Original Article

Monitor Mosquito Activity Using Standard Ovitraps in Kashan County, Central Iran (April to Late September 2019)

Tahereh Sadat Asgarian[1], Seyed Hassan Moosa-Kazemi[1], Rouhullah Dehghani[2], Abdolreza Mirolyaei[3], Mohammad Mehdi Sedaghat[1]

[1]Department of Medical Entomology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran, [2]Department of Environment Health, School of Public Health, Kashan University of Medical Sciences, Kashan, Iran, [3]Department of Vector-borne Diseases, CDC, Iran

ORCID:

Tahereh Sadat Asgarian: 0000-0002-1694-9312
Seyed Hassan Moosa-Kazemi: 0000-0001-6073-2594
Mohammad Mehdi Sedaghat: 0000-0001-6073-2594

Abstract

Aims: The aim of this study was to monitor mosquito activity with emphasis on *Aedes* species in Kashan County. **Materials and Methods:** This investigation was carried out in Kashan County, from April to late September 2019. The location coordinates of the sampling sites were marked and registered with Global Positioning System deviceand mapped using Arc Map 10.5 software. In this study, mosquitos' larvae were collected from 120 standard ovitraps, which had been placed in 15 locations in Kashan, Qamsar, and Niasar. *Cyndon dactylon* at 50% concentration was used as an oviposition attractant (250 ml/trap). Ovitraps were visited regularly to check for eggs or larvae. The mosquito's larvae were identified using a systematic key. **Results:** A total of 535 mosquitos' larvae were collected from ovitraps and were identified under two genera *Culex* and *Culiseta*. The collected species were *Culex pipiens* Linnaeus, 1758 (72.4%), *Culex theileri* Theobald, 1903 (18.3%), *Culex torrentium* Martini, 1925 (0.2%) and *Culiseta longiareolata* (Macquart, 1838) (9.1%). This is the first report of present *Cx. torrentium* Martini, 1925 in Kashan County. *Culex pipiens* Linnaeus, 1758 was found at 13 locations from 15. In this study, there were 4.46 larvae per ovitrap. **Conclusions:** Although no samples of *Aedes* mosquitoes were captured in this preliminary study, further investigations using different methods of mosquito collection should be done to accurately comment on the current fauna of Kashan *Aedes*.

Keywords: *Culex pipiens*, *Culex torrentium*, mosquitoes, ovitrap

Introduction

Vector-borne diseases account for around 17% of the estimated global burden of communicable diseases and disproportionately affect poorer populations. 80% of the world's population lives in areas where there are a risk of at least one or more vector-borne disease. More than half of the world's population is at risk of two or more vector-borne diseases, and Over 700,000 deaths occur by vector-borne diseases each year. The risk of infection is high for some viral pathogens, especially in areas where there are favorable habitats for *Aedes* and *Culex* mosquitoes to proliferate and are in close contact with humans. Vector-borne diseases impede economic development through direct medical costs and indirect costs such as loss of productivity and tourism.[1] Vector-borne diseases are also a major health problem in different parts of Iran. Mosquitoes are most important arthropods in health.[2-4] The mosquitoes are from the order Diptera, sub order Nematocera, and the family Culicidae.[5] Due to recent outbreaks of dengue, chikungunya and West Nile fever in Pakistan, dengue and rift valley fever in Saudi Arabia, and West Nile Fever in Iraq,[6-8] Iran is at serious risk of these diseases. *Mansonia uniformis* was added

Received: 27-Apr-2021 **Revised:** 07-Sep-2021
Accepted: 07-Sep-2021 **Published:** 30-Dec-2021

Access this article online

Quick Response Code:

Website: http://iahs.kaums.ac.ir

DOI: 10.4103/iahs.iahs_75_21

Address for correspondence: Dr. Seyed Hassan Moosa-Kazemi, Department of Medical Entomology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.
E-mail: moosakazemi@tums.ac.ir
Prof. Mohammad Mehdi Sedaghat, Department of Medical Entomology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.
E-mail: sedaghat@hotmail.co.uk

How to cite this article: Asgarian TS, Moosa-Kazemi SH, Dehghani R, Mirolyaei A, Sedaghat MM. Monitor mosquito activity using standard ovitraps in Kashan County, Central Iran (April to Late September 2019). Int Arch Health Sci 2021;8:274-9.

to Iranian mosquito checklist thus there are 70 species and 8 (or 12) genera depending the classification of the tribe Aedini.[9] The agents of 15 vector-borne diseases are transmitted by mosquitoes in Iran, including seven arboviral diseases, two bacterial diseases, four filarial and two protozoal diseases.[2,10] Confirmation of the presence of *Aedes albopictus* (Skuse, 1895) in Sistan and Baluchestan Province[11] and neighboring countries is an alarm for international health regulations and vector control in Iran.

