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LETTER

Epidemiological Evidence of Mosquito-Borne Viruses among Persons and Vectors in Iran: A Study from North to South

Short title: Seroprevalence of DENV, WNV and CHIKV in Iran

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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 Ramírez 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. Euroimmune 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 seropositve 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 DNEV infection. In our report, DNEV 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 *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 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. pipienss.l*, was found in Gilan, Mazandaran, Golestan and East Azerbaijan (Eybpoosh *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 (Pouriayevali *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. 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; Vongpunsawad *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.

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COMPLIANCE WITH ETHICS GUIDELINES

Conflict of Interest The authors declare that they have no conflict of interest.

Animal and Human Rights Statement

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).

SUPPLEMENTARY MATERIAL

The online version of this article contains supplementary material, which is available to authorized users.

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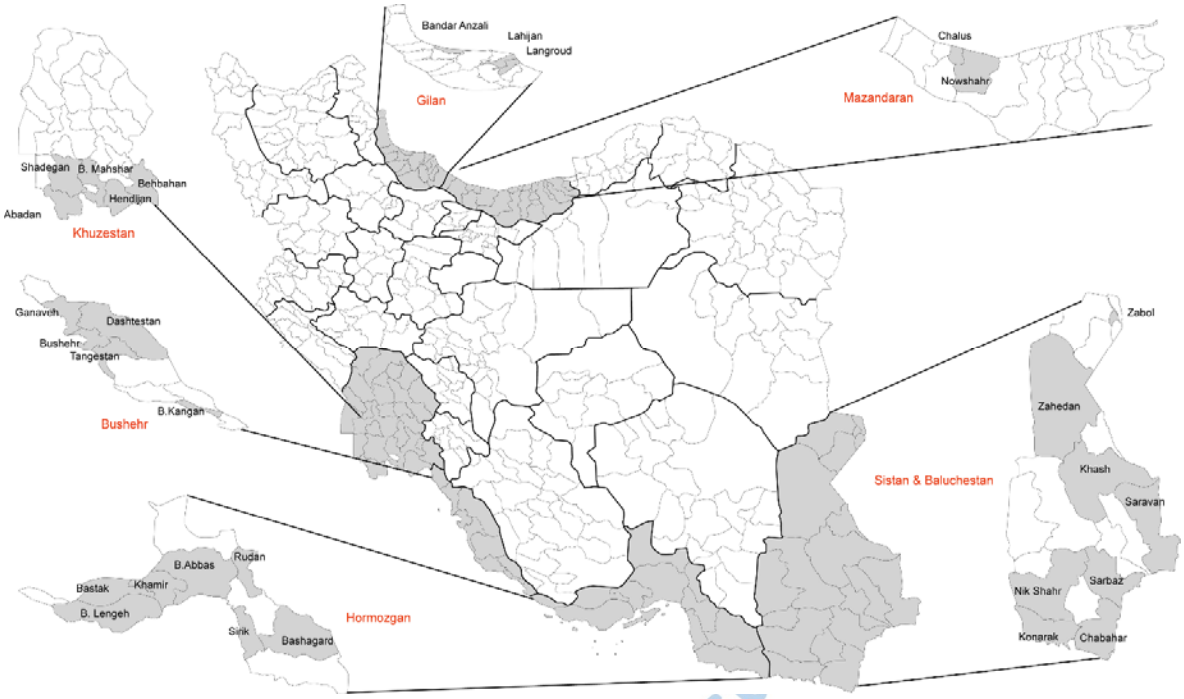

Figure 1. The map of the sampling regions in this cross-sectional study. The sampling areas are highlighted in grey.

Table 1. Study populations' demographic characteristics and WNV, DENV and CHIK 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

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.

