East African Medical Journal Vol: 94 No. 8 August 2017
IGG-ANTIBODY SEROPREVALENCE OF WEST NILE VIRUS AMONG BLOOD DONORS IN NAIROBI AND NAKURU REGIONAL BLOOD TRANSFUSION TESTING CENTERS IN KENYA
C.J Soi, Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62,000 – 00200 Nairobi, Kenya, S. Kibet, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya, A. Mbugua, Kenyatta National Hospital/University of Nairobi, P. Maturi, Lu Hiyan, Futan University of Shanghai China

IGG-ANTIBODY SEROPREVALENCE OF WEST NILE VIRUS AMONG BLOOD DONORS IN NAIROBI AND NAKURU REGIONAL BLOOD TRANSFUSION TESTING CENTERS IN KENYA

C.J. SOI, S. KIBET, A. MBUGUA, P. MATURI and L. HIYAN

ABSTRACT

Background: **West Nile Virus (WNV) is an arbovirus transmitted by infected mosquitoes which cause most of its incidence (CDC, 2015). It is transmitted by the culex mosquito which is prevalent in Kenya.**
Objective: **To determine and compare the sero prevalence of WNV among blood donors in Nairobi and Nakuru Regional blood transfusion testing centers in Kenya.**
Study design: **A cross-sectional study**
Setting: **It was carried out in two Regional Blood Transfusion Centers (RBTCs) which are based in Nairobi and Nakuru. These two centers are associated with possible low and high prevalence respectively.**
Subject: **A total of 180 blood samples were randomly selected over a period of one month. These blood samples were tested for WNV IgG using ELISA. Results: Majority of the donors were below 35 years of age and were predominantly male. WNV IgG prevalence was 15% in blood donors (95% CI 10-20.5%). Prevalence of cross infection of TTI and WNV was 8.3% (95% CI 4.4-12.2%). The prevalence of WVN IgG was highest in the 19-35 years' age group (16.5%) and females (21.6%) though the results were not statistically significant. There was no difference in the IgG positivity between the different centers.**
Conclusion**: Infection with WNV should be of public health concern because about a fifth of those infected with WNV develop illness. About 10% of those who develop neurological symptoms succumb to the disease.**

INTRODUCTION

West Nile Virus (WNV) is an arbovirus transmitted by infected mosquitoes which cause most of its incidence (1). Birds are known to be the natural hosts of WNV while humans are the incidental hosts. As a result, there is a mosquito-bird-mosquito cycle for WNV which consequently results to human infection. The Culex species of mosquitoes is primarily involved in the spread of WNV (2). Transmission starts when a mosquito bites an infected bird or an animal and gets the virus while feeding on the animal's blood. The infected mosquito can then transmit the virus to another bird or animal when it feeds again (1). In 1937, the first case of WNV was identified in East Africa in a female Ugandan patient after which the virus has since spread continentally to Australia, USA, Europe and Asia.

Its spread has been linked to ecological factors, population growth, inadequate vector control and climatic changes (3). Most people involved in outdoor activities in the affected regions are more exposed and at risk of the virus.

The incubation period of WNV ranges from two to fourteen days based on the medical conditions of the infected patients. Approximately 80% of people who are infected with WNV will not show any symptoms at all. Patients may recover after months or weeks based on the severity of the disease. Individuals over sixty years of age and those with other medical conditions such as cancer and kidney diseases are at more risk of severe disease following infection by WNV.

The diagnosis of WNV is based on clinical signs and symptoms. Serology is the most used laboratory tests in diagnosis of WNV.Other tests may include viral cultures and tests to detect the viral RNA being done on the serum, CSF and tissue specimen. This specimen is collected in the early stages of illness and an infection is confirmed by a positive test result. Another test that can be performed is immuno histochemistry which detects WNV antigen in formalin-fixed tissue. WNV has no known vaccine (4).

