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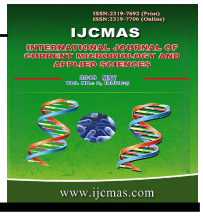

Original Research Article

Seroprevalence of Chikungunya Infection in Pyretic Children Seeking Treatment in Alupe District Hospital, Busia County Kenya

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A B S T R A C T

Chikungunya fever is a viral disease transmitted to humans majorly by a bite of infected *Aedes aegypti* mosquitoes. Studies have revealed that other species of *Aedes* mosquitoes equally transmit chikungunya virus (CHIKV). This study was conducted to determine the prevalence of CHIKV in febrile children seeking treatment in Alupe District Hospital, Busia Kenya. This was a hospital based cross-sectional study of 384 febrile children aged 1 to 12 years. A detailed clinical history and socio-demographic information of the study participants was taken by the clinician after signing a consent form. Whole blood of about 5 ml was collected in vacutainers, centrifuged to obtain serum for antibody detection. Enzyme-linked Immunosorbent Assay (ELISA) and Plaque Reduction Neutralisation Test (PRNT) tests were performed to detect the chikungunya antibodies. The median (Interquartile range) age (months) for the febrile children was 54 (30, 96) and majority 55.5% (213) were female. More than three quarters 76 .8% (295) were rural dwellers and 55% (211) attended school. Majority of the children 72.1% (277) were under the care of their mothers. The prevalence of CHIKV was 9.4% (36) and 11.5% (44) using ELISA and PRNT techniques, respectively. The wet month of September had the highest prevalence (40.9%) and febrile children from the rural areas were the most affected{79.5% (35/44)}. In the study population, the common symptoms and signs exhibited were malaise (33.1%), nausea (23.4%), stomachache (214%), headache (19.3%) and fever (100%), rash (40.6%) respectively. It is therefore recommended that if a patient is diagnosed with typhoid or other more prevalent infectious diseases, CHIKV should be tested for. Serology and molecular diagnosis should be used simultaneously for better case detection.

K e y w o r d s

Chikungunya fever; *Aedes aegypti*; febrile children; antibody detection; ELISA; Plaque Reduction Neutralisation.

Introduction

Chikungunya fever is an acute febrile illness caused by an arthropod-borne vector, Chikungunya virus in the genus alphavirus (CHIKV). The virus is primarily transmitted to humans via the

bite of an infected *Aedes* species mosquito. CHIKV was first recognized as a human pathogen during the 1950s in Africa, and since then, cases have been identified in many countries in Africa and Asia (Robinson, 1955; Jupp and McIntosh,1988). The diseases are manifested as mild febrile illness to severe polyarthritis and encephalitis (Kumar *et al.,* 2008).

Outbreak of CHIKV occurred in Lamu Island and Mombasa in Kenya in 2004 (Sergon, *et al*., and Kariuki, *et al.,* 2008) and the disease subsequently spread eastward, causing millions of disease cases throughout countries in and around the Indian Ocean (Powers and Logue,2007; Sergon etal 2007; WHO, 2007, Kariuki, *et al.,* 2008). The epidemics resulted in significant morbidity and taxed the health care and public health infrastructure in these regions. By 2007, CHIKV was imported into Europe, causing an outbreak of chikungunya fever in Italy (Rezza et al., 2007). This outbreak suggested for the first time the significant potential of the virus to move to novel ecological niches, including Europe, Australia, and the Western Hemisphere through returning travelers (Pialoux, *et al.,* 2007; Epstein, 2007; Enserink 2008; Simon, *et al*, 2008).

Western Kenya is a malaria endemic area and mosquito vectors for both malaria and CHIKV transmissions are prevalent (Korenromp. *et al*., 2003). Although 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 go undiagnosed and as such its prevalence is likely underestimated. This study was therefore designed to estimate the prevalence of Chikungunya fever in patients presenting fever at a health facility located near a transit point on the Kenya-Uganda border.

Materials and Methods

Study Site

The study was conducted in Alupe, Busia, Kenya. The study site consisted of two health facilities; Alupe District hospital and Kenya Medical Research Institute (KEMRI)-Centre for Infectious and Parasitic Disease Control Research (CIPDCR). The site is located within Busia County which falls within the Lake Victoria basin. The altitude varies from 1130m to 1375 m above sea level. The study area experiences bimodal rainfall pattern with an annual rainfall of 1200-1800mm. The long rain starts in March and continues to May/June while the short rain season starts in August and ends in October. This rainfall pattern supports two crop-growing seasons and is suitable for mosquito breeding. Adjacent to the site are two border crossing points into Uganda serving traders not only from neighbouring Uganda but also Rwanda and Democratic Republic of Congo.

