

Molecular Monitoring of Flavivirus Infections (West Nile Virus and Dengue Virus) Among Culicidae Mosquitoes in Southern Iran

Masoumeh Amin¹, PhD;
Hamideh Edalat², PhD;
Hamidreza Basseri², PhD;
Morteza Zaim², PhD; Kourosh
Azizi³, PhD; Mostafa Salehi-
Vaziri⁴, PhD; Sahar Khakifirouz⁴,
MSc; Saideh Yousefi⁵, PhD;
Mohsen Kalantari³, PhD;
Mozaffar Vahedi⁶, PhD;
Parisa Amin⁷, BSc

¹Department of Vector Biology and Control of Diseases, School of Health, Shiraz University of Medical Sciences, Shiraz, Iran

²Department of Vector Biology and Control of Diseases, School of Health, Tehran University of Medical Sciences, Tehran, Iran

³Research Center for Health Sciences, Institute of Health, Department of Vector Biology and Control of Diseases, School of Health, Shiraz University of Medical Sciences, Shiraz, Iran

⁴Department of Arboviruses and Viral Hemorrhagic Fevers, Pasteur Institute of Iran

⁵Sirjan School of Medical Sciences, Sirjan, Iran

⁶Student Research Committee, Department of Vector Biology and Control of Diseases, School of Health, Shiraz University of Medical Sciences, Shiraz, Iran

⁷Department of Physiotherapy and Rehabilitation, Yeditepe University, Istanbul, Turkey

Correspondence:

Hamidreza Basseri, PhD;
Department of Vector Biology and Control of Diseases, School of Health, Tehran University of Medical Sciences, Tehran, Iran

Email: basserih@tums.ac.ir

Received: 12 October 2024

Revised: 09 November 2024

Accepted: 16 December 2024

Abstract

Background: Mosquitoes (Diptera: Culicidae) are significant vectors of mosquito-borne diseases prevalent across temperate and tropical regions. Understanding the abundance and distribution of these vectors is essential for studying the eco-epidemiology of mosquito-borne diseases.

Methods: This cross-sectional study aimed to identify mosquito populations (Culicidae) and conduct molecular analysis for Flavivirus infections, explicitly focusing on West Nile Virus (WNV) and Dengue Virus (DENV) in Fars Province, Southern Iran. Mosquitoes were collected from 23 stations across urban and rural areas and migratory bird habitats in five counties, using hand catch with manual aspirators, CDC light traps with CO₂, and human and animal bait methods from April to December 2018. Molecular screening for Flavivirus RNA, including WNV and DENV, was performed using a specific PCR technique.

Results: 8212 adult mosquitoes were collected, representing four genera and nine species. The most prevalent species were *Culex pipiens* (54.20%), *Culiseta longiareolata* (30.40%), and *Culex sinaiticus* (10.25%). Molecular screening was conducted on the 8212 mosquitoes grouped into 150 pools based on sex, species, and trapping location. None of the pools tested were positive for Flavivirus RNA.

Conclusion: This study highlights the importance of monitoring mosquito species distribution and emphasizes the need for enhanced Flavivirus surveillance and long-term monitoring programs in the region to understand the risk of disease transmission better.

Please cite this article as: Amin M, Edalat H, Basseri HR, Zaim M, Azizi K, Salehi-Vaziri M, Khakifirouz S, Yousefi S, Kalantari M, Vahedi M, Amin P. Molecular Monitoring of Flavivirus Infections (West Nile Virus and Dengue Virus) Among Culicidae Mosquitoes in Southern Iran. J Health Sci Surveillance Sys. 2025;13(1):68-74.

Keywords: Fars, *Flavivirus*, Iran, Mosquito

Introduction

Mosquitoes (Diptera: Culicidae) are a widespread and prolific group inhabiting temperate and tropical regions globally. Understanding mosquito species' biology, abundance, and distribution is essential for accurately assessing the transmission dynamics of

mosquito-borne diseases and comprehending their ecoepidemiology.

