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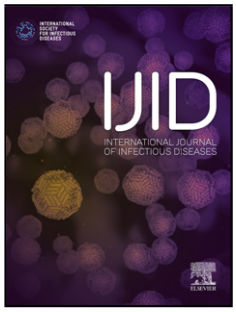

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Seroprevalence of West Nile Virus among Healthy Blood Donors from Different National Populations Residing in Qatar

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HIGHLIGHTS

- Prevalence of WNV IgG ranged from 0.9% in Lebanese to 37.0% in Sudanese.
- Prevalence of WNV IgM ranged from 0.0% in few nationalities to 10.0% in Sudanese.
- WNV is circulating in humans in different Middle East and North Africa countries.
- Seroprevalence appears highest in Sudan and Egypt and lowest in Qatar and the Levant.

1. ABSTRACT

Objective: To estimate the age- and nationality-specific West Nile virus (WNV) seroprevalence in select Middle East and North Africa (MENA) populations residing in Qatar.

Methods: Sera were collected from male blood donors attending Hamad Medical Corporation. A total of 1,948 sera were tested for anti-WNV antibodies using Serion ELISA classic IgG and IgM kits.

Results: Overall, seroprevalence estimates of WNV-specific IgG and IgM antibodies were 10.4% and 3.3%, respectively. Country-specific WNV-specific IgG seroprevalence was estimated to be 37.0% (34/92) in Sudanese, 33.0% in Egyptians (66/200), 13.0% (26/200) in Indians, 10.6% (11/104) in Iranians, 10.2% (14/137) in Yemenis, 9.2% (18/195) in Pakistanis, 7.0% (14/199) in Jordanians, 5.4% (6/111) in Filipinos, 2.5% (5/200) in Palestinians, 2.5% (5/200) in Syrians, 1.5% (3/200) in Qataris, and 0.9% (1/110) in Lebanese. Seroprevalence of WNV-specific IgM was lowest in Iranians (0/77), Lebanese (0/108), and Filipinos (0/107) at 0.0%, and was highest in Sudanese at 10.0% (8/80). While there seemed to be apparent trends in the prevalence of WNV-IgM and WNV-IgG antibodies, none of these trends were found statistically significant.

Conclusion: The findings support the circulation of WNV in human populations in the different countries of the MENA region. Seroprevalence was highest in Sudanese and Egyptians and lowest in Qataris and nationals of the Levant. The findings call for further animal, vector, and human studies, such as studying the actual prevalence of the viral RNA in blood donors to assess risk of

viral transmission through blood donation and for a better characterization of the epidemiology of this infection in this part of the world.

KEYWORDS: Arbovirus; prevalence; Vector-borne disease; Mosquito; West Nile Fever; Zoonosis; Qatar

2. INTRODUCTION

West Nile virus (WNV) was first isolated in 1937 in the West Nile district of Uganda and subsequently was found to cause outbreaks in Africa, Middle East, and western Asia (Chancey et al., 2015, Gould and Fikrig, 2004, Hayes et al., 2005b). Since then, outbreaks were also reported in Europe and North America in the 1990s (Chancey et al., 2015, Gould and Fikrig, 2004, Hayes et al., 2005b). Evidence shows that the circulation of WNV is primarily preserved in a zoonotic transmission cycle between infected birds and mosquitoes, mainly the *Culex* species, which is distributed around the world (Chancey et al., 2015, Hayes et al., 2005b, Paz, 2015). Nonetheless, other modes of transmission have been recognized: breast-feeding, blood transfusion, organ transplantation, and occupational exposure among laboratory workers (Hayes et al., 2005a, Kramer et al., 2008, Sampathkumar, 2003).