The two health facilities serve Busia county which according to the National census conducted in Kenya in 2009, It has a total population of 488,075 out of which 232, 075 were male and 256,000 female (m:f=1:1), (Kenya National Bureau of Statistics, Kenya, 2009).

Study Design

This study utilized a hospital-based cross-sectional design. Serum samples were collected from febrile children aged 1 to 12 years with symptoms or clinical features suggestive of CHIKV infection; these symptoms included fever, headache, myalgia, joint pain with or without

swelling, and the presence or absence of rash on the body. An acute case of CHIKV infection was defined as any case with clinical features consistent with Chikungunya fever and in which CHIKV infection was confirmed either by reverse transcriptase PCR or by real-time PCR or virus isolation.

Study Population

The study comprised of both rural and urban populations of febrile children (1 to 12 years old) who sought treatment at Alupe District Hospital, Kenya from January to December 2010. Criteria for inclusion in the study were: those Children whose parents or guardians gave consent to be included in the study, patients aged one year and above [to avoid maternal antibodies], all children who had clinical presentations suggestive of CHIKV infection and both sexes of patients

Sample Collection, Storage, Transportation and Processing

Upon signing of the consent forms by the clients, detailed history was taken to obtain information on socio-demographic and clinical manifestations. The clients underwent phlebotomy using standard precautions in the laboratory where 5 ml of whole blood was obtained in yellow capped vacutainer tubes. The blood sample was centrifuged for 5 minutes at 5000 r.p.m and in liquid nitrogen before being transported to KEMRI, CIPDCR where it was stored at -80^{o}C until laboratory screening for CHIKV was performed.

Laboratory Sample Analysis for CHIKV

Indirect ELISA screening test was performed according to the method described by Igarashi (2000) at KEMRI-CIPDCR. The plates were then read on an ELISA plate reader (Thermoscientific Multiskan ex. Version, Tokyo, Japan) at a wavelength of A492nm (Ascent software version 2.6-Deafulte.See, Shangai, China). The Optical Densities (OD) of positive-to-negative (P/N) ratios of >1.0 was considered positive, <0.5 was considered negative and ≥0.5 was considered borderlines. Presence of specific anti-CHIKV antibodies was confirmed by PRNT as described below.

Plaque Reduction Virus Neutralization Tests (PRNT)

A plaque reduction neutralization test was used to confirm the presence of specific neutralizing antibodies to the CHIKV in patient sera already regarded as positive or borderline by ELISA. Vero cells at a concentration 200,000 cells/ml were seeded into 6-well plates (Nunc) at a volume of 2 ml/well. Cells were cultured in Growth Medium for 1 day at 37ºC, 5% CO_2. Sera was diluted with Maintenance Medium (EMEM, 2% FCS, Penicillin/Streptomycin supplemented) in 1:20, 1:40 and 1:80 dilutions and then mixed with equal volume of standard virus solution (2000 PFU/ml). The virus-serum mixture was incubated for 1 hour at 37ºC. 100 µl/well of mixture was added to a mono layer of 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, 1.5% FCS, 1.2% Methylcellulose, penicillin/streptomycin supplemented) was added into each well, and the plates incubated at 37ºC, 5% CO_2 for 4-8 days. After the final day, 2 ml of 4% paraformaldehyde in PBS was poured over the overlay medium and incubated for 2 hours at room temperature, and the

wells then rinsed with water. Staining solution (0.5 ml of 1% Crystal Violet solution in water) was used to stain each for 20 minutes at room temperature, after which the dye was discarded, plates rinsed with water and air-dried at room temperature. Plaques were counted for each set of duplicate wells and the percentage reduction calculated by comparing with the control virus (100% plaque formation). The PRNT was determined by highest dilution that resulted in <50% of input plaque count.

Data presentation and analysis

Clinical and laboratory data was maintained as excel databases. Data was processed by a microcomputer using Genstat 4th edition. All qualitative data was summarized using frequency tables and charts.

Ethics statement

Approval for the study was obtained from the Ethical Review Committee at KEMRI (SSC PROTOCOL No. 2109-3RD REVISION) through the Centre of Infectious and Parasitic Control Centre Research, Alupe Busia, Kenya.

