European Journal of Applied Sciences – Vol. 11, No. 5
Publication Date: October 25, 2023
DOI:10.14738/aivp.115.15370
Mwongula, A. W., Siamba, D. N., Mwamburi, L. A., & Mailu, M. (2023). Concurrent Infections Among Pyretic Children Seeking Treatment at Alupe Sub County Hospital, Busia County, Kenya. European Journal of Applied Sciences, Vol - 11(5). 38-45.
SERVICES FOR SCIENCE AND EDUCATION

Concurrent Infections Among Pyretic Children Seeking Treatment at Alupe Sub County Hospital, Busia County, Kenya

Albert W. Mwongula
Department of Biological and Agricultural Sciences, School of Science, Technology and Engineering (SSTE), Alupe University P.O. Box 845, 50400, Busia, Kenya

Donald N. Siamba
Department of Biological and Environmental Sciences, Faculty of Science, Kibabii University, P.O. Box 1699 – 50200, Bungoma, Kenya

Lizzy A. Mwamburi
Department of Biological Sciences, School of Science, University of Eldoret, P.O. Box 1125 – 30100, Eldoret, Kenya

Matilu Mailu
Centre for Infectious Parasitic Diseases Control Research, Kenya Medical Research Institute, P.O Box 3 – 50400, Busia, Kenya

ABSTRACT

Fever is a frequently occurring medical symptom and may result from many divergent conditions ranging from mild to potentially serious. Children typically get high and fast – occurring fevers, reflecting the effects of the pyrogens upon an inexperienced immune system. Symptoms and signs of Chikungunya virus infections are quite similar to those of malaria and typhoid fever. Malaria and typhoid investigations are routinely carried out to establish the cause of pyrexia of unknown origin and treatment follows with complete neglect of Chikungunya virus infections. Thus, Chikungunya virus fever cases can sometimes be misdiagnosed or occur simultaneously with malaria, typhoid fever or both. This study was conducted to determine the concurrent infections of malaria and/or typhoid fever with Chikungunya virus, among febrile children aged 1 – 12 years seeking treatment in Alupe Sub County Hospital, Busia, Kenya. Blood smears were prepared for detection of malarial parasites and serum sample for widal test to detect typhoid fever. Enzyme-linked Immunosorbent Assay and Plaque Reduction Neutralisation Test were performed to detect the Chikungunya virus antibodies. The median age/interquartile range age for the febrile children was 4.5 years and 55.5% were female. Concurrent infections of Chikungunya virus with malaria or typhoid were 9.6% and 7%, respectively, using the Enzyme-linked Immunosorbent Assay technique and 10.5% and 9.9%, respectively, using Plaque Reduction Neutralisation Test. This supports the recommendation that Chikungunya virus should be tested for using both serological and molecular diagnostics in cases of patients presenting with fever.

Keywords: Malaria, Chikungunya virus and Typhoid fever

INTRODUCTION

Fever is a non – specific physiological mechanism of the immune system's attempt to gain advantage over infectious agents, such as viruses and bacteria. A fever makes the body less favorable as a host for replicating viruses and bacteria, which are temperature sensitive. Children typically get higher and fast – occurring fevers, reflecting the effects of the pyrogens upon an inexperienced immune system (1). Besides malaria and typhoid fever as the diseases which are associated with fever, Chikungunya virus according to sero-survey studies is an endemic disease in Africa that cause fever.

Previous studies reveal that in malaria holoendemic regions; such as Western Region in Kenya where Alupe is located, mosquitoes of *Aedes* species are found in large populations (2) The species of mosquitoes that transmit malaria in the Western province are competent vectors for Chikungunya virus transmission indicating a possibility of the virus circulating in Western Province.

Chikungunya infections go undiagnosed or delayed diagnosis at the health centre level due to lack of diagnostic tests and the children are usually treated with antimalarial drugs or antibiotics empirically. Clinical and epidemiological similarities with dengue fever, malaria and typhoid fever make Chikungunya diagnosis challenging (3), which may lead physicians to misdiagnose Chikungunya as dengue fever, malaria or typhoid (3); therefore, the incidence of Chikungunya occurrence may actually be higher than currently reported (3-5).