WNV can also be transmitted through blood transfusion where an infected blood transmitter is involved. It also occurs during organ transplants and during delivery, pregnancy or breastfeeding from mother to child (1,4). Risk of transfusion transmissible infections (TTIs) has been greatly reduced by improvements in donor screening and testing. Every blood screening programme faces ongoing challenges.

Reports of newly identified infections or re-emerging infections appear regularly in the scientific literature, including reports of their transmission through the route of transfusion. Examples include variant Torque Teno Virus, SEN virus, and West Nile virus (5).It is therefore necessary for blood transfusion services to develop contingency plans that ensure surveillance for emerging infections, assessment of their transmission by transfusion and the actual likelihood associated with the spread, and action to be taken to avoid further infections, including to pandemic level (1, 4).The spread of WNV can be prevented by avoiding mosquito bites through the use of mosquito nets and repellants and by wearing protective clothes with long sleeves.

Additionally, bush clearing as well as emptying standing water is necessary to prevent breeding. Screen windows and doors can be used to keep mosquitoes out (4). This study was intended to provide an in-depth insight on the factors that contribute to the spread of the virus by Culex mosquito and its impact on the health of Kenyans. It was also intended to contribute to lowering the spread of WNV as a TTI (6). It evaluated blood donations in a facility within the lake basin versus the capital city which is highly cosmopolitan and devoid of malaria. It was therefore anticipated that some donors are likely to be harboring WNV especially in Nairobi due to its cosmopolitan nature.

METHODS

Study site

The study was conducted at the Regional Blood Transfusion Testing Centers (RBTTCS) located in Nairobi and Nakuru.The samples used for the study were already processed and archived in plain evacuated vacutainer tubes (with no anticoagulant). Nakuru is located in the central part of Kenya and serves a wide variety of people.

This region serves a wide catchment area that includes: Baringo, Naivasha, Molo etc. all of which are known to have a contrasting variation of weather that spans from hot to cold. This therefore took care of the environmental variations experienced in the country and partly contributes to the seasonal outbreaks experienced in Kenya.

The virus is known to affect populations along the lake basin and these regions form a good representation of the target population. Nairobi is the capital city of the country with a population of about 5 million people. All communities are represented and the RBTC serves this population and the environs. The coverage of these areas in the study was intended to limit sampling bias in high and low prevalent areas for WNV.

Study design

This was a cross-sectional descriptive study.
Study samplesBlood was obtained for TTI testing from the regional blood transfusion testing centers of Nairobi and Nakuru.

Sample size

Using Fishers formula and a prevalence of 12.4%, the formula used to obtain the minimum sample size was

n=(z^2 P(1-p))/d^2
n = minimum sample size
z = Statistic for level of confidence on normal distribution
Critical value set at 1.96 which corresponds to 95% confidence interval
p =residual rate of 12.4%
d = degree of precision set at plus or minus 5%
n=(〖1.96〗 ^2×0.124(1-0.124))/ 〖0.05〗 ^2 =167 Subjects
The study analysed a total of 180 serum samples
Sampling method and processing
TTI samples were collected in 5ml plain evacuated vacutainer blood collection tube (with no anticoagulant) and span on arrival at the lab with a speed of 5000 revolutions per minute for 10 minutes. All collected samples meeting the inclusion criteria were considered for testing.

They were arranged randomly and a consecutive sampling technique applied (every second sample was picked for storage and analysis subsequently). This technique was applied until all the samples required for the study were obtained. Serum was harvested in the selected samples and was aliquoted into 2ml vials and stored at -20oc. Testing only commenced once all the samples had been obtained.

Sampling method and processing

TTI samples were collected in 5ml plain evacuated vacutainer blood collection tube (with no anticoagulant) and span on arrival at the lab with a speed of 5000 revolutions per minute for 10 minutes. All collected samples meeting the inclusion criteria were considered for testing. They were arranged randomly and a consecutive sampling technique applied (every second sample was picked for storage and analysis subsequently). This technique was applied until all the samples required for the study were obtained. Serum was harvested in the selected samples and was aliquoted into 2ml vials and stored at -20oc. Testing only commenced once all the samples had been obtained.

