***Virologica Sinica***

**Supplementary Data**

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

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**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](https://en.wikipedia.org/wiki/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](https://en.wikipedia.org/wiki/Iraq) and the [Persian Gulf](https://en.wikipedia.org/wiki/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, and 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 ≥ 1.1 were considered positive for WNV, DENV and CHIKV IgG antibodies. An OD value of ≤ 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.](http://www.mn-net.com/Products/DNAandRNApurification/RNA/NucleoSpinTriPrep/tabid/11113/language/en-US/Default.aspx)

**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.

**Reference**

Becker N PD, Zgomba M, Boase C,  Zgomba M, Boase C, Madon MB, Dahl C, Kaiser A (2010) Mosquitoes and their control (2nd Ed), Springer, Berlin. pp. 577.

Doosti S, Yaghoobi-Ershadi MR, Schaffner F, Moosa-Kazemi SH, Akbarzadeh K, Gooya MM, Vatandoost H, Shirzadi MR, Mosta-Favi E (2016) Mosquito surveillance and the first record of the invasive mosquito species *Aedes* (*Stegomyia*) albopictus (Skuse)(Diptera: Culicidae) in southern Iran. Iran J Public Health 45: 1064–1073.

Jacobson RH (1998) Validation of serological assays for diagnosis of infectious diseases. Rev Sci Tech 17: 469-486.

Schaffner F, Angel G, Geoffroy B, Hervy JP, Rhaiem A, Brunhes J (2001) The mosquitoes of Europe/Les moustiques d’Europe. IRD Editions & EID Méditerranée.

Yaghoobi-Ershadi M, Doosti S, Schaffner F, Moosa-Kazemi SH, Akbarzadeh K, Yaghoobi-Ershadi N (2017) Morphological studies on adult mosquitoes (Diptera: Culicidae) and first report of the potential Zika virus vector Aedes (Stegomyia) unilineatus (Theobald, 1906) in Iran. Bull Soc Pathol Exot 110:116–121.

**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 | rRT-PCR |
| *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 |
| *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 |

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|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species** | **Hormozgan** | | | | | | | | | | | | | | | **Total (N/%)** | | rRT-PCR |
| 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 |
| *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 |

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species** | **Bushehr** | | | | | | | | | | **Total (N/%)** | | rRT-PCR |
| 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 |
| *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 |

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species** | **Khuzestan** | | | | | | | | | | **Total (N/%)** | | rRT-PCR |
| 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 |
| *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 |

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species** | **Gilan** | | | | | | **Total (N/%)** | | rRT-PCR |
| 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) |
| *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** | | | | | | **Total (N/%)** | | rRT-PCR |
| Chalus | | | Nowshahr | | |
| 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) |
| *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.