The most recent taxonomy classifies mosquitoes (Diptera: Culicidae) into two subfamilies, 11 tribes, 41 genera, 186 subgenera, and 3563 species. Based on the latest checklist in Iran, seven genera have been identified, including *Anopheles*, *Uranotaenia*,

Culiseta, *Coquillettia*, *Culex*, *Aedes*, and *Ochlerotatus*. These encompass 16 subgenera, 64 species, and three subspecies.¹

Numerous studies have demonstrated that mosquitoes are key vectors for transmitting a variety of arboviruses belonging to families such as *Phenuiviridae*, *Togaviridae*, and *Flaviviridae*. The *Flaviviridae* family comprises 58 species, many posing significant threats to human health. Of these, 39 species are transmitted by ticks and mosquitoes.²

One prominent family member is West Nile Virus (WNV) (*Flaviviridae: Flavivirus*), prevalent in regions including Australia, Africa, and North and Central America. While both soft and hard ticks (*Arachnida: Ixodidae*) are known vectors, mosquitoes are particularly important in transmitting WNV. Wild birds, especially wetland species, serve as amplifying hosts for the virus.³

Several reports have documented the transmission of pathogens by mosquitoes in Iran, including *Dirofilaria immitis* and *D. repens*,⁴ various *Plasmodium spp.*,⁵ West Nile Virus⁶ (Bozorg-Omid et al. 2020), Dengue virus (DENV) (*Flaviviridae: Flavivirus*),⁷ and Rift Valley Fever Virus (RVFV) (*Phenuiviridae: Phlebovirus*).⁸ Despite the significant public health impact of viral diseases like WNV and DENV, limited information is available regarding their mosquito vectors in Iran. Moreover, no comprehensive vector control program for WNV has been implemented in the country. A previous serological study identified the presence of WNV antibodies in multiple regions of Fars Province in southern Iran.⁹

The objective of this research was to identify the mosquito species present in Fars Province and to screen them for West Nile Virus (WNV), Dengue Virus (DENV), and other Flaviviruses using real-time pan-flavivirus RT-PCR and virus-specific real-time RT-PCR techniques.

Methods

Study Area

This research was conducted in Fars Province, located at 29.62° N and 52.53° E, between April and October 2018. Fars Province is geographically bordered by Isfahan and Yazd provinces to the north, Kohgiluyeh & Boyer-Ahmad to the west, Bushehr and Hormozgan to the south, and Kerman to the east. Five diverse locations within Fars Province were selected for mosquito collection: Shiraz County, Sepidan County, Zarqan County, Marvdasht County, and Maharloo Wetland (Figure 1). Between April and December 2018, mosquito sampling was conducted at 25 fixed and variable stations strategically chosen within these locations.

Sample Collection and Processing

Adult mosquitoes were collected using multiple sampling methods, including CO₂-baited CDC light traps operated for 13 hours (from 6 p.m. to 7 a.m.), old tires, night landing catches on human and animal baits, and manual aspirators for daytime hand catches (8 a.m. to 5 p.m.) at resting sites (Figure 2).

After collection, mosquitoes were identified morphologically using established identification keys.⁴ Each specimen was pooled based on external morphological characteristics, sex, species, and collection location. Pools contained between 5 and 100 specimens, which were stored in cryovials. These cryovials were then placed in a liquid nitrogen tank for preservation and transported to the insectarium at the School of Health, Shiraz University of Medical Sciences, Shiraz, Iran, for further analysis.

Molecular Detection of Flavivirus RNA

Mosquito pools were homogenized, and 200 µL of the resulting homogenate was used for viral nucleic acid (NA) extraction using the Virus Extraction Mini Kit (QIAGEN). A 5 µL aliquot of the extracted NA was

