Seroprevalence of Chikungunya, Yellow fever and West Nile Viruses in Children at the Alupe District Hospital in Western Kenya

A Dissertation Submitted in Part Fulfillment for the Award of the Degree of Master of Medicine in Paediatrics and Child Health, at the University Of Nairobi

By

Dr. Mary Inziani Muyeku – Matilu, MBChB (University of Nairobi), MTropMed, (Nagasaki University, Japan)

Department of Paediatrics and Child Health, University of Nairobi

2011

Table of Contents

Declaration

I certify that this thesis is my own original work and has not been presented to any other university or institution for the award of a degree.

Signed: _____ Date: _____

Dr. Mary Inziani M. Matilu. MB.ChB (Nairobi), MTropMed (Nagasaki)

This dissertation has been submitted for examination with our approval as University supervisors.

Signed: _____ Date: _____

Professor Francis E. Onyango

Associate Professor of Paediatrics

Department of Paediatrics and Child Health

University of Nairobi.

Signed: _____ Date: _____

Dr. Elizabeth Maleche Obimbo

Associate Professor of Paediatrics

Department of Paediatrics and Child Health

University of Nairobi.

Signed: _____ Date: 10-11-2011

Dr. Matilu Mwau

Director, Centre for Infectious and Parasitic Diseases Control Research

Kenya Medical Research Institute, Alupe, Kenya

Signed: _____ Date: 10th November 2011

Dr. Shingo Inoue

Nagasaki University Institute of Tropical Medicine, Nairobi Research Station

Signed: _____ Date: 10th November 2011

Professor Masaaki Shimada

Nagasaki University Institute of Tropical Medicine, Nairobi Research Station

Signed: _____ Date: 10th November 2011

Professor Kouichi Morita

Nagasaki University Institute of Tropical Medicine, Nagasaki, Japan

Dedications

I dedicate this thesis to the children of Teso district, who I hope will benefit fully from the outcome of the research. And to my family, Matilu Mwau, " The Russian" Mwau Matilu, and "The Merovingian" Muyeku Matilu who gave me their full support and endured my absences during the research and thesis preparation.

Acknowledgement

I would like to acknowledge all the people, listed and not listed, whose contribution made this study possible. I am grateful to my supervisors, Professor F.O. Onyango and Professor E.M. Obimbo for their encouragement and constructive criticism, Dr. Florence Murila my mentor and Professor Ruth Nduati for her invaluable input.

My sincere thanks to Dr. Matilu Mwau, the Director CIPDCR who provided the overall leadership of this project and did the statistical analysis.

I am greatly indebted to Professor Kouichi Morita, the head of The Department of Virology at Nagasaki University, who was the sponsor and chief investigator of this study. He provided funding and strategic direction of the project. Dr. Shingo Inoue, Assistant Professor of Nagasaki University provided invaluable technical assistance and advises during the laboratory procedures. Dr. Toru Kubo of Nagasaki University who taught me the molecular methods used in this study. Prof. Masaaki Shimada, the Director of KEMRI-NUITM, who provided administrative assistance and advice.

Miss Janet Awando for her immense organizational skills and her technical assistance, Joyce Ngoi, Minayo Chahilu and Sheila Kageha for their technical support. Mr. Obura and Mr Francis Omao who were instrumental in the recruitment and data collection. Dr. Jimmy Wafula, the medical superintendent, and other staff of Alupe district hospital and the staff of KEMRI Alupe for allowing me to carry out this research. The parents/guardians and the children who agreed to participate in this study deserve special thanks.

The collaborative efforts between Kenya Medical Research Institute and Nagasaki University made this work possible.

Abbreviation

CHIKV	Chikungunya virus
CIPDCR	Centre for Infectious and Parasitic Diseases Control Research
DENV	Dengue virus
ELISA	Enzyme linked immunosorbent assay
EMEM	Eagle's Minimum Essential Medium
ERC	Ethical and Research Committee
FRNT	Focus reduction neutralization test
HI	Hemaglutinition inhibition
IgG	Immunoglobulin G
IgM	Immunoglobulin M
KEMRI	Kenya Medical Research Institute
KEPI	Kenya Expanded Programme on Immunization
NUITM	Nagasaki University Institute of Tropical Medicine
ONNV	O'Nyong' Nyong' virus
P/N	Positive value over negative value
PBS-T	Phosphate buffered saline-Tween
PCR	Polymerase chain reaction
PRNT	Plaque reduction neutralization test
RT-PCR	Reverse transcription PCR
WHO	World Health Organization
WNV	West Nile virus
YFV	Yellow fever virus

List of Tables

Abstract

Background: Arboviruses including Chikungunya virus, Yellow fever virus and West Nile virus are recognized causes of acute febrile illness in the tropics, and have been responsible for epidemic outbreaks in some parts of Kenya. During the past 20 years there has been a dramatic epidemic resurgence of a number of well-known arbovirus diseases thought to be effectively controlled or unimportant. Other viruses have expanded their geographic distribution such as the spread of Rift Valley fever virus in East Africa. However, these arbovirus diseases are not usually regarded as a differential diagnosis in children with fever in Kenyan health institutions because of lack of readily available and affordable diagnostic tests to detect them and a low index of suspicion.

The majority of arbovirus infections remain undiagnosed; as a result the frequency of arbovirus disease and the public health threat they pose is greatly underestimated. The non-specific nature of the clinical signs and symptoms of arbovirus infections makes it difficult to differentiate them from illnesses such as malaria, typhoid, dysentery and bacterial meningitis. Despite this, few surveys have been done to document the magnitude of infections from these viruses in children in Kenya.

Primary objective: To determine the seroprevalence of Chikungunya virus, Yellow fever virus and West Nile virus exposure in children attending Alupe District Hospital and KEMRI Alupe clinic.

Secondary objectives: To describe socio-demographic and clinical features observed in the exposed children, and the factors associated with seropositivity.

Study design: Hospital based cross sectional study.

Study sites: Alupe District Hospital and KEMRI Alupe Clinic.

Methodology: Children aged between one and twelve years whose parents/guardians gave informed consent were recruited consecutively into the study. Clinical and socio-demographic data was collected using a questionnaire and blood drawn for analysis.

Standard tests for malaria and typhoid were done for all children with reported or confirmed fever. A routine human immunodeficiency virus (HIV) test was also done for those who opted for it. Indirect Enzyme Linked Immunosorbent Assays were done to

screen for the presence of antibodies to the selected arboviruses. Data analysis was performed using STATA for Mac Version 9.

Results: Between August and December 2010, 425 eligible children were recruited into the study. 209 were males and 216 females. The Seroprevalence of arboviruses were as follows: Overall, 136(32%) were positive for at least one arbovirus exposure. Thirty one percent of 296 tested were positive for WNV, eleven percent of 298 tested were positive for CHIKV and seventeen percent of the 310 tested for YFV were positive. Having bushes, trees or forests near the home, photophobia, bruising of skin and dehydration and a positive malaria test were significantly associated with arbovirus seropositivity ($p<0.05$). Use of other mosquito control measures besides mosquito nets was associated with significant protection from West Nile virus and any arbovirus seropositivity

Conclusion: Children in Teso District are exposed to WNV, CHIKV, YFV and possibly other Arboviruses. Further studies are required to determine the prevalence and distribution of arboviruses in the wider Teso Community. Furthermore, virus isolation studies and virus characterization are required to determine specific strains circulating in the area and the role of arboviruses in the causation of fever in children. Personal protection from and vector control strategies are needed to control and prevent the transmission of arboviruses to non-immune populations like children. The use of other mosquito control measures besides mosquito nets confers significant protection from any arbovirus and WNV seropositivity but not from CHIKV or YFV seropositivity.

Literature Review

Introduction

Arboviruses, including Chikungunya (CHIKV), Yellow fever (YFV) and West Nile virus (WNV), which were of interest to this study, are arthropod borne viruses. By definition, arboviruses require a minimum of two hosts, a vertebrate and an arthropod, to complete their life cycle(1). Mainly mosquitoes, ticks, midges and sand flies transmit them. Arboviruses as a group have a global distribution, but the majorities are found in tropical areas where climate conditions permit year-round transmission by arthropods(1, 2). Arboviruses may have a focal geographic distribution that is limited by the ecologic parameters governing their transmission cycle. In general, the important limiting factors include temperature, rainfall patterns, and humidity, which in turn influence vegetation patterns and other ecologic parameters that determine the geographic distribution of arthropod vectors and vertebrate hosts. More than 100 arboviruses cause diseases in man(1).

Arboviruses are recognized causes of acute febrile illness in the tropics, and fever is a common presentation in children in Kenya. Several outbreaks of febrile illness have been associated with arbovirus infections(3-9). Studies have shown that CHIKV and O'Nyong' Nyong' virus (ONNV) are common causes of fever in certain parts of the country like Trans Nzoia, the Coast and Rift Valley(8, 10, 11). It is therefore conceivable that one of the causes of fever in Kenyan children may be arbovirus infection.

The majority of infections caused by arboviruses remain undiagnosed; as a result the frequency of arbovirus diseases and the public health threat they pose is greatly underestimated. A major factor for this situation is the nonspecific nature of the clinical signs and symptoms of arbovirus infections, which may be confused with illnesses such as malaria, typhoid, dysentery and bacterial meningitis(12). Majority of the infections are also subclinical and may present without fever. However, arbovirus infections are not usually regarded as a differential diagnosis in children with fever in Kenyan health institutions because of lack of readily available and affordable diagnostic tests to detect them and a low index of suspicion by clinicians who are not familiar with the clinical presentation of these viral diseases. Children with fever in malaria endemic areas like Teso District are therefore commonly treated empirically with antimalarial drugs and

antibiotics. This exposes them to unnecessary side effects; their parents and the government to unnecessary costs and increases the risk of losing essential antimicrobial drugs due to development of resistance.

During the past 20 years there has been a dramatic epidemic resurgence of a number of well-known arbovirus diseases thought to be effectively controlled or unimportant, such as WNV, DENV, YFV, Japanese encephalitis virus, Rift Valley fever virus, Ross River virus, to name just a few. Several viruses have also expanded their geographic distribution. WNV has spread into the United States of America while YFV and Rift Valley fever virus have spread in East Africa, beyond their earlier geographic locations(3, 6, 13, 14), which raises the potential for arbovirus disease outbreaks in new regions where the population is non-immune to the infecting virus.

Arboviruses such as YFV, DENV, ONNV, CHIKV, WNV and Rift valley fever virus have been responsible for epidemic outbreaks of public health significance in some parts of Kenya, that were characterized by significant morbidity and mortality(6, 7, 12, 13, 15, 16). For instance, during the first recorded outbreak of YFV in Northern Kenya, 54 cases were identified and 29 deaths occurred, an attack rate of 27.4 per 100,000 populations, and a case-fatality rate of 19%(6, 17). The outbreak of CHIKV disease in Lamu was associated with high fever and severe protracted arthralgias, and seroprevalence findings suggested that the outbreak was widespread, affecting 75% of the Lamu population(7). The first major epidemic caused by ONNV in East Africa in 1959 affected more than 2 million people, with attack rates of up to 70% in some areas. The patients presented with high fevers, headache, severe joint pain, skin rash, lymphadenitis and painful eyes(18). Despite evidence of these arbovirus outbreaks reported in Kenya and the morbidity associated with them, few surveys have been done to document the magnitude of infections from these viruses in children in Kenya.

Western Kenya is heavily forested and mosquito species that transmit arboviruses and other vector borne diseases like malaria, including *Aedes, Culex* and *Anopheles* are prevalent. Exposure to arbovirus infections is probably a commonplace occurrence in this area where man has encroached on forests for habitation or livelihood. Human activity occurs on the edge of the forest, ranging from bush clearing for farming, to cattle rearing to sugarcane farming. In this rural area, children are often involved in these activities. Even infants accompany their mothers, often tied on the back as the mother goes about

her farming, firewood-gathering chores amongst other activities. These human activities bring people and livestock into contact with mosquitoes and expose them to viral infections transmitted via mosquito bites. It is therefore probable that arboviruses are an important cause of fever or subclinical infection in Western Kenya, even in children(11). But this cannot be categorically stated without going out of the way to investigate them.

Anti-arbovirus immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies can be detected in the blood of healthy as well as febrile arbovirus infected children(19). It is therefore important to determine the prevalence of arboviruses in children; the clinical features and the factors associated with seropositivity. Detection of the presence of arbovirus antibodies in the blood of children can be achieved using serological tests such as enzyme-linked immunosorbent assay (ELISA), hemaglutinition inhibition (HI), complement fixation (CF), plaque reduction neutralization test (PRNT) and focus reduction neutralization test (FRNT)(19, 20). Determination of the seroprevalence of arboviruses in children, who are at most risk of infection in endemic areas is important in the early detection of arbovirus activity, in order to implement preventive measures before outbreaks occur.

Classification

Arboviruses are classified into 3 main families: *Togaviridae*, *Flaviviridae* and *Bunyaviridae*. Some arboviruses are antigenically distinct from the three main families and are classified separately. Viruses that cause similar disease in each family are then grouped together(19).

CHIKV and ONNV belong to the *Togaviridae* family, genus alphavirus(19, 21), whereas, YFV and WNV belong to the family *Flaviviridae*, genus Flavivirus(19, 22). Rift valley fever and Crimean Congo hemorrhagic fever viruses belong to the *Bunyaviridae* family(19).

Epidemiology of the Selected Arboviruses

Occurrence and outbreaks of selected arboviruses in Kenya

Dr. David Bylon first described CHIKV infection in 1799. Several epidemics in Africa, Arabia, India and Asia were described in the 19th century. The modern geographic distribution of CHIKV outbreaks has been in Sub-Saharan Africa, India and South East Asia(21). In Kenya, an outbreak of CHIKV occurred in Lamu Island beginning May 2004

and peaked in July 2004. After that outbreak, other associated outbreaks occurred in Mombasa, Kenya, between November and December 2004, and spread to other islands in the Indian Ocean, and in India in 2005 to 2006(7, 21).