Supplementary Materials

Methods

Study Area

This cross-sectional study was conducted in 6 provinces and 29 counties located in southern, eastern south and north of Iran that as depicted in **Fig. 1**. All of these data were extracted based on the 2016 general census data. Mazandaran and Gilan as north provinces, Sistan and Baluchestan as the eastern south province, Hormozgan, Bushehr and Khuzestan as southern and south-western provinces were included. Mazandaran and Gilan are among the most densely populated provinces in Iran, have a moderate climate that their maximum temperature is 40.2 °C and the minimum is −19 °C. Sistan and Baluchestan Province has a tropical climate with the maximum and minimum temperatures 43 °C and 22 °C. Hormozgan is south of the country facing Oman and The UAE. This province has a tropical climate with the maximum and minimum temperatures 51 °C and 22 °C. Bushehr Province a Persian Gulf coast of south-western of Iran with a sub- and mild tropical climate, almost Mediterranean. Its maximum and minimum temperature is 50 °C and 31 °C. Khuzestan Province is southwest of the country, bordering Iraq and the Persian Gulf. It is very hot and occasionally humid and summertime temperatures routinely exceed 45 °C. Regarding the fact that Iran has different types of climate, definitely, sampling time was different between sites in the north and south. In the north, the preferred seasons for sampling are just spring and summer, while in the south, sampling is done in all seasons. Therefore, we selected various provinces with different weather conditions.

Study Design and Sample Selection

From September 2017 to June 2018, a total of 1257 serum samples were collected after an agreement with the private and governmental public laboratories at six provinces including; Mazandaran, Gilan, Bushehr, Hormozgan, Sistan-Baluchestan and Khuzestan. Patients consulting private laboratories had heterogeneous conditions and mainly previous history of occasional fever, headache, body ache, arthralgia or rash illness. The eligibility criteria were acceptance to participate in the study and age over 15 years. To calculate the sample size, we estimated a prevalence of 20% with a variation of ± 10 and 80% power and finally cluster sampling was performed. At the time of initial sample collection, a verbal consent was obtained from the participants. For each serum sample, basic demographic information including age, sex, residential area and traveling history was obtained. Sera were stored at -20 °C until testing.

Detection of IgG Antibodies against Arboviruses Using ELISA

Commercially available Euroimmune ELISA kits (EUROIMMUN AG, Lübeck, Germany) were used to detect IgG antibodies against WNV, DENV and CHIKV (Andayi *et al.* 2014). Positive and negative control sera were provided by the *National Reference Centre for Arbovirus* or by the kits' manufacturers. For each serologic assay, a minimum of three positive controls was included, alongside three negative controls and three blank controls (normal saline), in accordance with the established standard protocols (Jacobson 1998). The assays were carried out at the Virology Department of Tehran University of Medical Science. For each sample, a ratio of the extinction value of the control or patient sample over the extinction value of the calibrator was calculated according to the manufacturer's instructions. Specimens with an optical density (OD) value of $\geq$ 1.1 were considered positive for WNV, DENV and CHIKV IgG antibodies. An OD value of $\leq$ 0.8 and $<$ 1.1 was considered as an equivocal result and an OD value $<$0.8 was determined to be negative. All samples with borderline results were tested twice.

Mosquito's Collection

Morphological identification of mosquitoes was carried out using the keys of Becker *et al.* (Schaffner *et al.* 2001; Becker *et al.* 2010). Specimens were identified in Medical Entomology, Department of Medical Entomology and Vector Control, Tehran University of Medical Sciences. Adult females and larvae mosquitoes were collected in six provinces from 232 sites based on previous studies (Doosti *et al.* 2016; Yaghoobi-Ershadi *et al.* 2017). The mosquitoes were classified into different species and then pooled according to the collection site, species and day of collection (all of the stages were performed on ice). The samples were then placed into cryovials, immersed in RNase blocking solution, and transported in a liquid nitrogen gaseous phase. Totally, 6212 mosquito larvae and 2668 adults were collected and pooled by sampling site, date and taxon comprising between 1 to 20 specimens per pool. Finally, 290 pools were kept at a -70 °C freezer for further analysis.

RNA Extraction

Mosquito pools were placed in chilled 15-mL falcons with 1 mL of cooled PBS then were homogenized using glass beads and vortexing for about 1 min. Mosquito homogenates were centrifuged for 5 min at 2500 × *g* at 4 °C and supernatants were collected. RNA was extracted from 200 µL of each mosquitoes' homogenate using the NucleoSpin® RNA Kit (MACHEREY-NAGEL GmbH & Co. KG, Germany) according to the manufacturer's instructions.