Laboratory testing

The assay of choice for this study was qualitative. An ELISA was used that targeted the immunoglobulin G in the sample. The expected result was either a positive or a negative. Dx Select ELISA kit that was used for analysis of the WNV is FDA approved and so further validations were not necessary. The principle of the test is based on the antibody capture technique.

Data analysis

All data was analyzed using SPSS version 21. The results were presented in percentages using tables and charts as well as ranges, mean and standard deviation.

Ethical consideration

Permission to conduct the study was sought from KNH/UON ethics review committee and from the director in charge of KNBTS CENTRES.Donors' privacy and confidentiality was strictly observed.

RESULTS

Blood samples for 180 blood donors were analyzed. The donors had a mean age of 31.4 years (SD 7.6 years) ranging from 19 to 58 years. More than three quarters (76.7%) were aged between 19 and 35 years. They were predominantly males (79.4%) and about a half (51.1%) were sampled from the Nairobi blood center.

Table 1

Demographic characteristics

Variables	Frequency (%)
Mean age (SD) years	31.4 (7.6)
Min-Max age years	19-58
Age categories	
19-35 years	138 (76.7)
36-45 years	32 (17.8)
46-55 years	9 (5.0)
>55 years	1 (0.6)
Gender	
Male (Total)	143 (79.4)
Nairobi	75(83.3)
Nakuru	68(75.6)
Female(Total)	37(20.6)
Nairobi	15(16.7)
Nakuru	22(24.4)
Blood centers	
Nairobi	90(50)
Nakuru	90(50)

Blood samples that were TTI positive made up 40% of the population. The TTI positive were mainly HIV (81.9%) while 12.5% were positive for hepatitis B.

Table 2

TTI

Variable	Frequency (%)
TTI	
Positive	72 (40.0)
Negative	108 (60.0)
Positive TTI (n=72)	
HIV	59 (81.9)
Hepatitis B	9 (12.5)
Hepatitis C	2 (2.8)
Syphilis	2 (2.8)

Seroprevalence of WNV IgG

WNV IgG prevalence in blood donors was at 15% with a 95% CI of between 10 and 20.5%. Prevalence of cross infection of WNV and TTI was 8.3% (95% CI 4.4-12.2%).

Table 3

Seroprevalence of WNV IgG

Variable	Frequency (%)	95% CI
WNV results		
Positive	27 (15.0)	10.0, 20.5
Negative	153 (85.0)	79.5, 90.0
Cross infection (WNV and TTI)		
Yes	15 (8.3)	4.4-12.2
No	165 (91.7)	87.8-95.6

Seroprevalence of WNV IgG by age, gender and blood center

Prevalence of WNV IgG was higher (16.5%) in donors in the age group between 19 and 35 years compared to those above 35 years (9.5%). However, the difference was not statistically significant (p=0.256). Similarly, though not statistically significant, females had a higher prevalence at 21.6% compared to males at 13.3%, p=0.206 Blood samples from the different centers did not show any difference in terms of IgG positivity with Nairobi having 14.1% of the blood donors positive, 16.3% in Nakuru and 12.5% in other centers (p=0.909).

Table 4

Seroprevalence of WNV IgG by age, gender and blood center

	WNV		P value
	Positive (%)	Negative (%)	
Age			
19-35	23 (16.7)	115 (83.3)	0.256
35	4 (9.5)	38 (90.5)	
Gender			
Male	19 (13.3)	124 (86.7)	0.206
Female	8 (21.6)	29 (78.4)	