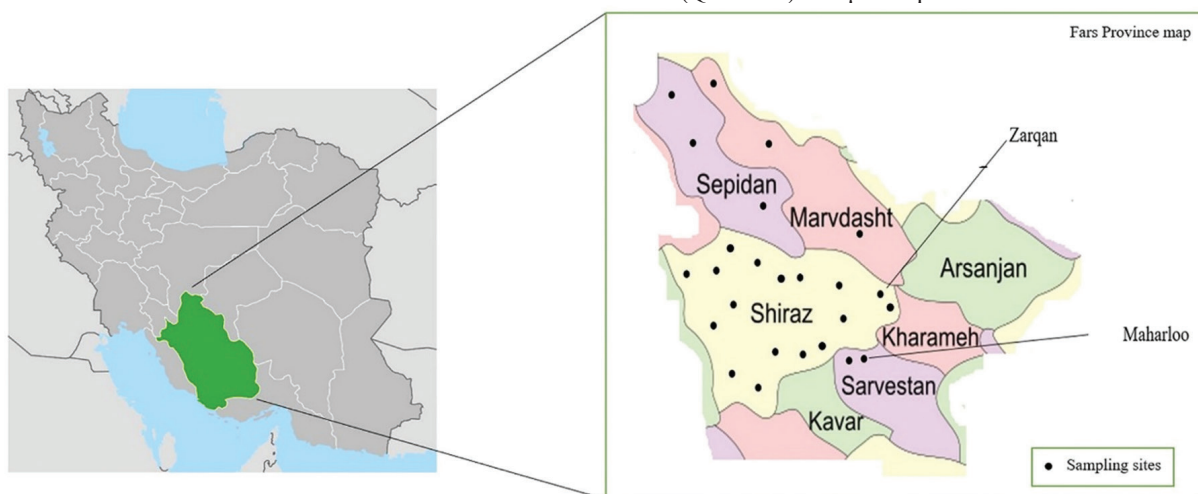


Figure 1: Study area and sampling sites in Fars Province, Southern Iran (Designed by author).



Figure 2: Ecosystems sampled in Fars Province, Southern Iran: a. migratory bird area, b. rice field, c. riversides, d. swamp, e. stable, f., and g. stagnant water, h. marsh (Original Fig.).

utilized for RT-PCR. The assay employed sense (ProC-F1: CCTGTGTGAGCTGACAACTTAGT) and reverse (ProC-R: GCGTTTTAGCATATTGACAGCC) primers, combined with the fluorogenic TaqMan probe (ProC-TM: 6FAM-CCTGGTTTCTTAGACATCGAGATCTTCGTGC-TAMRA). The reactions were performed using the GoTaq Probe 1-Step RT-qPCR kit (Promega).¹⁰

Total nucleic acid extraction was done with the Virus Mini Kit v2.0 (QIAGEN) and the BioRobot EZ1 system. For further analysis, another 5 μ L aliquot of NA was processed using the Real-Time Quanti-Tec SYBR-GREEN RT-PCR assay, following previously described one-step procedures.¹¹ The PCR test targeted a highly conserved region of the polymerase gene, facilitating the detection of a wide range of Flaviviruses.

Results

A total of 8,212 adult mosquitoes were collected from the research areas, representing four genera and nine species. The most prevalent species were *Cx. pipiens* (54.2%), *Cs. longiareolata* (30.40%), and *Cx. Sinaiticus* (10.25%). Additional species included *Cx. Deserticola* (3.41%), *Ur. Unguiculata* (1.23%), *Cx. Pusillus* (0.29%), *An. Claviger* (0.09%), *Cx. Perexiguus* (0.08%), and *An. Stephensi* (0.05%) (Table 1). The study area demonstrated high species diversity, with all nine species recorded. *Cx. pipiens* was the most widespread species collected from all five study areas. None of the 150 mosquito pools tested positive for West Nile Virus or Dengue Virus using real-time RT-PCR.

Discussion

This study collected 8,212 mosquito specimens, representing nine different species. The most prevalent species were *Cx. pipiens*, *Cx. Sinaiticus*, and *Cs.*

longiareolata. However, none of the samples tested positive for West Nile Virus (WNV) or Dengue virus (DENV).

Cx. pipiens, commonly known as the house mosquito, was found in all the sampling regions. This species is a known vector for several arboviruses, including TAHV, SINV, and WNV.¹⁰ *Cx. pipiens* is widely distributed across Africa, central North America, Europe, Asia, Australia, and South America.¹²⁻¹⁴ Previous studies have reported frequent sightings of *Cx. pipiens* in various regions of Iran, including Sanandaj,¹⁵ Zanjan,¹⁶ Ardebil,⁴ Isfahan,¹⁷ East Azerbaijan,¹⁸ and Kashan.¹⁹ Epidemiological evidence of WNV in different hosts has been documented in Iran,²⁰ and the circulation of WNV in the primary vector, *Cx. pipiens s.l.* and *Cx. pipiens*, has been confirmed in several studies.²¹

Cx. sinaiticus has been previously reported in several provinces of Iran, including Sistan and Baluchistan, Khuzestan, Bushehr, Fars, Hormozgan, and Kerman provinces. A few years ago, a study showed that this species prefers larval environments with immobile or temporary water, clear, muddy bottoms, and plants.^{19,22} In this research, *Cx. Sinaiticus* was one of the most significant populations, with 842 specimens (10.13%) collected.

