



Letter

Epidemiological evidence of mosquito-borne viruses among persons and vectors in Iran: A study from North to South



Dear Editor,

Arthropod-borne viruses are a group of the most important emerging pathogens. They cause a range of diseases in vertebrate hosts and threaten human health (Gan and Leo, 2014). The global distribution of arboviruses is associated with the vector which is strongly affected by changes in environmental conditions. Dengue virus (DENV) and Chikungunya virus (CHIKV), which cause high annual infected cases and have an increasing geographic distribution, are transmitted by *Aedes* spp. mosquitoes, in particular *Ae. albopictus* and *Ae. Aegypti* (Presti et al., 2014; Higuera and Ramirez, 2018). Although, the main vector of dengue virus, *Ae. aegypti*, was not detected in Iran, other possible important vectors such as *Ae. Albopictus* and *Ae. unilineatus* were recorded (Doosti et al., 2016; Yaghoobi-Ershadi et al., 2017). West Nile virus (WNV), a member of the genus *Flaviviruses*, is one of the most widespread arboviruses (Chancey et al., 2015). The epidemiological evidence of WNV in different hosts in Iran was found (Bagheri et al., 2015), and the circulation of WNV in the main vector, *Culex pipiens s.l.* and *Cx. pipiens*, has been proved (Shahhosseini et al., 2017). Due to limited information on the situation of CHIKV, DENV and WNV in Iran, we performed a wide geographical investigation to determine the prevalence of IgG specific antibodies in human samples as well as the genome of WNV, CHIKV and DENV in mosquitoes.

From September 2017 to June 2018, a total of 1257 serum samples were collected in six provinces (south area: Bushehr, Hormozgan, Sistan & Baluchestan, Khuzestan; north area: Gilan, Mazandaran) (Fig. 1). Patients with previous history of occasional fever, headache, body ache, arthralgia or rash illness and age over 15 years were included. Euro-immune ELISA kits were used to detect the IgG antibodies against WNV, DENV and CHIKV (Andayi et al., 2014). Adult female mosquitoes and larvae (10,488 adult mosquitoes and larvae) were collected from 190 pools in the above six provinces between March 2017 to March 2018 using light traps (Fig. 1). Morphological identification of mosquitoes was carried out using the keys of Becker et al. (Schaffner et al., 2001; Becker, 2010). RNA was extracted and Altona Real-time PCR kits were used to detect and amplify the genome of WNV, DENV and CHIKV (see Supplementary Material for detailed methods). All statistical analyses were conducted using IBM SPSS Statistics version 22 (IBM Corp, Armonk, NY). Logistic regression analysis using single and multiple univariate analysis was used to determine the relationship between the variables and seroreactivity of WNV, DENV and CHIKV.

The demographic characteristics of study participants are shown in Table 1. Results showed that 236 (18.8%) and 74 (5.9%) serum samples were reactive for WNV and DENV IgG antibodies, whereas IgG antibodies against CHIKV (22, 1.8%) were lower than WNV and DENV. According to the univariate analysis, WNV seroprevalence were significantly

associated with age (45–54 vs. 1–24, OR = 1.77, 95% C.I.: 1.03–3.02, $P < 0.05$; ≥ 55 vs. 1–24, OR = 1.93, 95% C.I.: 1.15–3.26, $P < 0.05$), and residential areas (Gilan vs. Bushehr; OR = 0.39, 95% C.I.: 0.12–0.71, $P < 0.001$). Also, DENV and CHIKV seroprevalences were significantly associated with residential areas (Hormozgan vs. Bushehr; DENV, OR = 0.09, 95% C.I.: 0.018–0.95, $P < 0.05$; CHIKV, OR = 8.5, 95% C.I.: 2.287–33.01, $P < 0.05$) (Supplementary Table S1).

