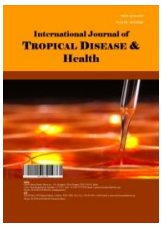

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First Evidence of West Nile Virus in Hodeidah, Yemen: Clinical and Epidemiological Characteristics

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Authors' contributions

This work was carried out in collaboration among all authors. Authors QY and AAM designed, wrote the protocol and managed the literature searches. Author AAA collected the data and analyzed the sample. Authors IAM and MAAK performed samples analysis, statistical analysis, wrote the first draft of the manuscript and managed the analyses of the study. Author ASK edited the article, made critical revisions and gave final approval of the version to be published All authors read and approved the final manuscript.

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ABSTRACT

Introduction: West Nile Virus (WNV) infection is an important arthropod-borne zoonosis viral disease. This virus is neglected in Yemen especially in Hodeidah.
Aim of the Study: The purpose of this study was to detect WNV infection, determine the epidemiological and clinical characteristics within febrile patients in Hodeidah city and to determine some risk factors associated with WNV infection.
Materials and Methods: 136 febrile patients in a hospital base study were diagnosed in Center of Tropical Medicine and Infectious Diseases (CTMID), Authority of General Al-Thawara Hospital,

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Hodeidah, Yemen from January of 2017 to December of 2017. WNV infection was detected by enzyme linkage immune sorbent assay (ELISA) on serum samples.

Results and Discussion: The results showed that 5 cases (3.67%) were WNV – positive namely IgM that was detected in winter and spring seasons, the most prevalent antibodies of WNV were IgG namely 75 cases (55.14%). Most common symptoms were fever, headache, fatigue, weakness, arthralgia, myalgia and photophobia. The treatment based on the intravenous therapy (IV) with anti-pyritic, plasma in some cases and all cases were recovered while mortality rate was 00%.

Conclusion: WNV was detected in Hodeidah which placed in Tehama "western Yemen", as first time by our preliminary study that confirmed the evidence of WNV IgM and IG antibodies presence on 2017, in order to increase safety of diagnosis of febrile diseases, it is essential to continue surveillance of this emerging infection, suggesting that this emergence has been transported by migratory birds from wintering areas to Tehama region.

Keywords: West Nile virus; Hodeidah; Yemen.

1. INTRODUCTION

West Nile virus (WNV) is an enveloped, icosahedral, positive-sense, single stranded RNA virus with a linear segmented genome of the family Flaviviridae, genus Flavivirus. It is a member of the Japanese encephalitis virus serocomplex, which involves several medically important mosquito-borne viruses that are the prominent causes of arboviral encephalitis in birds and vertebrate hosts, including humans equine, and avian [1-3]. WNV is mainly transmitted by *Culex pipiens* mosquitoes which serve as the primary enzootic vector [4] and passerine birds which serve as the amplification hosts of the virus [5,6]. WNV transmission is maintained by zoonotic cycles in nature comprising mosquitoes, birds and a sum of other vertebrates as incidental hosts such as horses and humans. Infected birds are the main reservoir of WNV; more than 200 species of birds have been found to be infected by the virus in the western hemisphere [7]. The intensity of transmission of WNV to human depends on the abundance and feeding patterns of infected mosquitoes, local ecology and behavior that influence human exposure to mosquitoes [8]. Only about 1 in 5 human infections are symptomatic but up to 1 in 150 develop severe illness from neuroinvasive disease [9]. The more common symptoms are fever, rash, muscle aches, and gastrointestinal complaints [10,11].

West Nile Virus has been widely reported through the world and in some Middle East countries starting from Egypt on 1950s [12], to Sudan on 1940s [13] and then Jordan, Lebanon, Saudi Arabia and UAE [14-18]. Outbreaks of WNV have been reported largely in Greece, Israel, Romania, Russia, and the USA. Outbreak sites are on major bird migratory routes [3]. In its original range, WNV was prevalent throughout Africa, parts of Europe, Middle East, West Asia, and Australia. Since its introduction in 1999 into the US, the virus has spread and is now widely established from Canada to Venezuela [6].

WNV infection is still quite unclear and not well documented in Yemen. This preliminary study was undertaken to identify evidences of WNV infection among febrile patients attending some hospitals in Hodeidah city to determine whether the emerging infection is a possible health risk in Tehama, western Yemen.

