding consistent with the findings from a previous study by LaBeaud et al. 2015 that found ONNV exposure to be common in Coastal Kenya between the years 2014 to 2015. The last documented ONNV outbreak in Kenya occurred in 1961; the outbreak spread to neighboring countries, eventually affecting more than 2 million individuals but without any recorded fatalities (Rezza et al. 2017). We did not detect any neutralizing antibodies against SINV or SFV in the measles negative samples but this doesn't rule out the possibility of these viruses circulating among undiagnosed measles cases. This is because these viruses have been detected in serological surveys carried out in Kenya (Tigoi et al. 2015, Ofula et al. 2016) and also during vector surveillance carried out by Ochieng et al. 2013.

The results of this study agree with the findings by Antonio et al. 2019, where CHIKV IgM and IgG were detected in negative measles cases from 2009 to 2015 in Mozambique. Other studies have also been carried out to determine the etiologies of fever and maculopapular rash, and these have yielded results indicating that some of the etiological agents were overlooked or left out. First, in Brazil by De Moraes et al. 2011, where dengue virus was detected in 22 cases. Two of these 22 cases met Brazil's clinical case definition for rubella, which would have been reported to be rubella, but these 2 cases were diagnosed as dengue infection. Second, in Belarus, a study by Yermalovich et al. 2014 found antibodies against enteroviruses, human herpes virus, adenovirus, and human parvovirus B19 in measles or rubella suspected cases among children. Lastly, in Rio de Janeiro, Brazil a study by Oliveira et al. 2001, showed that dengue fever, rubella, parvovirus B19, measles, and human herpes virus 6 were among the causes of rash between 1994 and 1998. Possibly, the main contributor to these occurrences could be the fact that arboviruses are overlooked because of unavailability of resources in terms of equipment for diagnostics assays and specialized laboratories.

In this study, cross-reactivity was observed in samples that had tested positive for anti-CHIKV IgG on ELISA neutralizing with ONNV on PRNT assay. This finding is consistent with other studies reported in Kenya and Cameroon, where cross-reactivity was observed between CHIKV and ONNV (Kuniholm et al. 2006, LaBeaud et al. 2015, Tigoi et al. 2015). This could be due to the fact that both viruses may have been co-circulating in the region and continued evolution of ONNV has made it antigenically more similar to CHIKV than previously reported (Chanas et al. 1979).

Conclusion

This study found two cases of chikungunya and seven cases of o 'nyong' nyong viruses in suspected measles cases from various counties in Kenya that had tested negative for measles. There is need for continuous awareness among clinicians and the need to improve surveillance of these epidemic-prone arboviruses for better detection, outbreak detection, and clinical management.

Our findings, indicate clinical diagnosis is not sufficient to detect febrile rash cases and laboratory confirmation is essential to determine other causes of febrile rash illnesses in Kenya.

Arboviruses are overlooked because diagnostic assays are not typically available at the county and sub-county levels. The tests are often performed in very few specialized laboratories at national level. A further reason is the lack of ELISA kits for other alphaviruses, which makes it difficult to detect these viruses causing illness. We strongly recommend molecular testing of arboviruses to detect these arboviral infections in Kenya and testing for additional pathogens should also be considered in measles/rubella control.

We consider this study a success; but at the same time, we acknowledge existing limitations. First, over 90% of the study participants are children, given the surveillance of measles and rubella is focused on children. Second, molecular testing of alphaviruses was not possible due to limited resources. Third, logistically speaking it would be difficult to obtain convalescent samples having worked on archived samples. Fourth, we were also not able to test for other pathogens due to

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limited testing panels, and finally, the travel history of the patients was not captured during sample collection.

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Author Disclosure Statement

No competing financial interests exist.

Funding Information

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References

Agha SB, Chepkorir E, Mulwa F, Tigoi C, et al. Vector Competence of populations of aedes aegypti from three distinct cities in Kenya for chikungunya virus. PLoS Negl Trop Dis 2017; 11:E0005860.

