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Sero-Activity of Chikungunya Virus at the Kenyan Coast after the 2004 Epidemic

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Abstract:*Chikungunya virus (CHIKV) is an alphavirus of Semliki Forest virus antigenic complex. Seroprevalence of CHIKVwas measured after the reported 2004CHIKV epidemic in Coastal Kenya and factors associated with seropositivity determined.The total anti-CHIKV antibodies were measured by in-house ELISA's and neutralizing antibodiesdetermined by focus reduction neutralization test (FRNT) in a cross sectional serosurvey, comparisons of means done using Student's t test, prevalence rates determined using descriptive statistics, tests of associations performed using Chi-square, and Fisher's exact test.Of 452 samples, 134 (29.6%) were seropositive for CHIKV antibodies by in-house ELISA; 105 (23.2%) had CHIKV neutralizing antibodiesby FRNT. Age, the month of July, and herding wereassociatedwith CHIKV seropositivity;OR=0.53 (P=0.051, 95% CI: 0.28-1.02), OR=0.14 (P=0.012, 95% C.I 0.02 – 0.8) and OR=6.34 (P= 0.014, 95% CI:1.55-30.61) respectively. Myalgia is likely present in CHIKV infection odds 3.72 (P=0.0363, 95% CI: 1.10-13.53). CHIKVis endemic at the Kenyan coastand poses a high risk on socio-economic development. There is need for frequent serosurveys to enhance targeted preventive public health interventions and new control paradigms.*

Keywords: *Chikungunya virus, seroprevalence, socio-demographic correlates.*

I. Introduction

There is evidence CHIKV is endemic in the Coastal Kenya, causing acute severe self-limiting febrile illness often misdiagnosed as malaria in the acute care setting[1]. CHIKV infects all age groups, causing disabling symptoms and immunocompromised people may get severe complications[2, 3].CHIKV is responsible for unexpected severe epidemics in countries of the Indian Ocean region [4]. It causes an arboviral disease: Chikungunya Fever (CHIKF).CHIKF is responsible of a two phase disease: acute and chronic[5-7]. CHIKF was first recognized in epidemic form in East Africa along the border between Newala, Tanzania and Mozambique 1952-1953 where CHIKV was first isolated [4, 8-10].

Chikungunya is a single stranded, positive sense RNA-enveloped belonging to the family Togaviridae, genus Chikungunya and belongs to the Semliki Forest virus antigenic complex whose members are associated with similar clinical manifestations and are closely related serologically. [11-14]. It is maintained in a sylvatic and urban cycle involving humans and mosquito species *Aedes aegypti* and *Aedes albopictus* [4, 12, 15-18].

CHIKV epidemiology is characterized by explosive outbreaks [16]. The first significant CHIKV outbreaks were documented in the early 1960s in Bangkok and from 1963 through 1973 in India [16, 18]. Minor outbreaks periodically occurred over the next 30 years with no major outbreaks were recorded until 2004, when the large epidemic started on the coast of Kenya [8]. It affected approximately 75% of the population of Lamu Island, and spread to the islands of Comoros, La Reunion and throughout the Indian Ocean islands and coastal region into Eurasia infecting over 2 million people with approximately 500,000 cases in East Africa [16].

This study was undertaken to measure the seroprevalence of CHIKV after the documented 2004 CHIKV epidemic in Coastal Kenya, assess CHIKV and link it to socio-demographic correlates and possible risk factors.

II. Materials and Methods

Ethics statement: Participants consented to participate in their own language. Written consent was obtained from all adults; children assented with parental written consent. The study was undertaken after approval from The Scientific Steering Committee (SSC) of the Kenya Medical Research Institute (KEMRI) and The National Ethical Review Committee (ERC) which grants approval for research studies involving human subjects in compliance to the Helsinki declaration.

