



# Species Composition and Geographic Distribution of Culicinae Mosquitoes and Their Possible Infection with West Nile Virus in Hormozgan Province, Southern Iran

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## Abstract

**Background:** West Nile virus (WNV) is a mosquito-borne virus that causes a febrile disease and may cause a fatal neurological illness in humans. We aimed to investigate the geographic distribution of Culicinae mosquitoes and their possible infection with WNV in Hormozgan Province, southern Iran.

**Methods:** Field studies were carried out from June 2017 to May 2019. Different mosquito sampling methods were used monthly to collect mosquitoes from 22 sites. The Real-Time PCR technique was used to detect the virus infection in the mosquitoes.

**Results:** Overall, 6165 mosquitoes were caught. The species were *Culex theileri* (33.25%), *Cx. pipiens* (20.45%), *Cx. quinquefasciatus* (10.51%), *Aedes caspius* (6.33%), *Cx. tritaeniorhynchus* (5.82%), *Ae. vexans* (4.10%), *Cx. sinaiticus* (3.62%), *Cx. antennatus* (3.29%), *Culiseta longiareolata* (2.81%), *Cx. perexiguus* (2.03%), *Cs. subochrea* (1.95%), *Cx. mimeticus* (1.49%), *Cx. pusillus* (1.38%), *Cx. univittatus* (1.27%), *Cx. modestus* (1.14%), and *Cx. sitiens* (0.57%). The molecular detection of virus infection in mosquitoes found to be negative for WNV.

**Conclusion:** The presence of many species of mosquito vectors and high population traffic increase the risk of disease transmission is very high. Therefore, the way to restrict WNV infection factors is increasing the knowledge for personal protection measures to prevent mosquito bites.

**Keywords:** West Nile virus; Culicidae mosquitoes; Iran

## Introduction

The Culicinae is the subfamily of Culicidae (Diptera: Culicidae). So far, more than 3,500 species of

Culicidae mosquitoes have been identified in 43 genera (1). A wide range of important arbovirus



diseases is transmitted by Culicinae mosquitoes, including yellow fever, dengue fever, chikungunya, encephalitis as well as West Nile fever. *Aedes* and *Culex* species play an important role in the transmission of these diseases (2-3).

West Nile virus (WNV) is a common arbovirus disease between humans and some animals, especially birds. About 300 species are known as the reservoir host of WNV. Corvus and Cyanocitta crows are considered as important reservoirs of the disease (4). Migratory birds play a very important role in transmission of the virus in world (5). Hormozgan, due to its numerous wetlands and mangrove forests, hosts a variety of birds every year from October to the end of May. *Culex* mosquitoes due to their ornithophilic feature are considered important vectors of WNV (6-7). Recently the presence of WNV was reported in mosquitoes in the northwestern (8) and southern part of Iran in Hormozgan Province. The finding indicated that WNV genome was present in *Culex pipiens* complex, (9).

We aimed to determine the species composition and geographical distribution of potential Culicines vectors as well as their possible infection

with WNV in Hormozgan Province, an area with a high risk of WNV transmission in the south of Iran.