2. MATERIALS AND METHODS

2.1 Study Area

Hodeidah city is the local coastal capital of the fourth largest governorate in Yemen, "Hodeidah governorate". It is located on a flat and narrow coastal plain between the foothills of the highlands and the Red Sea known as Tehama; the area is 181 km^2, involves three districts (Hok, Hali and Mina), with a population of 415,283. The female: male ratio is 1:1 and children below 15 years comprise 50% of population [19]. This region is known for its rich biodiversity of birds and mosquitoes including Passers and *Culex* species, respectively. Tehama also is considered a major migratory zone for a number of bird species on their long journey between their breeding grounds in Asia and their wintering areas in Africa. The main hospitals and health centers in Hodeidah city were chosen as sample collection sites.

2.2 Study Population and Design

A hospital base study was performed among hospitalized febrile patients namely an acute

systemic febrile illness that may include headache, myalgia, arthralgia, rash, or gastrointestinal symptoms. All cases admitted were collected from some hospitals and health centers in Hodeidah city from the period of January of 2017 to December of 2017 [19].

2.3 Data Collection

Data were collected using a specially-designed questionnaire including demographic data, symptoms, some risk related data, income status, house structure and educational level.

2.4 Sample Collection

Five ml of blood was collected in plain tubes, from each consenting subject by venipuncture, transferred into anticoagulant-free sterile bottle, and allowed to clot. Then centrifuged (3000 rpm, 5 min), and the serum was transferred into two separate Eppendorf tubes [17]; one was the original sample and the second was the backup sample. Serum samples were then stored at (-20°C). Twenty-four hours prior to performing the serological assay, samples were thawed in a refrigerator overnight at 4°C.

2.5 Serological Assay

Sera were analyzed for West Nile virus IgM and IgG by ELISA using commercial diagnostic kits (DRG, USA) according to the manufacturer's instructions. The reading was accomplished by imark microplate reader in 450 nm. The validation of controls check was performed. On the other hand , sera samples were analyzed for dengue virus IgM and IgG by enzyme-linked immunosorbent assay (ELISA) technique using commercial diagnostic kits (Calbiotech, USA) to differential WNV from dengue because the area is endemic for dengue infection [20–24].

2.6 Statistical Analysis

The data were entered and analyzed using IBM SPSS version 21. Chi square was performed at a 95% confidence interval and a significance level of 0.05 was used to determine the relationships between data collection and seropositive samples; *P* values of 0.05 or less was considered to be significant. Odd ratio was performed to assess associated risk factors.

3. RESULTS

3.1 General Characteristics of Patients

Serum samples were collected from 136 febrile patients attending Authority of General Al-Thawra hospital, Al-Salakana hospital, Al-Alufi hospital and Al-Askari hospital. The patients ranged in age from 1.5 to 80 years old with a median age of 23 years. The majority of study participants resided in were living in urban areas (76.5%) and had limited means (75.7%); only (42.6%) of them had a primary education. Most of them live in random houses (78.7%) (Table 1).

Table 1. General characteristics of patients (*n* = 136)

Personal data	Number	Ratio (%)
Age group (years)		
≤7	12	8.8
8-18	31	22.8
19-50	87	64.0
>50	6	4.4
Gender		
Male	95	69.9
Female	41	30.1
Educational level		
Illiterate	31	22.8
Primary	58	42.6
Secondary	43	31.6
University	4	3
Residency		
Urban	104	76.5
Rural	32	23.5
Economic level		
Moderate	33	24.3
Poor(low)	103	75.7
House structure		
Random	107	78.7
Apartment	29	21.3

WNV IgM was detected in 5 (3.7%) of the study participants and IgG from 75 (55.1%). WNV infection according to the general characteristics of patients are shown in Table 2. Males were more likely to have WNV infection than females in 69.3%, while this difference was not statistically significant (P = 0.884). However, patients aged from 8-18 were more susceptible to have recent infection of WNV in 60% of cases, previous WNV infection was high in patients ranging from 19-50 in 54.6% with a significant statistically difference (*P*=0.057). Participants who live in urban areas represented 64 (85.3%) of WNV old infection with a significant association (*P*=0.007). Moreover, participants with low economic level were more susceptible to have WNV IgG antibodies 73.3%. The higher frequency of WNV antibodies was noticed in people who live in random houses as shown in the same table.

To differential diagnosis of WNV from dengue [20], because the area is endemic for dengue infection. The samples were tested against anti-dengue IgM and IgG antibodies before testing for WNV antibodies, results were recorded and clarified in Table 3.

3.2 Symptoms of WNV Infection

Symptoms of WNV infection were sequencing from fever (3.7%), myalgia (3.3%), photophobia (3%) headache (4%), fatigue (4.1%), arthralgia (3.2%) and weakness (2.3%) from all WNV IgM positive cases shown in Table 4.

