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ORIGINAL ARTICLE

SEROPOSITIVITY OF YELLOW FEVER VIRUS AMONG ACUTE FEBRILE PATIENTS ATTENDING SELECTED HEALTH FACILITIES IN BORENA DISTRICT, SOUTHERN ETHIOPIA

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ABSTRACT

Introductio*: Yellow fever (YF) is a reemerging public health threat in Ethiopia; however, there is limited information on the seroprevalence of Yellow Fever Virus (YFV) in different parts of the country and the sociodemographic factors that may predispose individuals to infection. In this study the seroprevalence and associated risk factors of YFV infection were assessed in Borena district, southern Ethiopia..*

Methods: *An institution based cross-sectional study was conducted from May to August 2016. A total of 519 consecutive acute febrile patients attending the outpatient departments of Teltelle Health Center, Yabelo and Moyale Hospitals were enrolled. Data on socio-demographic and environmental risk factors were collected using a structured questionnaire. Blood samples were collected from all participants and screened for yellow fever virus exposure using the indirect immunofluorescent assay.*

Result: *The overall prevalence of anti-YFV IgG and IgM was 12.5% and 7.3% respectively. IgG seropositivity was significantly higher among male subjects compared to females (15.7% and 9.6% respectively, AOR=1.69, 95% Cl: 1.01-2.91, p-value=0.04). A relatively higher IgM titer was observed in females compared to males (8.8% and 6) but it did not attain statistical significance (COR=1.51, 95% CI:0.78-2.95, p=0.16) . Furthermore, there was no significant association of IgG and IgM seropositivity by age.*

Conclusion *Yellow Fever Virus has public health significance in the study area. Males and those with a history of recent mosquito bites were disproportionately affected by the virus. We recommend further systematic studies to determine the environmental and host factors that determine the extent of exposure to yellow fever virus infection in the district to inform appropriate intervention measures.*

Key words*: Borena, Yellow Fever Virus, Indirect Immunofluorescent Assay, Ethiopia*

INTRODUCTION

Yellow fever virus (YFV) is an arthropod-borne virus (arbovirus) that causes yellow fever (YF) a common disease in South America and Sub-Saharan Africa (1). The virus is transmitted by the bite of infected mosquitoes belonging to the *Aedes* species in Africa and *Haemagogus* species in South America. Most infections remain asymptomatic (2, 3). Clinical YF is characterized by acute onset of fever, chills, headache, backache, generalized muscle pain, nausea and vomiting (4).

In most instances the clinical manifestation follows three phases; acute, remission and toxic phases. Most cases improve and recover within 4 to 5 days. Some cases will undergo a temporary remission phase for 24 –48 hours in which patients start to feel relief from their symptoms, and up to 15% to 25% might enter into a toxic phase after 1 to 2 days of initial recovery (5).The confirmation of YF infection requires trained laboratory personnel and specialized laboratory facilities. Laboratory criteria for diagnosis are detection of neutralizing anti-YFV antibodies or yellow fever viral genome. The standard confirmatory tests are plaque reduction neutralization test (PRNT) and reverse transcriptase-polymerase chain reaction (rt-PCR) (5). The indirect immunofluorescence assay (IIFA) to detect IgG and IgM antibodies against YFV, based on Euroimmun Biochip technology was shown to have an overall correlation of 98.7% with presence of active infection(6).

Yellow fever is becoming a re-emerging public health threat in Africa (1, 7, 8). During 2011 and 2012, major outbreaks were reported in Sudan and Uganda (9). There is a documented history of YF outbreaks in Ethiopia which dates back to the 1960s (10,11). YF re-surfaced in the country in 2013 in the Southern Nations Nationalities and Peoples Regional (SNNPR) State. The areas affected in the 2013 outbreak were the same ones, or adjacent to those affected by the outbreak from 50 years ago (12).

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The most recent outbreak (2013) claimed 43 lives and necessitated a mass vaccination campaign that targeted over 527, 000 people (13).

Despite the fact that acute febrile illnesses are among the most common complaints in outpatient departments, knowledge on arboviruses including YFV is limited (14). Thus the aim of this study was to generate baseline data on the seroprevalence of YFV infection and associated risk factors in acute febrile patients visiting health facilities in the catchment area (Borena district, Oromia region, southern Ethiopia).

MATERIALS AND METHODS

Study design and site

A health facility based cross sectional study was carried out from May to August 2016 in Borena District. Three health facilities were included in the study, namely: Yabello Hospital, Moyale Hospital and Teltelle Health Center. The district is located in the southern part of Ethiopia, bordering Kenya. The climate of the district is semi-desert, the mean annual rain fall is 400-700 mm primarly from two rainy seasons (spring and autumn), and mean annual temperature ranges from 25-37^{o}C.