Results

Demographic Characteristics

Three hundred and eighty four patients aged between 1 and 12 years were recruited for the study. Of these patients, 55.5% (213) were females, 76.8% (295) of them came rural areas of the county and 55% (211) attending school. The majority (72.1%, 277) of the patients were under the care of their mothers whose median (IQR) age was 30 (24, 35) and 26% (100) had incomplete primary education (Figure 1).

Figure 2 shows the marital status of the care takers of the children recruited in the study. Majority of the primary care givers 85.2% were married, 8.6% never married, 1.6% separated and 4.7% widowed. In this study most of the primary care givers 29.9% (115) were unemployed (Figure 3).

Symptoms and clinical Signs in patients

Among the symptoms and signs presented by the recruited children, 33.1% (127) had malaise and 23.4% (90) had nausea while 100% (384) had fever, 40.6% (156) rash, among others as indicated in Figure 4 and 5 respectively. Majority of the symptoms and signs of Chikungunya infection are similar to those of malaria and typhoid which are routinely tested for in the laboratory. It is as a result of these manifestations that led to the screening for CHIKV using ELISA and PRNT techniques alongside malaria and typhoid to understand the exact cause of the illness.

Seroprevalence of Chikungunya virus in the study population

ELISA was used to give preliminary results by detecting CHIKV antibodies and samples that were regarded as border and positive were confirmed using PRNT. Borderlines were samples which could not be regarded as neither negative nor positive hence needed a more sensitive test to firmly conclude the results.

Figure.1 Education Level of the primary care givers who brought their febrile children for treatment in Alupe District hospital

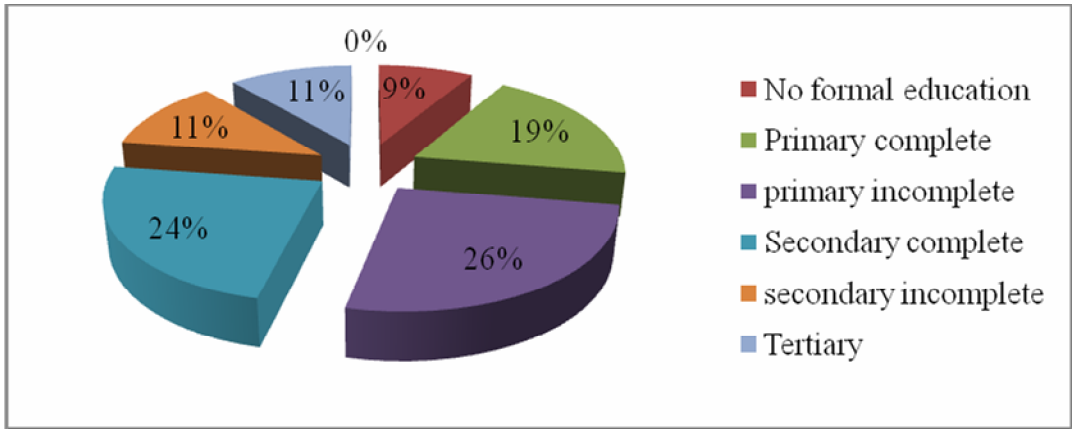


Figure.2 Marital status of the primary care givers who brought their febrile children for treatment in Alupe District hospital

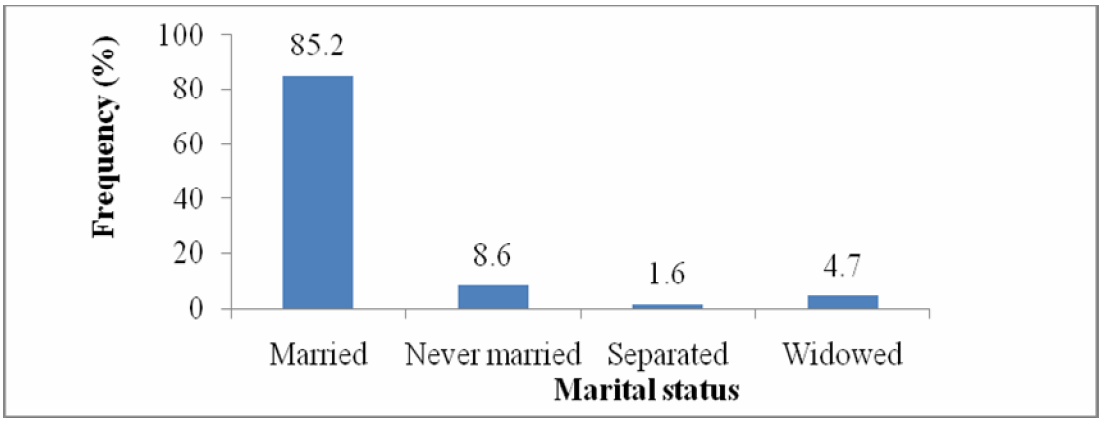


Figure.3 Occupation of the primary care givers who brought their febrile children for treatment in Alupe District hospital