It is important that survey the native mosquitoes of each area because globalization has increased susceptibility to mosquito-borne diseases. Economic and social factors, global trade, transport, and tourism have caused the spread of vectors and diseases transmitted by them.[12] The arrival of an exotic mosquito species in a new location is an important issue that leads to the spread of mosquitoes. Over the past three decades, the global spread of *A. albopictus* from Asia to other continents has led to the establishment of this species in Middle East, Africa, Southern Europe, America, and Oceania.[11]

About mosquitoes in Kashan County, a number of studies have been done in previous years. Zaim reported 14 species of Culicidae in this county.[13] Doroudgar *et al*. studied vectors of malaria in Kashan.[14] Dehghani *et al*., in two studies on fauna aquatic insects, reported 453 (43.89%), and 639 (49.7%) of caught larvae and pupae were Culicidae, collected from sewage maturation ponds of Kashan University of Medical Science and Kashan County, respectively. In other studies in this county, 70% of the adult insects caught from inside the houses were Culicidae.[15-17] In the newest study in Kashan County, Asgarian *et al*. studied fauna and larval habitat characteristics of Culicidae, and 13 species reported.[18]

Due to the construction of bird garden in Qamsar, and the entry of birds from 17 different countries into this area, the need to monitor mosquito activity with emphasis on *Aedes* species was felt in this county. Therefore, at the same time as studying the larval habitats of mosquitoes,[18] the *Aedes* mosquito surveillance program was carried out in Kashan County using ovitrop, which is a special trap for *Aedes* mosquitoes.

Due to the special behavior of *Aedes* mosquitoes, especially *Ae. albopictus* (Skuse, 1895) and *Ae. aegypti* (Linnaeus in Hasselquist, 1762), they cannot be collected effectively using conventional collection methods used in the surveillance of malaria vectors. Therefore, the use of new sampling methods is necessary according to the purpose of surveillance in entomological programs of *Aedes* mosquitoes.[19] Ovitraps are very simple and are considered effective for container-inhabiting *Aedes* species. Ovitrap consists of a small black plastic bucket (0.3–1.0 l, 2/3 filled with water and with a hole preventing overflowing and flooding the eggs) and an oviposition support (usually germination paper, a wooden stick, or a piece of polystyrene).[12]

The aim of this study was to monitor mosquito activity with emphasis on *Aedes* species in Kashan County, central Iran.

Materials and Methods

Kashan County is located at the center of Iran, in the northern part of Isfahan Province. The county is divided into four districts: The Central, Qamsar, Niasar, and Barzok Districts [Figure 1], and has seven cities. The climate of County is variable due to the low and high. The northern and eastern regions of Kashan County, including flat and wide plains with hot and dry desert climate, and the southern and western regions, including mountainous and foothill areas, have a temperate mountainous climate.[20]

This is a faunistic and descriptive-sectional study that was done in Central, Qamsar, and Niasar Districts in Kashan County. In order to monitor the population of arbovirus vectors in Kashan County, oviteraps were used as the most common monitoring tool. In this study, two types of ovitraps including Singapore ovitrap and standard black pot with a volume of 500 ml (with a hole preventing overflowing and flooding the eggs) and a wooden stick as an oviposition suppor) were used. *Cyndon dactylon* at 50% concentration was used as an oviposition attractant (250 ml/trap). 31.25 g of grass was added to 7.5 liters of tap water and kept for 7 days. Grass infusion solution was diluted with tap water to 10%, and transferred to ovitraps.[21,22] Ovitraps were positioned close to or under vegetation or near buildings and labeled, "Scientific study. Please do not remove" [Figure 2], and checked for mosquito eggs, larvae, or pupae.

In this study, 120 standard ovitraps were positioned in 15 locations in Kashan, Qamsar, and Niasar (including different topographical areas). Depending on the area, 5 or 10 ovitraps were placed. In Kashan, Qamsar, and Niasar cities, 60, 30 and 30 ovitraps were placed, respectively. These ovitraps were placed in human and animal places, near the plants, as well as near natural and artificial larval habitats since the beginning of the mosquito activity season in April and were regularly visited to late September depending on the weather conditions of the study areas in terms of heat and the possibility of water evaporation inside the ovitraps in tropical and cold regions. During the visits, the wooden stick inside the ovitraps were examined for the presence of eggs, the water in the ovitraps was also poured into containers and taken to *Aedes* insectary, Department of Medical Entomology and Vector control, School of Public Health, Tehran University of Medical Sciences (TUMS) to check for mosquito larvae and pupae, then ovitraps were again filled with grass infusion solution. The entomological survey was carried out in Kashan County from April to late September 2019. The larvae became transparent in lactophenol, and each larva was individually mounted on a microscope slide and identified to species using a systematic key.[23] Some mosquitoe specimens were deposited in the museum of medical entomology, TUMS.

