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RESEARCH ARTICLE

Survey of Usutu Virus Potential Vectors and their Diversity in Iran: A Neglected Emerging Arbovirus

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Abstract:

Introduction:

Usutu Virus (USUV) is a vector-borne flavivirus within the family of Flaviviridae; its reservoirs and vectors are birds and mosquitoes, respectively.

Methods:

At first, electronic databases were searched with a date range from 2000 to 2018 to find the *Culex* species that transmit the disease and then for determining the diversity of those, mosquito larvae were collected from six places in three main environmental categories using the dipping technique.

Results:

In total, 1369 specimens belonging to 10 different species were collected and identified, as follows: *Cx. hortensis* Ficalbi, *Cx. laticinctus* Edwards, *Cx. mimeticus* Noe, *Cx. perxigus* Theobald, *Cx. pipiens* Linnaeus, *Cx. modestus* Ficalbi, *Cx. sinaiticus* Kirkpatrick, *Cx. theileri* Theobald *Cx. torrentium* Martini and *Cx. tritaeniorhynchus* Giles. Four species involved as vectors of USUV in other countries are printed in bold. *Cx. pipiens*, as the main vector was the most frequent species in rural areas, share its larval habitats with *Cx. torrentium* (similarity > 0.9) and reached its peak in August. Overall, in the present study, there was a significant positive relationship between mean temperatures and abundance of mosquitoes ($r = 0.75$, $P = 0.005$).

Conclusion:

In the present study, some species involved as main vectors of USUV in other countries and their ecological features were recorded. Based on these results, the possibility of the emergence of USUV in Iran exists.

Keywords: Mosquitoes, Usutu virus, Species diversity, Flavivirus, Reservoirs, Rural areas.

Article History

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1. INTRODUCTION

Usutu Virus (USUV) is a culicid-borne flavivirus within the family of Flaviviridae; its reservoirs and vectors are birds and *Culex* mosquitoes, respectively [1]. Circulation and antigenic features of USUV is similar to West Nile Virus (WNV), since its identification in 1959 in Ndumu, South Africa, this culicid-borne virus has spread in Africa and Europe, reaching Austria in 2001 and countries of the Poland and Switzerland by 2006 [1, 2]. The virus has recently been identified in mosquitoes in northern Israel. [3]. USUV infection

in human cases was reported in European countries [4]. Some migratory infected bird species such as; *Passer domesticus* (house sparrows), *Gallus gallus*, and *Turdus merula* (the common blackbird) are responsible for the dissemination of USUV from Europe to other countries [5]. USUV had been detected in some culicid species from different parts of the world (i.e. *Culex pipiens* and *Cx. perexigus* in Israel and Spain, *Cx. torrentium* in Germany, and *Ae. albopictus* in Israel), *Cx. pipiens* and *Cx. neavei* are considered as the main vectors for this virus [3, 6 - 8]. These reservoirs and vectors have been reported from Iran [9, 10]. Co- transmission of USUV and WNV was reported in different countries, because of some bird/mosquito species are potential reservoirs/vectors for both viruses [5]. Sero-survey of WNV in the equine populations in Iran indicated the circulation of the virus in southwestern

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provinces [11]. Common coot (*Fulica atra*) was reported as a competent host for WNV in Iran [12]. As Iran experiences regional climate change, mosquitoes are possible to flourish better through expected conditions. Mosquito vectors are ectothermic and are thus specifically susceptible to climatic factors. Survival, behavior, and reproduction of vectors are affected by weather conditions and it can strongly affect pathogen development within vectors and also influence the availability of breeding sites for culicid vectors, because of their immature aquatic stages [13]. Therefore, the study on the dynamic of mosquitoes and surveillance for probable importation of vector-borne disease is essential.

Some culicid-borne viruses comprise the WNV, Sindbis virus, and the Dengue virus have been reported from Iran [14, 15]. According to a report from WHO, the risk of Japanese encephalitis and Rift Valley fever in the Eastern Mediterranean Region, comprising Iran, is remarkable [16]. Before USUV arrives in Iran, we should build upon information on the diversity of common mosquito's species. For this reason, the present study aims to gather baseline data on species richness and diversity of potential vectors of USUV in Iran.

2. MATERIALS AND METHODS

2.1. Search Strategy

Electronic databases (PubMed, Scopus, Google Scholar, EMBASE, the Chinese National Knowledge Infrastructure database (CNKI), the Chinese Biological Medical Literature database (CBM), were searched with a date range from 2000 to 2018 to find the vector species that transmits the disease using the free combinations of the terms "Usutu virus," "Mosquitoes," and "Culex".