Antonio VS, Amade NA, Muianga AF, Ali S, et al. Retrospective investigation of antibodies against chikungunya virus (Chikv) in serum from febrile patients in mozambique, 2009–2015: Implications For Its Prevention And Control. PLoS One 2019; 14:E0213941.

Chanas AC, Hubalek Z, Johnson BK, Simpson DI. A comparative study of o'nyong nyong virus with chikungunya virus and plaque variants. Arch Virol 1979; 59:231–238.

Chepkorir E, Tchouassi DP, Konongoi SL, Lutomiah J, et al. Serological evidence of flavivirus circulation in human populations in Northern Kenya: An assessment of disease risk 2016–2017. Virol J 2019; 16:65.

De Moraes JC, Toscano CM, De Barros EN, Kemp B, et al. Etiologies of rash and fever illnesses in campinas, Brazil. J Infect Dis 2011; 204 Suppl 2:S627–S636.

Ellis EM, Neatherlin JC, Delorey M, Ochieng M, et al. A household serosurvey to estimate the magnitude of a dengue outbreak in Mombasa, Kenya, 2013. PLoS Negl Trop Dis 2015; 9:E0003733.

Grossi-Soyster EN, Cook EAJ, De Glanville WA, Thomas LF, et al. Serological and spatial analysis of alphavirus and flavivirus prevalence and risk factors in a rural community in Western Kenya. PLoS Neglect Trop Dis 2017; 11:E0005998.

Kariuki Njenga M, Nderitu L, Ledermann JP, Ndirangu A, et al. Tracking epidemic chikungunya virus into the indian ocean from East Africa. J Gener Virol 2008; 89:2754–2760.

Konongoi SL, Nyunja A, Ofula V, Owaka S, et al. Human and entomologic investigations of chikungunya outbreak in mandera, Northeastern Kenya, 2016. PLoS One 2018; 13: E0205058.

Kuniholm MH, Wolfe ND, Huang CY, Mpoudi-Ngole E, et al. Seroprevalence and distribution of flaviviridae, togaviridae, and bunyaviridae arboviral infections in rural cameroonian adults. Am J Trop Med Hyg 2006; 74:1078–1083.

Labeaud AD, Banda T, Brichard J, Muchiri EM, et al. High rates of o'nyong nyong and chikungunya virus transmission in Coastal Kenya. PLoS Negl Trop Dis 2015; 9:E0003436.

Lanciotti RS, Ludwig ML, Rwaguma EB, Lutwama JJ, et al. Emergence of epidemic o'nyong-nyong fever in uganda after A 35-year absence: Genetic characterization of the virus. Virology 1998; 252:258–268.

Lutomiah J, Barrera R, Makio A, Mutisya J, et al. Dengue outbreak in Mombasa City, Kenya, 2013–2014: Entomologic Investigations. PLoS Negl Trop Dis 2016; 10:E0004981.

Lwande OW, Lutomiah J, Obanda V, Gakuya F, et al. Isolation of tick and mosquito-borne arboviruses from ticks sampled from livestock and wild animal hosts in Ijara District, Kenya. Vector Borne Zoonotic Dis 2013; 13:637–642.

Mbaika S, Lutomiah J, Chepkorir E, Mulwa F, et al. Vector competence of aedes aegypti in transmitting chikungunya virus: Effects and implications of extrinsic incubation temperature on dissemination and infection rates. Virol J 2016; 13:114.

Mease LE, Coldren RL, Musila LA, Prosser T, et al. Seroprevalence and distribution of arboviral infections among rural Kenyan Adults: A Cross-Sectional Study. Virol J 2011; 8:371.

Morrill JC, Johnson BK, Hyams C, Okoth F, et al. Serological evidence of arboviral infections among humans of Coastal Kenya. J Trop Med Hyg 1991; 94:166–168.