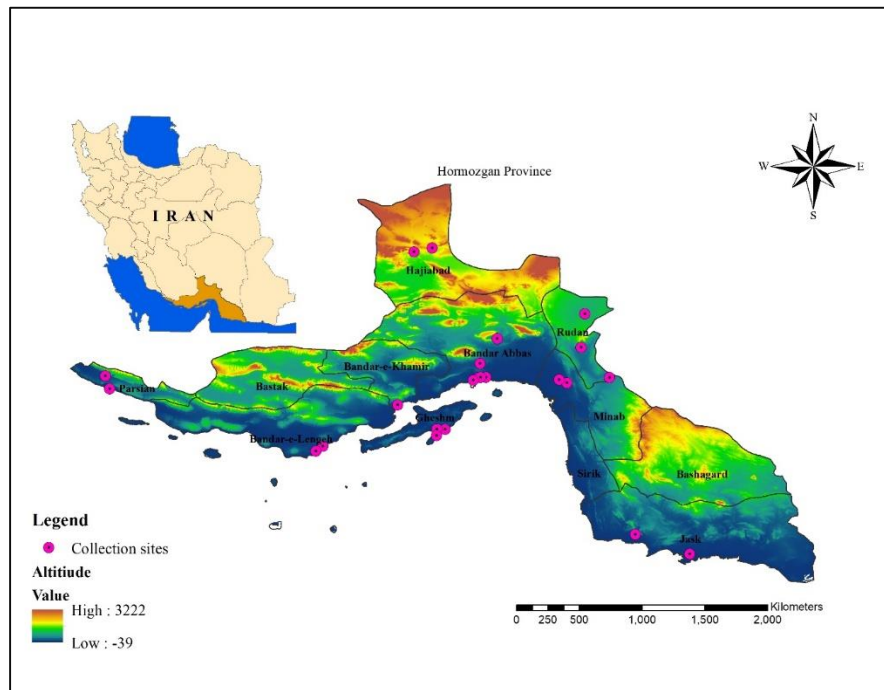
## Materials and Methods

### *Ethics approval*

This study was approved by the Ethics Committee of the Tehran University of Medical Sciences IR.TUMS.SPH.REC.1397.

### *Study area*

Hormozgan Province is located in the south of Iran bordering the Persian Gulf, with an approximate population of 1,776,000. the province with 71,000 km<sup>2</sup> located between latitude 25° 24'–28°53'N and longitude 52°44'–59°14' E (10). Out of 13 counties of the province, 8 cities and regions were selected, and entomological studies conducted monthly from June 2017 to June 2019 (Fig. 1).



**Fig. 1:** Selected areas for entomological survey in Hormozgan Province, Southern Iran

### **Mosquito collection**

Mosquitos' larvae were collected from the different natural and artificial larval habitats by dipper method collection. The breeding sites were categorized as temporary or permanent breeding places, vegetated water bodies, larval habitats with substrate of mud, stone, cement and sand bottom, water quality such as opacity or transparency and light status (sunny, partial shade, shade).

Adult mosquitoes were collected by different methods including CDC light traps, human baited trap net, animal baited trap net, birds baited trap net, mosquito trap net with CO<sub>2</sub>, hand catch method from indoor places, and collecting mosquitoes from shelter pits. Finally, the collected mosquito species were identified using valid keys (11, 12).

### **Molecular assays for detection of West Nile Virus (WNV)**

To extract RNA from mosquitoes' specimens, each separated pool that prepared from mono species was homogenized in 300 µl of PBS buffer using the pestle, then 200 µl of mosquito homogenates were added to a new tube consisting of 600 µl of RLT buffer, and then the samples were mixed using a vortex for 15 seconds. Mosquito homogenates were centrifuged at 1300rpm. Total RNA extraction was purified according to the manufacturer's kit by the use of the RNeasy mini kit (QIAGEN) and stored at -70 °C until needed for WNV detection. For detection and amplifying of WNV genomes in the mosquitoes, One-Step Real-Time PCR Kit (QIAGEN) was used. Briefly, RT-PCR reaction was carried out in a final 20µl volume (5 µl of 5X QIAGEN One-Step RT-PCR Buffer, 1µl of dNTP, 1µl of QIAGEN One-Step RT-PCR Enzyme, 1 µl of Primer F(CAGACCACGCTACGGCG), 1 µl of Primer R

(CTAGGGCCGCGTGGG), 5.5 µl of RNase free water, 0.5 µl of probe and 5µl of extracted RNA as a template). The cycling conditions consisted of one cycle at 50°C for 30 min, one cycle at 95°C for 5 min, and 45 cycles at 95°C for 10 s and 60°C for 1 min (13).