3.3 Risk Factors Associated with WNV Infection

Some associated risk factors were studied evaluating the emergence of WNV infection, mosquito breeding sites such as bogs, tires, opened drums, wells, opened water containers, trash and sewages, they were shown to associate the presence of WNV IgM and IgG antibodies mostly wells and sewages in high percentages of 58.8% and 60%, although they have not shown a significant association with WNV infection clarified in Table 5. Another risk factor that was strongly and statistically significant with WNV infection was mosquito abundance (P = 0.000) and OR of 3.714 (1.82 - 7.59) in a confidence Interval of 95%.

3.4 Seasonality of WNV Infection

Seasonality of WNV infection as four seasonal distribution, WNV was found through the whole year. However, winter and spring seasons were in the cases emerged namely 3 cases in winter and 2 cases in spring having recent infection of WNV in Table 6; on the other mean, the cases emerged during December, February, March and April (Fig. 1).

Table 2. Ratio of WNV infection and characteristics of patients in Hodeidah, Yemen

Patients characteristics	Number of IgG positive (%)	*P*- value	Number of IgM positive (%)
Age group (years)			
≤7	10(13.3)	0.057	2(40.0)
8-18	20(26.6)		3(60.0)
19-50	41(54.6)		0(0.00
>50	4(5.3)		0(0.0)
Gender			
Male	52(69.3)	0.884	3(60)
Female	23(30.7)		2(40)
Educational level			
Illiterate	19(25.3)	0.479	2(40)
Primary	33(44.0)		3(60)
Secondary	20(26.7)		0(0.0)
University	3(4.0)		0(0.0)
Residency			
Urban	64(85.3)	0.007	4(80)
Rural	11(14.7)		1(20)
Economic level			
Moderate	20(26.7)	0.469	4(80)
Poor(low)	55(73.3)		1(20)
House structure			
Random	61(81.3)	0.402	4(80)
Apartment	14(18.7)		1(20)

Table 3. Differential diagnosis of WNV infection from with dengue virus infection (*n*=136)

Dengue virus antibodies	Total		WNV positive IgG		WNV positive IgM	
	Number	%	Number	%	Number	%
Dengue virus positive IgG	58	42.6	26	44.8	1	1.7
Dengue virus positive IgM	25	18.4	15	60.0	2	8.0

Table 4. Symptoms of WNV infection (*n*=136)

Symptoms	WNV positive IgM		Total	
	No	%	No	%
Fever	5	3.7	136	100
Headache	5	4.0	124	91.2
Fatigue	4	4.1	98	72.0
Weakness	1	2.3	43	31.6
Myalgia	4	3.3	120	88.2
Photophobia	3	3.0	101	74.2
Arthralgia	3	3.2	92	67.6

4. DISCUSSION

The data we presented were the first serological evidence of WNV existence in Hodeidah city/ western Yemen, our results were close to a study that performed in Hadramoat by Qassem & Jawaal 2014, we found that WNV IgG antibodies present in about 75(55.1%) and WNV IgM antibodies were found in 5 (3.7%), their findings showed that WNV IgM antibodies were 2(5%) [25].

People aged from 8-18 years old were more likely to have WNV recent infection (60%), while people aged from 19-50 years old were more likely to have WNV old infection (54.6%), our findings concurred with a study in Egypt [26].

No significant association was observed between West Nile virus infection and gender, although males were more susceptible to have WNV infection, our result agreed with a study in Zambia [27].

Subjects who had attained primary level of education were (44%) more likely to have WNV infection compared to participants who had attained higher level of education, this finding has agreed with a study in Arizona [28].

Moreover, local movements of resident birds and long range travel of migratory birds may both contribute to the spread of WNV [29]. Various studies have provided indirect evidence that WNV is transported by migratory birds, especially via their migration routes from breading areas of Europe to wintering areas in Africa [30,31].

WNV is neglected by health authorities in Yemen, Although many indications of its presence occur. It has only payed attention in Hadramoat [25]. No previous studies have examined the seroprevalence of WNV antibodies in the human population in Tehama, although no clinical reports of infections have been made. Screening for the WNV seroprevalence is very

