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Research Article

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Seroprevalence of West Nile Virus and Associated Factors in Borena District, Southern Ethiopia

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Abstract

West Nile virus is a member of genus Flavivirus that cause emerging disease. It is transmitted with mosquitoes, Culex species. Still now, no studies carried out to determine the seroprevalence of WNV antibodies in Ethiopia. The aim of this study was to assess the WNV seroprevalence and its associated factors in health facilities in the Borena District. An institutional based cross-sectional study was conducted from May to August, 2019. 519 consecutive acute febrile patients attending the outpatient departments of Teltelle Health Center, Yabello and Moyale Hospital were participated. Data on socio-demographic and associated factors were collected using a structured questionnaire. 3-5 blood samples were collected from all participants and screened for WNV antibodies using indirect immunofluorescence assay. The overall prevalence of anti-WNV IgG and IgM was 7.3% and 2.7% respectively. Above thirty-nine percent of the study participants were from Teltelle Health center, 36.6% were from Moyale Hospital, and the left were (23.7%) were from Yabello Hospital. Female participants (3.3%) had higher rate of WNV IgM compared to males (2%), but male was account higher in IgG antibody (8.8%). This study provides evidence that WNV infection is prevalent in the study area. The observed low awareness of participants underlines the urgent need for further community based studies to determine the associated factors that determine the extent of exposure to WNV infection in the study area for appropriate control and prevention.

Keywords: West Nile Virus, Indirect Immunofluorescence Assay, Ethiopia

Introduction

West Nile virus (WNV) is a member of Flaviviridae family, genus Flavivirus that includes small and enveloped viruses. The genome of the virus is a single-stranded, positive sense RNA by approximately 11000 bp in length [1,2]. The phylogenic lineage studies show that approximately 1000 years ago, WNV emerged as a distinctive virus and had developed into two distinct lineages. Lineage 1 was found to be the source of epidemic transmission in Africa and throughout the world, whereas lineage 2 was discovered in horses in sub-Saharan Africa and Madagascar for the first time, WNV was isolated in a woman from Uganda in 1937 [3-5].

WNV was distributed throughout Africa, the Middle East, Southern Europe, western Russia, south western Asia, and Australia which derives from its ability to infect many mosquitoes and avian species. Until the early 1990s, human outbreaks mainly associated with moderate febrile illnesses, were reported infrequently from Israel and Africa. Starting from this time, new viral strains with likely African origin have increased human disease incidence in parts of Europe, with large outbreaks of increased clinically severe infection occurred in Romania, Russia, Israel, and Greece [6,7].

Most patients with WNV related illnesses are unrecognized clinically. The presentation of clinical illness in human's ranges from asymptomatic to viral syndrome and neurological disease [8]. In the epidemic of WNV infection, about 80% of infections are asymptomatic and the left present with fever, headache, body aches, and sometimes a skin rash on the trunk and swollen lymph glands. It can be severe but is commonly self-limited and less than 1% of cases lead to neuroinvasive disease such as encephalitis, meningitis or polio-like flaccid paralysis [9].

WNV is an arbovirus transmitted to human beings by mosquitoes, majorly the Culex genus, specifically by Culex pipiens that serve as vector, birds serve as intensifying host, and humans and other mammals can be dead-end host [9,10]. WNV also transmitted through transfused platelets, red blood cells, and fresh frozen plasma as well as via heart, liver, lung, and kidney transplants [11]. Zoonotic WNV circulates in natural transmission cycles comprising mosquitoes and avian and a range of other vertebrates, like

horses and human beings, are incidental hosts. WNV is maintained in mosquito populations via vertical transmission (adults to eggs); birds are an major reservoir of WNV in the environment [12]. WNV disease at or shortly after birth, indicate the possibility of intrauterine infection or infection at the time of delivery. Other rare or suspected routes of transmission include breast milk transmission, percutaneous or conjunctival exposure to laboratory workers, and by unknown means in patients undergoing dialysis and workers at a breeder farm [13].

As present, no studies conducted in Ethiopia regarding the seroprevalence of the WNV in the human being and no clinical reports have been made. The aim of this study was to estimate the seroprevalence and factors associated with WNV among febrile patients in Borena District.

Methodology

A health facility based cross sectional study was conducted from May to August, 2019 in three health facilities in Borena District: Yabello Hospital, Moyale Hospital and Teltelle Health center. The district is located in southern part of Ethiopia, bordering Kenya. The climate of the district is arid and characterized by mean annual rain fall of 400-700 mm in two rainy seasons, and mean annual temperature ranging from 25-37oC. A study area is bushy woodland and infested with mosquitoes [14].

The study participants were all patients presenting with acute febrile illness visiting outpatient departments of the health facilities during the study period and fulfilled the inclusion criteria. The sample size was estimated to be 519 using single population proportion formula [15].