Real-time Reverse Transcription Polymerase Chain Reaction (rtRT-PCR)

Commercially Altona kits (RealStar® Dengue RT-PCR Kit, cat no: 282013, RealStar® WNV RT-PCR Kit, cat no: 321013 and RealStar® Chikungunya RT-PCR Kit, cat no: 012013, Altona Diagnostics GmbH, Germany) were used to detect and amplify DENV, CHIKV and WNV RNAs. The tests were performed using an Applied Biosystem step one plus real-time PCR machine (Applied Biosystem, CA, USA). Amplification of WNV RNA took place in a 50 µL single-tube, 21 µL master mix and 9 µL of extracted sample RNA or serially diluted positive control copy number that was provided by the kits. The cycling conditions consisted of one cycle at 55 °C for 10 min, one cycle at 95 °C for 2 min, and 40 cycles at 95 °C for 15 s, 55 °C for 1 min and 72 °C for 15 s. The test condition for detection of DENV and CHIKV RNAs was the same as above except for the amount of master mix.

Statistical Analysis

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 for anti-WNV, DENV and CHIKV. Adjusted odds ratios (OR) and 95% confidence intervals (CI) for multiple univariate analysis was used to determine independent factors associated with WNV, DENV and CHIKV seroprevalence. A *P*-value of less than 0.05 was considered to be statistically significant.

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Table S1. Univariate analysis of WNV, DENV and CHIK IgG seroprevalence by age, gender, residential area and travelling history to outside of country.

West Nile virus							
Characteristic	**Negative cases (N)**	**Positive cases (N)**	**Seropositivity (%)**	**OR**	**95%CI**		**P-value**
Age (years)							
1–24	176	27	13.3	Ref			
25–34	325	74	18.5	1.48	0.92	2.39	0.1
35–44	215	48	18.25	1.45	0.87	1.42	0.15
45–54	147	40	21.4	1.77	1.03	3.02	0.03
≥ 55	158	47	22.9	1.93	1.15	3.26	0.01
Gender							
Female	590	144	19.6	Ref			
Male	387	86	18.2	0.91	0.67	1.2	0.53
Residential area							
Bushehr	330	84	20.3	Ref			
Hormozgan	118	35	22.9	1.16	0.74	1.8	0.5
Sistan & Baluchestan	189	41	17.8	0.85	0.56	1.28	0.44
Gilan	150	15	9.1	0.39	0.121	0.71	0.000
Mazandaran	83	12	12.6	0.56	0.29	1.08	0.08
Khuzestan	151	49	24.5	1.27	0.85	1.9	0.23
Travelling history (780)							
Yes	174	56	24.3	Ref			
No	601	149	19.9	1.29	0.91	1.8	0.14
Dengue virus							
Age (years)							
1–24	194	9	4.4	Ref			
25–34	385	14	3.5	0.78	0.33	1.68	0.57
35–44	243	20	7.6	1.77	0.79	3.98	0.16
45–54	177	10	5.3	1.2	0.48	3.8	0.67
≥ 55	184	21	10.2	2.4	1.06	5.5	0.21
Gender							
Female	700	34	4.6	Ref			
Male	435	38	8.0	1.79	1.1	2.94	0.01
Residential area							
Bushehr	388	26	6.3	Ref			
Hormozgan	152	1	0.7	0.09	0.018	0.73	0.02
Sistan & Baluchestan	220	10	4.3	0.67	0.32	1.1	0.3
Gilan	154	11	6.7	1.06	0.51	2.2	0.86
Mazandaran	90	5	5.3	0.82	0.31	2.2	0.7
Khuzestan	179	21	10.5	1.75	0.95	3.19	0.06
Travelling history (780)							
Yes	213	17	7.4	Ref			
No	709	41	5.5	1.38	0.76	2.47	0.28
Chikungunya virus							
Age (years)							
1–24	202	1	0.5	Ref			
25–34	388	11	3.0	5.78	0.73	44.6	0.09
35–44	261	2	0.8	1.5	0.13	17.1	0.72
45–54	183	4	2.1	4.4	0.48	39.8	0.18
≥ 55	201	4	2.0	4.02	0.44	36.2	0.21
Gender							
Female	720	14	1.9	Ref			
Male	466	7	1.5	0.77	0.3	1.9	0.58
Residential area							
Bushehr	411	3	0.7	Ref			