***Study population*:** The study examined seroprevalence of anti-CHIKV antibodies in a population residing in the catchment area of Coast Provincial General Hospital (CPGH) in the year 2012. The population is composed of high-to mid-to low socioeconomic status of both professionals in different occupations and casual workers.Their homes range from permanent, semi-permanent to shanty dwellings.Their economic activities include: farming, herding cattle, burning charcoal, collecting firewood and hunting birds. Socioeconomic status and type of houses were used to define the poverty index of the population.The sample population included only individuals with fever of $\geq 39^0$C diagnosed as"pyrexia of unknown origin"who had not been immunized against yellow fever. Venous blood was collected from consenting individuals, sera separated and bio-banked.

***Test antigen produced from Chikungunya virus S27 strain*:**CHIKVS27 was cultured in Vero Biken (African Green Monkey Kidney derived) cells and concentrated to 80 ELISA units (EU) [19, 20]. The dilution to give 120FFU/100µl was 1:253 as determined by Focus assay (FA)[21-23].

***In-house Indirect IgG ELISA Testing*:** The assay utilized purified CHICKVS27 antigens inastandard Indirect ELISA protocol[19, 20].Positive samples required a titer ≥ 1:30,000 above that of the negative control for each plate.

***In-house IgM capture ELISATesting*:** The in–house IgM capture ELISA was performed for anti-CHIKV IgM antibodies using published protocol. Positive samples required a positive control sample OD 492 /negative control OD 492 (P/N) ≥ 2.[24, 25].

Focus Reduction Neutralization Testing: All ELISA seropositive were subjected to FRNT confirmatory testing to verify ELISA results and determine whether seropositivity was due to CHIKV by detecting presence of specific neutralizing antibodiesusing published protocols[26, 27].

***Data Analysis*:**Data was compiled using Microsoft excel, analyzed using STATA MP 13.0 and R software's. Categorical data was tabulated. Meanand standard deviation were used to summarize the data. Comparisons of means were done using Student's t test. Prevalence rates were determined using descriptive statistics. Descriptive statistics were used to describe immunological characteristics.Tests of associations were performed using Chi-square test for categorical variables, and Fisher's exact test for nominal variables. The *P* valuesof less than 0.05 were considered significant. Logistic regression models were used to determine variables associated with seropositivity. Data was presented using bar charts and tables.

III. Results

Of the 452 study subjects, males were 244 (54.0%), females 206 (45.6%) and 2 (0.4%) gender was not indicated. They were aged zero (0) to seventy-two (72) years. A total of 427 (94.5%) of the study subjects were usual residents of Mombasa County and only 25 (5.5%) from other Counties, hence the findings are a true representation of CHIKV sero-activity at the Kenyan coast. The 452 study subjects were categorized into three age groups based on the last reported CHIKV epidemic at the Kenyan coast which lasted from 2004 – 2007 as follows: pre-epidemic [≥9yrs], Intra-epidemic [>4 ≤ 9yrs] and Post-epidemic [≤4yrs]. Seroprevalence rate of CHIKV was measured against variables which included age, months, socioeconomic status and risk factors. Clinical presentationsof CHIKV were assessed.

Of 452 in-house ELISA results, 114 (25.2%) were seropositive for IgG, 31 (6.9%) seropositive for IgM and 11(2.4%) seropositive for both IgG and IgM. Seropositivity for anti-CHIKV antibodies byELISA was 134(29.6%) and sero-negativity 318 (70.4%). The 134 samples seropositive by ELISA were subjected to FRNT as a confirmatory test,105 (23.2%) of subjects had neutralizing antibodies against CHIKV. Of these 105 sera samples with CHIKV neutralizing antibodies 99 (94.3%) had IgG antibodies, 15(14.3%) had IgM antibodies and 10(9.5%) had both IgG and IgM antibodies specific to CHIKV. IgM used as a measure of recent infections and IgG of past infections, our findings suggest there has been past exposure to CHIKV and transmission is currently ongoing (Table 1).

There is evidence of year-round CHIKV transmission. The months of July, August, January, June, March and October had the highest prevalence's: 2.4%, 2.4%, 2.7%, 2.9%, 3.1%, and 3.5% respectively (Fig.1). Exposure to CHIKV is likely to occur in the months of July OR= 6.14, *(P=0.00, 95% C.I 1.25 – 47.75)*(table2).