Cs. longiareolata has been reported from all regions of Iran, including Sistan-Baluchistan, Kerman, Kurdistan, and Kermanshah provinces.^{1, 18, 23} It has been identified as a dominant species of culicid mosquitoes in these areas.^{24, 25} Larvae of this species are typically found in various water sources, including wells and canals, with peak activity occurring during June and July.²⁶ In this study, *Cs. longiareolata* was found in all the sampling regions and was one of the dominant culicine mosquitoes.

Re-emerging and newly emerging arboviral diseases are recognized as significant global health

Table 1: Details of mosquito specimens collected by study areas (April–December 2018)

Species	Collection site	N	Total N (%)
<i>Culex pipiens</i>	Shiraz county	2134	4450 (54.2%)
	Marvdasht county	760	
	Zarqan county	648	
	Sepidan	476	
	Maharloo Wetland	432	
<i>Culex quinquefasciatus</i>	Shiraz county	132	280 (3.41%)
	Marvdasht county	70	
	Zarqan county	45	
	Sepidan	0	
	Maharloo Wetland	33	
<i>Culex Sinaiticus</i>	Shiraz county	352	842 (10.25%)
	Marvdasht county	0	
	Zarqan county	245	
	Sepidan	97	
	Maharloo Wetland	148	
<i>Culex pusillus</i>	Shiraz county	24	24 (0.29%)
	Marvdasht county	0	
	Zarqan county	0	
	Sepidan	0	
	Maharloo Wetland	0	
<i>Culex previous</i>	Shiraz county	0	7 (0.08%)
	Marvdasht county	7	
	Zarqan county	0	
	Sepidan	0	
	Maharloo Wetland	0	
<i>Culiseta longiareolata</i>	Shiraz county	1150	2496 (30.40%)
	Marvdasht county	425	
	Zarqan county	596	
	Sepidan	200	
	Maharloo Wetland	125	
<i>Anopheles stephensi</i>	Shiraz county	4	4 (0.05%)
	Marvdasht county	0	
	Zarqan county	0	
	Sepidan	0	
	Maharloo Wetland	0	
<i>Anopheles claviger</i>	Shiraz county	8	8 (0.09%)
	Marvdasht county	0	
	Zarqan county	0	
	Sepidan	0	
	Maharloo Wetland	0	
<i>Uranotaenia unguiculata</i>	Shiraz county	0	101(1.23%)
	Marvdasht county	0	
	Zarqan county	0	
	Sepidan	0	
	Maharloo Wetland	101	
Total			8212 (100%)

concerns, with approximately 30% of all newly emerging infectious diseases over the past decade attributed to arboviruses.²⁷ Among these, West Nile Virus (WNV) stands out due to its broad range of vectors, including species of *Culex*, *Aedes*, and occasionally *Anopheles* mosquitoes, while birds serve as the primary vertebrate reservoirs of the virus. WNV fever has become widespread across many parts of the world. Given the spread of the disease in neighboring countries such as Pakistan, Afghanistan, Turkmenistan, Iraq, and the Republic of Azerbaijan, and the movement of people between these nations, Iran faces a significant risk of WNV transmission.²⁸

Until the summer of 1999, the West Nile Virus (WNV) was primarily known to circulate in Africa,

Europe, and Southwest Asia. However, the first reported case of WNV in North America occurred in August 1999, around New York. Since then, the virus has spread significantly across various parts of the world.²⁹ WNV has been reported in regions including Africa, the Middle East, Southern Europe, Western Russia, Southwest Asia, and Australia. The global epidemiology of West Nile Virus has undergone dramatic changes over the past few decades, particularly with its introduction and spread in the Americas. This expansion has been accompanied by increased reported virus activity in Europe.^{30, 31}

The Middle East region, located at the crossroads of several continents, is particularly susceptible to the spread of zoonotic diseases, including West

Nile Fever. The virus has been reported in various countries across the region, such as Egypt, Lebanon, Iraq, the United Arab Emirates, Iran, Pakistan, India, and Russia.³²