Multiple univariate analysis showed significant association between WNV seroreactivity and age (45–54 vs. 1–24, OR = 1.82, 95% C.I.: 1.8–1.02, $P < 0.05$; ≥ 55 vs. 1–24, OR = 3.52, 95% C.I.: 1.98–6.26, $P < 0.01$). The association was also found between WNV seroreactivity and residential areas (Gilan and Khuzestan vs. Bushehr; OR = 0.25, 95% C.I.: 0.121–0.52, $P < 0.001$ and OR = 1.57, 95% C.I.: 1.01–2.45, $P < 0.05$). Also, DENV and CHIKV seroprevalences were significantly associated with residential areas (Hormozgan vs. Bushehr; OR = 0.12, 95% C.I.: 0.18–0.95 and OR: 9.0, 95% C.I.: 2.21–36.6, $P < 0.05$) (Table 2).

The mosquitoes collected in this study belonged to 4 genera and 23 species, including 13 *Culex*, 8 *Aedes*, 1 *Culiseta* and 1 *Uranotaenia* genera (Supplementary Table S2). In Sistan and Baluchestan Province, the highest detection frequency species of mosquito larvae and adults were *Cx. quinquefasciatus* (44%) and *Ae. vexans* (78%). The species of mosquito larvae with highest detection frequency in other regions were: *Cs. Longiareolata* in Hormozgan, *Cx. pipiens complex* in Khuzestan, Gilan and Bushehr. *Ae. Albopictus* species was only detected in Sistan and Baluchestan Province, but *Ae. Caspius* and *Cx. pipiens complex* were detected in all of the provinces. All species were screened for the presence of WNV, CHIKV and DENV, but RNA of three arboviruses were not detected.

In our study, there were two groups of cases: the ones who had not travelled to dengue-endemic areas and those who had travelled to east of Asia, Saudi Arabia. That was in parallel with previous studies in Iran (Chinikar et al., 2013; Aghaie et al., 2014). The previous study reported that there was no evidence of DENV seroprevalence in Iran before 2000 (Saidi, 1974), but positive cases in this decade have been reported (Aghaie et al., 2014; Heydari et al., 2018; Tavakoli et al., 2020). Our results showed a number of DENV seropositive cases from southern regions, Khuzestan and Bushehr. Those regions are in close proximity to Saudi Arabia and Pakistan, and thousands of Iranian travel there as pilgrims annually, which may increase the probability of DENV infection. In our report, DENV seroprevalence was not correlated with patients who had any travel history. It is possible that these cases might be infected through contact with imported cases. Another plausible explanation is that the seropositivity of these cases might be caused by infected vectors. Pakistan country, which is near Sistan and Baluchestan province of Iran, has the largest number of confirmed cases among countries in the Middle East and North Africa (MENA) during all DENV outbreaks (Chinikar