The location coordinates of the sampling sites were identified and recorded with the Global Positioning System device. Arch Map 10.5 software was used to create a geographic database of ovitraps and mapping the sites of collection and distribution of

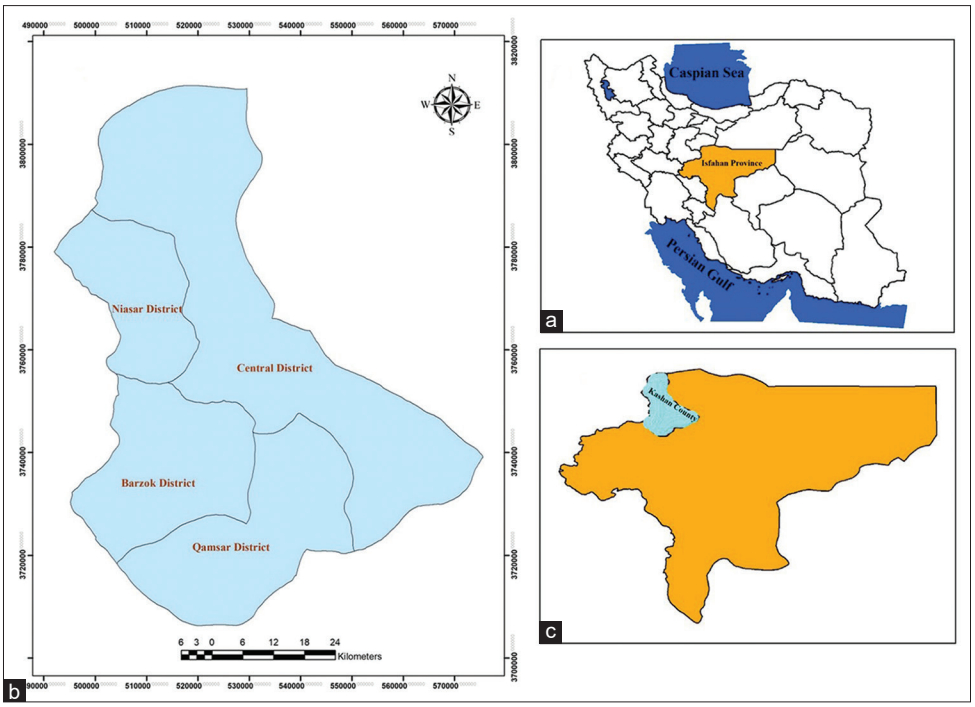

Figure 1: Maps of (a) Iran (b) Kashan County (c) Isfahan Province

Figure 2: Ovitraps: (a) Standard black pot and (b) Singapore model

the most important species of medicine [Figure 3 and Table 1]. Data analysis was performed with software of SPSS Statistics 26. A significance level of $\alpha = 0.05$ was used when using ANOVA for means comparison.

Results

No *Aedes* eggs and larvae were found on wooden sticks and waters in the ovitraps. However, a total of 535 mosquito' larvae were collected from waters and identified morphologically as *Culex pipiens* Linnaeus, 1758 (72.4%), *Culex theileri* Theobald, 1903 (18.3%), *Culex torrentium* Martini, 1925 (0.2%), and *Culiseta longiareolata* (Macquart, 1838) (9.1%). *Culex* spp. and *Culiseta* spp. mosquitoes lay their eggs on the surface of the water. But the *Aedes* mosquitoes lay their eggs on the inner, wet walls of containers with water above the waterline. Therefore, this is the reason for the presence of larvae in the water despite the presence of eggs on the wooden sticks.

The most prevalent species was *Cx. pipiens* followed by *Cx. theileri* in ovitraps. *Culex pipiens* was found at 13 locations from 15. The highest number of larvae of this species was in ovitraps placed in bird garden, fish farming and wastewater treatment, respectively.