Additionally, Iran is at risk for this disease due to the presence of suitable mosquito vectors and favorable environmental conditions for mosquito proliferation. Iran's wetlands serve as wintering grounds for migratory birds that arrive annually from northern Siberia. West Nile Virus is endemic in regions like western Siberia and southern Russia, where several epidemics have been reported in cities such as Volgograd, Astrakhan, and the Krasnodar region. Many of these migratory birds have previously been exposed to different strains of WNV in various locations along their migratory routes.²⁹

West Nile Virus has a broad range of vectors, with mosquitoes of the genus *Culex* being the primary carriers.³³ However, other genera, including *Aedes*, *Anopheles*, *Coquilletidia*, *Culiseta*, *Deinocerites*, *Mansonia*, *Psorophora*, *Orthopodomyia*, and *Uranotaenia*, can also transmit the virus.³⁴

So far, 69 species of *Culicid* mosquitoes, including 17 genera and 35 subgenera, have been identified, some of which are known vectors for WNV. Studies on the genetic transmission of the virus in vector mosquitoes have reported transmission rates of up to 26% in *Cx. pipiens* mosquitoes.³⁵

West Nile fever follows a natural cycle involving wild birds and several types of arthropods. The virus can infect arthropods when the virus level in the blood of wild birds is high enough. This viral activity typically becomes evident during the spring, when mosquitoes from the previous year that were carrying the virus become active again or when infected migratory birds arrive in the area. The most critical period for human and mammal infections occurs during late summer and early fall.^{36,37}

The presence of rice fields, wetlands, and ponds, which migratory birds frequent, may serve as potential breeding grounds for mosquitoes and could contribute to the outbreak of new viral epidemics, including WNV.³⁸ Fars Province is also a popular tourist destination, attracting travelers worldwide each year, which could increase the risk of imported cases of mosquito-borne diseases. Furthermore, *Cx. pipiens*, the primary vector of WNV, is the dominant mosquito species in the region.

This study represents the first attempt to screen mosquitoes in Fars Province for Flavivirus. An improvement that could be made to the present study is incorporating light traps and hand-catch methods for mosquito sampling, which could provide a more comprehensive collection of species. Additionally, collecting, anesthetizing, identifying, and preparing

specimens for molecular procedures are time-consuming tasks, and the sample mass could be a potential limitation for the study's ability to identify viruses. Furthermore, using birds, such as chickens or pigeons, as bait may increase the sampling rate of ornithophilic species (potential vectors for WNV), thereby improving the chances of detecting the virus.³⁹ Given the lack of a confirmed vector surveillance program for mosquito-borne viruses in Iran, it is recommended that such surveys continue to enhance our understanding and management of virus transmission in the region.

Conclusion

Although the species captured in this study can potentially transmit *Flaviviruses*, molecular tests did not show *Flavivirus* contamination. However, the presence of anti-virus antibodies in residents' blood in the study areas indicates the existence of a disease transmission cycle in the region. Continuous monitoring is recommended given the presence of primary carriers for West Nile Virus and Dengue in the study areas. Public health authorities should implement ongoing surveillance activities on arbovirus diseases. Establishing entomological surveillance as an early warning system to prevent future outbreaks of these diseases is essential.

Authors' Contribution

Masoumeh Amin: Conceptualization; investigation; methodology; project administration; supervision; validation; visualization; writing-original draft; writing-review and editing. Hamideh Edalat: Conceptualization; funding acquisition; data curation; formal analysis; methodology; project administration; resources; validation; visualization. Hamid Reza Basseri: Conceptualization; data curation; formal analysis; methodology; project administration; validation; visualization. Morteza Zaim: Conceptualization; project administration; methodology; validation; visualization. Kouros Azizi: Conceptualization; methodology; validation; visualization. Mostafa Salehi-Vaziri: Supervision; methodology; data curation; validation; visualization. Sahar Khakifrouz: Validation; methodology; resources; visualization. Saideh Yousefi: Conceptualization; project administration; supervision; visualization; methodology; writing-original draft preparation; writing-review and editing. Mohsen Kalantari: formal analysis; writing-original draft preparation; writing-review and editing; Mozaffar Vahedi: Writing-Original Draft Preparation; writing-review & editing; data curation. Parisa Amin: Writing-Original Draft Preparation; writing-review & editing.

