Iran J Public Health, Vol. 52, No.4, Apr 2023, pp.809-817

Original Article

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

Abbas Poudat [1,2], Hamideh Edalat [3], Morteza Zaim [3], Farhad Rezaei [4], Yaser Salim Abadi [5], *Hamid Reza Basseri [3]

1. *Department of Medical Entomology and Vector Control, School of Public Health, International Campus, Tehran University of Medical Sciences, Tehran, Iran*
2. *Infectious and Tropical Diseases Research Center, Hormozgan Health Institute, Hormozgan University of Medical Sciences, Bandar Abbas, Iran*
3. *Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
4. *Department of Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
5. *Department of Health Services and Health Promotion, School of Health, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*

***Corresponding Author:** Email: basserih@tums.ac.ir

(Received 19 Nov 2021; accepted 06 Feb 2022)

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^2 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).

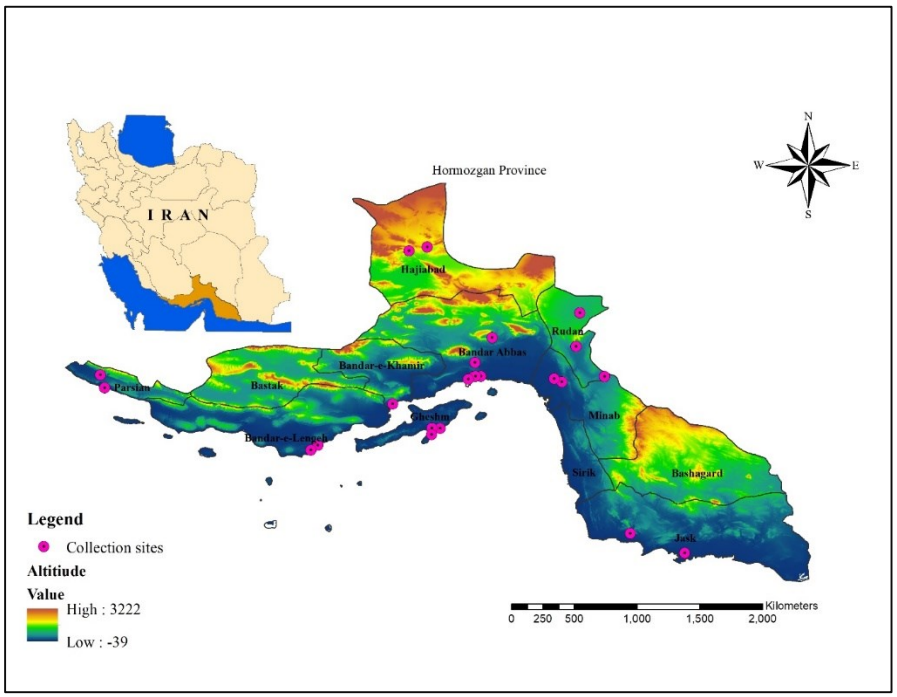

Fig. 1: Selected areas for entomological survey in Hormozgan Province, Southern Iran