## **Results**

### **Culicinae species**

During the current study, 6165 female culicine mosquitoes were collected included 3 genera and 16 species. The species were *Culex theileri*, *Cx. pipiens*, *Cx. quinquefasciatus*, *Cx. tritaeniorhynchus*, *Cx. antennatus*, *Cx. perexiguus*, *Cx. mimeticus*, *Cx. pusillus*, *Cx. univittatus*, *Cx. modestus*, *Cx. sitiens*, *Cx. sinaiticus*, *Aedes caspius*, *Ae. vexans*, *Culiseta longiareolata* and *Cs. subochrea*. *Culex theileri* with 33.25% and *Cx. pipiens* with 20.45% were dominant species (Table 1). The predominant species based on the collected site is summarized in Table 1. Totally, 2377 mosquitoes were collected in Bandar Abbas County; the most abundant species was *Cx. pipiens* (22.09%). *Aedes* mosquitoes were collected only in certain months of the year during the rainy season. The number of collected female mosquitoes in Minab County was 1180, and the dominant species was *Cx. theileri* species (30.34%). In Hajiabad County, 473 mosquitoes were collected and the most abundant species was *Cx. theileri* (53.49%). In Parsian County, 473 mosquitoes were collected and the most abundance was related to *Cx. theileri* (76.92%). In Bandar Lengeh County, the most abundant species was *Cx. theileri* (40.40%). In Rudan County, the most abundant species was *Cx. theileri* (45.75%). In Jask County, the most abundant mosquito was *Cx. theileri* (31.41%). In Qeshm County, the dominant species was *Cx. pipiens* 180 (39.91%).

**Table 1:** Distribution of female Culicinae mosquitoes in study area in Hormozgan, Southern Iran during June 2017 to May 2019