2.2. Study Area

To develop the study, six sites were selected in the different areas [Urban Areas (UA), Rural Areas (RA) and uninhabited area (UNA)] of the north of Bushehr province (50° 31' 1E, 29° 34' 45N), in the Southwest of Iran. Two sites for each of these areas were selected. During this period of study (January – December 2017), maximum and minimum temperatures were 48 °C and 1°C in July and February, respectively. The distance between urban and rural areas, uninhabited and rural areas and between urban and uninhabited areas were 30, 17, and 12 km, respectively.

2.3. Sampling Methods and Taxonomic Identification

To study the ecology of mosquitoes, sampling was carried out by dipping technique with a metal dipper for collecting larvae. Four dips were taken from each breeding site (350 ml each). Larval investigation was conducted two times a month during the study period.

All samples were brought to the laboratory of the Entomology department, Tehran University of Medical Sciences, Iran. The mosquito larvae were preserved in 75% ethanol and the microscopic slides were prepared using the chloral gum mounting. The microscope was used for the taxonomic study and identification, up to the species level using taxonomic keys available in the literature [17].

2.4. Biodiversity and Statistical Analysis

Diversity studies (alpha diversity) were conducted separately for each category (rural, urban and uninhabited areas) by calculating classic diversity indexes like Margalef's $(S-1) / \ln N$ [S = total number of species and N = total number of individuals) and Simpson's indexes ($\lambda = 1 - \sum pi^2$, where $pi = ni/N$ [ni is the number of individuals of taxon i]) [18]. Dominance = $1 - \text{Simpson index}$. Ranges from 0 (all taxa are equally present) to 1 (one taxon dominates the community completely). Shannon diversity index ($H' = -[\sum(pi \ln pi)]$) is commonly used to characterize species diversity in a community, accounting for both abundance and evenness of the species present [9, 18]. The equitability index measures the evenness with which individuals are divided among the taxa present.

Jaccard's and Whittaker indices were used to illustrate similarity and dissimilarity [18]. Data were analyzed using PAST software version 3.14 (Paleontological Statistics Software Package). Non-parametric tests were used to evaluate differences in species abundances and weather-related variables between months.

3. RESULTS

3.1. Monthly Abundance

A total of 1369 specimens belonging to 10 different mosquito species were collected and identified. Viz.; *Cx. hortensis* Ficalbi, *Cx. laticinctus* Edwards, *Cx. mimeticus* Noe, *Cx. perexiguus* Theobald, *Cx. pipiens* Linnaeus, *Cx. modestus* Ficalbi, *Cx. sinaiticus* Kirkpatrick, *Cx. theileri* Theobald *Cx. torrentium* Martini, and *Cx. tritaeniorhynchus* Giles. Four species involved as vectors of USUV in other countries are printed in bold. *Culex theileri* (28.0%), *Cx. pipiens* (26.1%), *Cx. tritaeniorhynchus* (23.1%) were predominated, respectively. The greatest number of mosquitoes were collected from RA (723 specimens) and the lowest in the UA (209 specimens), *Cx. pipiens* and *Cx. torrentium* were more frequent in RA, while *Cx. hortensis* was only caught in UNA and *Cx. perexiguus* was more frequent in UNA (Fig. 1). The greatest number of mosquitoes were collected in August (379) and September (370 specimens). *Culex pipiens*, *Cx. torrentium*, and *Cx. hortensis* were more active in August, while *Cx. perexiguus* was in October (Fig. 2). Depending on the month, the difference was not significant in mosquito abundances according to the Kruskal-Wallis test ($P = 0.44$). Analysis with Kruskal-Wallis and Mann-Whitney tests showed that the difference between environments (UA, RA, and UNA) and abundance of mosquitoes was not significant ($P = 0.33$). Overall, in the present study, there was a significant positive relationship between mean temperatures and abundance of mosquitoes ($r = 0.75$, $P = 0.005$). In contrast, abundance of mosquitoes decreased with increasing precipitation ($r = -0.42$, $P = 0.16$).

Among the four species mentioned above, *Cx. torrentium* and *Cx. hortensis* prefers mostly permanent water resources without vegetation.