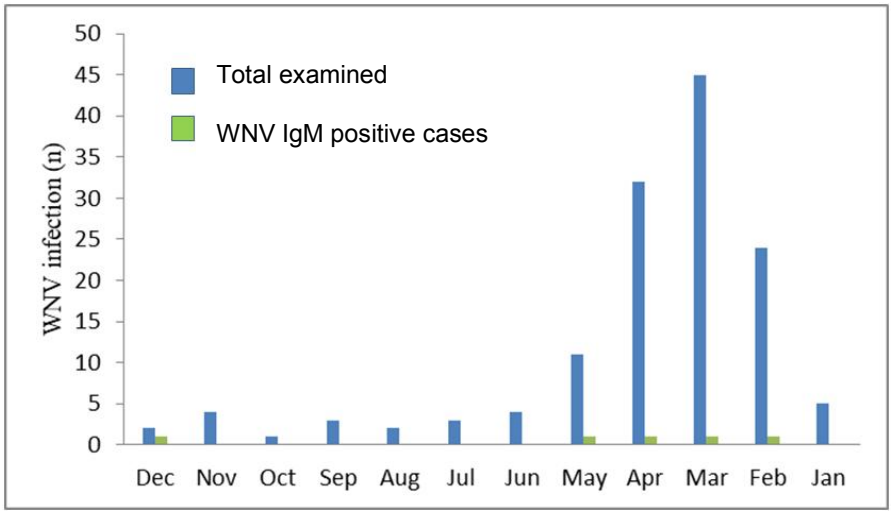

Fig. 1. Monthly seasonality distribution of WNV antibodies among suspected febrile patients in Hodeidah city, Yemen

Table 5. WNV infection with some risk factors associates the infection (*n*=136)

Associated risk factors	WNV positive IgG		Total		*P*-value	OR with 95% CI	WNV positive IgM	
	Number	%	Number	%			Number	%
Bogs	18	54.5	33	24.2	0.936	0.968 (0.441-2.129)	1	3.0
Tires	18	50.0	36	26.4	0.469	0.754(0.351-1.619)	2	5.6
Opened drums	20	58.8	34	25.0	0.619	1.221(0.556-2.680)	0	0.0
Wells	3	60.0	5	3.6	0.597	1.229(0.199-7.601)	5	100
Opened water containers	45	60.0	79	58.0	0.616	1.191(0.601-2.362)	3	3.8
Sewages	60	58.8	102	75.0	0.135	1.810(0.827-3.961)	4	4.0
Trash	40	54.0	74	54.4	0.779	0.908(0.460-1.790)	4	5.4
Mosquito abundance	53	68.8	77	56.6	0.000	3.714(1.817-7.591)	5	6.5

Table 6. Seasonality of WNV infection among febrile patients (*n*=136)

Seasonality	WNV positive IgM		Total	
	Number	%	Number	%
Winter	3	4.8	63	46.3
Spring	2	3.6	56	41.2
Summer	0	0.0	9	6.6
Autumn	0	0.0	8	5.9
Total	**5**	**3.67**	**136**	**100**

important because of many risk factors associate the presence of WNV in Tehama, such as the abundance of *Culex* mosquitoes as the main vector and a high degree of biodiversity, including migratory birds that serve as a natural reservoir of the virus, besides it is a tropical and temperate region.

Our findings indicate a strongly significant association with mosquito abundance and WNV infection and a possible risk factor which confirms that mosquitoes are the main risk of emerging WNV in the area. Sewages, tires and trash's distribution in Hodeidah city helps in spreading of the infection though they are proper habitat of mosquitoes to live in.

Bad water supply in this area increases the risk of WNV circulation, most people live in random houses and suffer from water intake so they keep water in containers which sometimes left opened and are a suitable environment for breading of mosquitoes. Besides the climatic nature of Tehama which makes it a temperate region and suitable for bird migration from wintering places, migratory birds contribute in the circulation of WNV from country to another specially that they are the main reservoir hosts of the virus.

Males have showed to be infected by WNV more than females because they spend most of their times outdoors which makes them more likely to be bitten by mosquitoes also they wear opened clothes not like females who go out quietly covered and less likely for mosquitoes exposure. Single participants were infected more than married ones and this may refer to mosquito exposure because they spend longer time in outdoors than those who have families inside, this status showed a significant association with WNV infection.

5. CONCLUSION

Our preliminary study carried out the first report indicating the presence of WNV infection among human in Hodeidah city and the results provided evidence of WNV circulation in the western region of Yemen. Moreover, the epidemiological importance of our findings should be considered in light of other parameters such as climatic, ecological patterns, birds and mosquitoes conditions are all favorable for WNV emergence in the area. Furthermore, longer scale studies are necessary to evaluate the possible risks of WNV infection in Yemen, also protection strategies against mosquitoes by equipping the area with programs of vector-born-diseases control and to increase the level of awareness in diagnosing viral diseases.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethics statement of the study acknowledged distinctive consent from committee of Biology Department, Faculty of Sciences, attained approval from the Ethics Committee of Sana'a University and of Center of Tropical Medicine and Infectious Diseases Center (CTMID), Authority of General Al-Thawra Hospital, Hodieah, Yemen.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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