| City & Collection Site       |                                     | Species & Number of Female Mosquitoes |                     |                              |                             |                        |                     |                     |                          |                    |                    |                       |                       |                    |                   |                      |                          | Number of female mos- quitos |
|------------------------------|-------------------------------------|---------------------------------------|---------------------|------------------------------|-----------------------------|------------------------|---------------------|---------------------|--------------------------|--------------------|--------------------|-----------------------|-----------------------|--------------------|-------------------|----------------------|--------------------------|------------------------------|
| City                         | Collection sites                    | <i>Cx. pipiens</i>                    | <i>Cx. theileri</i> | <i>Cx. tritaeniorhynchus</i> | <i>Cx. quinquefasciatus</i> | <i>Cx. poroclyptus</i> | <i>Cx. modestus</i> | <i>Cx. pusillus</i> | <i>Cx. annulirostris</i> | <i>Cx. sitiens</i> | <i>Cx. minutus</i> | <i>Cx. antennalis</i> | <i>Cx. sinaiticus</i> | <i>Ae. caspius</i> | <i>Ae. vexans</i> | <i>Cx. subochrea</i> | <i>Cx. longiareolata</i> |                              |
| Bandar Abbas                 | Daneshkadeh                         | 32                                    | 75                  | 8                            | 10                          | 0                      | 0                   | 0                   | 3                        | 2                  | 5                  | 3                     | 10                    | 12                 | 6                 | 0                    | 6                        | 172                          |
|                              | Behdasht                            |                                       |                     |                              |                             |                        |                     |                     |                          |                    |                    |                       |                       |                    |                   |                      |                          |                              |
|                              | Mahaleh sang kan                    | 70                                    | 110                 | 18                           | 80                          | 21                     | 15                  | 15                  | 1                        | 3                  | 12                 | 12                    | 16                    | 35                 | 29                | 18                   | 19                       | 474                          |
|                              | Khour shilat                        | 85                                    | 80                  | 17                           | 55                          | 25                     | 21                  | 22                  | 5                        | 2                  | 17                 | 9                     | 17                    | 36                 | 27                | 37                   | 17                       | 472                          |
|                              | Mohaleh posht shahr hormoudar Rural | 69                                    | 35                  | 16                           | 28                          | 0                      | 2                   | 14                  | 6                        | 2                  | 6                  | 12                    | 16                    | 35                 | 25                | 31                   | 16                       | 313                          |
|                              | khorgoo Rural                       | 45                                    | 150                 | 30                           | 32                          | 14                     | 15                  | 12                  | 10                       | 0                  | 20                 | 10                    | 20                    | 64                 | 30                | 18                   | 26                       | 496                          |
| Minab                        | City of Minab                       | 49                                    | 75                  | 16                           | 48                          | 21                     | 17                  | 22                  | 12                       | 6                  | 18                 | 11                    | 26                    | 68                 | 25                | 16                   | 20                       | 450                          |
|                              | Haji balouchi                       | 96                                    | 108                 | 23                           | 57                          | 12                     | 0                   | 0                   | 7                        | 3                  | 0                  | 15                    | 36                    | 0                  | 33                | 0                    | 0                        | 390                          |
|                              | Bolboli                             | 88                                    | 150                 | 25                           | 71                          | 6                      | 0                   | 0                   | 9                        | 4                  | 0                  | 17                    | 30                    | 0                  | 38                | 0                    | 0                        | 438                          |
| Hajiabad                     | Haji Abad City                      | 75                                    | 100                 | 35                           | 44                          | 10                     | 0                   | 0                   | 7                        | 5                  | 0                  | 11                    | 25                    | 0                  | 40                | 0                    | 0                        | 352                          |
|                              | Tejerj                              | 65                                    | 150                 | 10                           | 28                          | 0                      | 0                   | 0                   | 0                        | 0                  | 0                  | 12                    | 0                     | 0                  | 0                 | 0                    | 0                        | 265                          |
| Parsian                      | Parsian                             | 35                                    | 103                 | 25                           | 32                          | 0                      | 0                   | 0                   | 0                        | 0                  | 0                  | 13                    | 0                     | 0                  | 0                 | 0                    | 0                        | 208                          |
|                              | ziyarat                             | 20                                    | 220                 | 8                            | 7                           | 0                      | 0                   | 0                   | 0                        | 0                  | 0                  | 7                     | 0                     | 0                  | 0                 | 0                    | 6                        | 268                          |
| Bandar Lengeh                | Bandar Kong                         | 45                                    | 200                 | 9                            | 7                           | 0                      | 0                   | 0                   | 0                        | 0                  | 0                  | 8                     | 0                     | 0                  | 0                 | 0                    | 9                        | 278                          |
|                              | Rudan                               | 72                                    | 120                 | 27                           | 12                          | 0                      | 0                   | 0                   | 0                        | 0                  | 0                  | 12                    | 0                     | 0                  | 0                 | 0                    | 54                       | 297                          |
|                              | ziyarat ali                         | 45                                    | 98                  | 10                           | 17                          | 0                      | 0                   | 0                   | 10                       | 3                  | 0                  | 12                    | 14                    | 0                  | 0                 | 0                    | 0                        | 209                          |
| Bandar Jask                  | bahmadi                             | 75                                    | 112                 | 18                           | 5                           | 0                      | 0                   | 0                   | 12                       | 5                  | 0                  | 10                    | 13                    | 0                  | 0                 | 0                    | 0                        | 250                          |
|                              | zar abad                            | 68                                    | 73                  | 12                           | 40                          | 0                      | 0                   | 0                   | 0                        | 0                  | 0                  | 6                     | 0                     | 0                  | 0                 | 0                    | 0                        | 199                          |
| Qeshm                        | Mesen                               | 47                                    | 47                  | 33                           | 42                          | 5                      | 0                   | 0                   | 0                        | 0                  | 0                  | 9                     | 0                     | 0                  | 0                 | 0                    | 0                        | 183                          |
|                              | Direstan                            | 58                                    | 15                  | 7                            | 10                          | 2                      | 0                   | 0                   | 2                        | 0                  | 0                  | 8                     | 0                     | 62                 | 0                 | 0                    | 0                        | 164                          |
|                              | Shib Draz                           | 74                                    | 10                  | 10                           | 15                          | 9                      | 0                   | 0                   | 4                        | 0                  | 0                  | 2                     | 0                     | 40                 | 0                 | 0                    | 0                        | 164                          |
|                              |                                     | 48                                    | 19                  | 2                            | 8                           | 0                      | 0                   | 0                   | 4                        | 0                  | 0                  | 4                     | 0                     | 38                 | 0                 | 0                    | 0                        | 123                          |
| Number of female mosqui- tos |                                     | 1261                                  | 2050                | 359                          | 648                         | 125                    | 70                  | 85                  | 92                       | 35                 | 78                 | 203                   | 223                   | 390                | 253               | 120                  | 173                      | 6165                         |
| Abundance (%)                |                                     | 20.45                                 | 33.25               | 5.82                         | 10.51                       | 2.03                   | 1.14                | 1.38                | 1.49                     | 0.57               | 1.27               | 3.29                  | 3.62                  | 6.33               | 4.1               | 1.95                 | 2.81                     | 100                          |

**Sampling female mosquitoes**

The number of collected mosquito species based on different sampling methods was represented in Table 2. The majority mosquitoes were collected by hand catch method (43.70%), followed by animal bait (13.12%) and exit traps were placed over the air vents of septic tanks (12.68%). The light trap (0.92%) collected the lowest number of mosquitoes. The most abundant species collected by hand catch method was *Cx. theileri* (35.89%), but

no *Aedes* and *Culiseta* species captured. The number of mosquitoes captured by the human baited trap was 360, and with dominance of *Cx. pipiens* (34.72%). Furthermore, 809 Culicinae mosquitoes collected using animal baited trap nets, the dominant species was *Cx. pipiens* (49.69%). The number of mosquitoes captured by the CDC light traps method was relatively very low and only 57 mosquitoes were captured and mostly *Cx. theileri* (78.94%).