WNV is widespread in Africa, North America, Europe, the Middle East and India. It occurs in both endemic and epidemic transmission patterns. All age groups are affected in epidemics, but children are affected more often in endemic areas. It was first isolated in 1937 from the blood of a febrile patient in Uganda(23). It has caused both urban and sub-urban outbreaks in several countries, including Egypt, South Africa and Kenya(24) in Africa. Outbreaks tend to occur during heavy rainfall and summer months, with high attack rates as occurred in South Africa in 1974(25).

Yellow fever was first recognized as a disease in the 17th century in North America. It is endemic in Africa and South America. It was first isolated in a patient in Ghana. Yellow fever remains an important disease of international public health significance, but especially in Africa and South America(20). Yellow fever occurs in two major forms: urban and jungle or sylvatic yellow fever. The potential geographic distribution of urban Yellow fever is any areas infested with *Aedes aegypti* mosquitoes(20, 22, 26). The majority of reported human YFV cases come from Africa and South America. In endemic areas, young children are the ones who are more susceptible to disease whereas all age groups will be susceptible in an urban Yellow fever outbreak(20, 26). Although there is some evidence to suggest past outbreaks have occurred, the first documented epidemic of YFV in Kenya was reported between 1992 and 1993 in Kerio Valley(6, 27).

Seroprevalence of Selected Arboviruses in Kenya

The seroprevalence of arboviruses has been found to vary during non-outbreak and outbreak periods, and in different parts of the country(3, 28). Studies done in Kenya found the seroprevalence of CHIKV to be as low as 0.7% during a non-outbreak period along the Kenyan coast in 1987(5) and 75% after the outbreak in Lamu Island in 2004(7). A serosurvey conducted during September 1987 for evidence of human arbovirus infections in the Coast Province of Kenya revealed the following seroprevalence: RVF, 2.8%; Sindbis, 2.6%; Dugbe, 2.1%; DEN-2, 1.0%; WNV, 0.9%; CHIKV, 0.7% and Nairobi sheep disease, 0.3%(5).

During the first recorded YFV outbreak in Kerio valley, seroprevalence was found to be 47%(6, 27), and the mortality rate in this outbreak was 19%. The outbreak ended when mass vaccination of almost one million persons in the affected districts of Keiyo, Baringo and Koibatek was carried out(20). A surveillance system involving 13 sentinel sites and lasting about a year was established six months after the epidemic was over. An earlier surveillance in North Eastern province had estimated the prevalence of YFV in this area at 14%(29, 30).

In 2004, Rodney LC. *et al*., performed surveillance for presence of IgG against CHIKV, DENV, Rift Valley fever, WNV, and YFV among 820 patients admitted with acute febrile illnesses in three district hospitals in Kenya, (Alupe 127, Malindi 458 and Isiolo 215). CHIKV was detected in 7.06%, DENV in 6.93%, Rift Valley fever in none, WNV in 2.19% and YFV in 1.22% of these patients. However, significant regional differences in antibody rates were noted, given the vastly different ecologies of the districts under study. Little anti-arbovirus IgG was detected in semi-arid Isiolo, while Alupe and Malindi demonstrated smaller proportions of IgG positive patients for all tested arboviruses except DENV, which was found almost exclusively in Malindi(8).

Most seroprevalence studies for arboviruses in Kenya have been done in adults. However, one survey of school children aged 5 to 15 years from 26 schools south of Kisumu was done in May and June 1969, for hemaglutinition inhibition (HI) antibodies to selected arboviruses. Sera tested from 559 12 year olds revealed that 65% had antibodies to ONNV virus, 36% to CHIKV and 19% to Nyando virus(31). Antibodies to Sindbis, WNV besides others were also found in this age group. HI antibodies to CHIKV were found in 35% and ONNV in 38% of the 4 and 5 year old children(31). A serological survey of selected arboviruses in the age group 0-14 years was also conducted in Kenya in November 1966 to April 1968, in 3 three different districts, namely Central Nyanza near Lake Victoria, (neighboring present day Teso district), Kitui District formerly in Central Kenya and Malindi District in the Coast Province. The HI tests on the sera yielded varying results in the three areas. Due to cross reactivity and similar prevalence rates, ONNV and CHIKV were therefore considered together. CHIKV/ONNV was found in 55% of 176 children examined in Central Nyanza, and YFV and WNV in 1%. CHIKV/ONNV was found in 0.2%, YFV in 0.0% and WNV in 10% of the 441 children tested from Kitui District. CHIKV was found in 33%, YFV in 9.4% and WNV in 37.4%

of 265 children tested from Malindi. DENV was tested in 20 children from Central Nyanza, in 42 from Kitui and in 33 from Malindi. 3% were positive for DENV in Malindi but none were positive in Central Nyanza or Kitui(3).

The data from the studies above indicate clear evidence of arbovirus exposure in children, and suggest that several arboviruses could be circulating amongst certain Kenyan populations below the outbreak thresholds. However, there are no current studies that have documented the prevalence of arboviruses in febrile children in Kenya to date.

Transmission

Arbovirus transmission is cyclic. The virus replicates alternately in vertebrate and hematophagus hosts. Arthropods become infected following a blood meal on a viraemic vertebrate host and remain infected for life. Infections in humans are incidental and are usually acquired through the bite of an infected vector during a blood meal on a susceptible human. In rare cases such as DENV and YFV, humans can serve as the principle source of virus amplification and vector infection in the urban setting, for further spread to other humans(19, 22). Four new modes of WNV transmission to humans were identified in 2002: blood transfusion, tissue transplantation, transplacental transfer and breast-feeding(32).

The transmission of mosquito borne arboviruses is highly seasonal(33). It is highest in the rainy season, with high ambient temperatures, when the breeding of mosquito vectors is also highest. The *Aedes aegypti* that transmit urban YFV and DENV breed all year round in domestic water storage containers, but vector density is increased during the rainy season, when rainwater fills puddles, discarded containers, old tyres, sewers and open flower pots. Transmission of arboviruses is therefore highest during the rainy season and warmer months.

Reservoirs

Reservoirs for most arboviruses are unknown. Trans ovarian transmission of Ross River virus has been demonstrated in *Aedes vigilax* and YFV in *Aedes aegypti*, making an insect reservoir a possibility. It is thought that similar transmission cycles may occur with other Alphaviruses(19) like CHIKV and ONNV. Birds are the reservoirs for WNV(34). Jungle YFV is maintained between the natural reservoir, monkeys and forest

or canopy mosquitoes, while in the urban cycle it is maintained between man and *Aedes aegypti* mosquitoes(26).

Mosquito vectors

CHIKV virus is transmitted by *Aedes aegypti* and possibly others(21). *Aedes aegypti* is a peridomestic-breeding mosquito and is found in the urban and peri-urban environment. *Aedes africanus* is the vector implicated for the sylvatic transmission of YFV in Kenya(22, 26), while *Aedes aegypti* has been implicated in urban transmission of YFV in West Africa(22, 26). WNV is transmitted by various species of *Culex* especially *Culex univittatus,* identified in southern Africa(21, 25, 34).

Risk factors and susceptibility

The vector, virus, wild vertebrate host, humans and environmental factors influence risk factors for infection with arboviruses. Exposure to mosquito vectors infected by an arbovirus is a risk factor for infection by arboviruses. Availability of non-immune susceptible human hosts and an increased number of infected vectors increases the risk for infection(19, 21). Human activities that bring humans into contact with forest mosquitoes like hunting and gathering, clearing forests for agricultural activities and visiting forests increases the risk of exposure. Changing lifestyles renders people susceptible to arthropod bites. For instance urbanization, accompanied by poor drainage and poor waste disposal with open sewers and discarded containers that collect rainwater, water storage practices, construction of irrigation schemes and dams enhances mosquito vector diversity and provides additional breeding habitats and renders people susceptible to bites by domesticated *Aedes species* of mosquitoes that breed in these sites. Visiting endemic areas is also a risk factor to tourists and other visitors, while increased air travel leads to transportation of susceptible persons into endemic areas as well vectors and viruses into new habitats. Mosquitoes can bite children while playing near forested areas, herding cattle, fetching firewood, or sleeping without a mosquito net.

All age groups and both sexes are susceptible to infection by CHIKV, YFV and WNV. The elderly and the immuno-compromised, due to HIV AIDS, organ transplant recipients, diabetics and those on chemotherapy are more susceptible to clinical disease from arbovirus infection. In CHIKV infection, unapparent infections are common especially in children, among whom overt disease is rare(19, 21). In epidemic

polyarthritis caused by these two viruses, arthritis occurs most frequently among adult female patients. All age groups are susceptible to YFV and WNV infections, although the elderly are at increased risk of severe central nervous system disease caused by WNV infection. However, asymptomatic illness and mild infections are common in all these arbovirus infections, but lasting homologous immunity usually follows recovery. Therefore, the most susceptible age group in endemic areas is mainly young children who have no immunity to the arboviruses(19).

Incubation period

The incubation period for CHIKV, and WNV on average ranges between 3 and 12 days(21). For YFV the incubation period ranges between 3 to 6 days(22, 26).

Clinical presentation of arbovirus infections

Arboviruses cause a range of clinical syndromes in humans depending on the infecting virus, that range from a self-limiting, febrile illness of short duration to life threatening encephalitis or hemorrhagic fever. The commonest presentation for most arboviruses is benign.

Four major clinical syndromes caused by arboviruses are:

1. Acute central nervous system illness ranging in severity from mild aseptic meningitis to encephalitis with coma, paralysis and death.
2. Acute benign fevers of short duration with or without exanthem. Some may give rise to more serious illness with central nervous system involvement or haemorrhages
3. Haemorrhagic fevers including acute febrile diseases with extensive haemorrhage and jaundice.
4. Polyarthritis and rash with or without fever of variable duration, benign or with arthralgic sequelae lasting several weeks to months.

CHIKV and ONNV both cause fever, arthralgia and rash. Hemorrhage also occurs rarely in infections with CHIKV leading to gum bleeding and petechiae. Subclinical infection is common especially in children(19). In a study focusing on children in Bangkok, the most common presenting symptoms of CHIKV infection were vomiting (35%) and abdominal pain or anorexia (18%). Arthritis and arthralgia were less

prominent. Pharyngitis (70%) and facial flushing (24%) were the most frequent conditions. No rash was seen in the children(35).

Majority of cases of WNV infection present as mild febrile illness that resolves within two to five days, especially in children in endemic areas. The disease commonly presents with fever, headache, malaise, arthralgia or myalgia. Occasionally anorexia, nausea, vomiting and diarrhea occur. Conjunctivitis, ocular pain, photophobia and pharyngitis may also occur. A maculopapular, non-desquamating rash of the trunk, face and extremities is common in WNV infection. Meningoencephalitis is an occasional complication of WNV infection especially in the elderly where it may be fatal, though increasing numbers of cases in children have been reported. Convalescence is rapid in children but somewhat more prolonged in adults(22, 34).

Yellow fever is an acute infectious disease of short duration and varying severity. Mildest cases may be difficult to clinically recognize as YFV infection. Yellow fever disease is characterized by sudden onset fever, chills, headache, backache, generalized muscle pains, prostration, nausea and vomiting. The pulse may be slow and weak out of proportion to the elevated temperature. Jaundice is mild early in the disease but later intensifies. Leukopenia occurs commonly. Albuminuria and anuria with renal failure may occur(4, 22, 26). The case fatality rate may reach 20% to 80%; however, these figures are based on the most severe cases that are hospitalized and the overall case fatality rate is lower(2).

Antibody response to arbovirus infection

Following infection with an arbovirus, IgM appears in blood in the first three to five days and may last up to six months to a year. IgM is considered a marker for a recent infection. IgG appears within the first 2 weeks and may persists for many years to a lifetime. It is a marker of exposure to the specific arbovirus, indicating past infection(19).

In the case of YFV infection, antibodies appear in blood within the first week, and transient passive immunity occurs in infants born to immune mothers and persists for six months. Anti YFV IgM lasts about two months following infection(22, 26).

Diagnosis

Diagnostic tests for arbovirus infections are expensive and are not readily available in the clinical setting in Kenya. However, some of the tests are available in a research setting at KEMRI. These tests are described below:

Virus isolation

Virus isolation is the most definitive test for arbovirus diagnosis. This is done through cell culture such as *Aedes albopictus* derived C6/36 cells and African green monkey kidney derived Vero cells, or inoculation of serum collected within 24 to 48 hours of disease onset into susceptible animal models like suckling mice. The results are released after 1-2 weeks. Virus isolation is carried out in biosafety level (BSL) 2 or 3 laboratories to reduce the risk of laboratory infection.

Nucleic Acid Amplification Test

Tests such as reverse transcription-polymerase chain reaction (RT- PCR) techniques and reverse transcription loop mediated isothermal amplification assay (RT-LAMP) are available for diagnosing most arboviruses. They detect viral nucleic acid in patient's acute phase serum. PCR results are released after 1-2 days, whereas LAMP method can yield results within a few hours.

Serological diagnosis

For serological diagnosis, an acute phase serum must be collected immediately after clinical onset of disease and a convalescent phase serum 10-14 days after the disease onset. If onset of fever is designated as day 0, sero-diagnosis rests on demonstrating a fourfold increase in IgG antibody titer between the acute phase (day 5 to 7) and convalescent phase sera (day 8 to 14). However, getting paired sera is usually not practical. Alternatively, the demonstration of IgM antibodies specific for a given virus in acute-phase sera is used in instances where paired sera cannot be collected. Commonly used tests are the IgM capture enzyme-linked immunosorbent assay (MAC-ELISA) and the indirect IgG Enzyme Linked Immuno-sorbent Assay. Results of MAC-ELISA are ready in 2-3 days. Cross-reaction with antibodies of other alphaviruses occurs in the ELISA tests, such as between CHIKV and ONNV and Semliki Forest viruses; and with other flaviruses such as between YFV, WNV and DENV. Confirmation of the

specific virus can be done by neutralization tests such as PRNT and focus reduction neutralization test (FRNT). A positive virus culture together with a positive neutralization test is taken as definitive proof of the presence of a virus. A positive PCR is diagnostic.