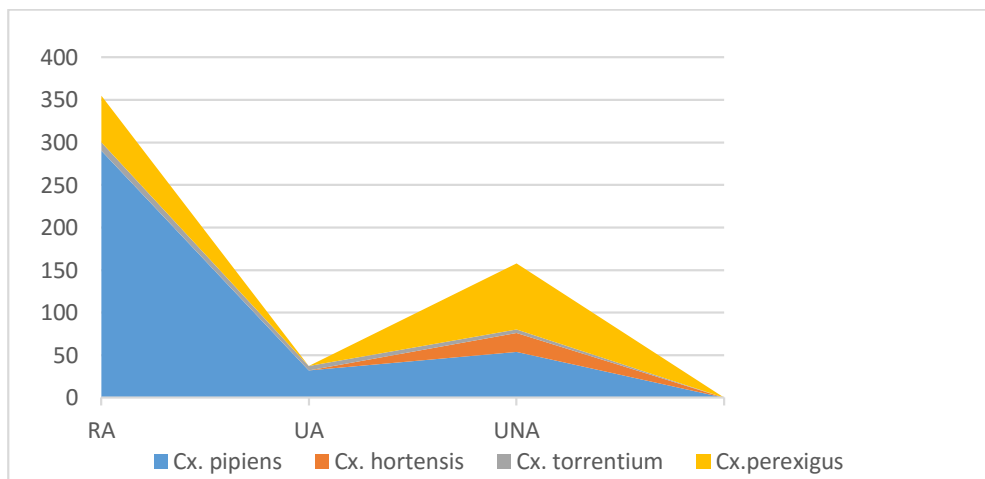


Fig. (1). Abundance of Usutu virus potential vector species found in rural (RA), urban (UA), and uninhabited (UNA) areas in southwest Iran.

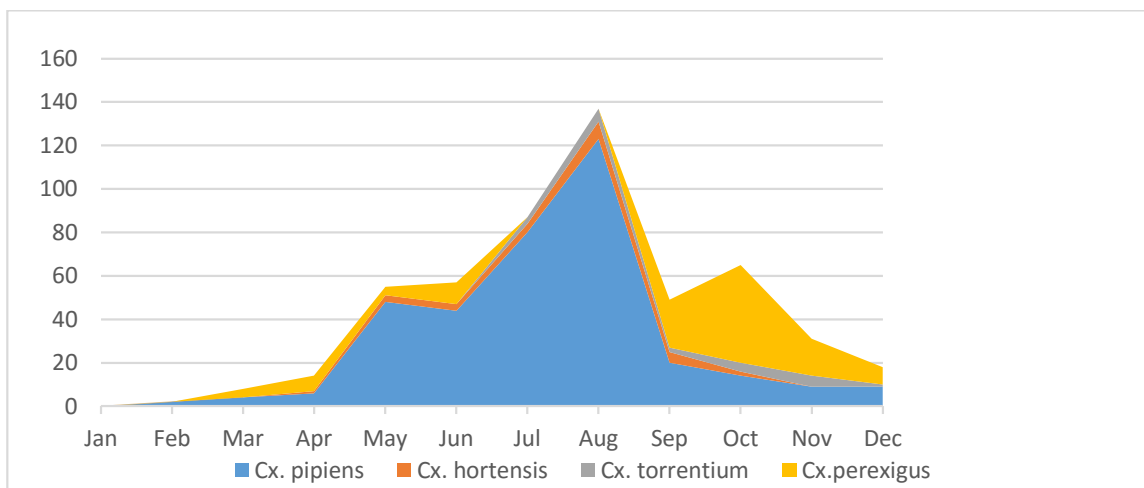


Fig. (2). Monthly abundance of Usutu virus potential vector species in southwest Iran, in 2017. Notes: January (Jan)-February (Feb)-March (Mar)-April (Apr)- May (May)-June (Jun)- July (Jul)- August (Aug)- September (Sep)- October (Oct)- November (Nov)-December (Dec).

3.2. Mosquito Species Diversity

Greater species richness was found in RA (M=1/21), while UA has the lowest (M=0.93) (Table 1). The average diversity indices for the three environment types ranged from 1.50 to 1.64 for the Shannon index and from 0.730 to 0.738 for the Simpson index. Shannon index was highest in UNA and lowest in UA. While Simpson index in UA was highest (Table 1). The T-test showed no statistically significant difference between the Shannon index (t=1.17, p=0.24) and Simpson index (t=0.39, p=0.69) in RA and UA. Similarly, there was also no statistically significant difference between the Shannon index (t=-1.36, p=0.17) and Simpson index (t=0.20, p=0.83) in RA and UNA. But, there was a significant difference between the Shannon index in UNA and UA (t=-2.26, p=0.024). However, the difference between the Simpson index was not significant in these areas (t=-0.15, p=0.87). Greater evenness was observed in UA because the most dominant species do not show such a strong influence as in the two other environments. Where, *Cx. theileri* in UNA and *Cx. pipiens* and *Cx. tritaeniorhynchus* in RA were dominant and showed a strong

influence.

The analysis of β biodiversity shows that RA and UNA are the closest categories in their specific composition (Whittaker index= 0.17), while UNA and UA are the farthest categories (Whittaker index= 0.28). *Culex pipiens* and *Cx. torrentium* share their larval habitats with each other and similarity was more than 0.9 according to the Jaccard's index, while *Cx. hortensis* had little similarity to other species.

Table 1. Alpha biodiversity estimators for rural (RA), urban (UA), and uninhabited (UNA) areas.