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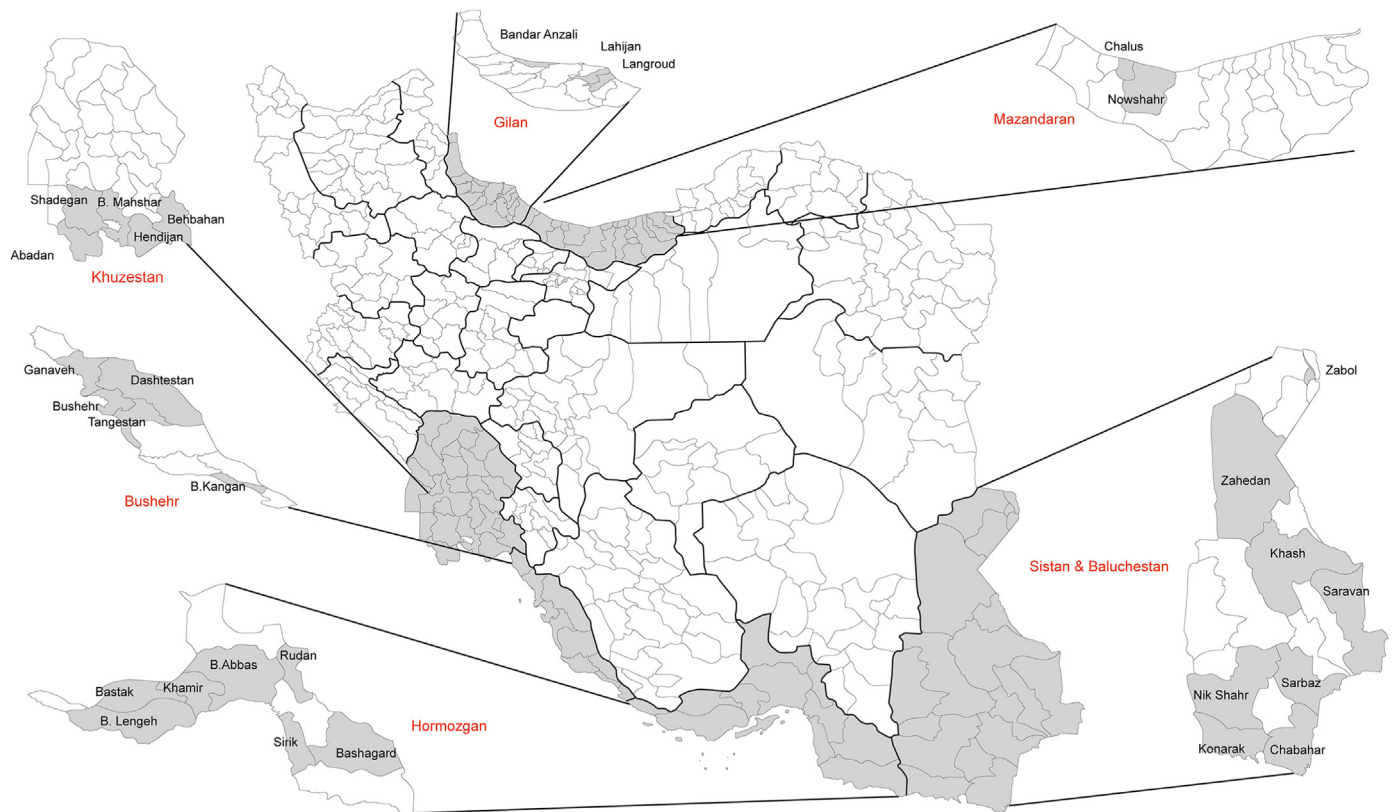


Fig. 1. The map of the sampling regions in this cross-sectional study. The sampling areas are highlighted in grey.

et al., 2013; Humphrey et al., 2016). And our report has shown the potential for the presence of DENV vector, *Ae. albopictus* and *Ae. unilineatus*, in Iran (Doosti et al., 2016; Yaghoobi-Ershadi et al., 2017).

An earlier report showed that the prevalence of WNV in humans in

Table 1

Study populations' demographic characteristics and WNV, DENV and CHIKV IgG seroprevalence.

Characteristic	Total Count	Percent (%)
Age (years) (n = 1257)		
1–24	203	16.2
25–34	399	31.7
35–44	263	20.9
45–54	187	14.9
≥55	205	16.3
Gender (n = 1207)		
Female	734	60.8
Male	473	39.2
Residential area (n = 1257)		
Bushehr	414	32.9
Hormozgan	153	12.2
Sistan & Baluchestan	230	18.3
Gilan	165	13.1
Mazandaran	95	7.6
Khuzestan	200	15.9
Travelling history (n = 980)		
Yes	230	23.5
No	750	76.5
Seroprevalence (n = 1257)		
West Nile virus (WNV)	236	18.8
Dengue virus (DENV)	74	5.9
Chikungunya virus (CHIKV)	22	1.8
Coinfection (n = 1257)		
WNV + DENV	67	5.3
WNV + CHIK	14	1.1
DENV + CHIK	4	0.3
DENV + WNV + CHIK	4	0.3