Hormozgan	144	9	5.9	8.5	2.287	33.01	0.001
Sistan & Baluchestan	228	2	0.9	1.2	0.199	7.245	0.841
Gilan	160	5	3.0	4.2	1.011	18.123	0.048
Mazandaran	94	1	1.1	1.4	0.150	14.167	0.74
Khuzestan	198	2	1.0	1.3	0.22	8.3	0.72
Travelling history (780)							
Yes	225	5	2.2	Ref			
No	741	9	1.2	1.83	0.6	5.5	0.28

Notes: Ref, the data was set as reference

Table S2. Distribution of collected mosquito's species in 6 province of Iran.

Species	Provinces and Counties																Total (N/%)		Assay
	Sistan and Baluchestan																		
	Konarak		Sarbaz		Khash		Chabahar		Saravan		Zahedan		Nikshahr		Zabol				
Larvae/Adults	L	A	L	A	L	A	L	A	L	A	L	A	L	A	L	A	L	A	
Cx. perexiguus	0	ND	5	ND	0	ND	70	ND	30	ND	7	ND	9	ND	0	ND	121 (4.64)	ND	
Cx. pipiens complex	0	0	0	ND	0	ND	36	29	1	67	18	ND	0	ND	0	0	55 (2.1)	96 (5.1)	
Cx. theileri	0	ND	0	ND	0	ND	38	ND	0	ND	99	ND	9	ND	ND	ND	146 (5.6)	ND	
Cx. laticinctus	ND	0	ND	ND	ND	ND	ND	19	ND	10	ND	ND	ND	ND	ND	0	ND	29 (1.5)	
Cx. mimeticus	0	ND	0	ND	0	ND	4	ND	71	ND	0	ND	0	ND	ND	ND	75 (2.9)	ND	
Cx. sinaiticus	0	ND	10	ND	0	ND	0	1	0	3	0	ND	0	ND	ND	0	12 (0.46)	4 (0.2)	
Cx. sitiens	271	ND	0	ND	0	ND	113	ND	54	ND	0	ND	2	ND	ND	ND	438 (16.8)	ND	
Cx. tritaeniorhynchus	5	0	22	ND	0	ND	184	5	93	2	0	ND	7	ND	ND	0	311 (11.9)	7 (2.4)	
Cx. quinquefasciatus	0	ND	300	ND	0	ND	270	ND	410	ND	94	ND	71	ND	ND	ND	1145 (44)	ND	
Cx. bitaeniorhynchus	0	ND	0	ND	0	ND	0	ND	1	ND	0	ND	0	ND	ND	ND	18 (0.7)	ND	rRT-PCR
Cs. longiareolata	25	0	1	ND	118	0	12	0	0	3	1	ND	2	ND	ND	0	159 (6.1)	3 (0.2)	
Ae. caspius	0	3	0	ND	0	ND	104	150	0	1	9	ND	0	ND	ND	45	113 (4.34)	199 (10.6)	
Ae. vexans	0	75	0	ND	0	ND	2	1390	0	0	0	ND	0	ND	ND	0	2 (0.08)	1465 (78)	
Ae. falvescens	0	ND	0	ND	0	ND	0	ND	0	ND	2	ND	0	ND	ND	ND	2 (0.08)	ND	
Ae. caballus	0	ND	0	ND	0	ND	1	ND	0	ND	0	ND	0	ND	ND	ND	1 (0.04)	ND	
Ae. unlineatus	ND	0	ND	ND	ND	ND	ND	1	ND	0	ND	ND	ND	ND	ND	ND	ND	1 (0.1)	
Ae. detritus	ND	12	ND	ND	ND	ND	ND	18	ND	0	ND	ND	ND	ND	ND	0	ND	30 (1.6)	
Ae. albopictus	0	0	3	ND	0	ND	0	6	0	0	0	ND	2	ND	ND	0	5 (0.19)	6 (0.3)	
Total	301	128	341	ND	118	0	834	1619	677	86	230	ND	102	ND	0	45	2603	1878	