DISCUSSION

This study showed a relatively high prevalence of WNV among blood donors at 15%. Similar proportions have been reported in previous studies. (7) reported 12.4% prevalence in a population sampled in the lake basin areas of Baringo, Naivasha and Tana River. Higher prevalence was reported in patients sampled in Western Kenya which showed 31% of them were positive for WNV (3). However, a study in New Zealand showed a lower prevalence of 2.1% in blood donors who had previously travelled to WNV endemic regions (8). A study on Iranian donors reported that all were negative for WNV-specific antibody (9).A number of sociodemographic factors of blood donors were analyzed. It emerged that there were no age differences associated with prevalence of WNV. However, a higher number of blood donors in the age group between 19 and 35 years were infected (16.5%) compared to those above 35 years (9.5%). This could be explained by the fact that WNV is a vector-borne disease that is transmitted by culex mosquitoes and individuals in this age group may be more likely to be involved in outdoor activities hence exposure to mosquito bites (1,10)

In addition, current examination of the mosquito immune response is starting to reveal corresponding proteins and pathways. The mosquito antiviral response is thought to consist of two pathways, the innate immune pathway and the RNA interference (RNAi) pathway. Not much is known regarding the role of mosquito AMPs in antiviral immunity, though their expression is often induced by viral infection (14).

It is also similar to data that shows more young people donate blood in low- and middle income countries (4). Despite males being the majority in this study, females had a higher prevalence (21.6%). This could be that women are more involved in outdoor activities such as washing laundry or near water bodies that put them at risk of infection. It was the opposite in a U.S. study where positive results for WNV were 63% higher among male than female donors (11). It could be that males in this population are more likely to be involved in activities or in areas where they are a higher risk of infection.

Similar results were reported in a North Dakot study where the rate of infection was greatest for younger persons and men (12). This large proportion of males corresponds to the WHO data where globally 70% of blood donations are given by men (4). Though a US study showed that 53% of the donations were from men (11), they were still the majority. However, there was no significant association found with age and sex of the donor in this study similar to an Italian study (10).

In this study the IgG positivity from different centers did not show any differences. This could be because of the cosmopolitan nature of the study sites. However, a U.S. study showed that positive blood donations clustered according to WNV epidemic activity and the catchment areas of participating blood collection networks (11). The transfusion transmissible infections (TTIs) positive were mainly HIV in this study, others included Syphlis, HEP B and Hep C. This was higher than WHO data considering the prevalence of TTIs is 0.2% and 1.08% in lower middle-income countries and low-income countries respectively (4).Though the risk of (TTIs) has been greatly reduced by improvements in donor screening and testing, reports of newly identified infections or re-emerging infections such as West Nile virus (WNV) cause ongoing challenges (1,4).

West Nile virus can be transmitted through transfusion with blood from an infected donor (1) and because large volumes of blood or blood components are given to patients during transfusion therapy, the result is such that even a blood unit with low viral load may cause infection in the patient (1, 2,3,4,7).

It is noted that co-occurrence of viral infections correlates with the optimal climatic conditions for the respective vector; cooler-adapted mosquitoes tend to co-occur with the same virus in cooler conditions than their warmer-adapted counterparts. This raises the need for blood transfusion services, for example in Nairobi and Nakuru, to develop contingency plans to ensure surveillance for emerging infections, assessment of their transmissibility by transfusion, actual likelihood of transmission and diseases associated with transmission (1,13). This is because the cosmopolitan nature of these areas results in some donors harboring the WNV. Infection with WNV should be of public health concern because about a fifth of those infected with WNV develop illness with symptoms such as body aches, vomiting and skin rash. A smaller number of these develop severe illness with symptoms ranging from coma, meningitis, encephalitis and paralysis. About 10% of those who develop neurological symptoms succumb to the disease. Those over the age of 50 years, those with weakened immune functions, those with medical conditions like cancer and kidney disease are at the highest risk for getting severely ill when infected with WNV (1).

It is therefore important to screen for WNV seeing that in low-income countries, up to 65% of transfusions are for children under the age of five years which contrasts with high-income countries where 76% of all transfusions are in patients over 65 years of age (4).