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_2, 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 & Nomber of Female Mosquitoes*																*Nomber of female mosquitos*
City	Collection sites	*Cx. pipiens*	*Cx. theileri*	*Cx. tritaeniorhynchus*	*Cx. quinquefasciatus*	*Cx. pervigilans*	*Cx. modestus*	*Cx. pusillus*	*Cx. mimeticus*	*Cx. sitiens*	*Cx. univittatus*	*Cx. antennatus*	*Cx. sinaiticus*	*Ae. caspius*	*Ae. vexans*	*Cs. subochrea*	*Cs. longiareolata*	
Bandar Abbas	Daneshkadeh Behdasht	32	75	8	10	0	0	0	3	2	5	3	10	12	6	0	6	172
	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	69	35	16	28	0	2	14	6	2	6	12	16	35	25	31	16	313
	hormoudar Rural	45	150	30	32	14	15	12	10	0	20	10	20	64	30	18	26	496
	khorgoo Rural	49	75	16	48	21	17	22	12	6	18	11	26	68	25	16	20	450
Minab	City of Minab	96	108	23	57	12	0	0	7	3	0	15	36	0	33	0	0	390
	Haji balouchi	88	150	25	71	6	0	0	9	4	0	17	30	0	38	0	0	438
	Bolboli	75	100	35	44	10	0	0	7	5	0	11	25	0	40	0	0	352
Hajiabad	Haji Abad City	65	150	10	28	0	0	0	0	0	0	12	0	0	0	0	0	265
	Tejerj	35	103	25	32	0	0	0	0	0	0	13	0	0	0	0	0	208
Parsian	Parsian	20	220	8	7	0	0	0	0	0	0	7	0	0	0	0	6	268
	ziyarat	45	200	9	7	0	0	0	0	0	0	8	0	0	0	0	9	278
Bandar Lengeh	Bandar Kong	72	120	27	12	0	0	0	0	0	0	12	0	0	0	0	54	297
Rudan	roudan	45	98	10	17	0	0	0	10	3	0	12	14	0	0	0	0	209
	ziyarat ali	75	112	18	5	0	0	0	12	5	0	10	13	0	0	0	0	250
Bandar Jask	bahmadi	68	73	12	40	0	0	0	0	0	0	6	0	0	0	0	0	199
	zar abad	47	47	33	42	5	0	0	0	0	0	9	0	0	0	0	0	183
Qeshm	Mesen	58	15	7	10	2	0	0	2	0	0	8	0	62	0	0	0	164
	Direstan	74	10	10	15	9	0	0	4	0	0	2	0	40	0	0	0	164
	Shib Draz	48	19	2	8	0	0	0	4	0	0	4	0	38	0	0	0	123
Nomber of female mosquitos		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 dominancy 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	***Cx. Pipiens***		***Cx. theileri***		***Cx. Tritaeniorhynchus***		***Cx. quinquefasciatus***		***Cx. perexiguus***		***Cx. modestus***		***Cx. pusillus***		***Cx. mimeticus***		***Cx. sitiens***		***Cx. univittatus***		***Cx. antennatus***		***Cx. sinaiticus***		***Ae. caspius***		***Ae. vexans***		***Cs. subochrea***		***Cs. longiareolata***	
Collection methods	Number & (Percentage)		Number & (Percentage)		Number & (Percentage)		Number & (Percentage)		Number & (Percentage)		Number & (Percentage)		Number & (Percentage)		Number & (Percentage)		Number & (Percentage)		Number & (Percentage)		Number & (Percentage)		Number & (Percentage)		Number & (Percentage)		Number & (Percentage)		Number & (Percentage)		Number & (Percentage)	
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 was 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

Species		*Cx. pipiens*	*Cx. theileri*	*Cx. tritaeniorhynchus*	*Cx. quinquefasciatus*	*Cx. mimeticus*	*Cx. Sinaiticus*	*Ae. caspius*	*Ae. vexans*	*Cs. subochrea*	*Cs. longiareolata*	***Total***
Total number of collected larvae		123	110	29	15	5	14	240	176	120	173	1005
water Habitat type	Permanent with running water	33	20	0	0	0	0	0	11	6	4	74
	Permanent with stagnant water	50	59	0	0	0	0	84	45	10	15	263
	Temporary with running water	15	3	0	0	0	0	0	0	25	64	107
	Temporary with static water	25	28	4	15	5	14	156	120	79	90	536
Vegetation status	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
Floor type	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
Water condition	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
The state of sunlight	sunny	0	15	4	0	0	0	30	8	6	20	83
	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
Habitat type	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	
Cx. pipiens	7	5	2	2	2	3	3	4	28
Cx. theileri	10	7	5	8	2	4	3	1	40
Cx. tritaeniorhynchus	2	2	1	1	1	1	1	1	10
Cx. quinquefasciatus	5	3	3	1	1	1	1	1	15
Cx. perexiguus	1	1	0	0	0	0	1	1	4
Cx. modestus	2	0	0	0	0	0	0	0	2
Cx. pusillus	2	0	0	0	0	0	0	0	2
Cx. mimeticus	1	1	0	0	0	1	0	1	4
Cx. sitiens	1	1	0	0	0	1	0	0	3
Cx. univittatus	1	0	0	0	0	0	0	0	1
Cx. antennatus	1	1	1	1	1	1	1	1	8
Cx. sinaiticus	2	2	0	0	0	1	0	0	5
Ae. caspius	5	0	0	0	0	0	0	3	8
Ae. vexans	3	3	0	0	0	0	0	0	6
Cs. subochrea	3	0	0	0	0	0	0	0	3
Cs. longiareolata	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 Culicinae 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 samples. *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 Northwestern 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 Northwestern 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.