All of four mosquito species were found from ovitraps in Qamsar bird garden. *Culex torrentium* was only found in July from one of the ovitraps in the bird garden. This is the first report of the present *Cx. torrentium* in Kashan County. The highest number of larvae was caught from ovitraps placed in Qamsar bird garden, with 12 larvae per ovitrap. In the locations of railway station and Ayatollah Madani Park, there were no eggs and larvae. In this study, there were 4.46 larvae per ovitrap. *Culiseta longiareolata* larvae was detected from five locations, that the highest number was reported from ovitraps in Khonb village [Table 1].

Discussion

The ovitrap was first designed in 1966 to monitor the *Aedes* population.[24] We did not catch *Aedes* eggs or larvae from ovitraps. At the same time, in a study that conducted at the larval habitats of mosquitoes in Kashan County, also no *Aedes* larvae were caught.[18] Zaim did not catch *Aedes* larvae in Kashan, but adults of *Aedes caspius* (Pallas, 1771) s.l. and *Ae. pulcritarsis* (Rondani, 1872) reported only once in early May from Aliabad (Aran and Bidgol County) and July from

Table 1: Coordinates of the entomological surveillance sites and mosquito larvae identified from ovitraps in Kashan County, Central Iran, 2019

Locality	District	Longitude	Latitude	Numbers of ovitraps	Mosquito larvae identified	Number of larvae/number ovitrap
Wastewater treatment plant	Central	51.39998	34.04713	10	*Cs. longiareolata* (7), *Cx. pipiens* (43), *Cx. theileri* (21)	7.1
Railway station	Central	51.47173	33.98554	5	-	0
Ayatollah madani park	Central	51.41199	34.00444	5	-	0
City roof park	Central	51.36662	33.93139	5	*Cx. pipiens* (24)	4.8
Fin garden	Central	51.37195	33.94604	10	*Cx. pipiens* (15)	1.5
Depot tires and trash	Central	51.6167	33.99601	5	*Cs. longiareolata* (2), *Cx. pipiens* (15), *Cx. theileri* (15)	6.4
Kashan University of Medical Sciences	Central	51.4065	34.01705	10	*Cs. longiareolata* (12), *Cx. pipiens* (30), *Cx. theileri* (14)	5.6
Ravand - fish farming	Central	51.37237	34.02696	5	*Cx. pipiens* (54)	10.8
Khonb	Central	51.36897	33.87091	5	*Cs. longiareolata* (16), *Cx. pipiens* (10)	5.2
Bonroud	Qamsar	51.42037	33.72226	10	*Cx. pipiens* (29), *Cx. theileri* (10)	3.9
Bird garden	Qamsar	51.46272	33.75846	10	*Cs. longiareolata* (12), *Cx. pipiens* (92), *Cx. theileri* (15), *Cx. torrentium* (1)	12
Doctor's building	Qamsar	51.45249	33.75347	10	*Cx. pipiens* (10)	1
Health center	Niasar	51.15264	33.97264	10	*Cx. pipiens* (21)	2.1
Waterfall	Niasar	51.14709	33.97244	10	*Cx. pipiens* (20), *Cx. theileri* (11)	3.1
Majd Abad	Niasar	51.45249	33.75347	10	*Cx. pipiens* (24), *Cx. theileri* (12)	3.6
Total				120	535	4.46

Cs. longiareolata: Culiseta longiareolata, Cx. pipiens: Culex pipiens, Cx. theileri: Culex theileri, Cx. torrentium: Culex torrentium

Dehnar, respectively.[13] Dehghani *et al*. reported that *Aedes* adults were observed in houses, but in their study, the species of *Aedes* were not mentioned.[17]

The results of our study are consistent with other studies in Iran. Doosti did not catch *Aedes* from ovitraps and reported two species of *Culex,* including *Cx. perexiguus* Theobald, 1903 and *Cx. pipiens* Linnaeus, 1758.[25] In Dorud County, Lorestan Province*, Cx. pipiens* Linnaeus, 1758 and *Anopheles stephensi* Liston, 1901 were catched from ovitraps.[26] Therefore ovitraps are very suitable for catching species of mosquitoes that prefer small habitats and stagnant waters. Furthermore, none of the ovitraps were positive for *Aedes* in Mazandaran Province.[27]

In the present study, *Culex* mosquitoes were the most abundant species founded in ovitraps all of the study sites. So most *Culex* mosquitoes are likely to lay eggs in stagnant water.[28] In our study, the most common species in ovitraps were *Cx. pipiens*. This species can be found in both urban and sub-urban temperate and tropical regions across the world.[29] The habitat of *Cx. pipiens* larvae can be divided into natural and artificial. This species was also most abundant collected from larval habitats of mosquitoes in Kashan County. It is a very active species in Kashan County and causes the most inconvenience to people in cities and villages.[13,18]