Acknowledgement

We want to express our gratitude to all employees of the

Department of Medical Entomology and Vector Control, School of Health, Shiraz University of Medical Sciences, Shiraz, Iran.

Funding

This paper results from a research project (Project no: 97-01-27-37365) conducted by the first author and financially supported by the Vice Chancellor of Research, School of Health, Tehran University of Medical Sciences, Tehran, Iran (Code of ethics: IR.TUMS.SPH.REC.1396.4204).

Conflict of Interest: None declared.

References

- Zaim, M. and P.S. Cranston. 1986. Checklist and keys to the Culicinae of Iran. *Mosq. Syst.* 18: 233-45.
- Schweitzer B.K. and Chapman N.M, Iwen P.C. Overview of the Flaviviridae, emphasizing the Japanese encephalitis group viruses. *Laboratory medicine.* 2009; 40(8): 493-99. doi: 10.1309/LM5YWS85NJPCWESW.
- Hubálek Z. and Halouzka J. West Nile fever--a re-emerging mosquito-borne viral disease in Europe. *Emerg Infect Dis.* 1999; 5(5): 643-50. doi: 10.3201/eid0505.990505. PMID: 10511520; PMCID: PMC2627720.
- Azari-Hamidian S, Harbach, R.E.. Keys to the adult females and fourth-instar larvae of the mosquitoes of Iran (Diptera: Culicidae). *Zootaxa.* 2009; 2078(1): 1-33. doi: 10.11646/zootaxa.2078.1.1.
- Djadid N.D, Jazayeri H, Gholizadeh S, Rad S.P, Zakeri S. First record of a new member of Anopheles Hyrcanus Group from Iran: molecular identification, diagnosis, phylogeny, status of kdr resistance and Plasmodium infection. *J Med Entomol.* 2009; 46(5): 1084-93. doi: 10.1603/033.046.0515. PMID: 19769039.
- Bozorg-Omid F, Oshaghi M.A, Vahedi M, Karimian F, Seyyed-Zadeh S.J, Chavshin A.R. Wolbachia infection in West Nile Virus vectors of northwest Iran. *Applied entomol. and zool.* 2020; 55: 105-13. doi:10.1007/s13355-019-00658-6.
- Kourosh Azizi K, Dorzaban H, Soltani A, Alipour H, Jaberhashemi SA, Salehi-Vaziri M, et al. Monitoring of Dengue Virus in Field-caught Aedes Species (Diptera: Culicidae) by Molecular Method, from 2016 to 2017 in Southern Iran. *J Health Sci Surveillance Sys.* 2023; 11(1): 77-83. doi: 10.30476/jhsss.2022.94608.1548.
- Moin-Vaziri V, Charrel R.N, Badakhshan M, Lamballerie X, Rahbarian N, Bavani M.M, et al. A Molecular screening of mosquitoes (Diptera: Culicidae) for flaviviruses in a focus of West Nile virus infection in northern Iran. *J Arthropod-Borne Dis.* 2019; 13(4): 391-98. doi: 10.18502/jad.v13i4.2235.
- Amin M, Zaim M, Edalat H, Basseri H.R, Yaghoobi-Ershadi M.R, Rezaei F, et al. Seroprevalence Study on West Nile Virus (WNV) Infection, a Hidden Viral Disease in Fars Province, Southern Iran. *J Arthropod-Borne Dis.* 2020; 14(20): 173-84. doi: org/10.18502/jad.v14i2.3735.