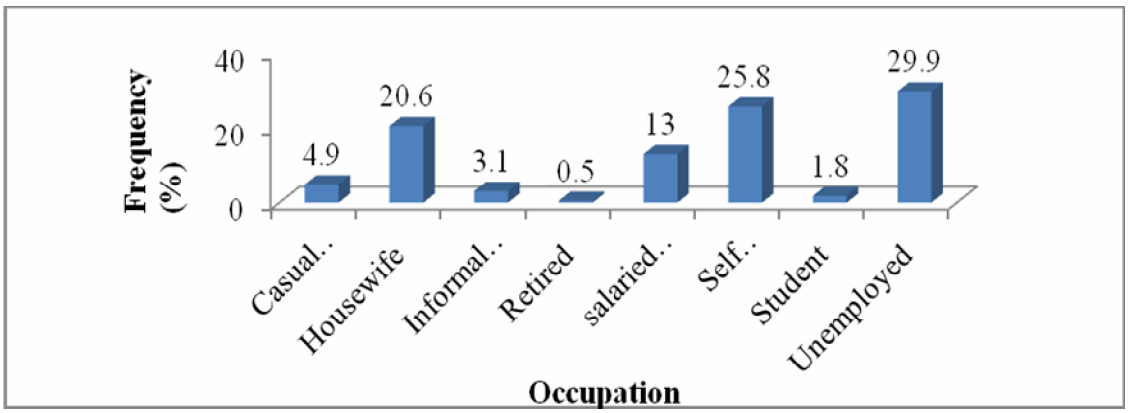


Figure.4 Symptoms amongst the febrile children seeking treatment in Alupe District hospital

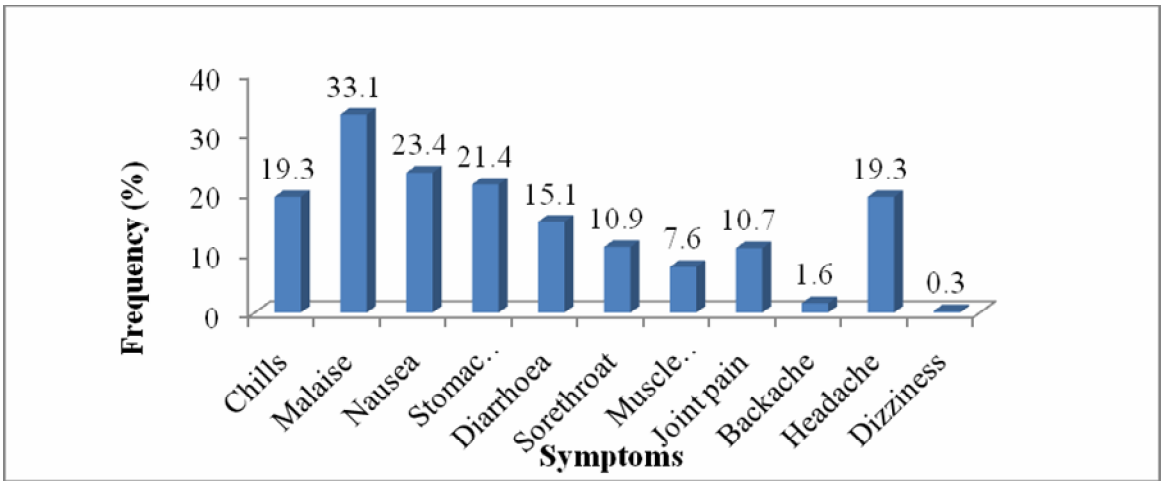


Figure.5 Clinical Signs amongst febrile children seeking treatment in Alupe District hospital