Culex pipiens Linnaeus, 1758 is a vector for many arbovirus diseases that can be found across many regions of the globe, same as of Rift Valley fever, Japan encephalitis, and West Nile virus (WNV).[30,31] *Culex pipiens* and *Cx. qinquefasciatus* have a prominent role in spreading West Nile virus.[32] In nature, WNV is held in a mosquito-bird-mosquito transmission cycle and *Culex* spp. are the main vectors.[33]

Culex torrentium Martini, 1925 belong to *Cx. pipiens* complex. The *Cx. pipiens* complex populations are distinct in behavior and physiology, which This greatly affects their ability for pathogen transmission and their vectorial capacity.[29] *Culex pipiens* may be associated with many species and share some larval sites with *Cx. torrentium*. *Culex pipiens* and *Cx. torrentium*, can be distinguished by Harbach's (1988) key using some features (seta 1-III-V, seta 1-M, seta 1-X, seta 1-C, and some other characters).[34] This is the first report of the present *C. torrentium* in Kashan County. This species has not been reported from Kashan larval habitats.[13,18] Leggewie *et al*. showed that *Cx. pipiens* biotype *pipiens* and *Cx. torrentium* mosquitoes native to Germany are susceptible to WNV infection at 25°C and 18°C incubation temperature.[35] *Culex torrentium* mosquitoes were the most permissive species and had maximum infection rates (96%) at 25°C. So, *Cx. torrentium* has been identified as a potent vector for WNV in central and northern Europe.[36] West Nile virus has spread in Africa, America, Southern Europe, Australia, and Asia. The virus has been reported from some of Iran's neighboring countries.[37] Fereidouni *et al*. provided first evidence of the prevalence of WNV antibody in water birds in Iran.[38] Chinikar *et al*. determined the seroprevalence of WNV in human and equine sera in Iran.[39] In a study in the north of Iran *Cx. pipiens* infection was reported with this virus.[40]

In our study, *Cx. theileri* Theobald, 1903 was the most aboundant species after *Cx. pipiens* that was collectad from

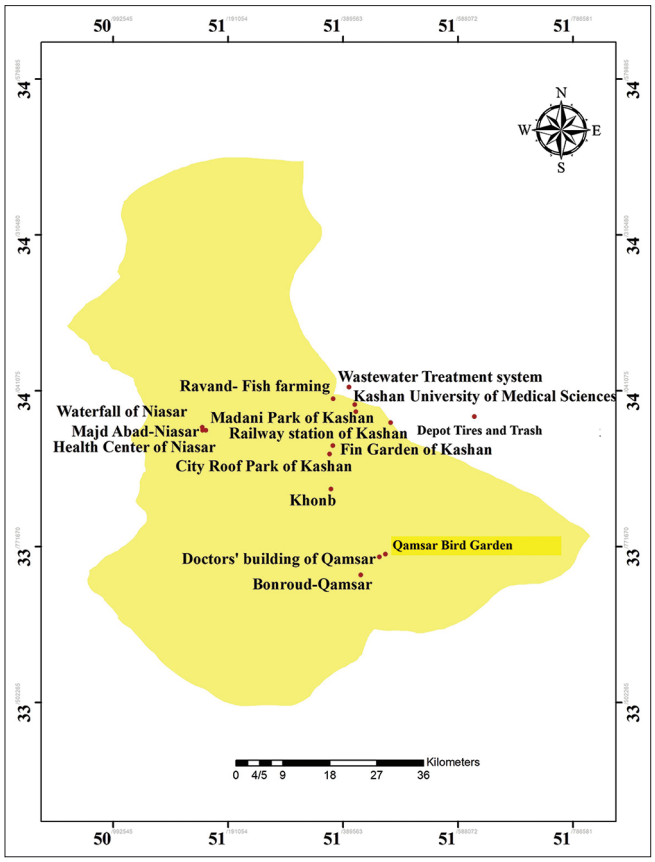

Figure 3: Map of ovitrap installation sites in Kashan County, Central Iran, 2019

ovitraps. *Culex theileri* is found in the most parts of the world and has been recorded in all provinces of Iran.[41-44] *Culex theileri* is a major vector of Rift Valley fever virus (Bunyaviridae: Phlebovirus) in South Africa[45] and it plays a role in the transmission of *Dirofilaria immitis* nematode. In Iran, the third stage of *Cx. theileri* larva, naturally was infected with *D. immitis*, may be the main vector of this parasite.[44,46]