In humans, 80% of WNV infections are asymptomatic, 20% develop a flu-like illness, and less than 1% go on to develop neurologic disease, which predominates in the elderly and immunocompromised (Pealer et al., 2003). Symptomatic disease is mainly characterized by mild febrile illnesses, such as headaches, nausea, and/or rashes (Gould and Fikrig, 2004, Hayes et al., 2005b, Pradier et al., 2012). Neurologic disease, which can be severe, can manifest as neurologic inflammation-causing viral encephalitis, meningitis, and/or seizures (Gould and Fikrig, 2004, Hayes et al., 2005b, Pradier et al., 2012).

With infected birds and mosquitoes being the reservoir and vector for the transmission of WNV infections, factors related to climate and environmental conditions impact the dynamics of this pathogen-reservoir-vector relationship (Chancey et al., 2015, Kramer et al., 2008, Paz, 2015). Mild winters followed by drought have been associated with WNV (Chancey et al., 2015, Hayes et al., 2005a, Paz, 2015). Longer durations of spring temperature and wind patterns affect bird

migration, whereas extensive rainfall and humidity have been found conducive to mosquitoes breeding sites (Chancey et al., 2015, Paz, 2015). In view of the extent of geographical dispersal and the large vector/host range, WNV continues to circulate worldwide, and specifically in Africa, Middle East, and western Asia (Hayes et al., 2005a, Petersen and Roehrig, 2001).

Recent data on the epidemiology of WNV in the Middle East and North Africa (MENA) region are limited. Qatar, a Middle Eastern peninsular country bordering the Arabian Gulf with a 2.8 million population size, has an expatriate population that constitutes 88% of the total population (Qatar Ministry of Development Planning and Statistics, 2015). Although Qatar's climate features sporadic rainfall and high temperature, a large proportion of the expatriate population originates from WNV-endemic countries with environmental conditions contributing to WNV transmission, including Iran, India, Egypt, and Sudan (Kardousha, 2015, Qatar Ministry of Development Planning and Statistics, 2015). Qatar is also a geographic convergence of Africa, Middle East, and western Asia for global travel and importation of goods and animals (Haroun et al., 2017). The growing activity of buying and trading wild birds and the annual regional and international equine competitions may increase the circulation of WNV in Qatar, thus posing a possible public health challenge. Against this background, this study reports the estimated age- and nationality-specific WNV antibody prevalence (seroprevalence) in healthy blood donors residing in Qatar.

3. METHODS

3.1 Study design

This cross-sectional study consisted of data collected on volunteer blood donors who participated in national blood donation campaigns at Hamad Medical Corporation (HMC) in Qatar,

a common practice among both Qatari nationals and resident expatriates of varying socioeconomic strata. The study sample was collected previously and used in other studies (AbuOdeh et al., 2015, Al-Qahtani et al., 2016, Dargham et al., 2018, Nasrallah et al., 2020, Nasrallah et al., 2017, Nasrallah et al., 2018) between June 2013 and June 2016 and consisted of blood specimens and basic demographic data on nationality and age from blood donors. Participants in these specific blood donation campaigns were men, as these campaigns were targeting men. All specimens were de-identified at the blood donation facility and provided to the study investigators. This research work was approved by the ethics boards at Qatar University, HMC, and Weill Cornell Medicine-Qatar.

The study sample consisted of Qatari and expatriate blood donors aged 18 years and above (n=1,948) from different origin countries, including both MENA and non-MENA nationals. The origin countries included Egypt (N=200), India (N=200), Iran (N=104), Jordan (N=199), Lebanon (N=110), Pakistan (N=195), Palestine (N=200), Philippines (N=111), Qatar (N=200), Sudan (N=92), Syria (N=200), and Yemen (N=137). An effort was made to include equal samples from these origin countries as feasible. Serological testing was conducted to detect both WNV-specific IgG and IgM antibodies.