**Table 2:** Abundance of Culicinae species collected with different methods in study area in Hormozgan Province, Southern Iran during June 2017 to May 2019

| Species                              | <i>Cx. pipiens</i>     | <i>Cx. theileri</i>    | <i>Cx. Tri-taeniorhynchus</i> | <i>Cx. quinquefasciatus</i> | <i>Cx. perexiguus</i>  | <i>Cx. modestus</i>    | <i>Cx. pusillus</i>    | <i>Cx. mimeticus</i>   | <i>Cx. sitiens</i>     | <i>Cx. univittatus</i> | <i>Cx. antennatus</i>  | <i>Cx. sinaiticus</i>  | <i>Ae. caspius</i>     | <i>Ae. vexans</i>      | <i>Cs. subochrea</i>   | <i>Cs. longiareolata</i> |
|--------------------------------------|------------------------|------------------------|-------------------------------|-----------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|--------------------------|
| Collection methods                   | Number & (Percent-age) | Number & (Percent-age) | Number & (Percent-age)        | Number & (Percent-age)      | Number & (Percent-age) | Number & (Percent-age) | Number & (Percent-age) | Number & (Percent-age) | Number & (Percent-age) | Number & (Percent-age) | Number & (Percent-age) | Number & (Percent-age) | Number & (Percent-age) | Number & (Percent-age) | Number & (Percent-age) | Number & (Percent-age)   |
| Hand catch                           | 449 (35.61)            | 967 (47.17)            | 242 (67.41)                   | 475 (73.30)                 | 74 (59.20)             | 43 (61.43)             | 53 (62.35)             | 30 (32.61)             | 22 (62.86)             | 46 (58.97)             | 166 (81.77)            | 127 (56.95)            | 0 (0.00)               | 0 (0.00)               | 0 (0.00)               | 0 (0.00)                 |
| Human bait                           | 125 (9.91)             | 120 (5.85)             | 15 (4.18)                     | 10 (1.54)                   | 0 (0.00)               | 0 (0.00)               | 0 (0.00)               | 0 (0.00)               | 0 (0.00)               | 0 (0.00)               | 0 (0.00)               | 0 (0.00)               | 75 (19.23)             | 15 (5.93)              | 0 (0.00)               | 0 (0.00)                 |
| Animal bait                          | 402 (31.88)            | 352 (17.17)            | 20 (5.57)                     | 35 (5.40)                   | 0 (0.00)               | 0 (0.00)               | 0 (0.00)               | 0 (0.00)               | 0 (0.00)               | 0 (0.00)               | 0 (0.00)               | 0 (0.00)               | 0 (0.00)               | 0 (0.00)               | 0 (0.00)               | 0 (0.00)                 |
| Light trap                           | 10 (0.79)              | 45 (2.20)              | 0 (0.00)                      | 2 (0.31)                    | 0 (0.00)               | 0 (0.00)               | 0 (0.00)               | 0 (0.00)               | 0 (0.00)               | 0 (0.00)               | 0 (0.00)               | 0 (0.00)               | 0 (0.00)               | 0 (0.00)               | 0 (0.00)               | 0 (0.00)                 |
| Adult collection of artificial ponds | 67 (5.31)              | 158 (7.71)             | 18 (5.01)                     | 15 (2.31)                   | 0 (0.00)               | 12 (17.14)             | 10 (11.76)             | 5 (5.43)               | 0 (0.00)               | 10 (12.82)             | 0 (0.00)               | 26 (11.66)             | 75 (19.23)             | 62 (24.51)             | 0 (0.00)               | 0 (0.00)                 |
| Adult collection from Septic tank    | 85 (6.74)              | 298 (14.54)            | 35 (9.75)                     | 96 (14.81)                  | 51 (40.80)             | 15 (21.43)             | 22 (25.88)             | 52 (56.52)             | 13 (37.14)             | 22 (28.21)             | 37 (18.23)             | 56 (25.11)             | 0 (0.00)               | 0 (0.00)               | 0 (0.00)               | 0 (0.00)                 |
| By collecting larvae                 | 123 (9.75)             | 110 (5.37)             | 29 (8.08)                     | 15 (2.31)                   | 0 (0.00)               | 0 (0.00)               | 0 (0.00)               | 5 (5.43)               | 0 (0.00)               | 0 (0.00)               | 0 (0.00)               | 14 (6.28)              | 240 (61.54)            | 176 (69.57)            | 120 (100)              | 173 (100)                |
| Total & Percentage                   | 1261 (20.45)           | 2050 (33.25)           | 359 (5.82)                    | 648 (10.51)                 | 125 (2.03)             | 70 (1.14)              | 85 (1.38)              | 92 (1.49)              | 35 (0.57)              | 78 (1.27)              | 203 (3.29)             | 223 (3.62)             | 390 (6.33)             | 253 (4.10)             | 120 (1.95)             | 173 (2.81)               |