Analysis of the 2004-2007CHIKV epidemic age related categories revealed the pre-epidemic [≥9yrs] were 178(39.4%) of which 43(24.2%, 9.5% of total) were seropositive for anti-CHIKV antibodies confirmed by FRNT;(90.7% (39/43) had IgG antibodies, 16.3% (7/43) had IgM antibodies and 9.3 %(3/43) had both IgG and IgM antibodies). The intra-epidemic [>4 <9 yrs.] group the subjects were 124(27.4%) of which 22(17.7%, 4.9% of total) were seropositive for anti-CHIKV antibodies by FRNT (95.5% (21/22) had IgG antibodies, 27.3% (6/22) had IgM antibodies, and 22.7% (5/22) had both IgG and IgM antibodies. Subjects in the post-epidemic [≤4yrs] were 150 (33.2%) of which 40(26.7 %, 8.8% of total) were positive for anti-CHIKV antibodies by FRNT (97.5% (39/40) had IgG antibodies, 5% (2/40) had IgM antibodies and 2.5% (1/40) had both IgG and IgM antibodies. The prevalence of CHIKV as calculated across 2004-2007 epidemic related age categories was 26.7%, 24.2% and 17.7% for the post-epidemic, pre-epidemic and intra-epidemic groups respectively (Fig.2).CHIKV transmission is more likely to be associated with the intra-epidemic group than the other epidemic related groupsOR=0.53, *(P=0.05, 95% C.I 0.28-1.02)* (table 3).

The mean age of Chikungunya virus exposed subjects was 17.5 years. Grouping the ages at 5-year interval, highprevalence of CHIKV were observed in children aged 0-5yrs, 6-10yrs and young adults aged 31-35yrs; of 42.9%, 16.2%, and 9.5% respectively (Fig. 3)

Males were 244(54.0%), females 206 (45.6%) females and for 2 (0.4%) of the study subjects gender was not indicated from questionnaires. Seropositive males were 23.3% (56/244) ofwhich 94.6% (53/56) had IgG, 21.4% (12/56) had IgM and 17.9% (10/56) had both IgG and IgM antibodies against CHIKV. Seropositive females were 23.8% (49/206) of which 93.9% (46/49) had IgG, and 6.1% (3/49) had IgM antibodies against CHIKV. Females were less likely to be seropositive for both IgM and IgG antibodies against CHIKV *(P = 0.00, 95% C.I. 0.72 – 1.05).*In the recent past more men are likely to have been exposed to CHIKV than females (Fig. 4).Herding is significantly associated with CHIKV seropositivity. OR=6.34,*(P= 0.01, 95% CI: 1.55-30.61)* (table 4). Social economic status of study subjects was not significantly associated with exposure to CHIKV *(P>0.05).*

All study subjects had presented with complaints of fever of ≥39.0^{0}C for 1-4 days among other symptoms and had been diagnosed with pyrexia of unknown origin after batteries of laboratory investigations were done to rule out known causes of fever. The complaint of myalgia was significantly associated with CHIKF OR=3.72, *(P=0.04, 95% CI: 1.10-13.53)* (table 5).

IV. Discussion

Chikungunya virus exposure is common at the Kenyan coast despite little public health attention. In this study, sera positive for CHIKV antibodies by ELISA was found to have neutralizing antibodies against Chikungunya virus. OR to Chikungunya virus when one is seropositive for CHIKV IgM abs was 0.18, *(P = 0.00, 95% C.I. 0.103 - 0.299),* CHIKV IgG abs was 14.29, *(P=0.00, 95% C.I. 6.64 - 30.74),* and both CHIKV IgG and IgM abs was 0.09, *(P= 0.00, 95% C.I 0.05 - 0.18)* (table 1). Seropositivity of IgM antibodies could not be used to calculate incidence rate of CHIKV since CHIKV unlike exposure to other infectious agents in which IgM antibodies produced lasts for up to 7 days, anti-CHIKV specific IgM abs are raised rapidly after onset of illness and persist after illness for longer periods up to 18months [16]. Therefore, seropositivity for IgM could have been due to CHIKV infection in the recent past two years.