In addition, local transmission of Chikungunya into previously nonendemic areas by travelers with viremia has been reported in tropical or subtropical areas of Africa (6). Chikungunya has been responsible for significant human morbidity for several hundred years; yet in spite of its prevalence; there are few studies which have looked into the epidemiology, mechanisms of virulence and pathogenesis of Chikungunya especially in regions endemic for the potential vectors (7).

Early recognition of local transmission followed by prompt, aggressive vector control and other public health measures might prevent long-term establishment of the virus in new areas (5). So far, no studies have been conducted to establish the prevalence and incidences of Chikungunya in high-risk areas of Western Kenya which borders Uganda and served by Busia and Malaba border crossing points.

METHODS

Study Site

The study was conducted in Alupe, Busia County, Kenya. This research was conducted in two health facilities; Alupe Sub County Hospital and the Centre for Infectious and Parasitic Disease Control Research (CIPDCR), Kenya Medical Research Institute (KEMRI).

Busia County has two border crossing points into Uganda – Busia and Malaba towns. The main economic activity is trade with neighbouring Uganda which is extended to Rwanda and Democratic Republic of Congo, with Busia and Malaba towns being the cross-border centers.

According to the National census conducted in Kenya in 2009, Busia County had a total population of 488,075 out of which 232, 075 were male and 256,000 female (m:f=1:1), (8).

Ethics Statement

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

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 Chikungunya infection; these symptoms include fever, headache, myalgia, joint pain with or without swelling, and the presence or absence of a rash on the body.

Study Population

The study comprised of both rural and urban populations of febrile children who sought treatment at Alupe Sub County Hospital 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 Chikungunya infection and both sexes of patients. Criteria for exclusion were: children who had no fever, children whose parent or guardians declined to consent or patients aged 13 years and above.

Sample Collection, Storage, Transportation and Processing

Upon signing of the consent forms by the client's, detailed history was taken to obtain information on socio-demographic and clinical manifestations by the study clinicians. 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 stored kept in liquid nitrogen before being transported to KEMRI, CIPDCR where it was stored at -80^{o}C until used.

Laboratory Sample Analysis of Chikungunya Virus

The study adopted an Indirect Enzyme Linked Immunosorbent Assay protocol as described by Mwongula. *et al*., (9). Presence of specific anti-Chikungunya antibodies was confirmed by PRNT done at Centre for Microbiology Research, KEMRI in Nairobi. The IgG, IgM, and IgA were measured for the determination of the sero-prevalence of Chikungunya in febrile children visiting the Alupe Sub County Hospital.

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. All sera that were positive and borderlines for Chikungunya indirect ELISA were further confirmed using the Plaque Reduction Neutralization Test (PRNT).

Plaque Reduction Virus Neutralization Tests (PRNT)

A plaque reduction neutralization test was used to confirm the presence of specific neutralizing antibodies to the Chikungunya in patient sera already regarded as positive or border by ELISA. The study adopted a PRNT protocol as described by Mwongula. *et al*., **(9).**

The PRNT was determined by linear regression (probit) analysis using either probit paper or a computer software program, or determine highest dilution that results in <50% of input plaque count.

Laboratory Sample Analysis of Endemic Diseases That Cause Fever

Giemsa Staining of Thick Blood Smear for Malaria Microscopy:

Thick film was dried completely and 10% Giemsa stain gently poured on the slide using a pipette on the staining rack for 15 minutes. The stain was gently flushed off the slide with a clean tap water and the slide placed on the drying rack. The slide was observed under oil immersion for malarial trophozoite parasites.

Widal Test:

A clean glass marked with circles was labeled “H” and "O”. One drop of undiluted serum was placed in the two circles with the help of a sterile pasture pipette.

One drop of H antigen was added to the first circle and one drop of O antigen was added in the second circle. With separate applicator sticks, serum and the antigen were mixed together and spread well to fill the whole of the individual circle. The slides were observed for agglutination.