Adults of *Cs. longiareolata* (Macquart, 1838) are often blood-sucking from birds, never enter human places, and rarely attack humans outside. This species can transfers diseases such as West Nile, encephalitis, brucellosis, avian influenza,[47] and its larvae are known as mosquito larvae predators.[48] This mosquito has a variety of larval habitats and is found in clear to turbid and polluted waters, as well as in fresh and salt water. Its larvae have also been caught from different habitats in Kashan County.[13,18]

Identifying the major vectors of pathogens and their impact on disease transmission rates is important for understanding the patterns of transmission. Many diseases transmitted to humans by vectors are zoonoses, including yellow fever, West Nile, St. Louis, eastern equine encephalitis, Lyme disease, rickettsia, and plague that have amplification cycles involving species other than humans. Their reservoirs are primates, small mammals and birds.[29,49] Surveillance is an important tool that provides the necessary knowledge about mosquitoes in an area, and it helps to determine if the pathogen is transmitted in the area.[12,50] Entomological surveillance, may provide the ability to detect early circulation of arbovirus in a particular area. Ovitrap is an environmentally-friendly method, cheap and fast for determining the density, frequency, seasonal activity, and geographical distribution of mosquito vectors.[51]

Conclusions

Among the study sites, the birth garden was the most important because all four species of mosquito larvae were in ovitraps positioned in this place, this supports the importance of birds as a major blood-meal host for mosquitoes. This is the first modeling study in Kashan for predicting areas with the possible presence of invasive *Aedes* species. Although no samples of *Aedes* mosquitoes were captured in this preliminary study, further investigations using different methods of mosquito collection should be continued to monitor the invasive *Aedes* in the future. Due to the pandemic of Covid-19, and in order to entomological surveillance programs, use of passive sampling methods which require less contact is recommended.

Ethics approval and consent to participate

Ethical clearance was earned from the Tehran University of Medical Sciences, Tehran, Iran. This work was supported as No.: IR.TUMS.VCR.REC.1397.1001, 2019-2-23.

Acknowledgment

Authors are grateful to Dr SM Asgarian for helping with field collections. We are also thankful Health Deputy, Kashan University of Medical Sciences, for Cooperation. The authors declare the ethical approval code as No.: IR.TUMS.VCR.REC.1397.1001.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Global Vector Control Response 2017-2030: World Health Organization (WHO); 2017. Available from: http://who.int. [Last accessed on 2017 Oct 02].
2. Azari-Hamidian S, Norouzi B, Harbach RE. A detailed review of the mosquitoes (Diptera: Culicidae) of Iran and their medical and veterinary importance. Acta Trop 2019;194:106-22.
3. Zaim M. Malaria control in Iran – Present and future. J Am Mosq Control Assoc 1987;3:392-6.
4. Zaim M, Manouchehri A, Yaghoobi-Ershadi M. Investigation of fauna of mosquitoes of Iran (Diptera: Culicidae) 1- Aedes. Iran J Health Sci 1984;13:3-10.
5. Gaffigan T, Wilkerson R, Pecor J, Stoffer J, Anderson T. Systematic Catalog of Culicidae 2018 Walter Reed Biosystematic Unit, Smithsonian Institution. Available from: http://www.wrbu.org/. [Last accessed on 2018 Jul 29].
6. Afzal MF, Naqvi SQ, Sultan MA, Hanif A. Chikungunya fever among children presenting with nonspecific febrile illness during an epidemic of dengue fever in Lahore, Pakistan. Merit Res J Med Med Sci 2015;3:69-73.
7. Rasheed S, Butlin R, Boots M. A review of dengue as an emerging disease in Pakistan. Public Health 2013;127:11-7.
8. Khan HA, Akram W, Shehzad K, Shaalan EA. First report of field evolved resistance to agrochemicals in dengue mosquito, *Aedes albopictus* (Diptera:

Culicidae), from Pakistan. Parasit Vectors 2011;4:1-11.