Mulwa F, Lutomiah J, Chepkorir E, Okello S, et al. Vector competence of aedes bromeliae and aedes vitattus mosquito populations from Kenya for chikungunya virus. PLoS Neglect Trop Dis 2018; 12:E0006746.

Nandy R, Handzel T, Zaneidou M, Biey J, et al. Case-fatality rate during a measles outbreak in Eastern Niger In 2003. Clin Infect Dis 2006; 42:322–328.

Natrajan MS, Rojas A, Waggoner JJ. Beyond fever and pain: Diagnostic methods for chikungunya virus. J Clin Microbiol 2019; 57:E00350–19.

Ochieng C, Ahenda P, Vittor AY, Nyoka R, et al. Seroprevalence Of infections with dengue, rift valley fever and chikungunya viruses in Kenya, 2007. PLoS One 2015; 10: E0132645.

Ochieng C, Lutomiah J, Makio A, Koka H, et al. Mosquito-borne arbovirus surveillance at selected sites in diverse ecological zones of Kenya; 2007–2012. Virol J 2013; 10: 140.

Ofula VO, Oundo J, Irura Z, Chepkorir E, et al. Evidence of presence of antibodies against selected arboviruses in Ijara And Marigat Districts, Kenya. Int J Infect Dis 2016; 45:188–189.

Oliveira SA, Siqueira MM, Camacho LA, Nogueira RM, et al. The aetiology of maculopapular rash diseases in Niterói, State Of Rio De Janeiro, Brazil: Implications For Measles Surveillance. Epidemiol Infect 2001; 127:509–516.

Paixao ES, Teixeira MG, Rodrigues LC. Zika, chikungunya and dengue: The causes and threats of new and re-emerging arboviral diseases. BMJ Glob Health 2018; 3:E000530.

Perry RT, Halsey NA. The Clinical Significance Of Measles: A Review. J Infect Dis 2004; 189 Suppl 1:S4–S16.

Rezza G, Chen R, Weaver SC. O'nyong-nyong fever: A neglected mosquito-borne viral disease. Pathogens And Global Health 2017; 111:271–275.

Sergon K, Njuguna C, Kalani R, Ofula V, et al. Seroprevalence Of Chikungunya Virus (Chikv) Infection On Lamu Island, Kenya, October 2004. Am J Trop Med Hyg 2008; 78:333–337.

Sigei F, Nindo F, Mukunzi S, ' ' Ng' ang' a Z, et al. Evolutionary analyses of sindbis virus strains isolated from mosquitoes in Kenya. Arch Virol 2018; 163:1–5.

Sutherland LJ, Cash AA, Huang YJ, Sang RC, et al. Serologic evidence of arboviral infections among humans in Kenya. Am J Trop Med Hyg 2011; 85:158–161.

Tigoi C, Lwande O, Orindi B, Irura Z, et al. Seroepidemiology of selected arboviruses in febrile patients visiting selected health facilities in the lake/river basin areas of Lake Baringo, Lake Naivasha, And Tana River, Kenya. Vector Borne Zoonotic Dis 2015; 15:124–132.

WHO. Chikungunya – *Mombasa, Kenya* [Online]. 2018. Available at: www.who.int/csr/don/27-february-2018-chikungunya-kenya/en/

WHO. Chikungunya–Kenya. 2016. Available at: www.who.int/csr/don/09-august-2016chikungunya-kenya/en/

WHO. *WHO African Regional Measles And Rubella Surveillance Guidelines* [Online]. 2015. Available at: www.afro.who.int/publications/who-african-regional-measles-and-rubella-surveillance-guidelines-0

WHO. Dengue Guidelines For Diagnosis, Treatment, Prevention And Control: New Edition. Geneva: World Health Organization, 2009.

Yermalovich MA, Semeiko GV, Samoilovich EO, Svirchevskaya EY, et al. Etiology of maculopapular rash in measles and rubella suspected patients from Belarus. PLoS One 2014; 9:E111541.

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