3.2 Laboratory analysis

A total of 10 µL of each patient's serum was used for testing for the presence of WNV-specific IgG and IgM. The testing was carried out using commercial microplate Enzyme-Linked Immunosorbent Assay (ELISA) kits, Virion\Serion Diagnostic ELISA classic IgG (Cat. No. ESR141G, Germany) and IgM (Cat. No. ESR141M, Germany). These kits are qualitative and quantitative for the detection of human antibodies to WNV present in the serum.

In the WNV-IgG ELISA kit, the microwell plates were pre-coated with two specific WNV polypeptide antigens (prepared from the WNV protein M and E) and the specimens were tested following the manufacturer's instructions. The testing procedure to detect WNV-specific IgM antibodies is identical to that of IgG detection except that the specimens are pretreated with rheumatoid factor absorbent (RFA) during the preparation of specimen dilutions. In both cases, after the addition of stopping solution, the optical density (OD) is measured at 405 nm using BioTek Epoch 2 Microplate Reader. Once the ODs recorded, the results are interpreted, according to the algorithm (automated excel sheet) provided by the company, as positive, negative, or borderline. For IgG, sera with OD index values <11 U/ml were considered negative, values >15 U/ml were considered positive, and values ranging between 11 and 15 U/ml were considered borderline (SERION Diagnostics, 2020). For IgM, sera with OD index values <10 U/ml were considered negative, values >15 U/ml were considered positive, and values ranging between 10 and 15 U/ml were considered borderline (SERION Diagnostics, 2020).

WNV Serion ELISA IgM and IgG kits are CE-marked for *in vitro* diagnosis. According to the manufacturer (SERION Diagnostics, 2020), the Serion ELISA WNV IgM has >99% sensitivity and specificity, while the WNV IgG kit reported 96.2% specificity and 92.6% sensitivity (SERION Diagnostics, 2020). However, cross-reactions with other flavivirus antibodies (e.g. dengue) are well known to occur (Ergunay et al., 2010, Lustig et al., 2018, Mansfield et al., 2011).

3.3 Statistical analysis

Age was categorized into eight five-year cohorts: ≤24, 25-29, 30-34, 35-39, 40-44, 45-49, 50-54, and ≥55. Borderline results were considered negative. Overall, age-specific, and nationality-specific seroprevalence measures of WNV-specific IgG and IgM were estimated. Chi-square tests (or Fisher's exact test when expected cell counts fell below 5) were performed to assess

the crude associations of WNV-specific IgG and IgM with age and nationality. Logistic regressions were conducted to estimate and report crude odds ratio (OR) and their 95% confidence intervals (CI). Significance level was defined at $\alpha = 0.05$. Data was analyzed using the Statistical Package for the Social Sciences (SPSS) version 25.

4. RESULTS

A total of 1,948 specimens from male blood donors residing in Qatar were included in this study. All specimens were tested for WNV-specific IgG; 203 specimens were identified as positive, 94 as borderline, and the remaining as negative. With insufficient serum for 130 specimens to conduct the IgM serological testing, only 1,818 specimens were tested for WNV-specific IgM; 60 specimens were identified as positive, 63 as borderline, and the remaining as negative. The estimated overall seroprevalence of WNV-specific IgG and IgM antibodies were 10.4% (95% CI 9.1%-11.9%) and 3.3% (95% CI 2.6%-4.2%), respectively.

Table 1 presents the age-specific seroprevalence estimates of WNV-specific IgG and IgM antibodies. Seroprevalence of WNV-specific IgG ranged between 8.0% (95% CI 5.8%-11.1%) among those aged 30-34 years and 13.3% (95% CI 10.0%-17.6%) among those aged 25-29 years. Seroprevalence of WNV-specific IgM ranged between 1.2% (95% CI 0.2%-6.5%) among those aged ≥55 years and 5.1% (95% CI 3.3%-7.8%) among those aged 30-34 years. No significant association or trend was found between age and WNV-specific IgG (p-value = 0.415) or IgM (p-value = 0.155) antibodies.