There is a year round exposure to CHIKV shown by cases of CHIKF reported monthly except for the month of December which had no CHIKV cases, a situation likely occasioned by the long holiday break, where sample collection was only done for a period of two weeks and most patients shun public hospitals during festivities. There is more likelihood of being exposed to CHIKV in the month of July than other months of the year, OR=*6.94 (P=0.00, 95% C.I. 0.25 – 47.75)*(table2). This could be due to the high humidity traditionally experience in this month which favors vector breeding.

Chikungunya virus, like other arboviruses depends on arthropods and vertebrate hosts for successful maintenance[28]. CHIKV infection is greatly affected by both demographic and environmental changes. CHIKV antibodies were detected in all age groups with a high positive ratio in children aged 0-5 years (Fig.3). The presence of anti-CHIKV IgG and IgM antibodies across all ages including children born after the documentedCHIKV epidemic that lasted from 2004 – 2007[13]indicates that CHIKV transmission has been and is ongoing in this region;yet it remains undetected or poorly reported within clinical settings.

The highpositive ratio of CHIKF cases observed among the children could be because: (i). They constitute the largest naïve populationnot exposed to CHIKV during the 2004-2007 CHIKV epidemic hence are highly susceptible to CHIKV infection. (ii). *Aedes* is a daytime feeder and it iscommon to find toddlers in daycare facilities, homes and even hospitals sleeping daytime unprotected from day time blood sucking mosquitoes.

Age is significantly associated with CHIKV infection: there is a likelihood that active transmission of CHIKV is among the intra-epidemic group composed of toddlers and children;OR= 0.53,*(P=0.05, 95% C.I 0.28-1.02*)(table 3).Theseare immunologically naïve, have underdeveloped immunity and are actively engaged

in outdoor activities as compared with other epidemic-related groups.Often, children play in the open field, also near dumping sites; suspectedurban habitats and breeding places for *Ae. Aegypti aegypti*[29].Lower seropositivity ratio was registered among the adults aged above 65 years (Fig.3). This could be due to: (i) herd immunity acquired overrepeated years of exposure to CHIKV following repeated mosquito bites and/or (ii) decline in their population following age related mortality.

Females had higher seroprevalence (23.8%) of CHIKV as compared to males (23.3%) (Fig.4) as documented in other studies[1, 30]. However, active transmission of CHIKV seems to be more actively ongoing among males thanfemales. For instance, 94.6% males were seropositive for anti-CHIKV IgG antibodies, 21.4% seropositive for anti-CHIKV IgM antibodies as compared to females who were 93.9% and 6.1% seropositive for anti-CHIKV IgG and IgM antibodies respectively. Furthermore, 17.9% of the cases were seropositive for both IgG and IgM antibodies, allwho were males (Fig.4). It is unlikely that females will have both IgG and IgM antibodies against CHIKV in their serumO.R = 0.87, *(P=0.00, 95% C.I. 0.72 - 1.05).* The unlikelihood that females will have both IgM and IgG CHIKV abs simultaneously and the likelihood that male will simultaneously mount IgG and IgM antibodies might be due to herd immunity in females and failure of males to mount herdimmunity against CHIKV, this phenomenon needs further investigations.

Findingsindicates that moremales (21.4%) were likely to have been exposed to CHIKV infection in the recent past than females (6.1%) (Fig.4).The lower rate of CHIKV transmission in females is probably due to the effect of herd immunity whereas high rates of CHIKV transmission in males might be due to gender related cultural norms and social habits in this region: Females stay around the homesteads indoors, and their dress code cover at least 95% of their bodies whereas males are in the fields, tending to animal herds, hunting, fishing, burning charcoal or busy earning livelihoods in more urban areas;and their dress code leaves most of their bodies exposed to the epidemic *Aedes* which are highly anthropophilic and crepuscular feeders.

IgM antibodies raised following CHIKV infection persists for several months [16], the presence of both IgG and IgM anti-CHIKV antibodiesin males could be due to:(i) recent or past exposuresto CHIKV or both thus both antibodies are in circulation, (ii) repeated exposures to mosquitoes bites due to endemic circulation of CHIKV with additive risk of exposure.