Data Presentation and Analysis

All data was recorded in purpose designed questionnaires. 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. Difference in the prevalence of Chikungunya, malaria and typhoid were analyzed using Pearson Chi-square test. The results were assumed to be significant when $P \leq 0.05$.

RESULTS

Demographic Characteristics

Three hundred and eighty-four patients aged between 1 and 12 years were recruited for the study. The characteristics of the study population were as described by Mwongula. *et al*., 2013. (9)

Misdiagnosis and Concurrent Infections

Symptoms and signs of Chikungunya infections are quite similar to those of other febrile illnesses such as malaria and typhoid fever. Malaria and typhoid investigations are routinely carried out to establish the cause of pyrexia of unknown origin (P.U.O) and treatment follows with complete neglect of for Chikungunya infection. Chikungunya fever cases can sometimes be misdiagnosed or occur simultaneously with malaria or typhoid fever.

Out of the 270 children that tested negative for malaria, 9.3% (25) tested positive for Chikungunya. Among 114 children that tested positive for malaria, 9.6% (11) were positive for

Chikungunya as shown in Table 1. The P value is greater than 0.05 and therefore there is no significant relationship between Chikungunya and malaria.

Table 1: Proportion of Children with Chikungunya using ELISA and Malaria amongst the febrile children seeking treatment at Alupe Sub County Hospital from January to December 2010

Malaria test results	Chikungunya		
	Border	Negative	Positive
Negative	20 (7.4%)	225 (83.3%)	25 (9.3%)
Positive	8 (7%)	95 (83.3%)	11 (9.6%)
NNT: No Neutralisation and NT: Neutralisation.			
$\chi^2 = 0.00$ DF=1 P=1.000			

Among the 70 children that tested negative for typhoid, 20% (14) tested positive for Chikungunya (Table 2). Out of 314 children that tested positive for typhoid, 7% (22) tested positive for Chikungunya virus. The P value is less than 0.05 and therefore there is a significant relationship between typhoid and Chikungunya virus.

Table 2: Proportion of Children with Chikungunya using ELISA and Typhoid amongst the febrile children seeking treatment at Alupe Sub County Hospital from January to December 2010

Typhoid test results	Chikungunya		
	Border	Negative	Positive
Negative	8 (11.4%)	48 (68.6%)	14 (20%)
Positive	20 (6.4%)	272 (86.6%)	22 (7%)
NNT: No Neutralisation and NT: Neutralisation.			
$\chi^2 = 13.43$ DF=1 P<0.001			

Out of 270 children who tested negative for malaria, 11.9% (32) tested negative for Chikungunya. Among 114 children who tested positive for malaria, 10.5% (12) tested positive for Chikungunya as shown in Table 3. The P value is greater than 0.05 and therefore there is no significant relationship between Chikungunya virus and malaria.

Table 3: Proportion of Children with Chikungunya using PRNT and Malaria amongst the febrile children seeking treatment at Alupe Sub County Hospital from January to December 2010

Malaria test results	Chikungunya	
	NNT	NT
Negative	238 (88.1%)	32 (11.9%)
Positive	102 (89.5%)	12 (10.5%)
NNT: No Neutralisation and NT: Neutralisation.		
$\chi^2 = 0.14$ DF=1 P=0.709		

Out of 70 children who tested negative for typhoid, 18.6% (13) tested negative for Chikungunya. Among 314 children who tested positive for typhoid, 9.9% (31) tested positive

for Chikungunya as shown in Table 4. The P value is less than 0.05 and therefore there is a significant relationship between typhoid and Chikungunya virus.

Table 4: Proportion of Children with Chikungunya using PRNT and Typhoid amongst the febrile children seeking treatment at Alupe Sub County Hospital from January to December 2010

Typhoid test results	Chikungunya	
	NNT	NT
Negative	57 (81.4%)	13 (18.6%)
Positive	283 (90.1%)	31 (9.9%)
NNT: No Neutralisation and NT: Neutralisation.		
$\chi^2 = 4.27$ DF=1 P=0.039		

DISCUSSION

Misdiagnosis of Chikungunya Virus infection as Malaria and Typhoid or Both

In the present study, 9.3% (25 out of 270) of children who tested negative for malaria were reported to have Chikungunya and 20% (14 out of 70) of children who tested negative for typhoid fever had Chikungunya using the ELISA technique. In addition, 11.9% (32 out of 270) of children who tested negative for malaria had Chikungunya and 18.6% (13 out of 70) of children who tested negative for typhoid fever were Chikungunya positive using PRNT technique.