Table 2 and Figure 1 present the nationality-specific seroprevalence estimates of WNV-specific IgG and IgM antibodies. Seroprevalence of WNV-specific IgG was lowest among Lebanese at 0.9% (95% CI 0.2%-5.0%) and highest among Sudanese at 37.0% (95% CI 27.8%-

47.2%). Seroprevalence of WNV-specific IgM was lowest among Iranians at 0.0% (95% CI 0.0%-4.8%), Lebanese at 0.0% (95% CI 0.0%-3.4%), and Filipinos at 0.0% (95% CI 0.0%-3.5%), and was highest among Sudanese at 10.0% (95% CI 5.2%-18.5%). The results identified a statistically-significant association among nationality and WNV-specific IgG (p-value < 0.001) and IgM (p-value < 0.001) antibodies.

5. DISCUSSION

West Nile virus is one of the most common and widespread arboviruses in the world, and yet its recent epidemiology in Qatar and MENA countries is poorly characterized. To our knowledge, this is the largest study of WNV seroprevalence in MENA, in which age- and nationality-specific seroprevalence measures are reported. Overall, seroprevalence estimates of WNV-specific IgG and IgM antibodies among male blood donors residing in Qatar were 10.4% and 3.3%, respectively.

Despite an apparent decreasing trend in the prevalence of WNV-IgM antibodies with age and an apparent increasing trend in the prevalence of WNV-IgG antibodies with age, none of these trends were found statistically significant. On the other hand, a significant association was observed between WNV seropositivity and nationality, indicating evidence of variation in WNV infection exposure by nationality. Seroprevalence was highest in Sudanese and Egyptians, both originating from Nile valley countries, consistent with a higher level of exposure in these countries (Eybpoosh et al., 2019) and supporting evidence that indicated major outbreaks as early as 1950s in this regions (Taylor et al., 1956); and seroprevalence was lowest in Qatar and nationals of the Levant, consistent with lower exposure in these countries (Eybpoosh et al., 2019).

These findings are similar to the findings of an anti-dengue and anti-chikungunya antibody seroprevalence survey we previously conducted (Humphrey et al., 2019). The same samples tested in this study were earlier also tested for anti-dengue and anti-chikungunya (Humphrey et al., 2019). Comparing the individual-level results for WNV and dengue indicated a positive percent agreement of 51.0% (99/194; 95% CI 44.0%-58.0%) and a negative percent agreement of 79.4% (1346/1696; 95% CI 77.4%-81.2%). Comparing the individual-level results for WNV and chikungunya indicated a positive percent agreement of 7.2% (14/194; 95% CI 4.4%-11.8%) and a negative percent agreement of 96.2% (1631/1696; 95% CI 95.2%-97.0%). Co-circulation of multiple arboviruses likely explains these similarities, as well as serologic cross-reactions among flaviviruses (e.g. dengue, Zika) (Ergunay et al., 2010, Humphrey et al., 2019, Lustig et al., 2018, Mansfield et al., 2011). Overall, our results suggest the circulation of WNV in human populations in the different countries of the region and argue for further human, animal, and vector studies for a better characterization of the epidemiology of this arbovirus in this region.

The observed nationality-specific WNV seroprevalence levels were overall consistent with those previously reported in the literature in the respective countries, as detailed recently in a systematic review of the epidemiology of WNV in the MENA region (Eybpoosh et al., 2019). However, the seroprevalence levels reported here tend to be lower than those previously published (Eybpoosh et al., 2019). The recent systematic review also noted that several countries had no published data on WNV infection. As such, the nationality-specific WNV seroprevalence measures reported herein provide, for the first time, preliminary insight into the potential epidemiology of WNV of several countries in the MENA region, including Palestine, Qatar, Syria, and Yemen.