West Azerbaijan and Khuzestan was 0% (Saidi et al., 1976). However, several studies have shown the prevalence of WNV in humans and in mosquitoes (*Culex* and *Aedes*) in recent years (Chinikar et al., 2012; Shahhosseini et al., 2017). In the studies reported in 2010 and 2016, the prevalence of WNV in Khuzestan and Sistan and Baluchestan provinces were 5% and 17.96%, respectively (Sharifi et al., 2010; Aghaie et al., 2016). In a recent study, the seroprevalence of WNV in Khuzestan Province is 23.8% (Kalantari et al., 2019). Another study in the northwest of Iran showed the presence of WNV RNA in *Ae. Caspius*, a vector of WNV (Bagheri et al., 2015). Also, the evidence of WNV infection in mosquitos, such as *Cx. pipiens.l*, was found in Gilan, Mazandaran, Golestan and East Azerbaijan (Eyboosh et al., 2019). Despite the evidence for existence and circulation of WNV, no clinical cases have been described in Iran until now. Our data showed that WNV IgG was positive in patients, but WNV RNA was not detected in vectors.

As the first study of Iran, our results showed that CHIKV seroprevalence was about 1.8% for humans, but there was no RNA detected in mosquitoes, which demonstrated that individuals might have likely been only exposed to CHIKV. Serologic evidence of CHIKV transmission has been identified in the countries surrounding the Red Sea, such as Pakistan (Ali and Dasti, 2018) and Saudi Arabia (Hussain et al., 2013). Also, a newly CHIKV imported case from Sistan and Baluchistan Province of Iran was reported (Pouriayevalei et al., 2019). In this report, the patient had a recent travel history to Pakistan, where a widespread epidemic of the disease was ongoing at the time of the study.

In recent study, age showed independently association with WNV and CHIKV seropositivity, and a significant association of WNV seroreactivity with the increase of age was found (Mease et al., 2011; Ang et al., 2017; Shaibi et al., 2017; Humphrey et al., 2019). The observed rate was higher in the people with 45 or older compared to those who are below 45, which may be related to a higher probability of exposure to WNV among older people in life period. These findings are consistent with another study (Gómez-Dantés and Willoquet, 2009). A significant relationship was found between the residential area and WNV/CHIKV seroreactivity.

Table 2
Multiple univariate analysis for the assessment of factors associated with these arboviruses seropositivity.