Prevention

Prevention of alphaviruses and flaviviruses focuses on vector control and protection of individual from mosquito bites, through destruction of breeding sites, insecticide sprays, insecticide treated nets (ITNs), insect repellants and screening of house windows and doors to prevent mosquitoes from entering houses(21, 22). Regular surveillance of susceptible vectors and vertebrate hosts is used to detect arbovirus activity early so that preventive measures can be put in place early before outbreaks occur(36). There is an effective attenuated YFV vaccine available for immunization against the virus(20, 22, 26). A formalin-inactivated CHIKV vaccine and a live attenuated CHIKV vaccine have been developed but are not yet in clinical use(37, 38). Vaccines for the other arboviruses in humans are still undergoing experimentation.

Treatment

There is no specific antiviral drug for the treatment of arbovirus infections. Management for these infections is mainly supportive and symptomatic. Analgesics are given for arthralgia, antipyretics like paracetamol for fever and any other necessary symptomatic treatment. Bed rest is also recommended. Intravenous fluid replacement is the mainstay of treatment for hemorrhagic fever. Blood transfusion may be required in severe hemorrhagic fever(19).

Justification:

Arboviruses such as YFV, DENV, Rift Valley fever virus besides others are emerging or re-emerging and expanding their geographic locations to new areas, with potential to cause epidemics. Outbreaks of several arboviruses have been recorded in Kenya over the years and children are at highest risk of arbovirus infections in endemic areas. However, no recent surveys to document the magnitude of arbovirus exposure in children have been attempted in Kenya to date, therefore the current seroprevalence of arbovirus infections in children in Kenya remains unknown. Arboviruses and malaria both cause similar nonspecific clinical features including fever yet fever in this region is commonly ascribed to malaria or typhoid even when a malaria test is negative, and these children are usually treated with antimalarial drugs or antibiotics empirically. Arbovirus infections therefore go undiagnosed.

This study determined the seroprevalence of CHIKV, YFV and WNV in children, described clinical features and socio-demographic features observed in the exposed children and the factors associated with seropositivity. The data generated has greatly contributed to new data on the magnitude of arbovirus exposure in children in Alupe. This information forms a basis to stimulate the planning and conduction of further widespread prevalence studies and detailed studies to determine the role of arboviruses in the etiology of febrile illnesses in children and in the planning and setting up of public health intervention programs for the prevention of arboviruses. It also informs clinicians to consider arboviruses as a differential diagnosis in febrile children. The increasing rate of YFV exposure shown by this study exposes the need to expand the coverage of the effective YFV vaccine already in use in some parts of the Rift Valley province to Alupe.

Objectives of the Study

Primary Objective

To determine the seropositivity of CHIKV, YFV and WNV among children presenting at Alupe District Hospital and KEMRI Alupe clinic.

Secondary Objectives

1. To describe the clinical and socio-demographic features observed in the children exposed to CHIKV, YFV and WNV.
2. To determine factors associated with arbovirus seropositivity among these children with emphasis on socio-demographic, clinical and environmental factors.

Materials and Methods

Study Design

This was a hospital based, cross-sectional study.

Study Area

This study was carried out at Alupe District Hospital and KEMRI Alupe clinic, which are located in Teso District of Western Province of Kenya, eight Kilometers north of Busia town. Teso district covers an area of 559 square kilometers(39). The altitude varies from 1130m to 1375m and Alupe is located at an altitude of 1250m above sea level. The climate of the district is modified equatorial characterized by a mean annual rainfall of 1500 mm divided between two rainy seasons; the long rains between March and June and the short rains between September and December. The annual maximum temperature ranges from 26°C to 30°C and minimum temperatures vary from 14°C to 18°C. The countryside is bushy woodland and infested with mosquitoes and tsetse flies. To the south of Alupe is the Budalangi Flood Plain. Teso district is traversed by rivers Sio and Malaba and several streams that feed into them. The flood plain and the rivers provide breeding grounds for mosquitoes.

Source Population

Study patients were drawn from the entire population of Teso district. The population of the district is 181,491 and the average population density is 325/sq. km(39). The population served here includes both Luhya and Teso ethnic groups, as well as migrants from Uganda. Only approximately 7% live in an urban area of Malaba town, the Chakol divisional headquarters (39). The rest of the population resides in rural areas comprising of market centres and villages with individual homesteads. Majority of the people live in mud walled grass thatched houses. Others live in mud walled houses with iron sheet roofing. Most of the permanent houses are found in the urban centres, market places, administrative centres and schools.

Mixed farming is the main economic activity in the District, with a variety of livestock species, mainly cattle, sheep, goats and poultry reared. Limited commercial farming mainly of sugar cane is practiced, as well as subsistence farming of maize, beans, millet, sorghum, potatoes and cassava. Living standards are generally low and social

amenities like running water and electricity are not available to the majority of the residents in the district. Therefore, water for domestic use is collected from streams, ponds, rivers and rainwater and stored in containers at home, a practice which promotes breeding of *Aedes aegypti* mosquitoes, the vectors for YFV and DENV. In this poor rural population, children are involved in agricultural activities that bring them into contact with mosquitoes and expose them to arbovirus infections.

Study Sites

Alupe District Hospital and KEMRI Alupe clinic are located in the same compound as the Centre for Infectious and Parasitic Diseases Control and Research (CIPDCR), KEMRI, which houses KEMRI Alupe clinic. The hospital was initially set up as a leprosy hospital but later converted into the district hospital for Teso. It serves the entire population of the district. The average number of pediatric patients seen at Alupe District Hospital number about 30 each day and about 800 each month, of whom approximately 16 to 22% present with fever. Approximately 12 children are seen per day from Monday to Friday at the KEMRI Alupe clinic.

Study Population

Subjects recruited into the study included 1-12 year old children attending Alupe District Hospital and KEMRI Alupe clinic during the study period.

Inclusion Criteria

This study included children aged between 1 and 12 years, attending Alupe District Hospital or KEMRI Alupe clinic and had a written consent by a parent or guardian before recruitment into the study.

Exclusion Criteria

The study excluded children below 1 year or above 12 years of age and those whose parent or guardian was unable or unwilling to give written consent.

Study Period

This study was conducted between August and December 2010.

Definition

A patient exposed to arbovirus (seropositive) was defined as any child in whom arbovirus antibodies specific to CHIKV, YFV and WNV were detected in serum.

Sample size calculation

We suppose that the children attending Alupe District Hospital and KEMRI Alupe clinic during the study period represent the total population. Therefore sample size was determined using Cochran formula as follows(40, 41):

$$N = \frac{Z^2 P (1-P)}{D^2}$$

Where:

N = minimum sample size required

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

P = the prevalence of CHIKV recorded in children aged 0-14 years in the nearby Central Nyanza district in 1966 = 55% (3).

D = degree of precision = 5%

$N = 1.96^2 \times 0.55 \times (1-0.55)/0.05^2$

N = 380 = minimum sample size. Since experimental failure is expected in approximately 10% of samples, the sample size was adjusted to 418 = 420 children.

Study Procedure

Recruitment

The principal investigator and the two trained study clinicians recruited eligible children and collected data at the pediatric outpatient department, in-patient ward of the hospital, and at the KEMRI Alupe clinic on Monday to Friday, from 9am to 5pm. The interviewer introduced himself/herself and explained to the parents and guardians the purpose of the study. Informed verbal and written consent was sought from parents/guardians who allowed their children to take part in the study. The interviewees were assured of confidentiality of the data and requested to answer questions truthfully. Eligible children for whom consent was given were recruited consecutively until the desired sample size was achieved.

Data collection

Data was collected by taking a brief history from the person accompanying the patient, examining the patient's clinical records and the immunization card where available and examining the patient physically. A structured questionnaire was then administered. Information collected included age, sex, area of residence, type of house, parental/guardians' occupation, school attendance, status of primary vaccinations, history of vaccination for yellow fever, history of previous illnesses and hospital admissions, past history of fever, rash, joint pains or swelling, headache, jaundice and photophobia or during current illness, history of mosquito bites, the activities engaged in after school e.g. herding livestock, firewood gathering, fetching water, gathering fruits and herbs from the forest, besides others as per the questionnaire (Appendix 1). Vaccination status was confirmed from the immunization card where available. Due to logistical difficulties and limited funding, home visits were not made by a trained fieldworker to the households to evaluate the home environment and verify the information given, as had been anticipated.

Clinical Procedures

The principle investigator, or the study clinician performed a complete physical examination and took anthropometric measurements including weight, height, mid upper arm circumference (MUAC) and temperature (as per the proforma outlined in appendix 1.) as follows:

Weight

Infants' weights were measured using an infant beam balance scale with 100gm increment. The older children were weighed using a standing (adult type) beam balance scale also with 100gm increment. The scale was placed on a flat and firm stable surface. Children aged 2 years and above were required to have minimal clothing at the start and completely undressed for those below this age.

For infants, the scale was covered with paper. The kilogram and gram sliding beam weights was placed directly over their respective zeroes then the screw on the adjustable zeroing weight or counter weight was loosened. The screw was moved until the beam balanced, and then tightened on the counter-weight. The infant was then placed on his/her back or sitting on the tray of the scale, ensuring that the child was centered in the tray and not touching anything off the scale tray including other parts of the scale.

The kilogram weight was then moved until the first notch where the beam falls, then moved the weight back one notch. The gram weight was then slowly moved across the beam until it was balanced. Measurement was then be taken to the nearest 100 gram and recorded.

The same procedure was done for the older children, except that they were stepping onto the center of the platform of the weighing scale. Their weight was also taken to the nearest 100 grams.

The scale was zeroed before each weighing and calibrated after every eight-hour session.

Height/length

Height was measured using a portable wooden stadiometer. Recumbent length was measured in children younger than 2 years or under 85cm if age was not known or those who were too ill to stand. The child was made to lie parallel to the long axis of the board and the crown of the head placed against the fixed board. An assistant gently held the head so that the child was facing directly up with the line of sight (Frankfort plane) at a right angle to the board. The measurer then held the knees together and pushed them down against the measuring board with one hand to bring them to full extension. The movable board was then brought up against the heels with the other hand until in contact with the feet. The movable board was then secured as the feet were withdrawn from contact with the board. The length was read and recorded to the nearest 0.1 cm.

Standing height was measured in children above 2 years of age or taller than 85 cm, ensuring the mid axillary line was parallel to the measuring board and the head in the Frankfort position, feet flat on the footplate portion with back against stadiometer ruler and legs together, knees not bent, arms at sides, shoulders relaxed. The headpiece was lowered snugly to crown of head with sufficient pressure to press hair. The value was read at eye level to nearest 0.1 cm and recorded immediately on the data form.

Mid upper arm circumference (MUAC)

The MUAC was measured on the left arm with the upper arm hanging on the side of the trunk, by passing a linen tape midway between the acromion process of the scapula and the tip of the olecranon. The reading was recorded to the nearest 0.1cm. The measuring tape was checked against a standard metal tape at frequent intervals to avoid any errors due to stretching.

Temperature

Axillary temperature was measured using a digital thermometer. The thermometer was switched on, placed in the child's left axilla, held in place until a beep was heard. The temperature reading was then recorded to the nearest 0.1^0 centigrade. The thermometer was cleaned with alcohol swabs before use on the next patient.

Physical examination

A physical examination was then done, specifically looking for presence of a rash, bleeding stigmata, fever, jaundice, conjunctivitis, pharyngitis, stiff neck, altered consciousness, hepatomegaly, and joint swelling and/or tenderness and the findings recorded as indicated in the data collection form. The patient was then referred to the laboratory for blood sampling.

Blood sampling

A venous blood sample was obtained in a sterile procedure as follows: The veins in the antecubital fossa or dorsum of the hand were identified and a tourniquet applied to make the veins visible. The area was then cleansed with an alcohol swab and allowed to air dry. Approximately 2.5ml of blood was drawn from children without fever using a sterile needle and syringe. Four drops of the blood sample was used for HIV testing of children whose parents/guardians consented for the child to be tested for HIV. The rest was dispensed into a serum vacutainer tube with clot activating gel. Approximately 30 minutes was allowed for clotting of the blood sample at room temperature. An additional 2.5ml of blood was obtained from children with fever. Two drops of this blood was used for a standard malaria test, and the remaining was dispensed into a plain vacutainer for a standard widal test to exclude malaria and/or typhoid as a cause of fever.

Sample handling and transport

The blood samples for ELISA were centrifuged at 2,500 revolutions per minute (rpm), for 10 minutes, at 4^0 Celsius. Serum was obtained by pipetting and placed into plastic vacutainers. The serum samples were then transferred within one hour to KEMRI laboratory at Alupe, and immediately stored at -80^0 Celsius until tested. The HIV test was done on site by a trained counselor according to the ministry of health's guidelines and hospital policy on provider initiated testing and counseling. Two (2) drops of blood were directly applied on a slide to make a thick blood smear for a standard malaria test. The

blood slide and the blood in the plain vacutainer were transported to the KEMRI Alupe Laboratory for a standard malaria test and widal test for typhoid.

Laboratory Procedures

Arbovirus antibody specific for each of the 3 viruses were analyzed by ELISA at KEMRI laboratories as described in appendix 4(42). The blood slide was air dried, stained and subjected to microscopy in a standard malaria test. The blood in the plain vacutainer was used in a standard qualitative widal test for typhoid and the results recorded. (This test only identifies exposure to typhoid bacteria)

Data Storage and Analysis

All the data collected was coded, cleaned and entered into an excel file in a password-protected computer. The data was then converted into STATA version 9 for Mac for analysis. Overall seropositivity rates, as well as virus-specific seropositivity rates were calculated. Descriptive statistics were calculated for socio-demographic, clinical and environmental variables. Unadjusted logistic regression models were used to examine the effects of socio-demographic, clinical and environmental variables on the odds of seropositivity to arboviruses. *P* values less than 0.05 were considered significant.

Ethical Considerations

This study was approved by Ethical and Research Committees (ERCs) of Kenyatta National Hospital, KEMRI and Alupe District Hospital. The parent/guardian of the children enrolled in the study did not incur any cost in relation to the study; neither did they receive any monetary inducements to participate in the study. An informed written consent was sought from the parents/guardians of the children enrolled in the study. The questionnaire and consent in both English and Swahili were availed to study participants and guardians to read before consenting. The results of the malaria and widal tests done were made available to the primary clinician for appropriate treatment of the patient. The risk of fear, pain or bleeding from the venipuncture site and the benefits of the study to the community in helping the government to understand the magnitude of arboviruses in the community and designing appropriate interventions to minimize the impact of these infectious diseases were explained to the study participants. HIV testing was done with consent according to the Ministry of health guidelines, and positive patients were referred for appropriate care.