Although Qatar's climate is described as a very hot summer and a mild winter with few rainfalls, some areas are noted for surface depressions that are filled with run-off water, conducive for mosquito breeding sites (Kardousha, 2015). A published study reported that Qatar's north-eastern area has witnessed an increase in the number of reported *Culex* mosquitoes and has reported that *Cx. pipiens* molestus and *Cx. quiquefasciatus*, primary vectors of WNV, were the most prevalent larvae (Kardousha, 2015). The presence and increase of these mosquito populations in Qatar constitute a risk of a potential outbreak, highlighting the need of establishing surveillance capacity in Qatar and the broader MENA region for effective monitoring of WNV infection circulation.

Furthermore, WNV has become a growing concern in blood transfusion in the United States and Europe, but blood donations and organ donations in the United States are now screened for WNV RNA (Pealer et al., 2003, Pisani et al., 2016). In the present study, WNV-IgM antibodies were detected in 3.3% of the sampled population, suggesting the possibility of viremia and acute infection of the asymptomatic donors. Therefore, blood banks in Qatar might need to consider screening for WNV, particularly when transfusion is intended to immunocompromised patients. For better safety protocols of blood transfusion, further studies are warranted to look at the actual prevalence of the viral RNA among blood donors to estimate the risk of viral transmission through blood donations.

There are limitations in this study. Firstly, we were unable to confirm the antibody test results with neutralization testing due to resource constraints. Thus, we cannot exclude the possibility of cross-reactivity with other flaviviruses. The sample, restricted to male blood donors, may not be representative of the overall population due to possible selection bias. The nationality-specific WNV seroprevalence estimates may not reflect the estimates in their respective origin

countries, even though the majority of the migrants are recent and typically only temporary residents in Qatar. Having said so, seroprevalence studies on herpes viruses (infected/transmitted by different transmission modes) on this same sample have shown that the seroprevalence levels reflect the country of origin, and not the seroprevalence in Qatar (Dargham et al., 2018, Nasrallah et al., 2020, Nasrallah et al., 2018). Finally, the dataset only included basic socio-demographic variables, preventing the assessment of relevant associations such as with employment, location of residence, recent travel, and/or clinical symptoms. No data were also available for the duration of residence, and thus we could not generate inferences about the local transmission if any.

In conclusion, this study fills an epidemiologic knowledge gap on the overall, age- and nationality-specific WNV seroprevalence in MENA. Nonetheless, there is still a paucity of information and data on WNV infection and burden in several countries within the region. At present, the likelihood of eliminating WNV globally is scant. The broad range of the pathogen, reservoir, and vector ensures its extensive ecological widespread. As a result, WNV transmission will be difficult to prevent considering the inadequate logistics/infrastructure and the WNV circulation in human, animal, and vector populations.

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COMPETING INTERESTS

The authors have no conflicts of interest to disclose.

CONTRIBUTORS

GKN, SRD, and LJA designed the study and developed the research methodology. GKN provided the specimens. GKN and HMY led the laboratory component of this study including testing of all specimens. DWA, MA, and HK performed the laboratory testing and data documentation. HMY, GKN, and LJA provided the resources. SRD conducted the data analysis, interpreted the results, and wrote the first draft of the study. LJA led the data analysis and interpretation of the results. JMH critically reviewed and edited the manuscript. All authors contributed to the interpretation of the results, drafting of the manuscript, and revision of the article.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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TABLES and FIGURES

Table 1: Age-specific seroprevalence estimates of WNV IgG and IgM antibodies among male blood donors residing in Qatar