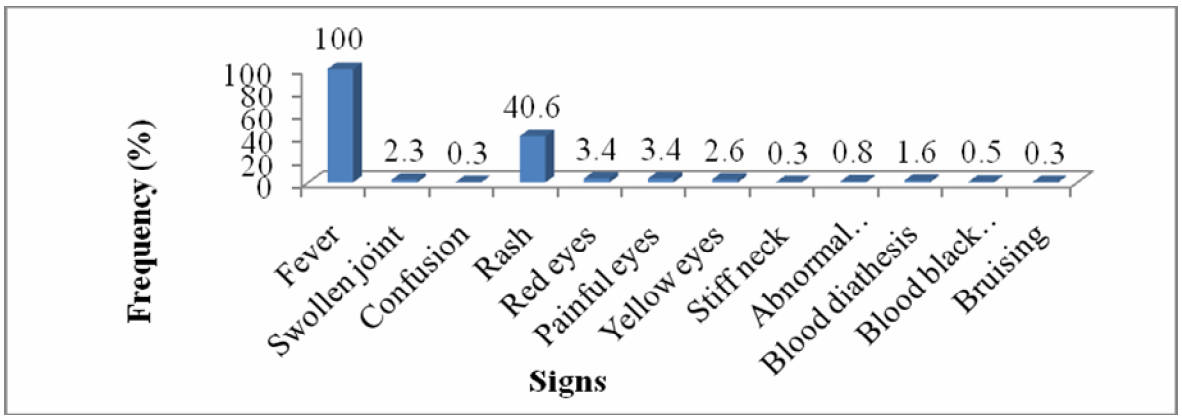
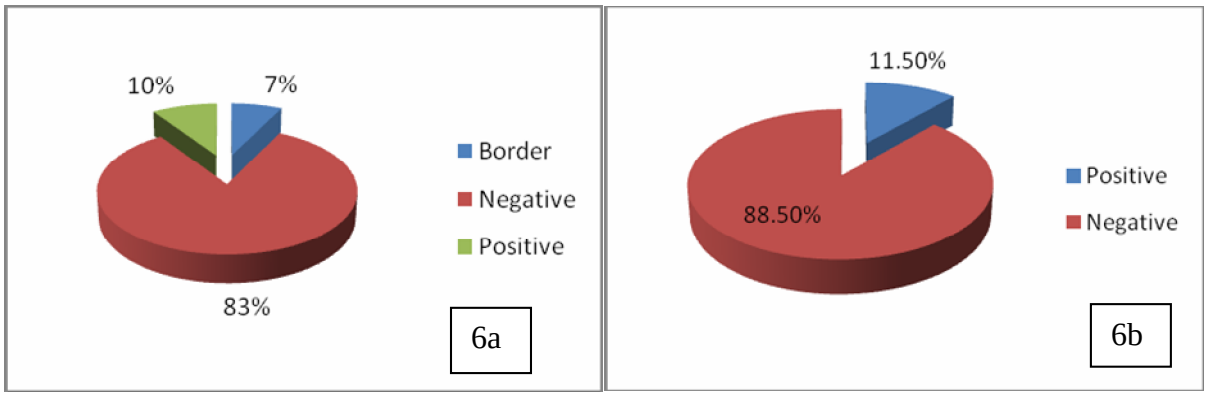


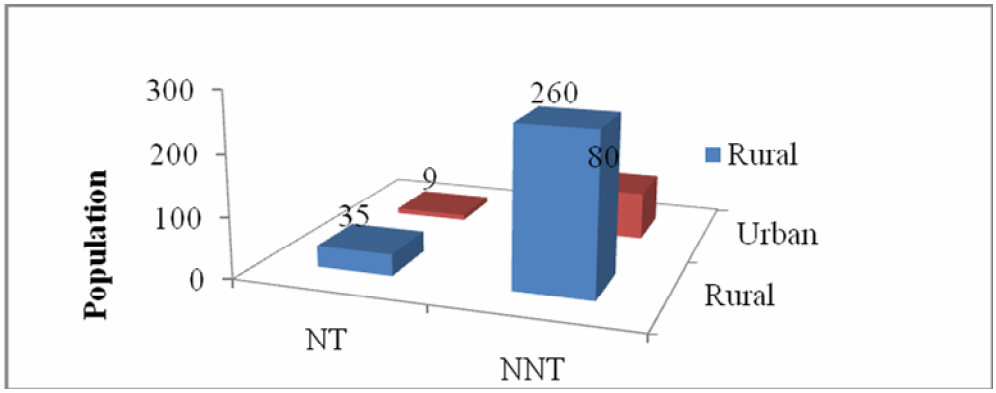
Figure. 6 Serostatus of Chikungunya infection using (a) Indirect Enzyme-linked Immunosorbent Assay and (b)Plaque Reduction Neutralisation Test amongst the febrile Children seeking treatment in Alupe District Hospital



ELISA and PRNT

Antibodies to CHIKV were detected in 36 out of 384 febrile children using ELISA indicating a prevalence of 9.4%. With ELISA, 28 cases were considered borderline (Figure6a). These were subjected to PRNT test and 11 cases were confirmed positive. Combining the ELISA and PRNT positive cases revealed a prevalence of 11.5% (Figure 6b). Out of 295 febrile children from the rural, 35 had Chikungunya antibodies while 89 who were from the urban areas, 9 had chikungunya antibodies detected as shown in Figure 7.