Continued

Species	Hormozgan														Total (N/%)		
	Bandar Abbas		Bashagard		Sirik		Rudan		Bastak		Bandar Khamir		Bandar Lengeh				
	L	A	L	A	L	A	L	A	L	A	L	A	L	A	L	A	
Cx. perexiguus	4	ND	0	ND	ND	ND	0	ND	ND	ND	0	ND	0	ND	4 (0.3)	ND	
Cx. arbieeni	0	ND	2	ND	ND	ND	ND	ND	ND	ND	0	ND	0	ND	2 (0.1)	ND	
Cx. laticinctus	ND	0	ND	2	ND	0	ND	0	ND	0	ND	0	ND	0	ND	2 (3.2)	
Cx. pipiens complex	3	0	215	19	ND	0	3	0	ND	0	11	0	0	0	235 (15.7)	19 (30.1)	
Cx. theileri	0	ND	0	ND	ND	ND	0	ND	ND	ND	0	ND	155	ND	155 (10.5)	ND	
Cx. mimeticus	1	ND	77	ND	ND	ND	3	ND	ND	ND	0	ND	0	ND	81 (5.5)	ND	rRT-PCR
Cx. sinaiticus	0	0	52	3	ND	0	0	0	ND	0	0	0	0	0	52 (3.5)	3 (4.8)	
Cx. tritaeniorhynchus	10	8	61	1	ND	0	0	0	ND	0	34	0	37	0	142 (9.6)	9 (14.3)	
Cx. quinquefasciatus	0	ND	125	ND	ND	ND	0	ND	ND	ND	1	ND	9	ND	135 (9.1)	ND	
Cs. longiareolata	0	ND	648	ND	ND	ND	0	ND	ND	ND	0	ND	0	ND	648 (43.8)	ND	
Ae. caspius	ND	0	ND	1	ND	5	ND	7	ND	0	ND	5	ND	5	ND	23 (36.5)	
Ae. vexans	ND	0	ND	2	ND	0	ND	0	ND	1	ND	0	ND	0	ND	3 (4.8)	
Ae. vittatus	ND	0	ND	0	ND	0	ND	0	ND	0	ND	0	ND	4	ND	4 (6.3)	
Ae. caballus	0	ND	1	ND	ND	ND	0	ND	ND	ND	0	ND	0	ND	1 (0.1)	ND	
Total	18	8	1207	28	ND	5	6	7	ND	1	46	5	201	9	1478	63	

Continued

Species	Bushehr										Total (N/%)		
	Tangestan		Dashtestan		Bandar Ganaveh		Bushehr		Bandar Kangan				
	L	A	L	A	L	A	L	A	L	A	L	A	
Cx. perexiguus	22	ND	20	ND	ND	ND	8	ND	1	ND	45 (4.8)	ND	
Cx. hortensis	1	ND	0	ND	ND	ND	0	ND	0	ND	1 (0.1)	ND	
Cx. pusillus	ND	ND	0	ND	ND	ND	63	ND	0	ND	63 (5.9)	ND	
Cx. pipiens complex	106	0	0	0	ND	6	126	ND	282	28	522 (49.2)	34 (12.1)	
Cx. theileri	0	ND	0	ND	ND	ND	16	ND	0	ND	16 (1.5)	ND	
Cx. mimeticus	22	ND	0	ND	ND	ND	0	ND	0	ND	22 (2.1)	ND	
Cx. laticinctus	0	ND	2	ND	ND	ND	0	ND	0	ND	2 (0.2)	ND	rRT-PCR
Cx. sinaiticus	ND	1	ND	0	ND	0	ND	ND	ND	0	ND	1 (0.4)	
Cx. sitiens	0	ND	0	ND	ND	ND	56	ND	0	ND	56 (5.3)	ND	
Cx. tritaeniorhynchus	0	ND	1	ND	ND	ND	0	ND	4	ND	5 (0.5)	ND	
Cx. quinquefasciatus	21	ND	1	ND	ND	ND	33	ND	25	ND	80 (7.5)	ND	
Cs. longiareolata	50	ND	0	ND	ND	ND	3	ND	9	ND	62 (5.8)	ND	
Ur. unguiculata	0	ND	2	ND	ND	ND	6	ND	0	ND	8 (0.8)	ND	
Ae. caspius	15	22	0	86	ND	0	150	117	0	2	165 (15.6)	227 (80.5)	
Ae. vexans	7	2	0	0	ND	0	0	ND	0	0	7 (0.7)	2 (0.7)	
Ae. detritus	ND	0	ND	0	ND	0	ND	ND	ND	18	ND	18 (6.3)	
Total	244	25	26	86	ND	6	461	117	321	48	1060	282	