Socio-demographic and other possible associated factors with viral infection, such as use of bed net, trees around compound, use of mosquito repellent, presence of stagnant water around compound, recent mosquito bite, and stay outside at night time were collected using structured questionnaire. Blood samples were collected, clotted and centrifuged at 1300r/minute to obtain serum. Separated sera were transported using liquid nitrogen of -170oc to Hawassa University Referral Hospital, and stored at -80ºc. Sera screened at AHRI laboratory for WNV IgG and IgM using EUROIMMUN biochips immunofluorescent assay kit (Medizinische Labordiagnostika AG-Germany) according to the manufacturer's manual [16].

Data were entered and analyzed using SPSS version 20 software. Simple frequency tables were generated, and categorical variables were compared. A univariate logistic regression analysis was used to identify risk factors associated with the prevalence of anti-DENV IgM and IgG antibodies. Those independent variables found $p < 0.25$ in univariate analysis were then used in multivariate logistic regression analysis. Odds ratios (ORs) at 95% confidence intervals (CIs) were calculated to measure the degree of association. A p-value < 0.05 was considered as statistically significant and data were presented in the form of tables.

Ethical clearance was obtained from the AHRI Ethics Review Committee. Before data collection, participants were informed about the aim and purpose of the study, about their right not to participate on the study or withdraw at any point in time. Personal privacy and dignity was respected. Data was collected after obtaining participants'/guardians' informed written consent. Assent was also sought in cases the study participants were children under 18 years old. All samples and forms containing patient information had no name or information that can identify a study participant; these were ascertained by using specific study codes. Confidentiality of the information was ensured by analyzing the data in aggregate and putting all documents bearing participants name in a key and lock system.

Results

Socio-Demographic Characteristics

519 patients were investigated during the study period. Around 40% of the study participants were from Teltelle Health center, 36.6% were from Moyale Hospital, and the left participants were from Yabello Hospital (Figure 2). The mean age of the participants was 25.5 years (range, 1 to 80 years, standard deviation 1.54), and those in the age range 30-44 years accounted 32.4%. Female participants accounted 52% with male to female ratio of 0.92:1. Substantial proportion of the study participants were rural dwellers (53.6%), illiterate (60.9%), and farmers by occupation (33.9%) (Table 1).

Table 1: Socio-demographic characteristics of the study participants in relation to IgM seropositivity, 2016

Characteristics	Number tested (%)	Number positive (%)	COR(95% CI)	P-value
Sex				
Male	249(48)	5(2)	0.36(0.19-1.79)	0.51
Female	270(52)	9(3.3)	1	
Age				
<14	29(5.6)	1(1)	0.71(0.04-9.58)	0.71
15-29	74(14.3)	6(3.5)	0.46(0.28-17.6)	0.46
30-44	168(32.4)	3(3)	0.59(0.19-18.44)	0.59
≥45	136(26.2)	1(1.6)	1	
Residence				
Rural	278(53.6)	5(1.8)	0.18(0.16-1.43)	0.09
Urban	241(46.4)	9(3.7)	1	
Education level				
Illiterate	316(60.9)	9(2.9)	0.75(0.09-5.81)	0.99
Primary	139(26.8)	4(2.9)	0.76(0.08-6.6)	0.75
Secondary	39(7.5)	0(0)	0.99(0.0-∞)	0.76
College and above	25(4.8)	1(4)	1	
Occupation				
Farmer	176(33.9)	6(3.4)	0.88(0.10-7.34)	0.69
Animal keeper	137(26.4)	1(0.7)	0.23(0.01-2.9)	0.88
Employee	57(11)	1(1.8)	0.56(0.03-7.12)	0.23
Student	67(12.9)	3(4.5)	1.13(0.11-11.35)	0.55
House wife	57(11)	2(3.5)	0.91(0.08-10.1)	0.92
Others	25(4.8)	1(4)	1	

COR (95%CI) = crudes odds ratio at 95% confidence interval,

Seroprevalence of WNV infection

The overall prevalence of exposure to WNV were found to be 2.7% and 7.3 for IgM and IgG antibodies respectively. The rate of exposure of IgM was higher among urban residents (3.7%), students (4.5%) and, had college and above education level (4%) (Table 1). Yabello Hospital accounts highest in both IgM (4.1%) and IgG (10.6%), and seroprevalence of antibodies were lowest in Moyale hospital (Table 2). Female participants (3.3%) had higher rate of WNV IgM antibody compared to males (2%), but male was account higher in IgG antibody (8.8%). Further, with respect to age, the prevalence of WNV IgG was highest (10.1%) in age group 30-44 years and lowest (5.9%) in age group 15-29 years (Table 3). No variable was showed association in bivariate analysis in both WNV antibodies.