- Alkan C, Alwassouf S, Piorkowski G, Bichaud L, Tezcan S, Dincer E, et al. Isolation, genetic characterization, and seroprevalence of Adana virus, a novel phlebovirus belonging to the Salehabad virus complex, in Turkey. *J Virol.* 2015; 89(8): 4080-91. doi: 10.1128/JVI.03027-14. PMID: 25653443; PMCID: PMC4442372.
- Moureau G, Temmam S, Gonzalez JP, Charre RN, Grard G, X de Lamballerie. A real-time RT-PCR method for the universal detection and identification of flaviviruses. *Vector Borne Zoonotic Dis.* 2007; 7(4): 467-77. doi: 10.1089/vbz.2007.0206. PMID: 18020965.
- Vinogradova E.B. *Culex pipiens pipiens* mosquitoes: taxonomy, distribution, ecology, physiology, genetics, applied importance and control. 2000. Pensoft Publishers.
- Mavridis K, Fotakis E, Kioulos I, Mpellou S, Konstantas S, Varela E, et al. Detection of West Nile Virus–Lineage 2 in *Culex pipiens* mosquitoes, associated with disease outbreak in Greece, 2017. *Acta Trop.* 2018; 182: 64-68. doi: 10.1016/j.actatropica.2018.02.024. PMID: 29474832.
- Assaid N, Mousson L, Moutailler S, Arich S, Akarid K, Monier M, et al. Evidence of circulation of West Nile virus in *Culex pipiens* mosquitoes and horses in Morocco. *Acta Trop.* 2020; 205: 105414. doi: 10.1016/j.actatropica.2020.105414. PMID: 32088277.
- Kazemi S.M, Karimian F, Davari B. Culicinae mosquitoes in Sanandaj county, Kurdistan province, western Iran. *J Vector Borne Dis.* 2010; 47(2): 103-7. PMID: 20539048.
- Khoshdel-Nezamiha F, Vatandoost H, Azari-Hamidian S, Bavani M.M, Dabiri F, Entezar-Mahdi R, et al. Fauna and larval habitats of mosquitoes (Diptera: Culicidae) of West Azerbaijan Province, northwestern Iran. *J Arthropod-Borne Dis.* 2014; 8(2): 163-173.
- Nikookar S, Moosa-Kazemi S, Oshaghi M, Yaghoobi-Ershadi M, Vatandoost H, Kianinasab A. Species composition and diversity of mosquitoes in Neka County, Mazandaran Province, northern Iran. *J Arthropod Borne Dis.* 2010; 4(2): 26-34. PMID: 22808397; PMCID: PMC3385557.
- Paksa A, Sedaghat MM, Vatandoost H, Yaghoobi-Ershadi MR, Moosa-Kazemi SH, Hazratian T, et al. Biodiversity of Mosquitoes (Diptera: Culicidae) with Emphasis on Potential Arbovirus Vectors in East Azerbaijan Province, Northwestern Iran. *J Arthropod-Borne Dis.* 2019; 13(1): 62-75. doi: 10.18502/jad.v13i1.933.
- Zaim M. The distribution and larval habitat characteristics of Iranian Culicinae. *J Am Mosq Control Assoc.* 1987; 3(4): 568-73. PMID: 2904967.
- Bagheri M, Terenius O, Oshaghi MA, Motazakker M,