Results

A total of 425 children were recruited into the study between August and December 2010. All 425 had at least one anti-arbovirus antibody test carried out on the serum. Due to shortages in virus antigens for carrying out ELISA tests, only 274 (64.5%) had their serum tested for all 3 arboviruses, that is WNV, YFV and CHIKV ELISA, 31 (7.3)%) had 2 tests done, while 120 (28.2%) were tested for one arbovirus antibodies. Of the 425, 310 were tested for YFV, 298 for CHIKV and 296 for WNV antibodies. 304 (74.8) were also tested for malaria, 313 (76.2%) had a widal test done, while 289 (67.8%) had an HIV test done.

At least (97.5%) of the study participants hailed from Western Province. Approximately 1% came from neighboring Eastern Uganda. More than half the recruited children (67.1%) were from Teso District.

Demographic Characteristics of Study Participants

Table 1. Demographic Characteristics of the Study Participants

Characteristic	Number N = 425	Percentage
Sex		
Male	209	49.2%
Female	216	50.8%
Age group (Years)		
1 - 3	174	40.9%
>3 - 6	133	31.3%
>6 - 9	58	13.6%
>9 - 12	60	14.1%
KEPI Vaccinated		
Yes	415	97.6%
Vaccines completed		
Yes	359	84.5%
Missing	3	0.7%
Yellow fever vaccinated		
Yes	4	0.9%
Ever admitted to hospital		
Yes	152	35.8%
School Attendance		
None	229	53.9%
Pre-school	80	18.8%
Lower primary	69	16.2%
Upper primary	46	10.8%
Missing	1	0.2%
Usual Residence		
Village	343	80.7%
Town	74	17.4%
Missing	8	1.9%
Respondent		
Parent	375	88.2%
Grand parent	27	6.4%
Other	21	4.9%
Missing	2	0.5%
Primary Care giver		
Parent	384	90.4%
Grandparent	24	5.6%
Other	16	3.8%
Missing	1	0.2%

Demographic information for the 425 children included in this study is shown in Table 1. The children were clustered into 3 year age groups. As seen in this table, study participants were drawn from both sexes and ages between 1 and 12 years. About 72% of study participants were between the ages of 1 to 3 years. The dataset had as many females (216, 50.8%) as it had males (209, 49.2%), male to female ratio of 1:1.

Four hundred and fifteen (98%) of these children had received at least some KEPI vaccinations, about eighty four percent (359) of these had completed the scheduled vaccines and the KEPI road to health card was available for 363 (57%) of study participants. Information on completion of the vaccines was missing for three of the participants. The younger children were more likely to have the KEPI card available compared to older children (84.8% for <1 year versus 24.1% for >5 years). About 1.7% of the 425 children had been vaccinated against yellow fever virus as well.

One hundred fifty two (36%) of the study participants reported having been admitted to hospital. About half (195) of the study participants were attending school, with three hundred and forty three (81%) residing in the Village. The respondent was the parent in 88% of cases whereas the primary care giver was the parent in 384 (90%) of cases.

Table 2. Summary of Anthropometric Values and WHO Z- scores of the study Participants (N=425)

Characteristic	Mean	Median
Age (Months)	57.4	48
Weight (Kg)	17.8	15
Height (Cm)	102.4	100
MUAC (Cm)	16.2	16
WAZ (n=368)	-0.25	
HAZ (n=416)	-0.50	
WHZ (n=401)	-0.17	
Temperature (°C)	37.1	36.9

WAZ=weight for age Z –score, HAZ= height for age Z –score, WHZ= weight for height Z –score

Anthropometric measures for study participants are summarized in Table 2. The mean age of the study participants was 54.4 months with a median of 48 months. The mean weight was 17.8kg, with a median of 15kg. Mean height was 102.4cm and the median height was 100cm. The mean MUAC was 16.2cm, range of 10-30cm, within 2.7 SD of the mean. About 25% (106) of the study participants had fever. The mean temperature of all study participants was 37.1^{o}C (median 36.9 ^{o}C, range 34.7 - 40.5 ^{o}C), within 1SD from the mean. The mean duration of fever was 4 days (range 1-62 days). The mean WHO Z-scores were within normal limits. The nutritional status of the study participants was also analyzed (Table not shown). Majority, 258 (64.3%) of the study participants were well nourished (WHZ >=1-, normal), 65 (16.2%) had mild malnutrition (WHZ -2 to <-1), 31 (7.7%) had moderate malnutrition (WHZ -3 to <-2), while 47 (11.7%) had severe malnutrition.

Demographic Characteristics the Primary Care Giver of Study Participants

Table 3. Demographic Characteristics of the Primary Caregiver

Characteristic	Primary Caregiver No. (%) N = 425	Caregiver's Partner No. (%) N = 425
Caregiver		
Parent	384 (90.4)	
Grand Parent	24 (5.6)	
Other	16 (3.8)	
Missing	1 (0.2)	
Age (Years)		
Mean	28.5	32.2
Median	27.0	32.0
Range	17 - 72	18 - 68
Marital status		
Married	377 (88.9)	358 (84.8)
Never Married	25 (6.0)	4 (1.0)
Separated	6 (1.4)	2 (0.5)
Widowed	16 (3.8)	-
None/deceased	-	2 (0.5)
Missing	1 (0.2)	59 (13.3)
Education level		
No formal education	38 (8.9)	62 (14.6)
Primary school incomplete	198 (46.6)	61 (14.4)
Primary school complete	3 (0.7)	80 (18.8)
Secondary school incomplete	88 (20.7)	43 (10.1)
Secondary school complete	59 (13.9)	118 (27.8)
Tertiary	38 (8.9)	58 (13.6)
Missing information	1 (0.2)	2 (0.5)
Deceased	-	1 (0.2)
Occupation		
Salaried formal employment	44 (10.4)	76 (17.9)
Informally employed	23 (5.4)	220 (51.8)
Self employed	112 (26.4)	53 (12.5)
Unemployed	244 (57.4)	73 (17.2)
Missing	2 (0.5)	2 (0.5)
Deceased	-	1 (0.2)

The demographic characteristics of the primary care giver are shown in Table 3. The parent (either mother or father) was the main primary care giver in this study (90.4%), followed by the grandparent (5.6%). The mean age of the primary care giver was 28.5 years while that of the partner (either the husband or boyfriend) was 32.2 years. Majority of these care givers were married (88.9%). The rest were never married, separated or widowed. Most of the primary caregivers had received incomplete primary school education 198 (46.6%), compared to their partners, majority of whom had completed secondary school education 118 (27.8%). Majority of primary care givers were

unemployed 244 (57.4%), followed by self-employment 112 (26.4%). Few primary caregivers were in salaried formal employment 44 (10.4%). On the other hand, most of the partners of the caregivers were informally employed 220 (51.8%); followed by salaried formal employment 76 (17.9%) and those unemployed were 73 (17.2%).

Environmental Exposures of Study Participants

Table 4. Selected Environmental Exposures

Characteristic	Number N = 425	Percentage
Type of House		
Grass thatch, mud wall	148	34.8
Iron roof, mud wall	256	60.2
Iron/tile roof stone wall	21	4.9
Cracks in the walls		
Yes	26	6.1
Vegetation around the house/home		
Yes	148	34.8
Vegetation type near the house		
Bushes, trees, forest	48	14.3
Grass only/None	287	85.6
Water bodies near the house/home		
Yes	182	42.8
Dumping site near the house/ home		
Yes	154	37.7
Domestic water stored in containers?		
Yes	422	99.9
Water containers covered		
Yes	419	98.8
Place of water storage		
Inside the house	375	88.2
Outside the house	3	0.7
Both	45	10.6
Unknown	2	0.5
Recent mosquito bites		
Yes	393	93.1
Times of mosquito bites		
Dawn	2	0.5
Dusk	388	92.6
Daytime	2	0.5
All times	24	5.7
Place of most mosquito bites (N=395)		
Indoors	330	77.7
Outdoors	80	18.8
Both	9	2.1
Unknown	6	1.4
Sleep under mosquito net		
Yes	407	93.9
Mosquito net treated		
Yes	407	95.5
Other mosquito control measures		
Yes	106	24.9

Respondents were asked about the environmental exposures associated with mosquito borne illnesses that were present where the child lived. The responses and their frequency are tabulated in Table 4. More than 60% lived in mud walled, iron roofed houses, with only about 6% of the houses reported to have cracks or holes in the walls.

Few had vegetation, especially bushes, trees and forest near the house. More than 40% had water bodies (including swamps, rivers, ponds, canals and lakes) near the house or home. Majority (more than 88%) stored domestic water in containers inside the house, where about 99% covered the containers. Ninety three percent of respondents reported recent mosquito bites, with most citing indoors (78%) as the place, and dusk (93%) as the time when most mosquito bites occurred. Ninety four percent (407) of the study participants were reported to sleep under mosquito nets, where 96% of the nets were insecticide treated. However, only 25% used other mosquito control measures including mosquito coils, insect repellants, insecticide sprays and herbs.

Clinical Features Observed in the Study Participants

Table 5. Proportion of study participants with observed Clinical Features

Characteristic (Symptoms)	Frequency No. (%) N = 425	Characteristic (Signs)	Frequency No. (%) N = 425
Reported Fever	165 (38.9)	Confirmed fever	136 (32.0)
Chills	55 (12.9)	Wasted	31 (7.3)
Feeling sick	172 (40.5)	Dehydrated	26 (6.1)
Rash	143 (33.7)	Pallor	10 (2.4)
Red eyes	20 (4.7)	Jaundice	2 (0.5)
Photophobia	9 (2.1)	Scleral hemorrhages	1 (0.2)
Painful eyes	10 (2.4)	Lymph nodes	83 (19.6)
Yellow eyes	8 (1.9)	Throat inflammation	87 (20.6)
Nausea or vomiting	115 (27.1)	Rash	143 (33.7)
Stomachache	95 (22.4)	Joint swelling, tenderness	17 (4.0)
Diarrhea	74 (17.4)	Hepatomegaly	5 (1.2)
Sore throat	29 (6.82)	Bleeding manifestations	3 (0.7)
Muscle aches	23 (5.4)		
Joint pains	41 (7.7)		
Swollen joints	7 (1.7)		
Backache	7 (1.7)		
Headache	81 (19.1)		
Dizziness	2 (0.5)		
Neck stiffness	1 (0.2)		
Abnormal movements	3 (0.7)		
Bleeding from orifices	3 (0.7)		
Bloody or black stools	4 (0.9)		
Bruising of skin	1 (0.2)		

The frequency of select symptoms and physical findings in study participants are recorded in Table 5. Feeling sick (172, 40.5%), fever (165, 38.9%), rash (143, 33.7%), nausea or vomiting (115, 27.1%) and stomachache (95, 22.4%) were the commonest symptoms reported in study participants. Chills, diarrhea and headache each were also reported by more than 10% of study participants. Rash (143, 34%), throat inflammation (87, 21%), and lymphadenopathy (83, 20%) were the commonest signs observed in the study participants. Vomiting blood, hard to arouse and confusion were not reported in any of the study participants.

Seroprevalence of Arboviruses in Children At Alupe

Table 6. Proportion of Children Exposed to Arboviruses

Virus	Seropositive			Seronegative		
	Males No (%)	**Females No (%)**	**Total No. (%)**	**Males No (%)**	**Females No (%)**	**Total No. (%)**
Any arbovirus (N = 425)	67 (15.8)	69 (16.2)	136 (32.0)	142 (33.4)	147 (34.6)	289 (68.0)
YFV (N=310)	25 (8.1)	26 (8.4)	51 (16.5)	127 (40.1)	132 (45.6)	259 (83.5)
WNV (N=296)	45 (15.2)	47 (15.9)	92 (31.1)	105 (35.5)	99 (33.4)	204 (68.9)
CHIKV (N=298)	15 (5.0)	19 (6.4)	34 (11.4)	128 (43.0)	136 (45.6)	264 (88.6)

Sera from the 425 study participants were analyzed for at least one or, more arbovirus antibody using an indirect ELISA for antibodies to YFV, WNV and/or CHIKV. Due to limitations in availability of antigens for the viruses of interest, we were not able to test all the serum samples for all the three viruses. Work is still ongoing to test the remaining samples as antigens become available. The arbovirus prevalence rates are shown in Table 7. Overall, 136 (32%) of all the 425 sera tested were positive for at least one arbovirus exposure. WNV antibodies were present in 31.1% of those tested for it. 16.5% of the 310 tested were positive for YFV and 11.4% of 298 were positive for CHIKV antibodies. Thirty-seven study participants were positive for both YFV and WNV, five were positive for both CHIKV and YFV, whereas three were positive for both WNV and CHIKV antibodies. Only three (2.2%) of those who were seropositive for any arbovirus came from outside Western Province, one each from Nyanza province, Rift Valley Province and Eastern Uganda. Only eleven children (1.7%) had been vaccinated for yellow fever, and of these, only one was positive for yellow fever antibodies. Of those who had reported body hotness, fifty seven per cent had an ELISA positive for YFV, and forty eight per cent for CHIKV.

Proportion of Study Participants with a Positive Malaria, Widal and HIV Tests

Table 7. Proportion of Children with Positive Malaria, Widal and HIV Tests

Test	Number	Percentage
Malaria test (N=304)		
Positive	199	65.5
Negative	105	34.5
Widal test (N=313)		
Positive	268	84.6
Negative	45	14.4
HIV Test (N=289)		
Positive	4	1.4
Negative	284	98.6

A malaria test, widal test and HIV test were also performed on the blood samples as per protocol (See table 7). A malaria test was done in 304 of the study participants and out of these, 199 (65.5%) tested positive for malaria parasites. Of the two hundred and eighty nine children tested for HIV, only four (1.4%) were positive. Three hundred and thirteen children were tested for the Widal test and two hundred and sixty eight of these (84.6%) were positive.