West Nile virus							
Characteristic	Negative cases (N)	Positive cases (N)	Seropositivity (%)	OR	95%CI		Adjusted P-value
Age (years)							
1–24	176	27	13.3	Ref			
25–34	325	74	18.5	1.35	0.82	2.21	0.22
35–44	215	48	18.25	1.45	0.82	2.42	0.152
45–54	147	40	21.4	1.82	1.8	1.02	0.04
≥55	158	47	22.9	3.52	1.98	6.26	0.00
Gender							
Female	590	144	19.6	Ref			
Male	387	86	18.2	0.732	0.497	1.04	0.53
Residential area							
Bushehr	330	84	20.3	Ref			
Hormozgan	118	35	22.9	1.29	0.8	2.07	0.29
Sistan & Baluchestan	189	41	17.8	0.85	0.56	1.28	0.44
Gilan	150	15	9.1	0.25	0.121	0.52	0.000
Mazandaran	83	12	12.6	0.56	0.29	1.06	0.08
Khuzestan	151	49	24.5	1.57	1.00	2.45	0.04
Dengue virus							
Age (years)							
1–24	194	9	4.4	Ref			
25–34	385	14	3.5	0.63	0.26	1.5	0.3
35–44	243	20	7.6	1.15	0.49	2.7	0.73
45–54	177	10	5.3	0.65	0.24	1.7	0.4
≥55	184	21	10.2	2.19	0.92	5.19	0.07
Gender							
Female	700	34	4.6	Ref			
Male	435	38	8.0	1.17	0.67	2.03	0.56
Residential area							
Bushehr	388	26	6.3	Ref			
Hormozgan	152	1	0.7	0.12	0.18	0.95	0.04
Sistan & Baluchestan	220	10	4.3	0.71	0.32	1.55	0.4
Gilan	154	11	6.7	0.77	0.32	1.8	0.54
Mazandaran	90	5	5.3	0.91	0.31	2.45	0.84
Khuzestan	179	21	10.5	1.9	0.95	3.7	0.057
Chikungunya virus							
Age (years)							
1–24	202	1	0.5	Ref			
25–34	388	11	3.0	5.4	0.68	43.2	0.1
35–44	261	2	0.8	2.07	0.18	23.7	0.55
45–54	183	4	2.1	5.7	0.54	60.3	0.14
≥55	201	4	2.0	4.9	0.44	53.7	0.18
Gender							
Female	720	14	1.9	Ref			
Male	466	7	1.5	1.05	0.37	3.02	0.91
Residential area							
Bushehr	411	3	0.7	Ref			
Hormozgan	144	9	5.9	9.0	2.21	36.6	0.001
Sistan & Baluchestan	228	2	0.9	1.3	0.21	8.15	0.77
Gilan	160	5	3.0	4.2	0.8	22.86	0.08
Mazandaran	94	1	1.1	1.4	0.14	13.89	0.74
Khuzestan	198	2	1.0	1.25	0.19	8.1	0.8

Notes: Ref, the group was set as reference.

People residing in Gilan Province had the lowest seroprevalence of WNV, but Gilan and Hormozgan had the highest seroprevalence of CHIKV antibodies compared with other regions. This is in agreement with the studies elsewhere (Ang et al., 2017; Vongpunawad et al., 2017).

In conclusion, our results revealed the seroprevalence of WNV, CHIKV and DENV in human population in Iran and no proof of viral RNAs was presence in vectors. Gilan and Hormozgan areas were high risk regions and the elderly persons were at higher risk of getting infected by WNV and CHIKV. These results help us to better understand the epidemiology of the infection and the ecology of the vectors in Iran. Therefore, considering the risk factors identified by this study, we recommend that the prevention and control strategies should be designed in the country.

Footnotes

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Research Development, Iran (Grand No. 940947). The authors declare that they have no conflict of interest. The informed consent have been obtained from all participants and the studies have been approved by the National Institute for Medical Research Development ethics committee (IR.NIMAD.REC.1394.940947).

Data availability

All the data generated during the current study are included in the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.virs.2022.01.005>.

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Abbas Ahmadi Vasmehjani

Department of Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, 14156446, Iran

Farhad Rezaei*

Department of Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, 14156446, Iran

Mohammad Farahmand

Department of Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, 14156446, Iran

Talat Mokhtari-Azad

Department of Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, 14156446, Iran

Mohammad Reza Yaghoobi-Ershadi

Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, 14156446, Iran

Mohsen Keshavarz

The Persian Gulf Tropical Medicine Research Center, The Persian Gulf Biomedical Sciences Research Institute, Bushehr University of Medical Sciences, Bushehr, 7514763448, Iran

Hamid Reza Baseri

Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, 14156446, Iran

Morteza Zaim

Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, 14156446, Iran

Mahmood Iranpour

Department of Entomology, University of Manitoba, Winnipeg, Manitoba, R3T 2N2, Canada

Habibollah Turki

Infectious and Tropical Diseases Research Center, Hormozgan Health Institute, Hormozgan University of Medical Sciences, Bandar Abbas, 7919693116, Iran

Mohammad Esmaeilpour-Bandboni

School of Nursing, Midwifery and Paramedicine, Guilan University of Medical Sciences, Rasht, 4188794755, Iran

* Corresponding author. Department of Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, 14156446, Iran.

E-mail address: rezaie@tums.ac.ir (F. Rezaei).