Study participants

All patients presenting with acute febrile illness at the outpatient departments of above mentioned health facilities during the study period were included in the informed consent. The sample size was estimated to be 519 assuming 9.2% for the prevalence of YFV (15); 3% precision in the estimate and 95% level of confidence.

Sample collection and Laboratory analysis

A pre-tested structured questionnaire was used to capture socio-demographic information. Venous blood was collected by trained health professionals at laboratory. Then the bloods samples were clotted and centrifuged at 1300 rpm (TDZ4-WS table top low speed centrifuge, Yorco). Separated sera were transported using liquid nitrogen (-196^{0}C) to Hawassa University Referral Hospital, and stored in a deep freezer (-80ºC). Sera were transported to Armaeur Hansen Research Institute (AHRI) in Addis Ababa using dry ice and screened for YFV IgG and IgM using EUROIMMUN IFT kit (Medizinische Labordiagnostika AG, Germany) according to the manufacturer's manual (16).

Data analysis

Data was double entered in REDCap data software (8.0.3.@2018, Vanderbilt University), and was analyzed using SPSS version 20 (Armonk, NY: IBM Corp).

The binary outcome variable was modeled using logistic regression. To control for possible effects of confounding, variables found to have an association with the outcome variable (P-value of 0.25) with bivariate analysis, they were entered into multivariable logistic regression model. Strength of associations between independent and outcome variables were summarized using odds ratio with corresponding 95% confidence intervals. P-value <0.05 was considered as indicator of a statistically significant association.

RESULTS

Demographic information

Out of 531 febrile patients approached, 12 were excluded 3 of whom reported a history of YF vaccination, and 9 of whom gave insufficient blood samples. Thus, 519 participants were enrolled: 39.7% from Teltelle Health Center, 36.6% from Moyale Hospital and 23.7% from Yabello Hospital. The mean age of the participants was 25.5 years (range 1 -80 years, standard deviation 15 years), and those in the age range of 15-24 years accounted for 32.4%. The proportion of female participants was 52%. A substantial proportion of the study participants were rural residents (53.6%), illiterate (60.9%), and farmers (33.9%).

Anti-YFV seropositivity segregated by socio-demographic characteristics

The overall IgG YFV seropositivity among study participants was 12.5%. IgG seropositivity of YFV was higher among males (15.7%) than among females (9.6%). Similarly, anti-YFV IgG positivity was highest (20%) among the elderly age group (above 65 years of age) and among urban residents (14.5%). (Table 1).

Overall seropositivity for anti-YFV IgM was 7.3%, higher in female subjects(8.8%) compared to male (6%) though this difference was not statistically significant (Table 2).

Furthermore higher anti-YFV IgG positivity was observed among patients recruited from Yabello (17.1%) and Moyale Hospitals (12.1%) whereas anti- YFV IgM positivity was higher in Teltelle Health Center though this did reach statistical significance (Table 3).

Table 1: Anti-YFV IgG seropositivity in relation to socio-demographic characteristics of participants (N=519) in Borena District, Southern Ethiopia, 2016.

Socio-demographic Characteristics	Number tested (%)	Number positive (%)	COR (95% CL)	AOR (95%CL)	P-value
Sex					
Male	249(48)	39(15.7)	1.74(1.02,2.95)*	1.69(1.01,2.91)*	0.04
Female	270(52)	26(9.6)	1	1	
Age (years)					
<5	29(5.6)	1(3.4)	0.14(0.01 ,1.51)	0.8(0.01,1.00)	0.06
6-14	74(14.3)	13(17.6)	0.85(0.21,3.45)	0.61(0.13,2.82)	0.52
15-24	168(32.4)	21(12.5)	0.57(0.15,2.19)	0.44(0.11,1.83)	0.26
25-34	136(26.2)	13(9.6)	0.42(0.10,1.69)	0.41(0.9,1.73)	0.23
35-44	51(9.8)	7(13.7)	0.64(0.14,2.84)	0.59(0.13,2.79)	0.51
45-54	28(5.4)	5(17.9)	0.87(0.17,4.27)	0.92(0.18,4.68)	0.92
55-64	18(3.5)	2(11.1)	0.50(0.07,3.48)	0.49(0.7,3.50)	0.48
>65	15(2.9)	3(20)	1	1	
Residence					
Rural	278(53.6)	30(10.8)	1.41(0.83,2.37)	0.87(0.45,1.68)	0.68
Urban	241(46.4)	35(14.5)	1		
Education level					
Illiterate	316(60.9)	34(10.8)	0.89(0.25,3.12)	1.07(0.24,4.81)	0.92
Primary	139(26.8)	20(14.3)	1.22(0.33,4.46)	0.99(0.23,4.32)	0.99
Secondary	39(7.5)	8(20.5)	1.89(0.45,7.95)	1.69(0.34,8.27)	0.51
College and above	25(4.8)	3(12)	1	1	
Occupation					
Farmer	176(33.9)	19(10.8)	0.89(0.24,3.24)	0.45(0.9,2.18)	0.32
Animal keeper	137(26.4)	15(10.9)	0.90(0.24,3.38)	0.47(0.10,2.21)	0.34
Employee	57(11)	7(12.3)	1.03(0.24,4.34)	0.54(0.10,2.93)	0.48
Student	67(12.9)	14(20.9)	1.94(0.51,7.42)	1.05(0.22,4.97)	0.95
Housewife	57(11)	7(12.3)	1.03(0.24,4.34)	0.79(0.15,4.26)	0.78
Others	25(4.8)	3(12)	1	1	