Table 2: Seroprevalence of WNV antibodies of participants by study site of Borena district, Southern Ethiopia, May to August, 2016

	Total number	WNV IgM	WNV IgG
Study sites	No(%)	Pos No(%)	Pos No(%)
Yabello Hospital	123(23.7)	5(4.1)	13(10.6)
Moyale Hospital	190(36.6)	3(1.6)	9(4.7)
Teltelle Health center	206(39.7)	6(2.6)	16(7.8)
Total	**519(100)**	**14(2.7)**	**38(7.3)**

WNV- West Nile virus

Table 3: Socio-demographic characteristics of the study participants in relation to IgG seropositivity, 2016

Characteristics	Number positive (%)	COR(95% CI)	P-value
Sex			
Male	22(8.8)	0.21(0.79-3.0)	0.15
Female	16(5.9)	1	
Age			
<14	8(7.8)	0.92(0.29-3.02)	0.92
15-29	15(5.9)	0.5(0.24-1.99)	0.50
30-44	10(10.1)	0.69(0.41-3.87)	0.69
≥45	5(8.2)	1	
Residency			
Rural	19(6.8)	0.65(0.44-1.66)	0.12
Urban	19(7.9)	1	
Education level			
Illiterate	20(6.3)	0.29(0.14-1.8)	0.29
Primary	11(7.9)	0.49(0.16-2.42)	0.49
Secondary	4(10.3)	0.83(0.17-.11)	0.83
College and above	3(12)	1	
Occupation			
Farmer	11(6.2)	0.66(0.19-12.95)	0.66
Animal keeper	11(8)	0.49(0.26-16.99)	049
Employee	5(8.8)	0.45(0.26-20.8)	0.46
Student	5(7.5)	0.56(0.22-17.4)	0.57
House wife	5(8.8)	0.46(0.26-20.8)	0.46
Others	1(4)	1	
Characteristics	Number positive (%)	COR(95% CI)	P-value
Sex			
Male	22(8.8)	0.21(0.79-3.0)	0.15
Female	16(5.9)	1	
Age			
<14	8(7.8)	0.92(0.29-3.02)	0.92
15-29	15(5.9)	0.5(0.24-1.99)	0.50
30-44	10(10.1)	0.69(0.41-3.87)	0.69
≥45	5(8.2)	1	
Residency			
Rural	19(6.8)	0.65(0.44-1.66)	0.12
Urban	19(7.9)	1	
Education level			
Illiterate	20(6.3)	0.29(0.14-1.8)	0.29
Primary	11(7.9)	0.49(0.16-2.42)	0.49
Secondary	4(10.3)	0.83(0.17-.11)	0.83
College and above	3(12)	1	

Occupation			
Farmer	11(6.2)	0.66(0.19-12.95)	0.66
Animal keeper	11(8)	0.49(0.26-16.99)	049
Employee	5(8.8)	0.45(0.26-20.8)	0.46
Student	5(7.5)	0.56(0.22-17.4)	0.57
House wife	5(8.8)	0.46(0.26-20.8)	0.46
Others	1(4)	1	

NB: COR; Crude Odd Ratio AOR; Adjusted Odd Ratio Others: merchants, day laborer
* = statistically significant (p-value < 0.05), COR (95%CI) = crudes odds ratio at 95% confidence interval

Associated Factors with Seroprevalence of WNV Antibodies

Regarding the general awareness about this virus infection, 9.6% of respondents were reported WNV is transmitted by mosquito. Study participants were asked about the environmental exposures associated with mosquito-borne illnesses in their residence areas. Those who reported the existence of stagnant water and trees nearby their dwelling were 31.2% and 64.2%, respectively. Three hundred and thirty-three (64.2%) of the respondents were reported that they slept under mosquito nets; of which 20.2% and 41.4% used bed nets always and sometimes, respectively. However, only 4% used mosquito repellents at the day or night time (Table 4).

The seropositivity of WNV IgG was 12% in those who responded that they were aware that mosquitoes transmit the WNV. The rate of exposure was observed to be 6.6% in those who utilized bed nets. A recent experience of having had a mosquito bite (9.8%) was the only factor that significantly associated with the rate of WNV IgG seropositivity in bivariate analyses from the knowledge and environmental related factors. However, use of mosquito repellant, awareness of WNV, knowledge of the route of transmission and presence of tree around the compound, and the use of bed nets were not showed significant association with history of exposure to WNV (p-value > 0.05). The association between a recent mosquito bite and WNV IgG infection was not showed significant association in a multivariable logistic regression analysis (p>0.05) (Table 4).