In 2004, Kenya experienced two outbreaks of Chikungunya fever. The first outbreak in Lamu resulted in an estimated 13,500 cases, which represents 75% of the population of the island (**10**). The outbreak was initially thought to be due to malaria, but eventually, laboratory screening determined that Chikungunya was the cause.

Symptoms and signs associated with Chikungunya infection such as fever, joint pains, and myalgias are non-specific that makes clinical diagnosis difficult because they are indistinguishable from other febrile diseases such as dengue, Rift Valley fever, malaria and typhoid. However, pronounced persistent severe joint pains that affect wrists, elbows, fingers, and knees in some patients should raise the suspicion of alphavirus infection, especially Chikungunya disease or O'nyong nyong fever, which also occurred in epidemic form in East Africa in the late 1990s (11-13).

Concurrent infection of Chikungunya Virus infection with Malaria or Typhoid or Both

In the current study, 9.6% (11 out of 114) of children who were positive for malaria had Chikungunya and 7% (22 of 314) children who tested positive for typhoid fever had Chikungunya using the ELISA technique. In addition, 10.5% (12 out of 114) of children that tested positive for malaria had Chikungunya and 9.9% (31 out of 314) of children who tested positive for typhoid fever had Chikungunya using PRNT technique.

There was no significant difference between Chikungunya infection and malaria. Chikungunya infection was positively correlated to typhoid. These may be explained by the fact that a disease could exist independently while in other conditions a disease may exist in association with other diseases. Death caused by Chikungunya infections appears to be rare. However, increases

in crude death rates have been reported during the 2004–2008 epidemics (14 - 16). Chikungunya infections, individuals with underlying medical conditions and individuals with co-infections appear to be more likely to suffer complications and to have a higher risk of death (17 -19).

In Kenya, febrile illnesses have been restricted to malaria and typhoid investigations and treatment normally done with complete neglect of Chikungunya due to lack of cost-effective diagnostics at the point of care.

Chikungunya infection was positively correlated with typhoid fever ($P<0.05$) and but negatively correlated with malaria ($P>0.05$).

Chikungunya infections were misdiagnosed with the common febrile illnesses; 25/36 cases as malaria and 14/36 cases as typhoid and 32/44 cases as malaria and 13/44 cases as typhoid using PRNT and ELISA, respectively. Concurrent infection of Chikungunya with malaria or typhoid was 9.6% and 7% using the ELISA technique and 10.5% and 9.9% using PRNT technique, respectively. A significant number of patients had co-infection with typhoid (ELISA; $P=0.001$ and PRNT; $P=0.039$).

Considerable number of patients had co-infection with malaria and typhoid hence even if patient is diagnosed with other more prevalent infections, Chikungunya should be tested for. The differential diagnosis of Chikungunya includes infections of other alphaviruses that cause the fever such as Sindibis virus, O'nyong nyong virus, Ross River. In addition, Chikungunya should be differentiated from dengue, malaria, typhoid fever or other more prevalent infectious diseases for proper treatment and management. Serology and molecular diagnosis should be used simultaneously for better case detection.

ACKNOWLEDGEMENT

The authors wish to express their gratitude to Kenya Medical Research Institute (KEMRI) – Busia, Kenya in collaboration with Nagasaki University, Japan for sponsoring this project. Also, technical assistance of Mr. T. Mokaya, Mrs. O. Makwaga, Carolyne Kirwaye, Najma Salim, Janet Awando, Lucy Okubi, Angella Mutuku and F. Adung'o is highly appreciated.

References

1. Axelrod YK, Diringer MN. Temperature management in acute neurologic disorders. Clinical neurology. 2008 May 1; 26(2):585-603.