9. Azari-Hamidian S, Abai MR, Nourouzi B. Mansonia uniformis (Diptera: Cu-licidae), a genus and species new to south-western Asia, with a review of its medi-cal and veterinary importance. Zootaxa 2020;4772:385-95.
10. Pouriayevali MH, Rezaei F, Jalali T, Baniasadi V, Fazlalipour M, Mostafavi E, *et al.* Imported cases of Chikungunya virus in Iran. BMC Infect Dis 2019;19:1-8.
11. Doosti S, Yaghoobi-Ershadi MR, Schaffner F, Moosa-Kazemi SH, Akbarzadeh K, Gooya MM, *et al.* Mosquito surveillance and the first record of the invasive mosquito species aedes (*Stegomyia*) albopictus (Skuse) (Diptera: Culicidae) in Southern Iran. Iran J Public Health 2016;45:1064-73.
12. Schaffne F, Versteirt V, Medlock J. Guidelines for the Surveillance of Native Mosquitoes in Europe: European Centre for Disease Prevention and Control (ECDC); 2014. Available from: https://www.ecdc.europa.eu. [Last accessed on 2012 Aug].
13. Zaim M. The mosquito fauna of Kashan, public health importance and control. Desert Sci Res 1987;18:1-41.
14. Doroudgar A, Dehghani R, Hooshyar H, Sayyah M. Epidemiology of malaria in Kashan. J Med Fac Guilan Univ Med Sci 2000;8:52-8.
15. Dehghani R, Miranzadeh MB, Yosefzadeh M, Zamani S. Fauna aquatic insects in sewage maturation ponds of Kashan University of Medical Science 2005. Pak J Biol Sci 2007;10:928-31.
16. Dehghani R, Zarghi I, Aboutalebi M, Barzegari Z, Ghanbari M. Fauna and habitat of aquatic arthropods of Kashan in 2010. Bangladesh J Med Sci 2014;13:306-10.
17. Dehghani R, Akbari H, Vazirianzadeh B. A prospective study on the seasonal frequencies of insect bites [Diptera: Culicidae and Phlebotominae] and the related environmental and protective method factors in the city of Kashan, central of Iran, 2009. Pak J Med Sci Q 2012;28:158-61.
18. Asgarian TS, Moosa-Kazemi SH, Sedaghat MM, Dehghani R, Yaghoobi-Ershadi MR. Fauna and larval habitat characteristics of mosquitoes (Diptera: Culicidae) in Kashan County, Central Iran, 2019. J Arthropod Borne Dis 2021;15:69-81.
19. Infectious Disease Management Center, Ministry of Health, Treatment and Medical Education. Practical instructions for *Aedes aegypti* and *Aedes albopicus* in Iran. In: Zoonose Office. Tehran. Ministry of Health, Treatment and Medical Education; 2015. p. 1-34.
20. Climate Profile of Kashan County: I.R. of Iran Meteorological Organization; 2014. Available from: http://www.kashanmet.ir. [Last accessed on 2015 Sep 15].
21. Sant'ana A, Roque R, Eiras A. Characteristics of grass infusions as oviposition attractants to *Aedes* (*Stegomyia*) (Diptera: Culicidae). J Med Entomol 2006;43:214-20.
22. Polson K, Curtis C, Seng C, Olson J, Chantha N, Rawlins SC. The use of ovitraps baited with hay infusion as a surveillance tool for *Aedes aegypti* mosquitoes in Cambodia. Dengue Bull 2002;26:178-84.
23. Azari-Hamidian S, Harbach RE. Keys to the adult females and fourth-instar larvae of the mosquitoes of Iran (Diptera: Culicidae). Zootaxa 2009;2078:1-33.
24. Reiter P, Amador MA, Anderson RA, Clark GG. Dispersal of *Aedes aegypti* in an urban area after blood feeding as demonstrated by rubidium-marked eggs. Am J Trop Med Hyg 1995;52:177-9.
25. Doosti S. Systematic study of *Aede*s mosquitoes and their natural infection with arbovirus caused by dengue fever by Real Time RT-PCR method in the south and some border provinces of east and west of Iran. Ph D dissertation, Tehran University of Medical Sciences; Tehran: 2015.
26. Hajivand Z. Investigation of faunistic status and possible contamination of Culicinae in Tireh and Cesar areas Dorud County, Lorestan Province. M Sc dissertation, Tehran University of Medical Sciences; Tehran: 2019.
27. Nikookar SH, Fazeli-Dinan M, Azari-Hamidian SH, Nasab SN, Aarabi M, Ziapour SP, *et al*. Fauna, ecological characteristics, and checklist of the mosquitoes in Mazandaran Province, Northern Iran. J Med Entomol 2018;55:634-45.