Table 2: Yellow fever virus IgM seropositivity in relation to age characteristics of participants (N=519) in Borena District, Southern Ethiopia, 2016.

Characteristics	Number(%) tested	Number(%) positive	COR(95% CL)	P-value
Sex				
Male	249(48)	16(6)	1	
Female	270(52)	22(8.8)	1.51(0.78-2.95)	0.16
Age (years)				
<5	29(5.6)	1(3.4)	1	
6-14	74(14.3)	8(10.8)	3.39(0.41-28.43)	0.26
15-24	168(32.4)	13(7.7)	2.35(0.29-18.67)	0.42
25-34	136(26.2)	9(6.6)	1.98(0.24-16.30)	0.52
35-44	51(9.8)	5(9.8)	3.04(0.34-27.41)	0.32
45-54	28(5.4)	2(7.1)	2.15(0.18-25.18)	0.54
55-64	18(3.5)	0(0)	0	
>65	15(2.9)	0(0)	0	
Residence				
Rural	278(53.6)	16(6)	1	
Urban	241(46.4)	22(8.8)	1.51(0.78-2.95)	0.65
Education level				
Illiterate	316(60.9)	21(6.7)	3.8(0.12-1.19)	0.10
Primary	139(26.8)	12(8.6)	0.49(0.14-1.67)	0.26
Secondary	39(7.5)	1(2.6)	0.14(0.01-1.32)	0.09
College and above	25(4.8)	4(16)	1	
Occupation				
Farmer	176(33.9)	11(6.2)	1.60(0.19-12.95)	0.66
Animal keeper	137(26.4)	10(7.3)	1.89(0.23-15.45)	0.55
Employee	57(11)	3(5.3)	1.33(0.13-13.48)	0.81
Student	67(12.9)	7(10.4)	2.80(0.33-23.99)	0.35
House wife	57(11)	6(10.4)	2.82(0.32-24.77)	0.35
Others	25(4.8)	1(4)	1	

Table 3: Seroprevalence of YFV, among study participants by health facilities in Borena district, Southern Ethiopia, 2016.

	YFV tested	YFV IgG		YFV IgM	
Study sites		Pos N (%)	COR95%CL	Pos N (%)	COR95%CL
Yabello	123	21 (17.1)	1.81 (0.95,3.48)	8 (6.5)	0.77 (0.33,1.85)
Moyale	190	23 (12.1)	1.21 (0.65,2.27	13(6.8)	0.82 (0.39,1.73)
Teltelle	206	21 (10.2)	1	17(8.3)	1

COR: Crude Odds Ratio AOR: Adjusted Odds Ratio

YFV associated factors

38.2% of the participants had heard about YFV, and 9.6% knew it is transmitted by mosquitoes. When asked about the environmental factors associated with mosquito-borne illnesses, 31.2% and 64.2% of participants respectively responded that the existence of stagnant water and trees nearby their dwelling areas were risk factors for exposure. 47.8% reported recent mosquito bites while they stayed outside during night time. Three hundred thirty (63.6%) reported they slept under mosquito nets, among these participants 20.2% and 41.4% stated they used bed nets consistently or sometimes respectively. However, only 4.1% used mosquito repellents during the day time or at night (Table 4).

Correlation between YFV seropositivity and its associated factors: YFV seropositivity was 15.7% in those who had heard about the virus and 24% in those who were aware that mosquitoes transmit the infection.

Equal positivity (14.9%) was observed among those who stated they stay out at night and those that used bed nets (Table 4). In bivariate analysis, a recent experience of having a mosquito bite (17.3%) and lack of knowledge on mode of transmission were the only factors that were significantly associated with YFV positivity. The association between recent mosquito bite and YFV infection was also statistically significant in a multivariable logistic regression analysis (AOR=3.34; 95% CI, 1.78-6.78, p=0.002). The association between lack of knowledge on mode of transmission and anti-YFV IgG seropositivity was of borderline significant (AOR= 3.01; 95%CI 0.65-1.09; p = 0.06) (Table 4).