	WNV IgG				WNV IgM			
Age group	**+/N**	**Prevalence (95% CI)**	**OR (95%CI)**	**P-value**	**+/N**	**Prevalence (95% CI)**	**OR (95%CI)**	**P-value**
<=24	13/159	8.2 (4.8-13.5)	Ref		4/149	2.7 (1.1-6.7)	Ref	
25-29	41/308	13.3 (10.0-17.6)	1.73 (0.90-3.32)	0.103	14/287	4.9 (2.9-8.0)	1.86 (0.60-5.75)	0.282
30-34	33/411	8.0 (5.8-11.1)	0.98 (0.50-1.92)	0.954	19/374	5.1 (3.3-7.8)	1.94 (0.65-5.80)	0.236
35-39	39/387	10.1 (7.5-13.5)	1.26 (0.65-2.43)	0.492	7/375	1.9 (0.9-3.8)	0.69 (0.20-2.39)	0.558
40-44	37/306	12.1 (8.9-16.2)	1.55 (0.80-3.00)	0.199	10/280	3.6 (2.0-6.5)	1.34 (0.41-4.36)	0.624
45-49	19/188	10.1 (6.6-15.3)	1.26 (0.60-2.65)	0.536	3/177	1.7 (0.6-4.9)	0.63 (0.14-2.84)	0.543
50-54	10/98	10.2 (5.6-17.8)	1.28 (0.54-3.03)	0.581	2/90	2.2 (0.6-7.7)	0.82 (0.15-4.59)	0.825
>=55	10/88	11.4 (6.3-19.7)	1.44 (0.60-3.43	0.411	1/83	1.2 (0.2-6.5)	0.44 (0.05-4.02)	0.469

*OR: odds ratio; CI: confidence interval

Table 2: Nationality-specific seroprevalence estimates of WNV IgG and IgM antibodies among male blood donors residing in Qatar

	WNV IgG				WNV IgM			
Nationality	**+/N**	**Prevalence (95% CI)**	**OR (95%CI)**	**P-value**	**+/N**	**Prevalence (95% CI)**	**OR (95%CI)**	**P-value**
Egypt	66/200	33.0 (26.9-39.8)	4.84 (2.75-8.54)	<0.001	9/193	4.7 (2.5-8.6)	1.85 (0.61-5.62)	0.279
India	26/200	13.0 (9.0-18.4)	1.47 (0.78-2.78)	0.236	5/163	3.1 (1.3-7.0)	1.20 (0.34-4.21)	0.780
Iran	11/104	10.6 (6.0-18.0)	1.16 (0.53-2.57)	0.708	0/77	0.0 (0.0-4.8)	N/A	0.997
Jordan	14/199	7.0 (4.2-11.5)	0.74 (0.36-1.54)	0.426	2/194	1.0 (0.3-3.7)	0.39 (0.08-2.06)	0.269
Lebanon	1/110	0.9 (0.2-5.0)	0.09 (0.01-0.69)	0.020	0/108	0.0 (0.0-3.4)	N/A	0.996
Pakistan	18/195	9.2 (5.9-14.1)	Ref		5/194	2.6 (1.1-5.9)	Ref	
Palestine	5/200	2.5 (1.1-5.7)	0.25 (0.09-0.69)	0.008	1/193	0.5 (0.1-2.9)	0.20 (0.02-1.70)	0.140
Philippines	6/111	5.4 (2.5-11.3)	0.56 (0.22-1.46)	0.237	0/107	0.0 (0.0-3.5)	N/A	0.996
Qatar	3/200	1.5 (0.5-4.3)	0.15 (0.04-0.52)	0.003	3/188	1.6 (0.6-4.6)	0.61 (0.14-2.60)	0.507
Sudan	34/92	37.0 (27.8-47.2)	5.76 (3.03-10.97)	<0.001	8/80	10.0 (5.2-18.5)	4.20 (1.33-13.26)	0.014
Syria	5/200	2.5 (1.1-5.7)	0.25 (0.09-0.69)	0.008	18/193	9.3 (6.0-14.3)	3.89 (1.41-10.70)	0.009
Yemen	14/137	10.2 (6.2-16.4)	1.12 (0.54-2.34)	0.764	9/128	7.0 (3.7-12.8)	2.86 (0.94-8.74)	0.065

*OR: odds ratio; CI: confidence interval

Figure 1: Seroprevalence of WNV by nationality for men blood donors residing in Qatar