- Asgari S, Dabiri F, et al. West Nile Virus in Mosquitoes of Iranian Wetlands . *Vector Borne Zoonotic Dis.* 2015; 15:750-4. doi: 10.1089/vbz.2015.1778. PMID: 26565610.
- 21 Shahhosseini N, Chinikar S, Moosa-Kazemi SH, Sedaghat MM, Kayedi MH, Lühken R, et al. West Nile Virus lineage-2 in *Culex* specimens from Iran. *Trop Med Int Health.* 2017; 22(10): 1343-1349. doi: 10.1111/tmi.12935. PMID: 28746985.
 - 22 Farhadinejad R, Mousavi M, Amraee K. The species composition of mosquitoes (Diptera: Culicidae) in the Mahshahr district, Khuzestan province, southwest of Iran. *Archives of Razi Institute.* 2015; 70(2): 89-95. doi: 10.7508/ari.2015.02.003.
 - 23 Soltanbeiglu S, Vahedi M, Mohammadi Bavani M, Chavshin A.R. Molecular characterisation of Cytochrome oxidase I and internal transcribed Spacer 2 fragments of *Culiseta longiareolata*. *Turkiye Parazit Derg.* 2020; 44(4): 191-196. doi: 10.4274/tpd.galenos.2020.6886.
 - 24 Paksa A, Vahedi M, Yousefi S, Saberi N, Rahimi S. Biodiversity of mosquitoes (Diptera: Culicidae), vectors of important arboviral diseases at different altitudes in the central part of Iran. *Turk J Zool.* 2023; 47(2):111-119. doi: 10.55730/1300-0179.3121.
 - 25 Moosa-Kazemi SH, Zahirnia AH, Sharifi F, Davari B. The fauna and ecology of mosquitoes (Diptera: Culicidae) in Western Iran. *J Arthropod-Borne Dis.* 2015; 9(1): 49-59.
 - 26 Aldemir A, Bosgelmez A. Population dynamics of adults and immature stages of mosquitoes (Diptera: Culicidae) in Gölbaşı District, Ankara. *Turk J Zool.* 2006; 30(1): 9-17.
 - 27 Gould E, Patterson J, Higgs S, Remi Charrel R, Xavier de Lamballerie. Emerging arboviruses: Why today? *One Health.* 2017; 4:1-13. doi: 10.1016/j.onehlt.2017.06.001.
 - 28 Rizzoli A, Lca Bolzoni L, A Chadwick E, Capelli G, Montarsi F, Grisenti M, et al. Understanding West Nile virus ecology in Europe: *Culex pipiens* host feeding preference in a hotspot of virus emergence. *Parasites & Vectors.* 2015; 8: 213-226.
 - 29 Napp S, Petrić D, Busquets N. West Nile virus and other mosquito-borne viruses present in Eastern Europe. *Pathog Glob Health.* 2018; 112(5): 233-248. doi: 10.1080/20477724.2018.1483567. PMID: 29979950; PMCID: PMC6225508.
 - 30 Leggewie M, Badusche M, Rudolf M, Jansen S, Börstler J, Krumkamp R. *Culex pipiens* and *Culex torrentium* populations from Central Europe are susceptible to West Nile virus infection. *One Health.* 2016; 2: 88-94. doi: 10.1016/j.onehlt.2016.04.001.
 - 31 Komar N. West Nile Virus: Epidemiology and Ecology in North America. *Adv Virus Res.* 2003; 61: 185-234. doi: 10.1016/s0065-3527(03)61005-5. PMID: 14714433.
 - 32 Benjelloun A, Harra EL, Calistri P, Loutfi C, Kabbaj H, Conte A, et al. Seroprevalence of West Nile virus in horses in different Moroccan regions. *Vet Med Sci.* 2017; 3(4): 198-207. doi: 10.1002/vms3.71. PMID: 29152314; PMCID: PMC5677775.
 - 33 Tantely LM, Cêtre-Sossah C, Rakotondranaivo T, Cardinale E, Boyer S. Population dynamics of mosquito species in a West Nile virus endemic area in Madagascar. *Parasite.* 2017; 24(3). doi: 10.1051/parasite/2017005.
 - 34 Schuffenecker S, Iteman I, Michault A, Murri S, Frangeul L, Vaney MC, et al. Genome Microevolution of Chikungunya Viruses Causing the Indian Ocean Outbreak. *PLoS Med.* 2006; 3(7): e263. doi: 10.1371/journal.pmed.0030263. PMID: 16700631; PMCID: PMC1463904.
 - 35 Nelms BM, Macedo P, Kothera L, Savage H, Reisen WK. Overwintering Biology of *Culex* (Diptera: Culicidae) Mosquitoes in the Sacramento Valley of California. *J Med Entomol.* 2013; 50(4): 773-90. doi: 10.1603/me12280. PMID: 23926775; PMCID: PMC3920460.
 - 36 Azari-Hamidian SH. Larval Habitat Characteristics of Mosquitoes of the Genus *Culex* (Diptera: Culicidae) in Guilan Province, Iran. *Iranian J Arthropod-Borne Dis.* 2007; 1(1): 9-20.
 - 37 Moosa-Kazemi SH, Vatandoost H, Nikoogar H, Fathian M. Culicinae (Diptera: Culicidae) Mosquitoes in Chabahar County, Sistan and Baluchistan Province, Southeastern Iran. *Iranian J Arthropod-Borne Dis.* 2009; 3(1): 29-35. PMID: 22808369; PMCID: PMC3385524.
 - 38 Fereidouni SR, Ziegler U, Linke S, Niedrig M, Modirrousta H, Hoffmann B, et al. West Nile virus monitoring in migrating and resident water birds in Iran: are common coots the main reservoirs of the virus in wetlands? *Vector Borne Zoonotic Dis.* 2011; 11(10): 1377-81. doi: 10.1089/vbz.2010.0244. PMID: 21923253.
 - 39 Komar N. West Nile virus surveillance using sentinel birds. *Annals of the New York Acad Sci.* 2001; 951: 58-73. doi: 10.1111/j.1749-6632.2001.tb02685.x. PMID: 11797805.