The diversity of collected species from artificial ponds was more than other methods and totally 458 adult mosquitoes were captured while the dominant species was *Cx. theileri* (34.49%). By replacing window exit-traps over the air vents of septic tanks, 782 mosquitoes were captured. The dominant species was *Cx. theileri*, but no any *Aedes* and *Culiseta* species were not collected from these places.

### Larval habitats

Totally three genera of *Aedes*, *Culex*, and *Culiseta* larvae were collected from different breeding places which included 10 (Table 3). *Culex pipiens* was the dominant species collected from running water while *Ae. caspius* was dominant in stagnant water. In temporary running water, *Cs. longiareolata* was the most frequent. However, these three species were found in temporary stagnant water. *Culex*

*pipiens* was found in non-vegetated habitats while in vegetated habitats, *Ae. caspius* was the most frequent species.

The largest number of Culicinae larvae were collected in mud-bottomed habitats and less in rock or cement bottom. The greatest number of larvae collected in habitats with sandy floor was *Cs. longiareolata*. *Ae. caspius* was the most frequent in clear water habitats and *Cx. pipiens* dominantly found in non-clear water. In sunny, semi-shady, shady habitats, different larval species of all three genera were collected in the areas. In the semi-shady larval habitats, all species has been collected except *Cx. tritaeniorhynchus* and *Cx. mimeticus*. In natural habitats, all species were collected except *Cx. mimeticus*, *Aedes* while *Culiseta* were the more abundant in natural habitats.

**Table 3:** Culicinae larval habitats by species in the study areas of Hormozgan Province, Southern Iran during June 2017 to May 2019