28. Yap H, Zairi J, Jahangir K, Adanan C. *Culex*: Mosquitoes that spread Japanese encephalitis. In: Mosquitoes and Mosquitoborne Diseases: Biology, Surveillance, Control, Personal and Public Protection Measures Malaysia; ASM Sci J; 2000. p. 73-9.
29. Farajollahi A, Fonseca DM, Kramer LD, Marm Kilpatrick A. "Bird biting" mosquitoes and human disease: A review of the role of *Culex pipiens* complex mosquitoes in epidemiology. Infect Genet Evol 2011;11:1577-85.
30. Vinogradova EB. *Culex*: Taxonomy, Distribution, Ecology, Physiology, Genetics, Applied *Pipiens pipiens* Mosquitoes Importance and Control. Russian Academy of Sciences: Pensoft Publishers; 2000.
31. Hamer GL, Kitron UD, Brawn JD, Loss SR, Ruiz MO, Goldberg TL, *et al. Culex pipiens* (Diptera: Culicidae): A bridge vector of West Nile virus to humans. J Med Entomol 2008;45:125-8.
32. Campbell GL, Marfin AA, Lanciotti RS, Gubler DJ. West Nile virus. Lancet Infect Dis 2002;2:519-29.
33. Turell MJ, Sardelis MR, Dohm DJ, O'Guinn ML. Potential North American vectors of West Nile virus. Ann N Y Acad Sci 2001;951:317-24.
34. Harbach R. The mosquitoes of the subgenus *Culex* in southwestern Asia and Egypt (Diptera: Culicidae). Contrib Am Entomol Inst 1988;24:1-240.
35. Leggewie M, Badusche M, Rudolf M, Jansen S, Börstler J, Krumkamp R, *et al. Culex pipiens* and *Culex torrentium* populations from central Europe are susceptible to West Nile virus infection. One Health 2016;2:88-94.
36. Jansen S, Heitmann A, Lühken R, Leggewie M, Helms M, Badusche M, *et al. Culex torrentium*: A potent vector for the transmission of west Nile virus in central Europe. Viruses 2019;11:E492.
37. Naficy K, Saidi S. Serological survey on viral antibodies in Iran. Trop Geogr Med 1970;22:183-8.
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;11:1377-81.
39. Chinikar S, Shah-Hosseini N, Mostafavi E, Moradi M, Khakifirouz S, Jalali T, *et al.* Seroprevalence of west Nile virus in Iran. Vector Borne Zoonotic Dis 2013;13:586-9.
40. Shahhosseini N, Chinikar S, Moosa-Kazemi SH, Sedaghat MM, Kayedi MH, Luhken R, *et al*. West Nile virus lineage-2 in *Culex* speciments from Iran. Trop Med Int Health 2017;22:1343-9.
41. Moosa-Kazemi SH, Firoozfar F. Bionomic studies of the mosquitoes (Diptera: Culicidae) in Kermanshah Province, Western Iran. Life Sci J 2016;13:50-5.
42. 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;9:49-59.
43. Abai MR, Azari-Hamidian S, Ladonni H, Hakimi M, Mashhadi-Esmail K, Sheikhzadeh K, *et al.* Fauna and checklist of mosquitoes (Diptera: Culicidae) of East Azerbaijan Province, northwestern Iran. J Arthropod Borne Dis 2007;1:27-33.
44. Azari-Hamidian S, Yaghoobi-Ershadi MR, Javadian E, Abai M, Mobedi I, Linton YM, *et al.* Distribution and ecology of mosquitoes in a focus of dirofilariasis in northwestern Iran, with the first finding of filarial larvae in naturally infected local mosquitoes. Med Vet Entomol 2009;23:111-21.
45. McIntosh BM, Jupp PG, dos Santos I, Barnard BJ. Vector studies on rift valley fever virus in South Africa. S Afr Med J 1980;58:127-32.
46. Santa-Ana M, Khadem M, Capela R. Natural infection of *Culex theileri* (Diptera: Culicidae) with *Dirofilaria immitis* (Nematoda: Filarioidea) on Madeira Island, Portugal. J Med Entomol 2006;43:104-6.
47. Maslov AV, Ward RA, Rao P. Blood-Sucking Mosquitoes of the Subtribe Culisetina (Diptera, Culicidae) in World Fauna. Washington, D.C.: Citeseer; 1989.
48. Shaalan E. Predation capacity of *Culiseta longiareolata* mosquito larvae against some mosquito species larvae. J Entomol 2012;9:183-6.
49. Kilpatrick AM, Kramer LD, Jones MJ, Marra PP, Daszak P, Fonseca DM. Genetic influences on mosquito feeding behavior and the emergence of zoonotic pathogens. Am J Trop Med Hyg 2007;77:667-71.
50. Calzolari M. Mosquito-borne diseases in Europe: An emerging public health threat. Rep Parasitol 2016;5:1-12.
51. Barreto E, Resende MC, Eiras AE, Demarco Júnior PC. Evaluation of the baited ovitrap with natural attractant for monitoring *Aedes* spp. in Dili, capital of East Timor. Cien Saude Colet 2020;25:665-72.