Table 4: Anti-YFV IgG seropositivity in relation to participants knowledge about YFV and environmental risks of participants (N=519) in Borena District, Southern Ethiopia, 2016.

Characteristics	Number(%) tested	Number(%) sero-positive	COR (95% CL)	AOR(95% CL)	P-value
Heard about YFV					
Yes	198(38.2)	31(15.7)	1.57(0.93,2.64)	1.04(0.56,1.92)	0.91
No	321(61.8)	34(10.6)	1	1	
Mode of transmission					
Mosquito	50(9.6)	12(24)	2.45(1.20,4.98)	3.01(0.65,1.09)	0.06
By blood	14(2.7)	1(7.1)	0.59(0.08,4.65)	0.62(0.08,5.07)	0.66
Do not know	455(87.8)	52(11.4)	1	1	
Stagnant water					
Yes	162(31.2)	19(11.2)	1.11(0.63,1.97)	1.01(0.54,1.86)	0.99
No	357(68.8)	46(12.9)	1	1	
Trees around compound					
Yes	333(64.2)	44(13.2)	1.19(0.69,2.08)	0.95(0.51,1.78)	0.88
No	186(35.8)	21(11.3)	1	1	
Stay outside at night					
Yes	248(47.8)	37(14.9)	1.52(0.90,2.57)	1.33(0.74,2.39)	0.34
No	271(52.2)	28(10.6)	1	1	
Recent mosquito bite					
Yes	300(57.8)	52(17.3)	3.32(1.76,6.27)	3.34(1.78,6.78)*	0.002
No	219(42.2)	13(5.9)	1	1	
Bed net use					
Yes	330(63.6)	37(11.2)	0.89(0.52,1.50)	1.18(0.67,2.07)	0.58
No	189(36.4)	28(14.8)	1	1	
Repellent use					
Yes	21(4.1)	2(9.5)	0.73(0.16,3.19)	0.56(0.11,2.82)	0.48
No	498(95.9)	63(12.7)	1	1	

COR: Crude Odds Ratio AOR: Adjusted Odds Ratio *: significant at 95% CL

DISCUSSION

The prevalence of exposure to YFV as measured by anti-YFV IgG positivity among acute febrile patients in the study area was 12.5%. This is similar to reports from the Central African Republic (13.3%) (17) but lower than what was reported in Cameroon (26.9%) (18) or the Kenyan ocean coast (42%) (19). Similar lower prevalence rates have been reported in Kenya (6%), Uganda (7.5%), Nigeria (8.7%) and Guinea (2%) (20-23).

These discrepancies may be due to difference in the distribution of risk factors and variable climatic conditions by geographical regions, diversity of the studied populations, and difference in diagnostic performance of the employed laboratory methods. Moreover, lack of studies on the actual burden of YFV infection in the Ethiopian context limits comparison to studies in this country to gain perspectives on trends over time.

This study showed that gender significantly influenced the rate of YFV exposure status as measured by anti-YFV IgG levels where male participants were disproportionately infected. However, unlike this report, gender has not been found to be associated with YFV infection elsewhere (17, 19). Our findings of nearly equal risk across age groups,though somewhat more in the less sedentary age group (6-44 years), and urban setting might indicate a peridomestic type of transmission. Interestingly our data is in contrast to observations elsewhere where YFV infection showing increased exposure with age (17). Our relatively high anti-IgM and IgG seropositivity in our cohort may imply recent introduction and/or ongoing YFV transmission in our study settings.

In addition, self recall to recent mosquito bites was significantly associated with YFV seropositivity, collectively reinforcing our inference that the infection circulates in urban and rural settings and is likely that there is active ongoing peridomestic transmission in the study area. Overall, this study showed low awareness of YFV infection and its public health consequences among participants.

Thus, we recommend a community-based survey in the study and adjacent communities to verify our findings and take appropriate public health measures to avoid potential outbreaks. Studies are urgently needed to determine the environmental and host factors that determine the extent of exposure to YFV infection in the district for appropriate control and prevention planning.

Limitation of the study

Although, EUROIMMUN IFT was claimed to be highly specific, to our knowledge no data exist to rule-out cross reactivity with other flaviviruses in an endemic setting. Moreover, we have no afebrile community controls or convalescent sera, and as any health institution based study we used consecutive volunteering cases only there for, the risk of introducing bias is unavoidable. Thus, the findings of this study may not be generalized to the population in the study area.

Ethical Consideration

Ethical approval was obtained from Hawassa University College of Medicine and Health Sciences (IRB/006/08 date 27/10/2015), Oromia Regional Health Bureau (BEFO/1-8/3998 date 18/11/2015) and AHRI/ALERT. Written informed consent was sought from participants or parents/guardians in the case of minors. In addition, assent was obtained for minors between the age of 11 and 18 years.

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Conflict of Interest:

Authors have no conflict of Interest to declare.

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