| <i>Species</i>                   |                                     | <i>Cx. pipiens</i> | <i>Cx. theileri</i> | <i>Cx. tritaeniorhynchus</i> | <i>Cx. quinquefasciatus</i> | <i>Cx. minimus</i> | <i>Cx. Sinaiticus</i> | <i>Ae. caspius</i> | <i>Ae. vexans</i> | <i>Cs. subochrea</i> | <i>Cs. longiareolata</i> | <i>Total</i> |
|----------------------------------|-------------------------------------|--------------------|---------------------|------------------------------|-----------------------------|--------------------|-----------------------|--------------------|-------------------|----------------------|--------------------------|--------------|
| Total number of collected larvae |                                     | 123                | 110                 | 29                           | 15                          | 5                  | 14                    | 240                | 176               | 120                  | 173                      | 1005         |
| Habitat type                     | Permanent water with running water  | 33                 | 20                  | 0                            | 0                           | 0                  | 0                     | 0                  | 11                | 6                    | 4                        | 74           |
|                                  | Permanent water with stagnant water | 50                 | 59                  | 0                            | 0                           | 0                  | 0                     | 84                 | 45                | 10                   | 15                       | 263          |
| Vegetation status                | Temporary water with running water  | 15                 | 3                   | 0                            | 0                           | 0                  | 0                     | 0                  | 0                 | 25                   | 64                       | 107          |
|                                  | Temporary water with static water   | 25                 | 28                  | 4                            | 15                          | 5                  | 14                    | 156                | 120               | 79                   | 90                       | 536          |
| Floor type                       | non-vegetated habitats              | 43                 | 20                  | 4                            | 0                           | 0                  | 0                     | 0                  | 0                 | 0                    | 30                       | 97           |
|                                  | vegetated habitats                  | 80                 | 90                  | 0                            | 15                          | 5                  | 14                    | 240                | 176               | 120                  | 143                      | 883          |
| Water condition                  | mud                                 | 85                 | 65                  | 4                            | 15                          | 0                  | 14                    | 240                | 150               | 98                   | 108                      | 779          |
|                                  | Sand                                | 38                 | 30                  | 0                            | 0                           | 0                  | 0                     | 0                  | 26                | 22                   | 65                       | 181          |
|                                  | Stone or cement                     | 0                  | 15                  | 0                            | 0                           | 5                  | 0                     | 0                  | 0                 | 0                    | 0                        | 20           |
| The state of sunlight            | non-clear water                     | 108                | 95                  | 4                            | 15                          | 5                  | 14                    | 30                 | 10                | 15                   | 25                       | 321          |
|                                  | clear water                         | 15                 | 15                  | 0                            | 0                           | 0                  | 0                     | 210                | 166               | 105                  | 148                      | 659          |
|                                  | sunny                               | 0                  | 15                  | 4                            | 0                           | 0                  | 0                     | 30                 | 8                 | 6                    | 20                       | 83           |
| Habitat type                     | semi-shady                          | 91                 | 73                  | 0                            | 15                          | 0                  | 14                    | 125                | 99                | 99                   | 118                      | 634          |
|                                  | shady                               | 32                 | 22                  | 0                            | 0                           | 5                  | 0                     | 85                 | 69                | 15                   | 35                       | 263          |
|                                  | natural                             | 88                 | 90                  | 4                            | 15                          | 0                  | 14                    | 205                | 141               | 95                   | 141                      | 793          |
|                                  | artificial                          | 35                 | 20                  | 0                            | 0                           | 5                  | 0                     | 35                 | 35                | 25                   | 32                       | 187          |

#### Determination of West Nile Virus (WNV)

A total of 145 pools of mosquito species collected in the study areas were subjected to RT-PCR molecular tests to determine WNV infection (Table

4). Examinations on all samples (female and male mosquitoes) from different regions showed that the samples were not infection with WNV.

**Table 4:** Number of pools prepared for molecular assays in the study areas of Hormozgan Province, southern Iran during June 2017 to May 2019

| Mosquitoes species           | Number of pools provided by County |       |          |         |               |       |             |       | Total |
|------------------------------|------------------------------------|-------|----------|---------|---------------|-------|-------------|-------|-------|
|                              | Bandar Abbas                       | Minab | Hajiabad | Parsian | Bandar Lengeh | Rudan | Bandar Jask | Qeshm |       |
| <i>Cx. pipiens</i>           | 7                                  | 5     | 2        | 2       | 2             | 3     | 3           | 4     | 28    |
| <i>Cx. theileri</i>          | 10                                 | 7     | 5        | 8       | 2             | 4     | 3           | 1     | 40    |
| <i>Cx. tritaeniorhynchus</i> | 2                                  | 2     | 1        | 1       | 1             | 1     | 1           | 1     | 10    |
| <i>Cx. quinquefasciatus</i>  | 5                                  | 3     | 3        | 1       | 1             | 1     | 1           | 1     | 15    |
| <i>Cx. perexiguus</i>        | 1                                  | 1     | 0        | 0       | 0             | 0     | 1           | 1     | 4     |
| <i>Cx. modestus</i>          | 2                                  | 0     | 0        | 0       | 0             | 0     | 0           | 0     | 2     |
| <i>Cx. pusillus</i>          | 2                                  | 0     | 0        | 0       | 0             | 0     | 0           | 0     | 2     |
| <i>Cx. mimeticus</i>         | 1                                  | 1     | 0        | 0       | 0             | 1     | 0           | 1     | 4     |
| <i>Cx. sitiens</i>           | 1                                  | 1     | 0        | 0       | 0             | 1     | 0           | 0     | 3     |
| <i>Cx. univittatus</i>       | 1                                  | 0     | 0        | 0       | 0             | 0     | 0           | 0     | 1     |
| <i>Cx. antennatus</i>        | 1                                  | 1     | 1        | 1       | 1             | 1     | 1           | 1     | 8     |
| <i>Cx. sinaiticus</i>        | 2                                  | 2     | 0        | 0       | 0             | 1     | 0           | 0     | 5     |
| <i>Ae. caspius</i>           | 5                                  | 0     | 0        | 0       | 0             | 0     | 0           | 3     | 8     |
| <i>Ae. vexans</i>            | 3                                  | 3     | 0        | 0       | 0             | 0     | 0           | 0     | 6     |
| <i>Cs. subochrea</i>         | 3                                  | 0     | 0        | 0       | 0             | 0     | 0           | 0     | 3     |
| <i>Cs. longiareolata</i>     | 3                                  | 0     | 0        | 1       | 2             | 0     | 0           | 0     | 6     |
| Total                        | 49                                 | 26    | 11       | 14      | 9             | 13    | 10          | 13    | 145   |