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

3. Queyriaux B, Simon F, Grandadam M, Michel R, Tolou H, Boutin JP. Clinical burden of chikungunya virus infection. The Lancet infectious diseases. 2008; 1(8):2-3.

4. Sourisseau M, Schilte C, Casartelli N, Trouillet C, Guivel-Benhassine F, Rudnicka D, Sol-Foulon N, Roux KL, Prevost MC, Fsihi H, Frenkiel MP. Characterization of reemerging chikungunya virus. PLoS pathogens. 2007 Jun;3(6):e89.

5. Jain M, Rai S, Chakravarti A. Chikungunya: a review. Tropical doctor. 2008 Apr; 38(2):70-72.

6. Enserink M. A mosquito goes global (2008). Science; 320: 864-866.

7. Pialoux G, Gaüzère BA, Jauréguiberry S, Strobel M. Chikungunya, an epidemic arbovirosis. The Lancet infectious diseases. 2007 May 1; 7(5):319-327.

8. KNBS. Kenya Population and Housing Census 2009.

9. Mwongula AW, Mwamburi LA, Matilu M, Siamba DN, Wanyama FW. Seroprevalence of chikungunya infection in pyretic children seeking treatment in Alupe Sub County Hospital, Busia County Kenya. International Journal of Current Microbiology and Applied Sciences; 2013; 2(5), 133-134.

10. Sergon K, Njuguna C, Kalani R, Ofula V, Onyango C, Konongoi LS, Bedno S, Burke H, Dumilla AM, Konde J, Njenga MK. Seroprevalence of chikungunya virus (CHIKV) infection on Lamu Island, Kenya, October 2004. The American journal of tropical medicine and hygiene. 2008 Feb 1;78(2):333-337.

11. Sanders EJ, Rwaguma EB, Kawamata J, Kiwanuka N, Lutwama JJ, Ssengooba FP, Lamunu M, Najjemba R, Were WA, Bagambisa G, Campbell GL. O'nyong-nyong fever in south-central Uganda, 1996–1997: description of the epidemic and results of a household-based seroprevalence survey. The Journal of infectious diseases. 1999 Nov 1;180(5):1436-1443.

12. Jeandel P, Josse R, Durand JP. Exotic viral arthritis: role of alphavirus. Medecine tropicale: revue du Corps de sante colonial. 2004 Jan 1;64(1):81-88.

13. Tesh RB. Arthritides caused by mosquito-borne viruses. Annual review of medicine. 1982 Feb;33(1):31-40.

14. Higgs S. The 2005-2006 chikungunya epidemic in the Indian Ocean. Vector-Borne & Zoonotic Diseases. 2006 Jun 1;6(2):115-116.

15. Beesoon S, Funkhouser E, Kotea N, Spielman A, Robich RM. Chikungunya fever, Mauritius, 2006. Emerging Infectious Diseases. 2008 Feb; 14(2):337-338.

16. Mavalankar D, Shastri P, Bandyopadhyay T, Parmar J, Ramani KV. Increased mortality rate associated with chikungunya epidemic, Ahmedabad, India. Emerging infectious diseases. 2008 Mar;14(3):412-415.

17. Lemant J, Boisson V, Winer A, Thibault L, André H, Tixier F, Lemercier M, Antok E, Cresta MP, Grivard P, Besnard M. Serious acute chikungunya virus infection requiring intensive care during the Reunion Island outbreak in 2005–2006. Critical care medicine. 2008 Sep 1;36(9):2536-2541.

18. Economopoulou, A., Dominguez, M., Helynck, B., Sissoko, D., Wichmann, O., Quenel, P. & Quatresous, I. (2009). Atypical Chikungunya virus infections: clinical manifestations, mortality and risk factors for severe disease during the 2005–2006 outbreak on Reunion. *Epidemiology & Infection*, *137*(4), 534-541.

19. Renault P, Solet JL, Sissoko D, Balleydier E, Larrieu S, Filleul L, Lassalle C, Thiria J, Rachou E, de Valk H, Ilef D. A major epidemic of chikungunya virus infection on Reunion Island, France, 2005-2006. The American journal of tropical medicine and hygiene. 2007 Oct 1;77(4):727-731.