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Seroprevalence of Chikungunya Virus in Febrile Patients in the Kenyan Coast

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

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I. Introduction

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

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

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

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

II. Material And Methods

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

Study setting and Sample collection

The study was conducted between January 2014 and December 2015. Patients seen at CPGH presenting with febrile symptoms of unknown causes such as malaria, typhoid and other known bacterial infections were eligible. Venous blood was collected from consenting individuals and sera separated and stored for testing.

Laboratory procedures

Indirect IgM Indirect IgGELISA

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

Focus Reduction Neutralization Tests (FRNT)

Focus reduction neutralization test (FRNT) was used to determine the presence of arbovirus specific neutralizing antibodies in test sera already screened as positive by ELISA. Briefly, Vero cells at a concentration 1.5 x 10 cells/ml were seeded into 96-well plates (Nunc) at a volume of 2 ml/well. Cells were cultured in

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

5% CO_2 for 4-8 days. After the final day, 2 ml of 3.7% formaldehyde solution was poured over the overlay medium and incubated for 3 hours at room temperature, and the wells then rinsed with water. Staining solution (1% NP - 40 in 1 x PBS) was used to stain each for 30 minutes at room temperature, after which the dye was discarded, plates rinsed with 1 x PBS and blocked using 100 ul block ace for 30 min. 100 ul of conjugate (500 x goat anti rabbit IgG, American Qualex) was added and incubated for 1 hr at. Substrate buffer (10 mg/ml DAB) was added and the plates incubated at room temperature for 5 to 10 min. Plates were washed once with 1 x PBS (-) and dried. Foci were counted for each set of duplicate wells and the percentage reduction calculated by comparing with the control virus (100% foci formation). More than 90% focus reduction was regarded as positive.

Data Analysis

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

III. Result

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

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

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

Amongst clinical manifestations, 11.11% of those with eye infection were seropositive, with the odds of seropositivity for being 1.226 (95% CI 0.67-2.24). Seroprevalence in people residing in Changamwe was

16.13%, with the odds of CHIKV seropositivity being 1.877 (95% CI 0.68 -5.16). These data are presented in Table 1 below.

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

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

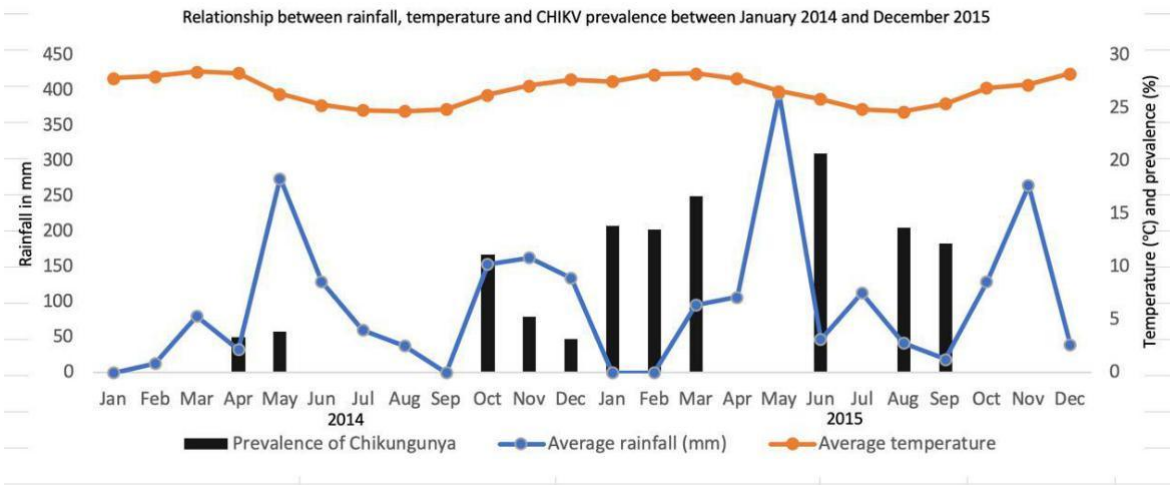

Table no 1: Seroprevalence (%) and odds ratios for presence of CHIKV-neutralizing antibodies.