There was a greater than 3-fold increase in both IgG and IgM anti-CHIKV antibody titers in majority of our study samples; 80/93 (86.0%) demonstrated >1: 90,000 and 8/16 (50%) demonstrated P/N>3 respectively indicating a likelihood of repeated mosquito exposures suggesting an endemic circulation of CHIKV with additive risk of exposure over time. Serumof a 33 years old male subject had IgG >1:256, 000 and IgM P/N =4 CHIKV specific neutralizing antibodies. This highlights theneed for further investigations to determine gender specific re-exposure to CHIKV in relation tolife-long immunity. It iswidely published that primary exposure to Chikungunya virus infection is the elicits and confers lifelong immunity against CHIKF[31, 32].

Sera of an11 months old malewith clinical history of three days'fever of 39.8^0C and arashwas seropositive for equivocal antibodies; IgG >1:256, 0000 and IgM P/N =16. This could be a case of re-exposureto CHIKVoran antigenically related alphavirus following repeated mosquito bites or a multi-exposure to CHIKV and/oran antigenically related alphaviruses(s)in a naïve immunocompromised subject. However, it was not easy toestablish whether CHIKV was responsible for the fever or an antigenically related alphavirus or both since the antibodies present did not neutralize CHIKV antigens. The high titersof antibodies observed in this case could be eitherneutralizing abs of an antigenically related alphavirus to CHIKV or CHIKV binding antibodies or both. Usually binding antibodies don't have neutralizing ability-or both.

Amongst the risk factors assessed, herding is significantly associated with exposure to CHIKV infection. This could be due to long hours spent in outdoor activities in mosquito habitats, herdsmen are usually haphazardly protected from the blood seeking *Aedes.* There was no correlation between socio-economic status and exposure to CHIKV.

The first case series of patients infected with CHIKV published in 1955 described patients with acute onset of high fever, severe joint pains and a rash[16, 17]. The arthralgia associated with infection is the most significant feature and has been reported to occur in approximately 70% of the cases[28, 32]. In this study, myalgia is significantly associated with CHIKF.

V. Conclusion/Recommendations

The findings suggest that at least 23.2% (105/452) of the population have been exposed to CHIKV. These demonstrate unreported ongoing transmission and circulation of CHIKV with an outbreak potential. It is likely that CHIKV continues to cause febrile illness currently ongoing undiagnosed. Of examined risk factors, Age, the month of July, and herding cattle are associated with seropositivity.

There is neither medications nor vaccines are currently available for treatment and protection against CHIKF. Thus, protection against mosquito bites and vector control methods are the elusive solutions for exposure to both CHIKV. There is need for civic education that protection against CHIKF relies on the use of mosquito repellents both day and night and other measures to limit skin exposure to mosquitoes like wearing

clothes that cover most parts of the body like long sleeved shirts, blouses and dresses, trousers, long dresses. Bed nets should not only be used during the nights but also in the day at home, in hospitals and even day-care facilities because *Aedes* mosquitoes are active all-day long. Control of both adult and larval mosquito populations would be the effective method of eliminating vectors. Breeding sites must be removed and destroyed, by frequently emptying water cans, burning cabbage at the dumping sites, clearing bushes and stagnant water and treating all suspected mosquito habitat and breeding places with insecticides. Besides vector control process being an endless, costly, and a labor-intensive measure; it is not always well accepted by local populations, whose cooperation is crucial. For proper control of CHIKV infection. Other than discovery of drugs for treatment of the disease will require development of vaccines in addition to individual protection from mosquitoes and other vector control programs. Surveillance is fundamental for early identification of cases and quarantine measures will contain transmission. To provide insight into the possible impact of future outbreaks in this region and the effectiveness of the proper interventions to prevent future explosive CHIKV epidemics, research should continue into the pathogenesis of persistent arthralgia's and possible therapeutics such as antivirals which can treat viremia and significantly reduce morbidity associated with CHIKV.