Proportion of Malaria positive and Widal Test Positive Children exposed to WNV, CHIKV and YFV

Table 8: Proportion of Malaria positive and Widal Test Positive Children exposed to WNV, CHIKV and YFV

Arbovirus	Malaria Negative	Malaria Positive	Total
WNV negative	53 (46.9)	113 (64.6)	**166 (57.6)**
WNV positive	60 (53.1)	62 (35.4)	**122 (43.4)**
Total	**113 (100)**	**175 (100)**	**288 (100)**
CHIKV negative	53 (86.9)	175 (91.6)	**228 (90.5)**
CHIKV positive	8 (13.1)	16 (8.4)	**24 (9.5)**
Total	**61 (100)**	**191 (100)**	**252 (100)**
YFV negative	60 (98.4)	168 (84.4)	**228 (87.7)**
YFV positive	1 (1.6)	31 (15.6)	**32 (12.3)**
Total	**61 (100)**	**199 (100)**	**260 (100)**
Arbovirus	**Widal Test Negative**	**Widal Test Positive**	
WNV negative	22 (64.7)	146 (73.7)	**168 (72.4)**
WNV positive	12 (35.3)	52 (26.3)	**64 (27.6)**
Total	**34 (100)**	**198 (100)**	**232 (100)**
CHIKV negative	33 (91.7)	189 (90.0)	**222 (92.2)**
CHIKV positive	3 (8.3)	21 (10.0)	**24 (7.8)**
Total	**36 (100)**	**210 (100)**	**246 (100)**
YFV negative	32 (86.5)	192 (89.3)	**224 (88.9)**
YFV positive	5 (13.5)	23 (10.7)	**28 (11.1)**
Total	**37 (100)**	**215 (100)**	**252 (100)**

The proportion of children who had both a malaria, typhoid or HIV test and an individual arbovirus antibody test done on their serum are recorded in table 8. One hundred and twenty two (43.4%) of two hundred and eighty eight children tested for both malaria and WNV antibodies were exposed to WNV. Of these, sixty two (35.4%) were positive for both malaria and WNV antibodies. Sixteen children (8.4%) of 191 children with a positive malaria test were exposed to CHIKV whereas 31 (15.6%) of 199 were exposed to YFV. Fifty two children (26.3%) of 198; 21 (10%) of 210 and 23 (10.7%) of children with a positive widal test were exposed to WNV, CHIKV and YFV respectively.

Factors associated with arbovirus antibody positivity

Association between arbovirus seropositivity and demographic factors

Table 9. Odds of Seropositivity by Demographic Characteristics of Study Participants (N = 425 if not stated)

Characteristic	Number (%)	Seropositive No. (%)	Seronegative No. (%)	OR (95% CI)	P Value
Sex (M: F = 1:1)					
Male	209 (49.2)	67 (49.3)	142 (49.1)	1.0 (0.7 - 1.5)	1.0
Female	216 (50.8)	69 (50.7)	147 (50.9)	1.0	
Age group (Years)					
1 - 3	174 (40.9)	52 (38.2)	122 (42.2)	1.3 (0.6 - 2.5)	0.4
>3 - 6	133 (31.3)	47 (34.6)	86 (29.8)	1.6 (0.8 - 3.2)	0.2
>6 - 9	58 (13.7)	22 (16.2)	36 (12.5)	1.8 (0.8 - 4.0)	0.1
>9 - 12	60 (14.1)	15 (11.0)	45 (15.6)	1.0	
KEPI Vaccinated					
Yes	415(97.6)	132 (97.1)	283 (97.9)	33.0 (12.2 - 89.2)	0.6
Vaccines completed (N=422)					
Yes	359 (85.1)	117 (87.3)	242 (84.0)	1.3 (0.7 - 2.4)	0.4
Yellow fever vaccinated					
Yes	4 (0.9)	0 (0.00)	4 (1.4)	0.0 (0 - 2.0)	0.1
Past hospital admission					
Yes	152 (35.8)	53 (39.0)	99 (34.3)	1.2 (0.8 - 1.9)	0.3
School Attendance					
Yes	195 (45.9)	62 (45.6)	133 (46.0)	1.0 (0.7 - 1.5)	0.9
Residence					
Village	344 (80.9)	110 (80.9)	234 (81.0)	1.0 (0.6 - 1.7)	1.0
Town	74 (17.4)	22 (16.2)	52 (18.0)	1.0	
Unknown	7 (0.2)	4 (2.9)	3 (1.0)	3.2 (0.7 - 13.7)	0.1
Respondent (N=423)					
Parent	375 (88.7)	116 (85.9)	259 (89.9)	1.0	
Grandparent	27 (6.4)	11 (8.1)	16 (5.6)	1.5 (0.7 - 3.4)	0.3
Other	21 (5)	8 (5.9)	13 (4.5)	1.4 (0.6 - 3.3)	0.5
Primary Care giver (N=424)					
Parent	384 (90.6)	122 (89.7)	262 (91.0)	1.0	
Grandparent	24 (5.7)	9 (6.6)	15 (5.2)	1.3 (0.6 - 3.0)	0.6
Other	16 (3.8)	5 (3.7)	11 (3.8)	1.0 (0.3 - 2.8)	1.0

In order to determine if socio-demographic, environmental and clinical features observed in the study participants were associated with arbovirus seropositivity, univariate analysis was carried out and the results recorded in tables 9, 10 and 11. Children who were seropositive for arbovirus antibodies were compared to those that were negative. As shown in table 9, socio-demographic characteristics of gender, age, immunization status and YFV vaccination were not associated with arbovirus seropositive status. There was also no difference in history of previous admission to

hospital, residence, school attendance and respondent to the interview or even the primary care giver. The odds of seropositivity increased with age except in the age group 9 -12 years as follows: for those in the age groups 1 - 3 years, the OR (95% CI) was 1.3 (0.6 - 2.5), >3 - 6 years, the OR (95% CI) was 1.6 (0.8 - 3.2), >6 – 9 years OR (95% CI) was 1.8 (0.8 - 4.0). However, there was no statistically significant difference in arbovirus seropositivity between the age groups (p values >0.13).

Association between arbovirus seropositivity and environmental factors

Table 10. Odds of Seropositivity by Environmental Exposures (N=425 if not stated)

Characteristic	Number (%)	Seropositive No. (%)	Seronegative No. (%)	OR (95% CI)	P Value
Type of House					
Grass thatch, mud wall	148 (38.2)	44 (32.4)	104 (36.0)	1.7 (0.5 - 6.7)	0.5
Iron roof, mud wall	256 (60.2)	88 (64.7)	168 (58.1)	0.6 (0.2 - 1.6)	0.3
Iron/tile roof stone wall	21 (4.9)	4 (2.9)	17 (5.9)	1.0	
Cracks in the walls					
Yes	26 (6.1)	12 (8.7)	1.9 (0.9 - 4.2)	0.1	0.6
Vegetation type near house					
Bushes, trees, forest	48 (14.3)	16 (32.7)	32 (11.2)	3.9 (1.8 - 8.2)	<0.001
Grass only/None	287 (85.7)	33 (67.3)	254 (88.8)	1.0	
Water bodies near the house					
Yes	182 (42.8)	61 (44.9)	121 (41.9)	1.1 (0.7 - 1.7)	0.6
Dumping site near the house					
Yes	154 (37.7)	56 (42.7)	98 (35.3)	1.4 (0.9 - 2.1)	0.2
Water stored in containers?					
Yes	422 (99.3)	132 (99.2)	287 (99.3)	0.9 (0.1 - 7.1)	1.0
Water containers covered					
Yes	419 (98.5)	135 (99.3)	283 (97.9))	0.9 (0.3 – 25.8)	1.0
Place of water storage					
Inside	375 (88.2)	120 (88.2)	255 (88.2)	1.0	
Outside	3 (0.7)	1 (0.7)	2 (0.7)	1.1 (0.1 - 15.1)	1.0
Both	45 (10.6)	14 (10.3)	31 (10.7)	1.0 (0.5 - 2.0)	1.0
Unknown	2 (0.5)	1 (0.7)	1 (0.3)	2.5 (0.2 - 20.5)	0.6
Recent mosquito bites					
Yes	393 (93.1)	130 (96.3)	263 (91.6)	2.4 (0.2 - 6.1)	0.1
Times of mosquito bites					
Dawn	2 (0.5)	0 (0.0)	2 (0.7)	0.0 (0.0 - 4.1)	0.3
Dusk	388 (93.3)	125 (95.4)	263 (92.3)	1.0	
Daytime	2 (0.5)	0 (0.0)	2 (0.7)	0.0 (0.0 - 4.1)	0.3
All times	24 (0.7)	6 (0.6)	18 (6.3)	0.7 (0.3 - 1.8)	0.5
Place of most mosquito bites (N=395)					
Indoors	330 (77.6)	109 (80.2)	221 (76.5)	1.0	
Outdoors	80 (18.8)	24 (17.6)	56 (19.4)	0.9 (0.5 - 1.5)	0.7
Both	9 (2.1)	2 (1.5)	7 (2.4)	0.6 (0.1 - 3.1)	0.7
Unknown	6 (1.4)	1 (0.7)	5 (1.7)	0.4 (0.0 - 3.6)	0.6
Sleep under mosquito net					
Yes	407 (95.8)	129 (94.9)	278 (96.2)	0.7 (0.3 – 2.1)	0.7
Mosquito net treated					
Yes	407 (95.5)	126 (94.7)	278 (96.2)	0.7 (0.3 - 2.1)	0.6
Other mosquito control measures					
Yes	106 (24.9)	34 (25.0)	72 (24.9)	1.0 (0.6 - 1.7)	1.0

Univariate analysis was carried out to determine whether arbovirus seropositivity was associated with environmental exposures. Table 10 shows that having a dumpsite near the home (OR 1.3, 95% CI 0.9 - 2.1, p value 0.2), not using mosquito nets at all (OR

1.4, 95% CI 0.5 - 3.5, p value 0.6), or not using insecticide treated mosquito nets (OR 1.4, 95% CI 0.6 - 2.0, p value 0.5) had increased odds of seropositivity that were not statistically significant. Of 393 participants reporting recent mosquito bites, 130 (33.1%) were seropositive compared to 5 (17.2%) who did not report recent mosquito bites and this was trending to statistical significance (OR 2.4, 95% CI 0.2 - 6.1, p value 0.08). Having bushes, trees or forests near the home was significantly associated with seropositivity compared to having grass only or no vegetation near the home, with an OR of 3.9, 95% CI 1.8 - 8.2 and a p value of <0.001.

Association between arbovirus seropositivity and clinical features

Table 11. Odds of Seropositivity by Clinical Symptoms

Characteristic	Number (%)	Seropositive No. (%)	Seronegative No. (%)	OR (95% CI)	P value
Reported Fever	165 (38.9)	49 (29.7)	116 (70.3)	0.7 (0.4-1.3)	0.3
Chills	55 (12.9)	21 (38.2)	34 (61.8)	1.8 (0.9-3.6)	0.1
Feeling sick	172 (40.5)	56 (32.6)	116 (67.4)	1.3 (0.7-2.1)	0.5
Rash	143 (33.7)	42 (29.4)	101 (70.3)	0.6 (0.3-1.1)	0.1
Red eyes	20 (4.7)	11 (55.0)	9 (45.0)	1.5 (0.5-4.4)	0.5
Photophobia	9 (2.1)	6 (66.7)	3 (33.3)	4.9 (1.4-17.5)	0.0
Painful eyes	10 (2.4)	6 (60.0)	4 (40.0)	2.6 (0.7-9.5)	0.2
Yellow eyes	8 (1.9)	3 (37.5)	5 (62.5)	0.8 (0.1-5.3)	0.9
Nausea or vomiting	115 (27.1)	39 (33.9)	76 (66.1)	1.2 (0.7-2.2)	0.5
Stomachache	95 (22.4)	34 (35.8)	61 (64.2)	1.3 (0.7-2.3)	0.5
Diarrhea	74 (17.4)	25 (33.8)	49 (66.2)	1.2 (0.6-2.3)	0.7
Sore throat	29 (6.82)	11 (37.9)	18 (62.1)	2.0 (0.8-4.7)	0.1
Muscle aches	23 (5.4)	11 (47.8)	12 (52.2)	1.7 (0.6-4.6)	0.3
Joint pains	41 (7.7)	16 (39.0)	25 (61)	1.5 (0.7-3.3)	0.3
Swollen joints	7 (1.7)	0 (0.0)	7 (100.0)	0.0 (0.0-3.2)	0.3
Backache	7 (1.7)	2 (28.6)	5 (71.4)	1.0 (0.2-6.3)	1.0
Headache	81 (19.1)	28 (20.6)	53 (65.4)	1.3 (0.7-2.5)	0.4
Dizziness	2 (0.5)	2 (100.0)	0 (0.0)	5.9 (0.6-57.6)	0.2
Neck stiffness	1 (0.2)	0 (0.0)	1 (100.0)	5.8 (0.6-56.6)	0.2
Abnormal movements	3 (0.7)	1 (33.3)	2 (66.7)	2.6 (0.4-23.1)	0.4
Bleeding from gums/nose/eyes	3 (0.7)	0 (0.00)	3 (100.0)	0.0 (0.0-7.6)	0.5
Bloody or black stools	4 (0.9)	0 (0.0)	4 (100.0)	0.0 (0.0-5.6)	0.4
Bruising of skin	1 (0.2)	1 (100.0)	0 (0.0)	Inf (1.5-Inf)	0.0