Continued

Species	Khuzestan										Total (N/%)		
	Abadan		Behbahan		Shadegan		Bandar Mahshahr		Hendijan				
	L	A	L	A	L	A	L	A	L	A	L	A	
Cx. perexiguus	0	ND	1	ND	0	ND	0	ND	0	ND	1 (0.2)	ND	
Cx. pusillus	0	ND	13	ND	12	ND	0	ND	0	ND	25 (5.1)	ND	
Cx. pipiens complex	31	ND	70	ND	86	ND	0	ND	0	ND	187 (33.8)	ND	
Cx. theileri	6	ND	7	ND	2	ND	0	ND	0	ND	15 (1.6)	ND	rRT-PCR
Cx. sitiens	6	ND	0	ND	0	ND	0	ND	0	ND	6 (1.1)	ND	
Cx. tritaeniorhynchus	0	ND	112	ND	34	ND	0	ND	11	ND	157 (28.4)	ND	
Cx. quinquefasciatus	0	ND	1	ND	57	ND	0	ND	0	ND	58 (10.5)	ND	
Ur. ungiuculata	0	ND	6	ND	0	ND	0	ND	0	ND	6 (1.1)	ND	
Ae. caspius	20	ND	34	ND	6	ND	41	ND	0	ND	101 (18.2)	ND	
Total	57	ND	232	ND	200	ND	41	ND	11	ND	553	ND	

Continued

Species	Gilan						Total (N/%)		
	Langarud		Bandar Anzali		Lahijan				
	L	A	L	A	L	A	L	A	
Cx. perexiguus	11	1	14	4	10	2	35 (11.11)	7 (7.53)	rRT-PCR
Cx. pipiens complex	37	11	57	21	75	24	169 (53.8)	57 (60.2)	
Cx. theileri	15	6	19	9	10	0	44 (12.42)	15 (16.12)	
Cx. mimeticus	0	ND	3	ND	1	ND	4 (1.3)	ND	
Cx. sitiens	4	1	7	3	2	0	13 (4.2)	4 (4.3)	
Cx. tritaeniorhynchus	1	ND	5	ND	2	ND	9 (2.55)	ND	
Cx. hortensis	15	3	20	5	5	2	40 (12.7)	10 (10.75)	
Cs. longiareolata	1	ND	2	ND	0	ND	3 (0.96)	ND	
Ae. caspius	0	ND	2	ND	0	ND	2 (0.64)	-	
Ae. vexans	0	0	0	1	1	0	1 (0.32)	1 (1.1)	
Total	84	22	129	43	101	28	314	93	

Continued

Species	Mazandaran						
	Chalus		Nowshahr		**Total (N/%)**		
	L	A	L	A	L	A	
Cx. perexiguus	37	11	22	6	59 (10.61)	17 (7.7)	
Cx. pipiens complex	115	46	158	71	273 (49.1)	117 (52.94)	
Cx. theileri	45	17	63	24	108 (19.42)	41 (18.55)	rRT-PCR
Cx. mimeticus	2	0	5	2	7 (1.26)	2 (0.91)	
Cx. sitiens	27	13	71	28	98 (17.63)	41 (18.55)	
Cx. hortensis	1	0	2	1	3 (0.54)	1 (0.45)	
Cs. longiareolata	3	1	0	0	3 (0.54)	1 (0.45)	
Ae. caspius	5	1	0	0	5 (0.9)	1 (0.45)	
Total	235	89	321	132	556	221	

Abbreviation: L: Larvae; **A: Adults**; r**RT-PCR:** Real-time Reverse Transcriptase-Polymerase Chain Reaction; **ND**: Not determined.