There are also no medications to treat nor vaccines to be used in the prevention of WNV (1,4).

CONCLUSION

Infection with WNV should be of public health concern because about a fifth of those infected with WNV develop illness. About 10% of those who develop neurological symptoms succumb to the disease. Further testing is recommended for all positive samples for confirmation as this is outside the scope of this study.

REFERENCES

1. CDC. West Nile virus: statistics and maps. Fort Collins, CO: US Department of Health and HumanServices,CDC;2015.Availableathttp://www.cdc.gov/westnile/statsmaps/index.html
2. Sue, H., Helen, B. and Ange, B. West Nile Virus Seroprevalence for blood donors in the Wellington Region.Ministry of Agriculture and Forestry. Arch Iran Med. 2010 Jan;13(1):1-4.
3. Lwande, O., Lutomiah, J. and Obanda, V. Isolation of tick and mosquito-borne arboviruses from ticks sampled from livestock and wild animal hosts in Ijara District, Kenya. Vector Borne Zoonotic Dis. 2013: 13:637–642
4. World Health Organization (WHO). Fact sheet: Blood safety and availability. 2017.Availablefrom:www.who.int/mediacentre/factsh12 .Carson, PJ., Borchardt, SM., Custer, B., Prince, HE., Dunn-Williams, J., Winkelman, V. et al. Neuroinvasive disease and West Nile virus infection, North Dakota, USA, 1999-2008. Emerging Infectious Diseases. 2012;18 (4): 684-686.eets/fs279/en/ [Accessed: 29th July 2017].
5. Komar N., Langevin S., Hinten S., et al. Experimental infection of North American birds with the New York 1999 strain of West Nile virus. Emerg Infect Dis 2003; 9:311–322 [PMC free article] [PubMed].
6. LaBeaud AD., Sutherland LJ., Muiruri S., et al. Arbovirus prevalence in mosquitoes, Kenya. Emerg Infect Dis 2011b; 17:133–241 [PMC free article] [PubMed].
7. Caroline Tigoi, Olivia Lwande, Benedict Orindi, Zephania Irura, Juliette Ongus, and RosemarSangSeroepidemiology ofSelected Arboviruses in Febrile Patients Visiting Selected Health Facilities in the Lake/River Basin Areas of Lake Baringo, Lake Naivasha, and Tana River, Kenya Vector Borne Zoonotic Dis.2015 Feb 1; 15(2): 124–132. 8, Cinnia Huang, *Brett Slater, *Robert Rudd, *Nandakishore Parchuri, †Rene Hull, *Michelle Dupuis, *and Alexander Hindenburg. First Isolation of West Nile
8. virus from a Patient with Encephalitis in the United States.Emerg Infect Dis. 2002 Dec; 8(12): 1367–1371
9. .Sharifi, Z., Shooshtari, M. M., Talebian, A. A study of West Nile virus infection in Iranian blood donors. Ach Iran Med. 2010; 13(1):1–4.
10. Pezzoti, P., Piovesan, C., Barzon, L., Cusinato, R., Cattal, M., Pacenti, M. et al. Prevalence of IgM and IgG antibodies to West Nile virus among blood donors in affected area of north-eastern Italy, summer 2009. Euro Surveill. 2011;16 (10): pii=19814.
11. Betsem, E., Kaidarova, Z., Stramer, SL., Shaz B., Sayers M., LeParc G.et al..

12. Correlation of West Nile virus incidence in donated blood with West Nile neuro invasive disease rates, United States, 2010-2012. Emerging infectious diseases. 2017;23(2): 212-219
13. Koka, S. H., Turell, M., Lutomiah, J., et al (2011). Evaluation of Kenyan mosquito species as vectors of West Nile Virus. The African Journal of Health Sciences.
14. Arjona A, Wang P, Montgomery RR, Fikrig E. 2011. Innate immune control of West Nile virus infection. Cell. Microbiol. 13:1648–1658 [PMC free article] [PubMed]