Acknowledgements

The authors would like to thank the School of Public Health, Tehran University of Medical Sciences, for their support in carrying out this research. This study was financially supported by the Deputy of Research of International Campus, Tehran University of Medical Sciences, with Grant No. 37360. In addition, the authors would like to appreciate the collaboration received from Dr. Alireza Sanei Dehkordi, and Abdolrasool Mojahedi and Masood Yerian of Hormozgan Health to provide required data for this study.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

1. Harbach R (2007). The Culicidae (Diptera): a review of taxonomy, classification and phylogeny. *Zootaxa*, 668 (1): 591-638.
2. Cleton N, Koopmans M, Reimerink J et al (2012). Come fly with me: review of clinically important arboviruses for global travelers. *J Clin Virol*, 55(3):191-203.
3. Adams LE, Martin SW, Lindsey NP et al (2019). Epidemiology of dengue, Chikungunya, and Zika virus disease in US states and territories, 2017. *Am J Trop Med Hyg*, 101(4):884-890.
4. Weingartl, H, Neufeld J L, Copps J et al (2004). Experimental West Nile virus infection in blue jays (*Cyanocitta cristata*) and crows (*Corvus brachyrhynchos*). *Vet Pathol*, 41(4): 362-70.
5. Komar N, Langevin S, Hinten S, et al (2003). Experimental infection of North American birds with the New York 1999 strain of West Nile virus. *Emerg Infect Dis*, 9(3): 311-22.
6. Turell, M.J., Sardelis M.R., Dohm D.J et al (2001). Potential North American vectors of west Nile virus. *Ann N Y Acad Sci*, 951(1): 317-24.
7. Amin M, Zaim M, Edalat H, et al (2020). Seroprevalence Study on West Nile Virus (WNV) Infection, a Hidden Viral Disease in Fars Province, Southern Iran. *J Arthropod Borne Dis*, 14(2):173–184.