Figure.7 Comparison of Chikungunya serostatus among febrile children in the Rural and Urban populations seeking treatment in Alupe District hospital



NT: Neutralisation ,NNT: No Neutralisation

Figure 8 shows those febrile children who were confirmed for CHIKV infection at the Alupe District hospital in the year. The prevalence of chikungunya infection ranged between 2.3 to 40.9. The month of September had the highest prevalence of CHIKV infection (40.9%), followed by October 22.7%, July and November both had a prevalenceof 9.1% while August had an incidence of 4.5%. The lowest months were January, February, April, May, June and December which had an average of 2.3% while March recorded no CHIKV infections.

Discussion

This study was undertaken to establish the prevalence of CHIKV among pyretic children seeking treatment in Alupe District hospital, Busia Kenya using ELISA and PRNT techniques. The point prevalence of Chikungunya infection was 9.4% and 11.5% using ELISA and PRNT respectively, in this cohort of children seeking treatment. This was because of the difference in specificity and sensitivity of the techniques employed. The PRNT yields more definitive results and is a reliable confirmatory method, although more time demanding, while ELISA is a rapid pre-screening procedure in serosurvey programs (Castillo-Olivares and Wood, 2004).

The prevalence rate of CHIKV infections in the present study (11.5%) was lower than in previous studies carried out in Kenya (Lamu), which reported a prevalence rate of 75% (Sergon, *et al.*, 2008), and 63% in the Union of Comoros (Sergon, *et al.*, 2007). The difference in prevalence rate could be attributed to

recruitment of patients aged 1 to 12 years in the present study while previous studies in Lamu Island, survey participants were randomly chosen irrespective of their age excluding the infants only (< 1 year old); few study sites and limited study period. Furthermore, the current study was conducted for a period of one year from January to December 2010 while the study at Lamu Island was carried out for five days (5-9) in October 2004 although it was a response to an outbreak of unknown febrile illness. In addition, the lower prevalence rate could be the effect of concurrent mosquito net campaigns in both pregnant mothers and infants which may have reduced exposure to mosquito bites in young populations.

The month of September had the highest prevalence and febrile children from the rural areas were the most affected. This may be explained by the geographical and ecological factors of the study site which lies in the Lake Victoria basin hence have high humidity and rainfall which will in turn support the breeding of mosquitoes.

Chikungunya infection was observed to occur more among the rural population (35 / 44) compared to the urban population (9 / 44) areas. This is in contrast with the findings of Mahadev. *et al*.,(2004) who found out that dengue and CHIKV are prevalent in both rural and urban areas., Mosquitoes can bite children while playing near forested areas or or sleeping without a mosquito net. Human activities that increase children contact with forest mosquitoes such as herding cattle, fetching firewood, clearing forests for agricultural activities farming practices in the forest increases the risk of exposure, (Kumar *et al.,* 2008).

Among the symptoms and signs presented by the recruited children, 33.1% (127) had malaise, 23.4% (90) nausea, 21.4% (82) stomachache, 19.3% (74) headache and 100% (384) had fever, 40.6% (156) rash, among others clinical presentations. This result is in agreement with findings in other previous Chikungunya fever studies which have reported; fever (89%–100%), joint pain (96%–100%) of of the cases, (Rezza. *et al*., 2007; Borgherini. *et al.*, 2007*;* Lakshmi. *et al.,* 2008) while joint swelling was less frequently reported, at between 32% and 40% (Borgherini. *et al.*, 2007*;* Lakshmi. *et al.,* 2008). Headache was found in 31%–51% of the cases, while rashes in 28%– 52%, (Rezza. *et al*., 2007; Borgherini. *et al.,* 2007*;* Lakshmi. *et al.*, 2008). The point prevalence of Chikungunya infection amongst pyretic children aged 1-12 years seeking treatment in Alupe District hospital in Busia County was 11.5%. This prevalence suggests that the condition is endemic in the study area and goes undetected is likely misdiagnosed as malaria.

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