Table 12. Odds of Positivity by Clinical Signs

Characteristic	Number (%)	Seropositive No. (%)	Seronegative No. (%)	OR (95% CI)	P value
Confirmed Fever	136 (32.0)	33 (24.3)	103 (75.7)	0.9 (0.6 - 1.5)	0.8
Wasted	31 (7.3)	8 (25.8)	23 (74.2)	1.4 (0.6 - 3.4)	0.5
Dehydrated	26 (6.1)	12 (46.2)	14 (53.8)	3.8 (1.7 - 8.7)	0.0
Pallor	10 (2.4)	5 (50.0)	5 (50.0)	1.0 (0.2 - 6- 3)	1.0
Jaundice	2 (0.5)	1 (50.0)	1 (50.0)	0.0 (0.0 - 11.4)	0.6
Scleral hemorrhages	1(0.2)	0(0.0)	1 (100.0)	0.0 (0.0 - 22.7)	0.7
Lymph nodes	83 (19.6)	31 (37.3)	51 (62.7)	1.0 (0.5 - 1.9)	1.0
Throat inflammation	87 (20.6)	27 (31.0)	50 (69.0)	1.0 (0.5 - 2)	0.9
Rash	143 (33.7)	42 (29.4)	101 (70.3)	0.6 (0.3 - 1.1)	0.1
Joint swelling, tenderness	17 (4.0)	7 (41.2)	10 (59.8)	1.9 (0.6 - 5.7)	0.3
Hepatomegaly	5 (1.2)	2 (40.0)	3 (60.0)	1.5 (0.2 - 10.0)	0.7
Bleeding manifestations	3 (0.7)	1 (33.3)	2 (66.7)	1.1 (0.1 - 8.2)	1.0

In order to determine if clinical symptoms and signs were associated with arbovirus seropositivity, univariate analysis was carried out comparing reported symptoms and observed clinical signs in seropositive and seronegative children. As shown in table 10 and 11, patients who had photophobia, painful eyes, dizziness, abnormal movements and neck stiffness had more than two times odds of positivity for arbovirus antibodies. Those with sore throat (OR 2.0 95% CI 0.8 - 4.7, p value 0.1), joint swelling and tenderness (OR 1.9, 95% CI 0.6 - 5.7, p value 0.3), chills (OR 1.8, 95% CI 0.9 - 3.6, p value 0.104), muscle aches (OR 1.7, 95% CI 0.6 - 4.6, p value 0.3), joint pains (OR 1.5, 95% CI 0.7 - 3.3, p value 0.3), red eyes (OR 1.5, 95% CI 0.5 - .4, p value 0.482) and hepatomegaly (OR 1.5, 95% CI 0.2-10.0, p value 0.7) had increased chance of seropositivity for arbovirus antibodies, though not statistically significant. Photophobia (OR 4.9, 95% CI 1.4 - 17.5, p value 0.0), bruising of skin (OR Inf, 95% CI 1.5 - Inf, p value 0.0) and dehydration (OR 3.8, 95% CI 1.7 - 8.7, p value 0.0) were significantly associated with seropositivity for arbovirus antibodies. However, fever was not associated with seropositivity (OR 0.9, 95% CI 0.6 - 1.5, p value 0.8).

Association Between Positive Malaria test, Positive Widal test and WNV, CHIKV and YFV Antibody Positivity

Table 13: Association between Positive Malaria test, Widal test and WNV, CHIKV and YFV antibody positivity

Arbovirus	Malaria Negative (No. %)	Malaria Positive (No. %)	OR (95% CI)	P value
	N=63	N=175		
WNV negative	60 (95.2)	113 (64.6)	1	
WNV positive	3 (4.8)	62 (35.4)	20.66(6.48 - 65.83)	0.00
	N=61	N=191		
CHIKV negative	53 (86.9)	175 (91.6)	1	
CHIKV positive	8 (13.1)	16 (8.4)	2.00 (0.86 - 4.67)	0.273
	N=61	N=199		
YFV negative	60 (98.4)	168 (84.4)	1	
YFV positive	1 (1.6)	31 (15.6)	31.00 (4.23-227.08)	0.003
Arbovirus	**Widal Test Negative (No. %)**	**Widal Test Positive (No. %)**		
	N=34	N=194		
WNV negative	22 (64.7)	142 (73.2)		
WNV positive	12 (35.3)	52 (26.8)	4.33 (2.31 – 8.12)	0.28
	N=36	N=210		
CHIKV negative	33 (91.7)	189 (90.0)		
CHIKV positive	3 (8.3)	21 (10.0)	7.00 (2.09 – 23.47)	0.76
	N=37	N=215		
YFV negative	32 (86.5)	192 (89.3)		
YFV positive	5 (13.5)	23 (10.7)	4.60 (1.75 – 12.10)	0.62

Only three (5%) of the 63 patients who tested negative for malaria had a positive antibody test for WNV compared to 62 (35%) of 175 with a positive malaria test. Thus patients with slide positive malaria had a significantly increased risk of testing positive for WNV OR=20.7 [(95% CI 6.5 - 65.8) p<0.001]. Only one (1.6%) patient with a negative malaria slide tested positive for YFV compared to 31 (15.6%) of 199 with a positive malaria slide. Thus patients with positive malaria test had a thirty one fold increased likelihood of testing seropositive for YFV OR=31.0 [(95% CI 4.23-227.08) p=0.003]. Prevalence of CHIKV among patients with a positive malaria test was 16 (8.4%) versus 8 (13.1%) with a negative malaria test. The difference was not significant on statistical testing (p=0.273).

A total of 12 (35.3%) of the 34 patients with a negative widal test tested positive for antibodies to WNV compared to 52 (26.8%) of 194 with a positive widal test OR=4.3 [(95% CI (2.3 - 8.1) p=0.3]. Thus patients with a negative widal test had a four-fold risk of testing positive for WNV. Only three (8.3) patients who had a negative widal test were seropositive for CHIKV compared to 21 (10.0%) with a positive widal test OR=7.00 [(95% CI (2.09 – 23.47) p=0.8]. Thus patients with a positive widal test had a seven-fold risk of testing positive for CHIKV but the difference was not statistically significant. The prevalence of YFV among patients with a positive widal test was 23 (10.7%) versus 5 (13.5%) among those with a negative widal test. Patients with a negative widal test had an increased risk of testing positive for YFV OR=4.6 [95% CI (1.75 – 12.10) p=0.6] that was not statistically significant.

Factors Associated with individual WNV, CHIKV and YFV Antibody Positivity of those Tested for all Three Viruses N=274

Table 14: Factors associated with individual virus antibody positivity

	Yellow fever Virus				Chikungunya Virus				West Nile Virus				Any Arbovirus N=274			
Variable	**YFV +ve n (%)**	**YFV -ve n (%)**	**OR (95% CI)**	**P Value**	**CHIKV +ve n (%)**	**CHIKV _ve n (%)**	**OR (95% CI)**	**P Value**	**WNV +ve n (%)**	**WNV - ve n (%)**	**OR (95% CI)**	**P Value**	**Any +ve n (%)**	**Any - ve n (%)**	**OR (95% CI)**	**P Value**
Vegetation type																
Bushes, trees, forest	7 (13.7)	30 (11.6)	1.2 (0.5 - 2.9)	0.7	3 (8.8)	34 (12.9)	0.7 (0.2 – 2.3)	0.8	16(17.4)	21 (10.3)	1.8 (0.9 – 3.7)	0.1	47 (45.2)	61(35.9)	1.5(0.9 - 2.4)	0.1
Grass only/None	44 (86.3)	229 (88.4)	1.0		31(91.2)	230(87.1)	1.0		76 (82.6)	183(89.7)	1.0		1.0			
Water bodies																
Yes	27 (52.9)	109 (42.1)	1.2 (0.8 – 2.8)	0.2	19(55.9)	113(42.8)	1.7 (0.8 – 3.5)	0.1	46(50.0)	88 (43.1)	1.3 (0.8 –2.1)	0.3	51 (49.0)	72(42.4)	1.3 (0.8 – 2.1)	0.3
Dumping site																
Yes	22 (44.9)	94 (37.5)	1.4 (0.7 - 2.5)	0.3	13(40.6)	100(39.2)	1.1 (0.5 - 2.2)	0.9	40(44.9)	73 (37.2)	1.4 (0.8 - 2.3)	0.2	43 (42.6)	61(37.4)	1.2(0.7-2.1)	0.4
Place of mosq. bites																
Indoors	41 (89.1)	193 (80.1)	1.0		26(81.3)	200(81.2)	1.0		76(86.4)	151(80.7)	1.0		84 (84.8)	125(81.2)	1.0	
Outdoors	5 (10.9)	43 (17.8)	0.5 (0.2 - 1.5)	0.2	5 (15.6)	38 (15.7)	1.0 (0.4 – 2.8)	1.0	11(12.5)	32 (17.1)	0.7 (0.3 – 1.4)	0.3	13 (13.1)	26 (16.9)	1.0 (0.2 - 6.1)	1.0
Both	0 (0.0)	5 (2.1)	-		1 (3.1)	4 (1.7)	1.9(0.2-17.9)	0.6	1 (1.1)	4 (2.1)	0.5 (0.1-4.5)	0.5	2 (2.0)	3 (1.9)	0.7 (0.4 -1.5)	0.4

Table 14 continued: Factors associated with individual virus antibody positivity

Variable	YFV +ve n (%)	YFV -ve n (%)	OR (95% CI)	P Value	CHIKV +ve n (%)	CHIKV _ve n (%)	OR (95% CI)	P Value	WNV +ve n (%)	WNV - ve n (%)	OR (95% CI)	P Value	Any +ve n (%)	Any -ve n (%)	OR (95% CI)	P Value
Other mosq. Control																
Yes	14 (27.5)	59 (22.8)	1.3 (0.7 - 2.5)	0.5	12 (35.3)	63 (23.9)	1.7 (0.8 – 3.7)	0.1	15 (16.3)	57 (27.9)	0.5 (0.3 – 0.9)	0.03	18 (17.3)	49(28.8)	0.5 (0.3 - 0.9)	0.03
Photophobia	5 (9.8)	3 (1.2)	9.3(2.1-40.2)	0.004	2 (5.9)	3 (1.1)	5.4(0.9-33.8)	0.1	5 (5.4)	3 (1.5)	3.9(0.9-16.5)	0.1	4 (3.8)	1 (0.6)	6.8(0.7-61.3)	0.070
Red eyes	6 (11.8)	9 (3.5)	3.7(1.3-10.9)	0.02	4 (11.8)	9 (3.4)	3.8(1.1-13.0)	0.04	7 (7.6)	8 (3.9)	2.0(0.7-5.7)	0.3	8 (7.7)	4 (2.4)	3.5(1.0-11.8)	0.063
Muscle aches	5 (9.8)	8 (3.1)	3.4(1.1-10.9)	0.045	5 (14.7)	7 (2.7)	6.3(1.9-21.2)	0.2	11 (12.0)	4 (2.0)	6.7(2.1-21.9)	0.001	7 (6.7)	2 (1.2)	6.1(1.2-29.8)	0.029
Joint pains	4 (7.8)	25 (9.7)	0.8(0.3-2.4)	1.0	6 (17.6)	22 (8.3)	2.4(0.9-6.3)	0.1	13 (14.1)	15 (7.4)	2.1(0.9-4.6)	0.08	14(13.5)	11 (6.5)	2.2(1.0-5.2)	0.08
Stomachache	10 (19.6)	63 (24.3)	0.8(0.4-1.6)	0.5	13 (38.2)	59 (22.3)	2.2(1.0-4.6)	0.04	26 (28.3)	47 (23.0)	1.3(0.7-2.3)	0.3	33(31.7)	36(21.2)	1.7(1.0-3.0)	0.051
Chills	10 (19.6)	36 (13.9)	1.5(0.7-3.3)	0.3	10 (29.4)	35 (13.3)	2.7(1.2-6.2)	0.01	17 (18.5)	27 (13.2)	1.5(0.8-2.9)	0.2	20(19.2)	20(11.8)	1.8(0.9-3.5)	0.09
Rash	20 (39.2)	70 (27.0)	1.7(0.9-3.3)	0.08	10 (29.4)	77 (29.2)	1.0(0.5-2.2)	1.0	28 (30.4)	64 (31.4)	1.0(0.6-1.6)	0.9	24(23.1)	55(32.4)	0.6(0.4-1.1)	0.1

A total of 274 patients were tested for all the three viruses, namely WNV, YFV and CHIKV. To determine whether environmental and clinical features were associated with individual arbovirus antibody positivity or any arbovirus antibody positivity of these 274 patients, a univariate analysis was done. As seen from table 14, 27.9% of patients who used other methods of mosquito control besides mosquito nets were negative for WNV compared to 16.3% of those who were positive for WNV. 28.8% of those who used other methods of mosquito control other than mosquito nets were negative for any arbovirus compared to 17.3 % who were positive for any arbovirus. Thus, patients who used other mosquito control measures besides mosquito nets had reduced chance of WNV positivity (OR=0.5 [95% CI (0.3 – 0.9) p=0.03]) and any arbovirus antibody positivity (OR=0.5 [95% CI (0.3 – 0.9) p=0.03]). The difference was statistically significant with a p value 0.03. No environmental factor was associated with any arbovirus, YFV, WNV or CHIKV antibody positivity. Photophobia OR=9.3[95% CI (2.1-40.2) p =0.004] and muscle aches OR=3.4 [95% CI (1.1-10.9) p=0.045] were significantly associated with YFV seropositivity. Red eyes OR=3.8 [95% CI (1.1-13.0) p=0.04], stomachache OR=2.2 [95% CI (1.0-4.6) p=0.04] and chills OR=2.7 [95% CI (1.2-6.2) p=0.01] were associated with CHIKV antibody positivity, while muscle aches OR=6.7 [95% CI (2.1-21.9) p=0.001] were the clinical features significantly associated with WNV. Muscle aches were also associated with any arbovirus seropositivity OR=6.1 [95% CI (1.2-29.8) p=0.29]. Photophobia OR=6.8(0.7-61.3) p=0.070, red eyes OR=3.5 [95%CI (1.0-11.8) p=0.063] and stomachache OR=1.7 [95% CI (1.0-3.0) p= 0.051] were clinical features that had increased chance of seropositivity for any arbovirus antibody, that was found trending towards statistical significance (p value <0.05-= or >0.07). These factors may be possibly statistically significant but may have been compromised by factors such as the small sample sizes.

Discussion

This study has generated current data on the seroprevalence of WNV, YFV and CHIKV in children attending a district hospital in Teso. The study found antibodies to WNV, CHIKV and YFV in children between one and twelve years. WNV antibodies were found in 31% of 296 children tested for the virus, YFV in 17% of the 310 children tested and CHIKV in 11% of the 298 children tested for the virus. Of 274 children tested for WNV, CHIKV and YFV at the same time, 62.0% were negative, 25.9% were positive for one arbovirus and 12% were positive for more than 2 arboviruses.