8. Bagheri M, Terenius O, Oshaghi MA, et al (2015). West Nile Virus in mosquitoes of Iranian wetlands. *Vector Borne Zoonotic Dis*, 15(12): 750-54.
9. Ziyaeyan M, Behzadi MA, Leyva-Grado VH, et al (2018). Widespread circulation of West Nile virus, but not Zika virus in southern Iran. *PLoS Negl Trop Dis*, 12(12): e0007022.
10. Hormozgan Province (2022). Available from: https://en.wikipedia.org/wiki/Hormozgan_Province
11. Azari-Hamidian S, Harbach RE (2009). Keys to the adult females and fourth-instar larvae of the mosquitoes of Iran (Diptera: Culicidae). *Zootaxa*, 2078(1): 1-33.
12. Shahgudian ER (1960). A key to the Anophelines of Iran. *Acta Med Iran*, 3:38-48.
13. Va´zquez A, Herrero L, Negredo A, et al (2016). Real time PCR assay for detection of all known lineages of West Nile virus. *J Virol Methods*, 236:266-70.
14. Moosa-Kazemi SH, Vatandoost H, Nikookar H, et al (2009). Culicinae (Diptera: Culicidae) mosquitoes in chabahar county, sistan and baluchistan province, southeastern Iran. *Iran J Arthropod Borne Dis*, 3(1):29.
15. Mousa-kazemi SH, Zaim M, Zahraii A (2000). Fauna and ecology of Culicidae of the Zarrin-Shahr and Mobarakeh area in Isfahan Province. Armaghan Danesh. *Journal of Yasuj University of Medical Sciences*, 5: 46- 54.
16. Azari-Hamidian S (2011). Larval habitat characteristics of mosquitoes of the genus *Culex* (Diptera: Culicidae) in Guilan Province, Iran. *Iran J Arthropod Borne Dis*, 5(1):37-53.
17. Moosa-Kazemi H, Zahirnia AH, Sharifi F, et al (2015). The Fauna and Ecology of Mosquitoes (Diptera: Culicidae) in Western Iran. *J Arthropod Borne Dis*, 9(1): 49-59.
18. Nikookar SH, Moosa-Kazemi SH, Yaghoobi-Ershadi MR, et al (2015). Fauna and larval habitat characteristics of mosquitoes in Neka County, Northern Iran. *J Arthropod Borne Dis*, 9(2): 253-66.
19. Salim-Abadi Y, Oshaghi MA, Enayati AA, et al (2016). High insecticides resistance in *Culex pipiens* (Diptera: Culicidae) from Tehran, capital of Iran. *J Arthropod Borne Dis*, 10(4): 483-92.
20. Liu X, Yue Y, Wu H, et al (2019). Breeding site characteristics and associated factors of *Culex pipiens* complex in Lhasa, Tibet, PR China. *Int J Environ Res Public Health*, 16(8): 1407.
21. Hazratian T, Paksa A, Sedaghat MM, et al (2019). Baseline Susceptibility of *Culiseta longiareolata* (Diptera: Culicidae) to Different Imagicides, in Eastern Azerbaijan, Iran. *J Arthropod Borne Dis*, 13 (4): 407-15.
22. Rutledge CR, Day JF, Lord CC, et al (2003). West Nile virus infection rates in *Culex nigripalpus* (Diptera: Culicidae) do not reflect transmission rates in Florida. *J Med Entomol*, 40(3): p. 253-258.
23. Sardelis MR, Turell MJ, Dohm DJ, et al (2001). Vector competence of selected North American *Culex* and *Coquillettidia* mosquitoes for West Nile virus. *Emerg Infect Dis*, 7(6): 1018-22.
24. Ahmadnejad F, Otarod V, Fallah M, et al (2011). Spread of West Nile virus in Iran: a cross-sectional serosurvey in equines, 2008–2009. *Epidemiol Infect*, 139(10):1587-93.
25. Shahhosseini N, Moosa-Kazemi SH, Sedaghat MM, et al (2020). Autochthonous Transmission of West Nile Virus by a New Vector in Iran, Vector-Host Interaction Modeling and Virulence Gene Determinants. *Viruses*, 12(12):1449.
26. Assaid N, Mousson L, Moutailler S, et al (2020). Evidence of circulation of West Nile virus in *Culex pipiens* mosquitoes and horses in Morocco. *Acta Trop*, 205:105414.
27. Christova I, Papa A, Trifonova I, et al (2020). West Nile virus lineage 2 in humans and mosquitoes in Bulgaria, 2018-2019. *J Clin Virol*, 127:104365.
28. Mavridis K, Fotakis EA, Kioulos I, et al (2018). Detection of West Nile Virus–Lineage 2 in *Culex pipiens* mosquitoes, associated with disease outbreak in Greece, 2017. *Acta Trop*, 182:64-8.