Characteristic	Number (%)	Seropositive (%)	Seronegative (%)	OR	95 CI	*P value*
Overall	488 (100)	48 (9.8)	440 (90.6)			
Age groups						
0-6	45 (9.2)	3 (6.67)	42 (93.33)	Ref	-	-
7-18 years	62 (12.7)	7 (11.29)	55 (88.71)	1.78	0.43-7.39	0.42
18-35	217 (44.5)	14 (6.45)	203 (93.55)	0.97	0.27-3.52	0.96
36-81	148 (30.3)	19 (12.84)	129 (87.16)	2.06	0.58-7.37	0.26
Adults and children						
Children under 18 years	107 (21.9)	10 (9.35)	97 (90.65)	Ref	-	
Adults	365 (74.79)	33 (9.04)	332 (90.96)	0.96	0.46-2.03	0.92
Gender						
Male	269 (55.1)	25 (9.3)	244 (50)	Ref	-	-
Female	213 (43.6)	23 (10.8)	190 (38.9)	1.23	0.670-2.24	0.51
Vaccination status						
Incomplete	27 (5.5)	3 (11.11)	24 (88.89)	Ref	-	-
Complete	90 (18.4)	6 (6.67)	84 (93.33)	0.57	0.13-2.48	0.45
Not vaccinated	364 (74.6)	39 (10.71)	325 (89.29)	0.96	0.28-3.34	0.95
Occupation						
Driver	29 (5.9)	3 (10.34)	26 (89.66)	Ref	-	-
Student	128 (26.2)	8 (6.25)	120 (93.75)	0.58	0.14-2.34	0.44
Fisherman	9 (1.8)	1 (11.11)	8 (88.89)	1.08	0.10-12.31	0.95
Farmer	45 (9.2)	2 (4.44)	43 (95.56)	0.40	0.1-2.64	0.33
Teacher	35 (7.2)	3 (8.57)	32 (91.43)	0.81	0.15-4.43	0.81
Others	186 (38.1)	26 (13.98)	160 (86.02)	1.40	0.39-5.1	0.61
Clinical manifestations						
Swollen joints	2 (0.4)	0 (0)	2 (100)	Ref		
Chills	19 (3.9)	1 (5.26)	18 (94.74)	0.45	0.05-3.92	0.46
Jaundice	73 (16.0)	7 (9.59)	66 (90.41)	0.95	0.41-2.20	0.90
Headache	288 (59.0)	27 (9.38)	261 (90.63)	0.88	0.48-1.62	0.68
Rash	106 (21.95)	7 (6.60)	99 (93.4)	0.58	0.25-1.33	0.19
eye infection	27 (5.5)	3 (11.11)	24 (88.89)	1.14	0.33-3.95	0.83
Sub counties						
Changamwe	31 (6.4)	5 (16.13)	26 (83.87)	Ref	-	-
Likoni	23 (4.7)	3 (13.0)	20 (86.96)	0.78	0.16-3.72	0.75

Kaloleni	9 (1.8)	1 (11.1)	8 (88.89)	0.65	0.06-6.63	0.71
Kisauni	143 (29.3)	12 (8.39)	131 (91.61)	0.48	0.15-1.48	0.19
Mvita	68 (13.9)	7 (10.29)	61 (89.71)	0.60	0.17-2.08	0.41
Nyali	43 (8.8)	3 (6.98)	40 (93.02)	0.39	0.08-1.82	0.21
Others	166 (34.0)	16 (9.64)	150 (90.36)	0.46	0.15-1.42	0.17
Rainfall						
Low rainfall	488 (100)	38 (7.79)	450 (92.21)	Ref		
High rainfall	488 (100)	10 (2.05)	478 (97.95)	0.73	0.07-7.28	0.79
Temperature						
<26.8	488 (100)	16 (3.28)	472 (96.72)	Ref		
≥26.8	488 (100)	32 (6.56)	456 (93.44)	2.78	0.28-27.21	0.36

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

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

IV. Discussion

Previous studies conducted on CHIKV occurred during outbreaks and were focused on febrile patients [8]. The prevalence reported was 23.2%. The point prevalence of 9.83% determined in our study was lower.

Women had a higher prevalence and a higher likelihood of having CHIKV infection than men. This is consistent with previous reports from the Comoros and Reunion islands [9, 10] which indicate that women are more prone to CHIKV infection. The higher prevalence in women may relate to gender differences in exposure to infection due to community specific habits, customs and behavior; also, most of the women and the elderly people spend most of their time home during the day, while men are at home less than half of the time. As the mosquitoes that transmit Chikungunya do not travel far and often stay within the same households for days, women and the elderly are at high risk of contracting the virus.

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

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

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

V.Conclusion and Recommendations

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

VI. Limitations Of Study

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

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VII. Ethical Statement

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

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