VI. Limitations

The study was a nested one and had limitations. Bio-banked sera collected for an ongoing parent study on the seroprevalence of DENV at the Kenyan coast was used. Samples had been repeatedly frozen and thawed for investigations in the parent study, this might have resulted into false negative IgM-capture ELISA results. The specificity of the in-house IgM-capture ELISA and indirect IgG ELISA were limited because of cross-reactivity with other alphavirus-related infections. We were not able to trap vectors for viral testing;hence we cannot associate vector type abundance or mosquito infection rates with human data in this study. Due to cost, time, and labor considerations FRNT could not be performed on the entire study sample set (134 of the 452 samples were tested).ELISA screening had several false positives detected by FRNT testing, thusthere may have been misclassification of some participants in the overall serosurvey sample using the CHIKV ELISA screening, which would bias our results to overestimation of CHIKV prevalence at 25.2% (134/452) if the 27.6% (29/105) false positive rate was applied to our total sample set instead of 23.2% (105/452). It is possible that nonspecific false positives from sticky serum samples could have occurred but these should have been corrected by FRNT testing. FRNT negatives might not be true false positives but individuals who might have been exposed to CHIKV but had not mounted neutralizing antibodies responses but rather binding antibody responses. Despite our limitations, we feel our conclusions are well supported by our sample size.

Acknowledgments

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Table 1: Univariate analysis of anti-CHIKV seropositivity by FRNT n=452

Neutralizing antibodies in sera	Frequency	Odds ratio	95% C.I.	P
IgM	15 (14.3%)	0.18	0.07-0.49	0.00*
IgG	99 (94.3%)	14.9	4.56-55.26	0.00*
IgM/IgG	10 (9.5%)	2.93	0.39-132.3	0.46

Table 2: Univariate regression analysis of how months are associated with CHIKF

Month	Odd ratio	95% C.I	P-value
January	0.34	0.01, 2.59	0.29
February	1.10	0.18, 4.68	0.89
March	0.72	0.01, 6.78	0.76
April	0.82	0.14, 3.3	0.77
May	1.83	0.03, 36.35	0.62
June	0.49	0.09, 1.86	0.27
July	6.94	1.25, 47.75	0.00*
August	1.01	0.26, 3.21	0.99
September	0.90	0.02, 9.61	0.93
October	0.64	0.11, 2.51	0.76
November	2.88	0.4, 18.22	0.16

Table 3: Multivariate regression analysis of Demographic and Socio-economic factors associated with Chikungunya virus infection

Factor	Odd ratio	95% C.I	P-value
Demographic:			
Age (ungrouped)	1.04	0.90-1.20	0.60
Age (5-year interval)	0.84	0.43-1.69	0.63
Age in developmental stage	1.43	0.81-2.52	0.23
Age as per Epidemic season	0.53	0.28-1.02	0.06
Intra-epidemic group	0.53	0.28-1.02	0.05*
Sex	0.92	0.59-1.43	0.71
Socio-economic:			
Occupation	1.07	0.45-2.48	0.87
Type of house	0.84	0.31-2.15	0.72

Table 4: Multivariate regression analysis of Risk factors associated with CHIKV seropositivity

Factor	Odd ratio	95% C.I	P-value
Herd cattle	6.34	1.55-30.61	0.01 *
Collect firewood	2.98	0.76-13.40	0.13
Burn charcoal	0.47	0.07-2.64	0.40
Hunt for birds	0.18	0.03-1.14	0.07
Type of house	0.85	0.22-3.27	0.81
Walls with holes/cracks	1.44	0.52-4.34	0.50
Vegetable surrounding the house	1.03	0.36-3.08	0.95
Water bodies near house	0.51	0.21-1.28	0.14
Dumping site near house	0.82	0.34-2.00	0.66
Sleep under net	1.16	0.38-3.96	0.81
Mosquito control activity	0.25	0.02-3.08	0.25
Time of Mosquito control activity	5.09	0.52-44.61	0.14
Animals sleep in the same house	1.08	0.35-3.20	0.89
Activities where mosquito bites	0.82	0.29-2.22	0.69
Time of mosquito bites	0.84	0.43-1.72	0.63
Season mosquito bite experienced	1.03	0.07-15.16	0.98