## Discussion

In the current investigation, the highest number of mosquito species belonged to *Cx. theileri* (33.25%) and *Cx. pipiens* (20.45%). *Cx. theileri* and *Cx. pipiens* are dominantly present in all the study areas. Other species such as *Cx. pusillus*, *Cx. univittatus*, *Ae. caspius*, *Ae. vexans* and *Cs. subochrea* either did not exist or had a very low distribution. In a study conducted in Sistan-Baluchestan Province, *Cx. pipiens* complex was one of the most collected species (14). Based on the study conducted in Isfahan Province, it was one of the most frequent species (15). Furthermore, Azari Hamidian et al showed that this species is one of the most abundant Culicidae mosquitoes in Guilan Province (16). In addition, in Kurdistan and Kermanshah (17), and Mazandaran provinces (18), *Cx. pipiens* is considered one of the most frequently collected sam-

ples. *Cx. pipiens* complex has a worldwide distribution and is also well adapted to different types of breeding site, including stagnant water and even sewage system of the houses (3, 19,20). *Cx. pipiens* can be dominant and abundant in different breeding places. Investigation of *Cx. pipiens* larval habitats and their characteristics can make it clear that a very high compromise of this species with different types of larvae habitats and different degrees of contamination is the reason for the high distribution and abundance of this species in Iran. In the current study except *Culex* genus were the most abundant species and a few numbers of *Cs. longiareolata* and *Cs. subochrea* mosquitoes were collected. In contrast, in East Azerbaijan (21), Kurdistan and Kermanshah (17) provinces. *Cs. longiareolata* is one of the most abundant species in North-western Iran. In our study, all three genera of collected mosquitoes were found in natural larval habitats having some vegetation. Among three

genera, *Culex* species approximately were collected from different types of larval habitats and this finding was similar to study conducted in North-western Iran (21). *Cx. pipiens* complex e.g., *Cx. quinquefasciatus* and *Cx. pipiens* are important vectors to transmit some arboviral diseases mainly WNV to humans in many areas. Moreover, *Cx. quinquefasciatus* plays a major role in virus transmission among birds (6-7,19,22,23).

Although, our molecular detection of WNV infection among the collected mosquitoes from Hormozgan Province was negative, but in a previous study, *Cx. pipiens* was found infected with the virus from different areas of this province (9) and in the equine population (24). Thus, WNV should be more considered as an important mosquito-borne disease.

In addition, *Cx. theileri* and *Cx. pipiens* were positive to WNV in Lorestan Province (25). WNV was detected in *Ae. caspius*, collected from West Azerbaijan Province (8). However, we could not detect WNV among 6165 collected mosquitoes. It seems that the circulation of WNV between vectors and reservoir occurs every few years, not seasonally or annually. Therefore, it is possible we done the present survey during the time which WNV circulation was silence. However, *Cx. pipiens* mosquitoes was found positive to WNV in Morocco (26) Bulgaria (27) Greece and the other countries (28).

## Conclusion

Although detection of WNV genome was not confirmed in our study due to circulation of WNV in Hormozgan Province, more studies and regular checking of mosquitos is recommended for accurate monitoring of the disease in future.

## Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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## Conflict of Interest

The authors declare that there is no conflict of interest.

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