A positive arbovirus status was associated with bushy vegetation, trees or forest near the home; photophobia; bruising of skin; dehydration and a positive malaria test. WNV seropositivity was associated with muscle aches, CHIKV seropositivity was associated with red eyes, chills and stomachache while YFV seropositivity was associated with photophobia and muscle aches. Use of other mosquito control measures besides mosquito nets was associated with protection from WNV and any arbovirus seropositivity that was statistically significant. A positive malaria test was associated with WNV and YFV seropositivity.

These seroprevalence results are different from those of a previous study done in November 1966 to April 1968 in Central Nyanza, Kitui District and Malindi District by Geser *et al.(3),* in children aged 1-14 years. CHIKV/ONNV was found in 55% of 176 children examined in Central Nyanza, while YFV and WNV were found in 1% each. CHIKV/ONNV was found in 0.2%, YFV in 0.0% and WNV in 10% of the 441 children tested from Kitui District. CHIKV/ONNV was found in 33%, YFV in 9.4% and WNV in 37.4% of 265 children tested from Malindi. The current study reveals higher prevalence rates for WNV and YFV compared to the earlier Geser study done in Central Nyanza (31% and 15% vs. 1% each). However, the prevalence of CHIKV of 12% is much lower in the current study compared to 55% for central Nyanza in the Geser study. The Geser study included children up to the age of 14 years versus 12 years for the current study. Given that arbovirus antibody prevalence increases with age, the older age group in the Geser study may explain the higher WNV prevalence, since older children are likely to play in bushy forested areas where they come into contact with vectors for YFV and WNV. The Geser study was also community based and may have been more

representative of the true arbovirus prevalence compared to our hospital based study which looked at a specific group from the population. The difference in CHIKV prevalence in the two studies may also be explained by the different periods in time when the two studies were carried out, reflecting changes in arbovirus prevalence over time due to climate change and other factors like encroachment on forests, increased travel and exportation of vectors to new areas. The fact that the Geser study used a HI test as opposed to ELISA that was used in our study, may also explain the difference in prevalence in the two studies.

There were also differences in prevalence of the arboviruses between Kitui and Malindi districts in the Geser study compared to the current Teso district study. Generally the prevalence for CHIKV, WNV and YFV were lower in Kitui compared to Teso, while CHIKV and WNV were higher in Malindi, with YFV prevalence being higher in Teso compared to Malindi. These differences can be explained by the different ecologies in the three areas and hence different distribution of the different virus vectors. Malindi and Teso have an almost similar wet and humid climate that favors breeding of mosquitoes while Kitui has a much drier climate.

In a different study, Surtees G *et al.(31)*, in 1969 tested sera from school children aged 5 to 15 years from 26 schools south of Kisumu, an area near Alupe with a similar climate. 36% of 559 12 year olds had antibodies to CHIKV. The prevalence of WNV was not quantified even though antibodies to this virus were also found in this age group. HI antibodies to CHIKV were found in 35% of the 4 and 5 year old children. The prevalence of CHIKV found by Surtees was higher than that in our study. This difference may be explained by the narrow age groups tested in the Surtees study compared to the wide range of age groups considered in the current study. The earlier study also tested school children that may have been more representative of the community while the current study looked at a specific population that had come to hospital and may be less representative of the community.

In 2004, Coldren R.L *et al*., found presence of IgG against CHIKV in 7.06%, WNV in 2.19% and YFV in 1.22% among 820 patients admitted with acute febrile illnesses in three district hospitals in Kenya, namely Alupe (N=127), Malindi (N=458) and Isiolo (N=215)(8). The prevalence of all the three viruses was much lower in Coldren's study compared to the current study, where we found higher prevalence rates

for all the studied viruses, with WNV having higher prevalence rates compared to CHIKV. This difference maybe explained by the fact that Coldren's study combined the prevalence of the arboviruses in the three different areas with different ecologies, with the drier Isiolo area likely to have lower arbovirus prevalence rates. The Coldren study was also confined to febrile patients admitted to hospital compared to all children with or without fever who presented to hospital in my study. WNV may also be resurging while CHIKV may be on the wane given the higher CHIKV prevalence rates compared to WNV found in earlier studies by Geser *et al*. (55% vs. 1% in Central Nyanza near Teso), Surtees *et al.* (36% for CHIKV vs. not quantified for WNV in 12 year olds and 35% vs. not quantified in 4 and 5 year old children from South of Kisumu) and Coldren *et al.* (7.06% vs. 2.19% in Alupe, Malindi and Kitui).

The Yellow Fever prevalence of 15% found in this current study is much higher than the 1% rate found in Central Nyanza in 1966 to 1968 by Geser A. *et al*. and the 1.22% rate found in three districts, including Alupe by Coldren R.L *et al.* in 2004. The higher rate may mean that the yellow fever virus may be broadening its prevalence from the Kerio valley where the rate was 47% during the first outbreak of YFV(6, 27). Alternatively the locally found YFV in the Western part of Kenya that was documented by Geser, Surtees and Coldren in the earlier studies may just be spreading locally in response to global warming and populations encroaching on forests where the *Aedes aegypti* mosquitoes are found. The current YFV prevalence rate is similar to the 14% rate found during an earlier surveillance in adults and children in North Eastern Province by Henderson BE *et al*. in 1968(29). However, the current YFV prevalence in North Eastern Province is unknown, hence we cannot tell whether there has been an increase or decrease in the prevalence.

The finding of anti-arbovirus antibodies in children below 5 years, and the increase in prevalence with age found in our study confirms that there has been recent exposure to these viruses in the Teso community. Maternal arbovirus antibodies generally disappear from the blood of infants from the age of six months and would be found to decrease with age but this was not the case in this study. The antibodies detected in these children are likely to be derived from their bodies' response to natural infection by arboviruses. This finding reaffirms the need for regular surveillance for these arboviruses to detect changing patterns early and avert outbreaks where they are likely to occur.

Having vegetation like bushes, trees and forests near the home was associated with seropositivity compared to having grass only or no vegetation around the house. This factor was significantly associated with seropositivity, and this is in keeping with the fact that forests/bushy environment are a risk factor for arbovirus infections transmitted by *Aedes* species of mosquitoes that rest in forests and bush and bite out doors during the day. *Aedes aegypti* mosquitoes species that transmit YFV breed in water filled containers around houses where they easily come into contact and bite human hosts(22, 26).

In this current study, the commonest symptoms reported by arbovirus antibody positive study participants were: Feeling sick 42%, fever 37%, rash 32%, stomachache 26%, nausea or vomiting 24%, headache 21%, diarrhea 19%, chills 16%, joint pains 12%. Red eyes 8%, muscle aches and sore throat (8% each), photophobia and painful eyes (5% each), were also reported, while the commonest signs observed were rash 32%, lymphadenopathy 23%, throat inflammation 20% and dehydration 9% and joint swelling with tenderness 5%. Muscle aches, stomachache and chills were the specific clinical features that were significantly associated with CHIKV antibody positivity in our study. Stomachache (abdominal pain) was also observed in a study focusing on children in Bangkok, where the most common presenting symptoms of CHIKV infection were vomiting (35%) and abdominal pain and anorexia (18%), while pharyngitis (70%) and facial flushing (24%) were the most frequent signs. Arthritis and arthralgia were less prominent features and no rash was seen in these children(35). WNV infection commonly presents with fever, headache, malaise, arthralgia or myalgia. Occasionally anorexia, nausea, vomiting and diarrhea occur. Conjunctivitis, ocular pain, photophobia and pharyngitis may also occur(21). However, in our study, only muscle aches were the clinical feature significantly associated with WNV seropositivity. Yellow fever disease is characterized by sudden onset fever, chills, headache, backache, generalized muscle pains, prostration, nausea and vomiting(26). In our study, photophobia and muscle aches were significantly associated with YFV seropositivity. Therefore muscle aches were in keeping with a known clinical feature of YFV infection. Although photophobia and dehydration were significantly associated with arbovirus antibody positivity, this being a seroprevalence study, we could not attribute the symptoms we observed in our study to either WNV infection or any of the arboviruses tested in this study. A number of children who had antibodies to arboviruses also tested positive for malaria and typhoid. These are

acute febrile illnesses that share similar characteristics with the YFV, WNV and CHIKV infections and make differentiating malaria, typhoid or any other febrile illness from arboviral illnesses a big challenge. The management of arbovirus infections at this moment in time is mainly supportive whereas malaria and typhoid require specific antimicrobial therapy.

The association between malaria and arbovirus exposure status may be explained by the fact that the patients are exposed to different species of mosquitoes that share the same environment and that transmit malaria and/or arboviruses. Therefore the same public health measures can be used to prevent both malaria and mosquito-borne arbovirus infections.

Strengths of the study

The study design was well suited to the primary objective of determining the seroprevalence of arboviruses.

Study Weaknesses

This was a cross sectional hospital based study. There is likely to have been selection bias since the study population comprised of children who were visiting the hospital for various reasons. This population was not representative of the community from which they came from; therefore the results cannot be extrapolated to the source population. Recall bias is also a likely weakness of this study since part of the data required recall of past events by patients and/or their caretakers, and home visits were not carried out to verify the reported environmental exposures. This study could not also determine a causal relationship between arbovirus antibody positivity and the associated clinical features.

Conclusion and Recommendations

The exposure rates for WNV, CHIKV and YFV in children at Alupe District Hospital provided by this study gives is baseline information on the current status of the selected arbovirus diseases in Teso District. This information has confirmed that exposure to YFV, CHIKV and WNV and possibly to other arboviruses in Teso District has occurred as recently as in the last 5 years or less. The study has highlighted possible changing patterns of distribution of the YFV, CHIKV and WNV. WNV and YFV prevalence appear to be increasing in the general Western Kenya region. This study forms a basis on which further large scale community surveys should be carried out to determine the real prevalence of these viruses in the community and further evaluate the epidemiology of these arboviruses and others. Periodic surveys need to be carried out to determine any changes in arbovirus prevalence so that outbreaks can be prevented before they occur. There is also need to carry out specific IgM antibody surveys, molecular virus detection tests and viral isolations tests to determine the real disease burden caused by the YFV, CHIKV and WNV and other arboviruses and their role in causation of fever in children. This would help inform physicians and the community of their possible differential diagnosis in febrile illness and may help save the costs incurred by empirical treatment of febrile illness with anti malarial drugs or antibiotics in some patients. There is need to introduce the YFV vaccine in the routine childhood KEPI schedule for all children in Kenya and catch-up YFV vaccination as recommended by WHO and UNICEF for Africa in 1988. The YFV vaccine was found to have a seven-fold efficacy in the reduction of deaths due to YFV disease.

This study found that arbovirus exposure was associated with having a bushy, forested environment around the home compared to having grass only or no vegetation around the house. It also found that the use of other mosquito control measures besides mosquito nets confers significant protection from any arbovirus seropositivity and WNV seropositivity but not from CHIKV or YFV seropositivity. Muscle aches, red eyes, photophobia and stomachache were associated with arbovirus seropositivity in this study. This study has described the factors associated with arbovirus seropositivity that should now stimulate further research to determine the clinical features associated with

confirmed cases of arbovirus infection in our setting. The information provided by this study is a basis which should stimulate research for cheaper, simpler and more accessible point of care diagnostic tests for arboviruses, in combination with an understanding of specific clinical features that may be associated with arboviruses in order to improve the diagnosis and hence management of fever in children in areas where arboviruses are prevalent. There is therefore need to carry out community education on the causes of arbovirus infections and how to protect themselves and their families from getting infected. Use of other mosquito control measures besides mosquito nets is recommended to prevent arbovirus transmission, and reduce the prevalence of arbovirus infections in endemic areas. Arbovirus exposure can be prevented in two major ways:

1. Personal protective measures to reduce contact with mosquitoes and
2. Public health measures to reduce the population of infected mosquitoes in the environment.

Personal protection measures include reducing time outdoors, particularly in early morning and evening hours, wearing long pants and long sleeved shirts, and applying mosquito repellent to exposed skin areas and clothing.

Public health measures are likely the best mode of prevention. They include elimination of larval habitats by removing tins, tyres, gourds bottles and other discarded containers that contain water around houses, draining of stagnant water puddles around buildings where mosquitoes can breed or cover them with oil to kill mosquito larvae or spraying of insecticides to kill mosquito larvae and adult mosquitoes. In emergency situations, wide area aerial spraying can be used to quickly reduce the number of adult mosquitoes. Other vector control strategies would include covering all domestic water storage containers so mosquitoes cannot breed in them and covering drainage systems in urban areas or spraying them with oil. All people and especially those who are ill with fever should always use a mosquito bed-net as the bed-net may help prevent mosquito-borne diseases including arboviruses from spreading to others.

People who have a fever and any other features suggestive of an arbovirus infection should be taken to a health facility immediately.

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Appendix 1: Questionnaire

A SEROPREVALENCE SURVEY FOR SELECTED ARBOVIRUS EXPOSURE IN CHILDREN AT ALUPE DISTRICT HOSPITAL AND KEMRI ALUPE CLINIC

Personal Details

Study Identification number…….Date of hospital visit ……

Place of interview: 1. Outpatient dept. 2. Ward 3. KEMRI clinic

Village …… Sub-location ……

Location ……. Division ……

District ……. Province ……

Usual residence: 1. Village 2. Town

Attending school: Yes ( ) No ( ) Class……

Date of birth …… Age (years) ……

Gender: Male ( ) Female ( )

Weight in Kgs …… Height/length in cms ……

Mid upper arm circumference (MUAC) in cms…….

Temperature in ^{0}C……

Have you ever been vaccinated for KEPI childhood vaccines? Yes ( ) No ( )

KEPI card available? Yes ( ) No ( )

Specify if: 1. Complete 2. Incomplete

Have you ever been vaccinated for Yellow fever? Yes ( ) No ( )

If Yes, when? Year:……Date:……Age:……..

Have you been admitted to hospital before: Yes ( ) No ( )

If yes, how many times……..

Respondent: 1. Parent 2. Grand parent 3. Other relative/care giver

(Specify)……..