Table 5: Multivariate regression analysis of symptoms predicting diagnosis of CHIKF

Symptom	Odd ratio	95% C.I	P-value
Chills	0.97	0.20-0.48	0.92
Rash	0.76	0.39-1.41	0.39
Eye infection	1.49	0.78-2.78	0.22
Jaundice	0.48	0.13-1.39	0.22
Nausea vomiting	0.59	0.24-1.38	0.24
Abdominal pain	1.43	0.69-2.90	0.32
Diarrhea	0.95	0.42-2.06	0.89
Sore throat	1.00	0.34-2.56	0.99
Muscle aches	3.72	1.10-13.53	0.04 *
Joint pains	0.41	0.10-1.32	0.17
Swollen joints	0.00	0.00 – 0.00	0.98
Backache	2.31	0.73-6.81	0.13
Headache	0.83	0.51-1.37	0.48
Meningitis meningoencephalitis	0.76	0.26-2.04	0.61
Bleeding gums	2.01	0.29-18.72	0.50
Blood in stool	0.74	0.08-4.68	0.77
Coughing blood	1.39	0.25-8.63	0.71
Dehydrated	0.87	0.31-2.31	0.78
Wasted	1.20	0.32-3.98	0.77

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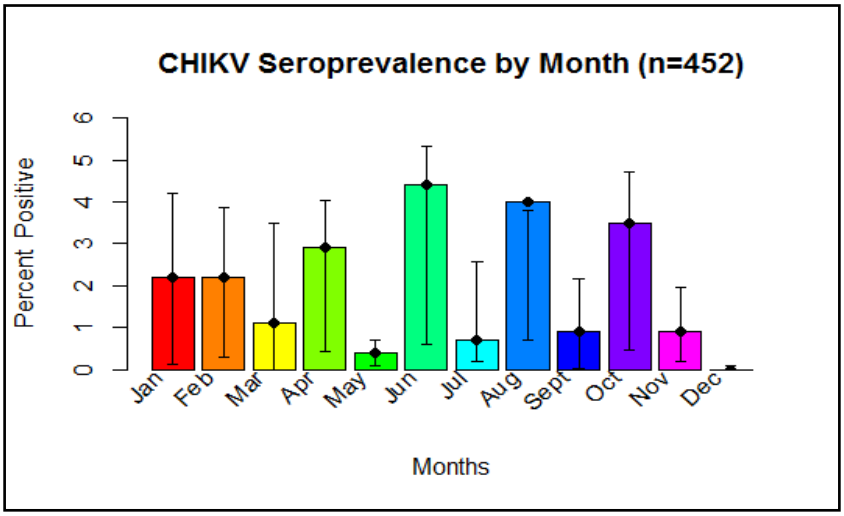