Table 4: Factors associated with the prevalence of anti-WNV IgG seropositivity among the study participants, 2016

Characteristics	Number (%) Tested	Number(%) positive	COR (95% CL)	AOR(95% CL)
Mode of transmission				
Mosquito	50(9.6)	6(12)	0.17(0.76-4.89)	
By blood	14(2.7)	2(14.3)	0.28(0.51-11)	
Do not know	455(87.7)	30(6.6)	1	
Stagnant water				
Yes	162(31.2)	8(4.9)	0.56(0.25-1.26)	
No	357(68.8)	30(8.4)	1	
Trees around compound				
Yes	333(64.2)	26(7.8)	0.57(0.6-2.5)	
No	186(35.8)	12(6.5)	1	
Stay outside at night				
Yes	248(47.8)	23(9.3)	1.75(0.89-3.42)	
No	271(52.2)	15(5.5)	1	
Recent mosquito bite				
Yes	300(57.8)	30(9.8)	2.15(1.02-4.53)*	1.87(0.86-4.1)
No	219(42.2)	8(3.8)	1	
Bed net use				
Yes	333(64.2)	22(6.6)	1	
No	186(35.8)	16(8.6)	1.33(0.68-2.6)	
Repellent use				
Yes	21(4)	1(4.8)	1	

No	498(96)	37(7.4)	1.6(0.21-12-29)	
Study site				
Yabello Hospital	123(23.7)	13(10.6)	1.4(0.65-3.03)	
Moyale Hospital	190(36.6)	9(4.7)	0.59(0.25-1.37)	
Teltelle Health center	206(39.7)	16(7.8)	1	

NB: COR; Crude Odd Ratio AOR; Adjusted Odd Ratio
* = statistically significant (p-value < 0.05), COR (95%CI) = crudes odds ratio at 95% confidence interval, AOR (95%CI) = adjusted odds ratio at 95% confidence interval

Discussion

To date, no studies have been carried out in Ethiopia regarding the seroprevalence of WNV antibody in human being and no clinical reports had been made. Investigating WNV seroprevalence is important because of many associated factors for the presence of WNV in Ethiopia, like appropriate climatic situation for propagation of vector mosquitoes. Since most WNV infections are asymptomatic, a seroprevalence survey was needed to examine the exposure of the population to the virus and to identify areas with high endemicity. This study assessed the WNV seropositivity and its associated factors in health facilities in the Borena District where febrile illness is known.

This study is conducted in Ethiopia for the first time. A total of 519 serum samples screened from three health facilities of Borena district, the results suggest that WNV has high prevalence (7.3% for IgG). This result is in agreement with findings reported from Kenya 6% [17]. However, the observed rate of WNV exposure was lower than results in Zambia 10.3%, Kenya 11.3%, Esrael 11.1% and Iran 11% [18-21]. In contrast, the prevalence of anti-WNV IgG seropositivity in this study was higher compared to the rates 1.2% in Malaysia and 4.3% in Turkey [22, 23]. These discrepancies may be due to the difference in the distribution of associated factors and the variable climatic conditions by geographical regions, the diversity of the studied participants, and the difference in the diagnostic technique of the employed laboratory methods, since this study performed by IIFA technique. The prevalence of anti-WNV IgM seropositivity was 2.7% which indicates recent infection with WNV. This result indicates that WNV recently circulate in the community in study areas. There was no any factor that has showed significance with anti-WNV antibodies in this study. But, the rate of reactive serum samples was in adult age group between 30 and 44 years, likely because people from this age group participate in a lot of outdoor activities such as hunting and working in the forest.

The data generated has greatly contributed to the new data on the magnitude of WNV exposure in the study area. This information forms a basis to stimulate the planning and conduction of further widespread studies and in the planning and setting up of public health intervention programs for the prevention of WNV. Although this is the first study of seroprevalence and associated factors with WNV infection in South Ethiopia, the study has several limitations. The findings in this study could be limited by the fact that the results were based on primary IgG and IgM antibody test. Plaque Reduction Neutralization Test (PRNT) was not done. This could have overestimated the seropositivity for WNV since the cross reactivity of arboviruses was not ruled out.

Conclusion

This study provides evidence that WNV infection is prevalent in Borena district, southern Ethiopia. Thus, it is recommending a community based survey in the study area and adjacent communities to verify our findings and take appropriate public health measures. Further community based studies should be employed to determine the environmental, and host factors that determine the extent of exposure to WNV infection in the area for appropriate control and prevention.

Abbreviations

AHRI: Armauer Hansen research institute.
ELISA: Enzyme Immunosorbent assay
IIFA: Indirect Immunofluorescent Assay
IgG: Immunoglobulin G
IgM: Immunoglobulin M
PRNT: Plague Reduction Neutralization test
WNV: West Nile virus

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