Parent/Caregiver Socio-demographic Data

Primary care giver: 1. Mother 2. Father 3. Sibling 4. Grandparent
5. Aunt/Uncle. 6. Other (Specify)……

	Mother/Primary care giver	**Father/Partner**
Age in years		
Marital status	1. Never Married	1. Never Married
	2. Married	2. Married
	3. Separated	3. Separated
	4. Widowed	4. Widowed
Level of education	1. No formal education	1. No formal education
	2. Primary (Incomplete)	2. Primary (Incomplete)
	3. Primary (Complete)	3. Primary (Complete)
	4. Secondary (Incomplete)	4. Secondary (Incomplete)
	5. Secondary (Complete)	5. Secondary (Complete)
	6. Tertiary education	6. Tertiary education
Occupation	1. Salaried formal employment	1. Salaried formal employment
	2. Informal employment	2. Informal employment
	3. Self employment	3. Self employment
	4. Casual worker	4. Casual worker
	5. Unemployed/Housewife	5. Unemployed/Housewife

Clinical Survey Form: Exposures

Describe the following in the environment the child lives in. Indicate in the last column if information given was verified on home visit.

Exposure	Present		Describe/Specify Type, when, where	Other remarks
	Yes	No		
Type of house			1. Grass thatched mud walled 2. Iron roof mud walled 3. Iron/tile roofed stone wall	
Vegetation surrounding the house/home			1. Grass 2. Bushes 3. Forest	
3 main crops grown near the house			1. …… 2. …… 3. ……	
Are there water bodies near the house/home?			1. Rivers 2. Swamps 3. Ponds 4. Canals 5. Lakes 6. Others……	Distance from house/home in meters? ……
Dumping site near the house/ home?				
Water collecting containers in the dumpsite and/or near the house/home			1. Tins and broken bottles 2. Broken pots/basins and dishes 3. Tyres 4. Potted plants 5. Others……	
Where do you store domestic water?			1. Pots 2. Jerry cans 3. Buckets/Basins 4. Drums 5. Others……	
Is stored domestic water covered?				
Where is domestic water stored			1. Inside house 2. Outside 3. Both	
Have you had recent mosquito bites?				
At what times do mosquitoes bite you?			1. Dawn (Morning) 2. Dusk (Evening) 3. Daytime 4. All times 5. Other……	
Where do mosquito bites occur most?			1. Inside the house 2. Outside the house 3. In the farm/forest	

During what farming activities/season do you experience increased mosquito bites			1. Planting: 2. Weeding: 3. Harvesting: 4. Rainy season 5. Dry season	
Do you sleep under a mosquito net?			Specify type: 1. Olyset^{R} 2. Permanet^{R} 3. Supernet^{R} 4. Other. ……	
Mosquito net treated?				
What form of mosquito control do you use?			1. Mosquito coils 2. Insect repellants 3. Insecticide sprays 4. Herbs 5. Other ……	
What activities does the child engage in after school?			1. Herding livestock 2. Tilling the farm 3. Fetching water from the stream/ pond etc. 4. Gathering firewood 5. Playing near the forest 6. Other…….	
Is there livestock in the Homestead?			1. Goats 2. Sheep 3. Cattle 4. Pigs 5. Duck/geese/chicken	
Does livestock sleep in same house as members of the family?				
How far is animal shed away form the house?			Specify distance in meters……..	

Home Visit done? Yes ( ) No ( )

By: Name:..... Designation.....

Date:.....

Clinical Survey Form: Symptoms

Do you have/have you had any of the following symptoms? If yes please indicate when.

	Now Present			**Past**		
Symptom	*Yes*	*No*	*Date of onset/duration*	*Yes*	*No*	*When*
Fever						
Chills						
Sick feeling						
Rash						
Red eyes						
Painful eyes to light						
Painful eyes						
Yellow eyes						
Nausea or vomiting						
Stomachache						
Diarrhea						
Sore throat						
Muscle aches						
Joint pains						
Swollen joints						
Backache						
Headache						
Confusion						
Dizziness						
Hard to arouse						
Neck stiffness						
Abnormal movements						
Vomiting blood						
Bleeding from gums/nose/eyes						
Bloody or black stools						
Bruising						

Clinical Survey Form: Physical Exam

Sign	**Present**		**Description and comments**
	Yes	*No*	
Wasted			
Dehydrated			
Pallor			
Jaundice			
Scleral hemorrhages			
Lymph nodes			1. Cervical 2. Axillary 3. Inguinal
Throat inflammation			
Rash			1. Papular 2. Maculo-papular 3. Erythematous 4. Hemorrhagic 5. Other……
Joint swelling, tenderness			Specify site/abnormality ……
Hepatomegaly			
Bleeding manifestations			1. Petechiae 2. Purpura 3. Ecchymosis 4. Other .....
Features of meningo-encephalitis			1. Stiff neck 2. Confusion 3. Altered consciousness
Other findings, remarks			

Form completed by:……...……Designation……

Signature …… Date …… Time ……

Appendix 2: Consent Forms For Minors in English

Seroprevalence of Selected Arboviruses in Children at the Alupe District Hospital in Western Kenya

Informed Consent for minors in English

My name is…….and I work in this health facility/ I am a doctor from Kenyatta National Hospital and University of Nairobi. We think that Chikungunya, O'Nyong' Nyong', West Nile virus, Yellow Fever and Dengue viruses are important causes of febrile and non-febrile illness in this area. In order to be sure about the exact magnitude of the problem, we are conducting a study to determine the prevalence of Chikungunya, O'Nyong' Nyong', West Nile virus, Yellow fever virus and Dengue viruses in children in this area. The information we gather is useful to the government and other policy makers who may consider preventative programs in this community or other communities in the future. We will summarize our findings from this study and disseminate it to various stakeholders including Alupe District Hospital, The Ministry of Health, University of Nairobi, Kenyatta National Hospital, Kenya Expanded Programme on Immunization (KEPI), KEMRI, and others. The Alupe District Hospital, Kenyatta National Hospital and KEMRI Ethical Review Committees have approved this study.

Research Procedures

If you allow your child to participate in this study, we will ask you questions regarding your child, his/her health, where he/she resides, his/her vaccination status and others. Then we will take $2^1/_2$ milliliters of blood during the procedure ($2^1/_2$ milliliters more if your doctor has requested other tests). We will use sterile and disposable instruments that are clean and safe. The extra blood taken from your child will be transported to the KEMRI laboratory in Nairobi for analysis or done here at the KEMRI laboratory in Alupe. The tests we conduct may identify the virus infections we have mentioned above. In order to ensure complete confidentiality of the test results, no names will be attached to the blood samples, but a number assigned to your child will be used to label the sample.

Risk/benefits

During this procedure there will be no long-lasting effect. However, your child may feel a brief moment of pain or fear and may bleed briefly from the venipuncture site. Your child will not be given any monetary benefits; neither will he/she incur any costs. The study will benefit your community since by helping us and the government to understand the problems your community is facing as a result of arboviruses, we will be able to recommend and design appropriate interventions to minimize the impact of these infectious diseases. If your child is found to have malaria or typhoid, the results of the tests will be availed to your primary clinician for appropriate treatment. If you allow your child to be tested for HIV and the test result is found to be positive, you will be referred to the appropriate clinic for appropriate care and follow up for the condition.

Your child's Rights

Your child's participation in this study is voluntary and if you disallow participation, you will not be denied any services that are normally available to your child.

Confidentiality

We will make every effort to protect your child's identity. Your child will not be identified in any report or publication of this study or its results.

Contact Information

If you have questions now or in the future regarding your child's rights or this study, you may ask any of the field officers involved in this study or contact Dr. Mary Inziani Matilu (Principal Investigator) of University of Nairobi on 0722523370. Or the Chairman, KNH/UON-ERC, Prof. K.M. Bhatt. Tel. 020726300-9. P.O. Box 20723, Nairobi. Email: KNHplan@Ken.Healthnet.org.

Consent for the individual for blood sample

May I now ask if you will allow your child to participate in the study?

The above details about the study and the basis of participation have been explained to me and **I allow my child** to take part in the study. I understand that I am free to allow my child to be part of the study. I also understand that if I do not want him/her to go on with the study, I can withdraw at any time. **I give my consent** for my child's blood to be tested for Chikungunya, West Nile and Yellow fever viruses.

Please sign here or put your right hand thumb mark if you agree:

Signature/ Thumb mark----------Date ----------

Appendix 3: Consent Forms For Minors in Swahili

Seroprevalence of Selected Arboviruses in Children at the Alupe District Hospital in Western Kenya

Ridhaa ya watoto walio chini ya miaka 18, kwa Kiswahili

Jina langu ni…….Mimi ni mfanyakazi wa kituo hiki cha afya/mimi ni daktari kutoka Hospitali kuu ya Kenyatta na Chuo kikuu cha Nairobi. Tunafanya utafiti juu ya ugonjwa wa arbovirus unaosababishwa na virusi vya Chikungunya, O'Nyong' Nyong', West Nile, Yellow fever na Dengue, ambayo tunafikiri ni mojawapo ya magonjwa yanayoambukiza jamii hii. Ili tujue umuhimu wa arbovirus huku, tumeamua kufanya utafiti. Matokeo ya utafiti huu yatasambazwa kwa Hospitali ya wilaya ya Alupe na kwa wadau kama Wizara ya Afya, Hospitali kuu ya Kenyatta, Chuo kikuu cha Nairobi, KEPI, KEMRI na wengineo, ambao watatengeneza miradi mbalimbali itakayolenga kupunguza maambukizi na kuboresha huduma za walioathirika katika jamii hii, na nyingine hapo baadaye. Utafiti huu umeruhusiwa na kamati zinazohusika na utoaji wa vibali vya utafiti za Hospitali ya Alupe, Hospitali kuu ya Kenyatta na KEMRI.

Utaratibu:

Ikiwa utakubali mtoto wako ahusike na utafiti huu, kwanza tutakuuliza maswali machache juu ya umri wake, anapoishi, na kama ameshapewa chanjo ya yellow fever na chanjo zingine za utotoni. Baadaye, tutatoa kiasi cha mililita mbili na nusu ($2^1/_2$mls) cha damu, (mililita mbili na nusu zaidi kama daktari wako amekueleza kuwa mtoto wako atatolewa damu kupima magonjwa mengine), zoezi ambalo litachukua muda mfupi tu. Tutatumia vifaa visafi na salama ambavyo vitafunguliwa mbele ya macho yako, na vitatumika kwako tu na kutupwa mara tu baadaye. Damu tutakayotoa kwa huu utafiti itapelekwa maabara kwa upimaji wa arbovirus. Ili kuhakikisha usiri wa jina la mtoto wako katika utafiti huu, jina na maelezo yake hayataandikwa kwenye sampuli ya damu, bali sampuli itatambulishwa na nambari tu.

Faida/Mapungufu:

Mtoto wako atahisi maumivu kidogo ama woga wakati atadungwa sindano ama atatoa damu kidogo baada ya kutolewa damu, lakini hatapata maumivu ya muda mrefu na kutokwa damu kutazuiliwa. Hatapata malipo yoyote ya kifedha, na pia hutatumia pesa

zako katika utafiti huu. Utafiti huu utasaidia jamii yako kwa sababu tukifahamu matatizo ya jamii hii, tutaweza kushauri na kutengeneza miradi mbalimbali ya kupunguza athari za arbovirus. Mtoto wako akipatikana na malaria au typhoid, majibu yatolewa kwa daktari wake ili apate matibabu yanayostahili. Ukikubali mtoto wako apimwe ukimwi, na apatikane nayo, utaelekezwa kwa cliniki ambako atapata usaidizi anaostahili kupunguza makali ya ugonjwa huu.

Haki za mtoto wako:

Ushiriki wa mtoto wako katika utafiti huu ni wa hiari kabisa. Ukikataa ashiriki, mtoto wako hatanyimwa huduma zinazotolewa kwa kawaida.

Usiri/Utunzaji wa taarifa:

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

Mawasiliano:

Iwapo utakuwa na swali kuhusiana na haki za mtoto wako ama utafiti huu, unaruhusiwa kuwasiliana na afisa yeyote wa utafiti, au Mkuu wa utafiti huu, Dr. Mary Matilu wa Chuo kikuu cha Nairobi, Nambari ya simu 0722523370. Au mwenyekiti wa kamati inayohusika na utoaji wa vibali vya utafiti ya hospitali kuu ya Kenyatta (KNH/UON-ERC), Profesa K.M. Bhatt, nambari ya simu 020726300-9. Barua pepe: KNHplan@Ken.Healthnet.org. Sanduku la posta 20723, Nairobi.

Ridhaa ya kutolewa damu

Ningependa kukuuliza ridhaa yako ya ushiriki wa mtoto wako katika zoezi la utoaji damu.

Nimeelewa maelezo ya hapo juu yanayohusu utafiti huu, na ninakubali mtoto wangu ashiriki katika zoezi hili. Naelewa kuwa ushiriki wa mtoto wangu ni wa hiari, na pia kama sitakubaliana muda wowote naruhusiwa kumtoa katika zoezi hili. Natoa ridhaa damu ya mtoto wangu itumike katika upimaji wa virusi vya Chikungunya, West Nile na Dengue.

Sahihi/dole gumba……Tarehe……

Appendix 4: Laboratory Procedures

Indirect IgG Enzyme Linked Immuno-Sorbent Assay

An in-house assay using purified antigens of CHIKV, WNV and YFV in a standard indirect ELISA as previously described by Bundo and Igarashi *et al.*(42) was used to identify antibodies specific to CHIK, WNV and YFV. This assay did not differentiate between IgG and IgM. Patient sera were tested according to well-established protocols with slight procedural modifications to suit the local environment. The optical density (OD) of the final ELISA reaction product was measured using an ELISA plate reader (Thermolab systems Multiscan JX, China), at a wavelength of 492 nm using Ascent Software Version 2.6 (Thermo Scientific, Tokyo, Japan). The OD specific for any arbovirus was calculated as: (Mean OD of virus coated wells) – (Mean OD of PBS-F coated wells). If the specific OD reading was more than 1.0, that serum was regarded as anti-arbovirus antibody positive.