Figure 1: Monthly Prevalence of Chikungunya virus at the Kenyan Coast

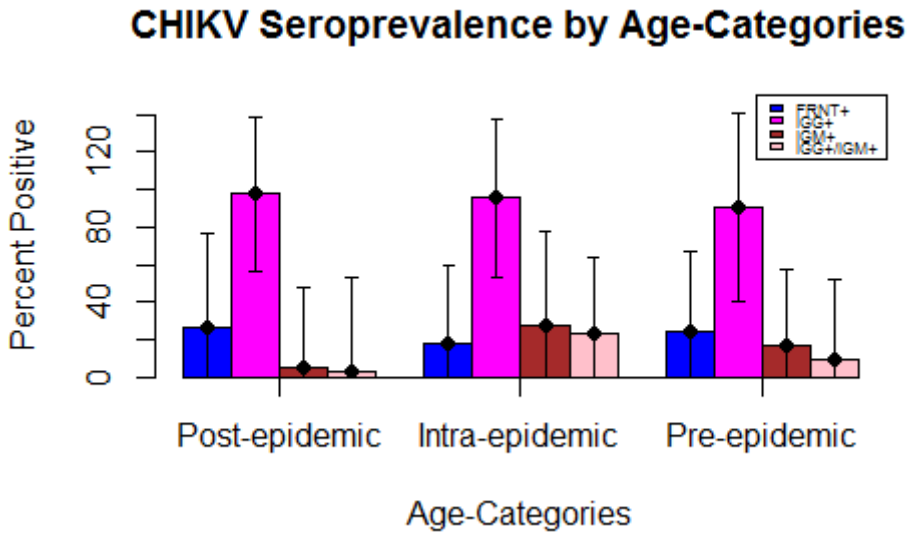

Figure 2: Sero-activity of Chikungunya viruses across CHIKV epidemic related age groups.
(After the 2004 - 2007 outbreak as measured by FRNT 95% CI indicated by bars)

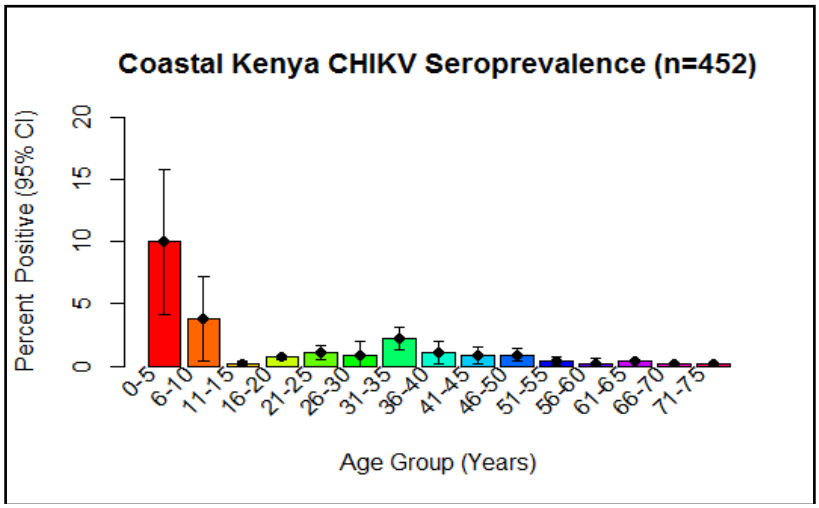

Figure 3:seroprevalence of CHIKV across age groups as measured by FRNT

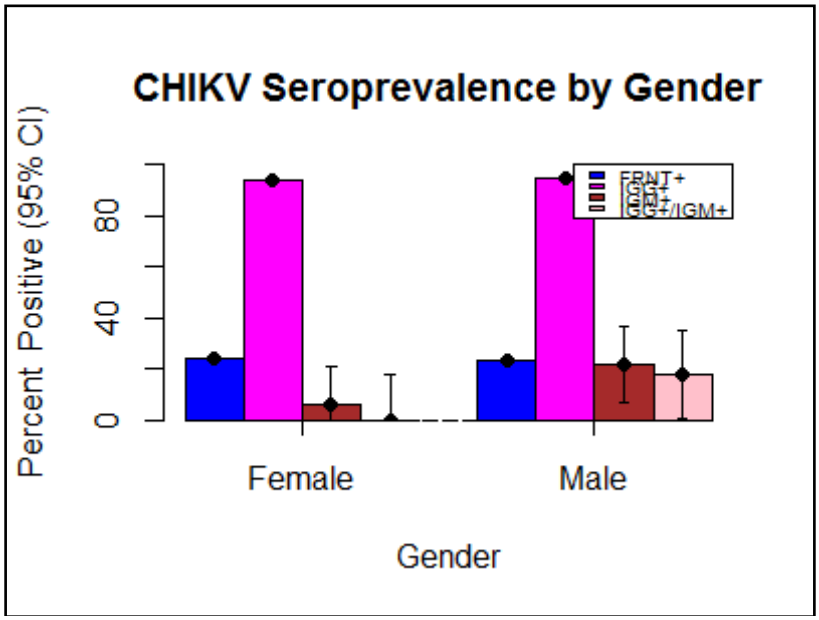

Figure 4: Sero-prevalence of Chikungunya viruses across gender. *As measured by ELISA and FRNT. 95% CI indicated by bars*

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