UNA	UA	RA	-
8	6	9	Taxa
437	209	723	Individuals
0.265	0.261	0.269	Dominance
1.64	1.50	1.56	Shannon index (H')
0.734	0.738	0.730	Simpson index (λ)
1.15	0.93	1.26	Margalef index (M)
0.78	0.83	0.71	Equitability

4. DISCUSSION

In the present investigation, 10 species of mosquitoes were identified. Among them, four species (*Cx. pipiens*, *Cx. hortensis*, *Cx. torrentium*, *Cx. perexigus*) involved as vectors of USUV in other countries [3, 6 - 8, 19].

Culex pipiens have two biotypes: the *molestus* and the *pipiens*, these two biotypes are morphologically indiscernible, both forms have been recorded from Iran [20, 21]. The *pipiens* biotype feeds mainly on birds, prefer aboveground habitats and frequently found in rural areas [20, 22]. We found similar findings in our research, and this species mostly caught from rural areas with aboveground habitats. In the present study, Shannon and equitability indices show that in RA, *Cx. pipiens* intensely dominate the rest of the species present in the community. Something similar occurs in the case of UNA, where *Cx. theileri* develop a strong influence. *Passer domesticus*, *Turdus migratorius*, and *Zenaidra macroura* are the most common feeding sources for *Cx. pipiens* [23]. *Culex pipiens* are considered as a major vector for USUV in Israel, Spain and Germany [3, 6 - 8]. Co-occurrence of this virus and WNV in *Cx. pipiens* have been reported from Europe [5, 7]. The same reservoir and vector for both flaviviruses represent that USUV has the potential to import to other countries where only WNV exists. Therefore, Iran is in threat of entering because WNV has already been reported from the country [12]. Although no study has been conducted to verify the presence or absence of this virus in Iran.

The high temperature accompanied by rainfall is desirable for *Cx. pipiens* and affect on vector competence to WNV [24]. Temporary water sources are its favor and could explain why this species is related to precipitation [25]. This is similar to the present study. Therefore, water resources are vital for both birds and *Cx. pipiens* and it may increase the risk of disease transmission [24]. In the present study, we caught this species from March to December and a peak in August was observed, when the mean temperature was 37.1°C and there was no rain. However, in this research, the presence of irrigation canals had created favorable habitats.

Culex hortensis has been reported as another vector of USUV in Central Europe [19]. This species feeds on both birds (ornithophilic) and reptiles (herpetophilic) [25]. This mosquito tends to grow in permanent water resources, in contrast to the *Cx. pipiens* species [25]. This is similar to the present study. The two above-mentioned mosquitoes had similar dynamics in Italy [25]. This is in contrast to our study and *Cx. hortensis* was caught only from July to September. The attempt to isolate WNV from *Cx. hortensis* in Iran was not successful, but it was detected from *Ae. (Och.) caspius* [26]. *Culex hortensis* is an endemic species in Iran, and more studies are needed on its hosting preferences and other behavioral features.

Culex torrentium is an ornithophilic species and involved in the transmission of both WNV and USUV [8, 27]. According to several studies, this species breeding in artificial sites and found it to be more frequent in clean water to turbid breeding sites [28, 29], which is supported by this study. This species may be sharing its larval habitats with *Cx. pipiens* [29]. This is similar to this study and similarity was more than 0.9

according to the Jaccard's index.

Culex perexigus is considered a vector of both WNV and USUV [4]. It is a common species in Iran and breeding in a wide range of places such as seepages, irrigation ditches and temporary ponds [9]. In the present study, it was more frequent in clean and temporary water. Birds are favor for feeding of *Cx. perexigus* and it is more frequent between June and August in Spain, where both WNV and USUV have been reported [7]. This contrasts with our study, in the present study, *Cx. perexigus* showed two peaks in June and October, while it was not caught in August and July.

In the end, it can be said that species that have been introduced as a vector of virus are indigenous in Iran. Thus, isolation of the virus from the *Fulica atra*, other migratory birds, culicid species mentioned above and also *Ae. (Och.) caspius* is essential.

CONCLUSION

In our research, some possible vectors of USUV were identified. In the present study, *Cx. pipiens* as the main vector of the disease was more active between May and August. Potential reservoirs and vectors of USUV have been reported from Iran, therefore, the possibility of the emergence of the virus in Iran exists. Biodiversity analysis indicated that species diversity in rural, urban, and uninhabited areas is somewhat similar. Therefore, attention to all areas in vector control programs is essential.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

Not applicable

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The datasets generated and/or analyzed during the current study are available at Baqiyatallah University of Medical Sciences at <https://www.bmsu.ac.ir>.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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REFERENCES

- [1] Vazquez A, Jiménez-Clavero M, Franco L, et al. Usutu virus: potential

- risk of human disease in Europe. *Euro Surveill* 2011; 16(31): 19935. [PMID: 21871214]
- [2] Aberle SW, Kolodziejek J, Jungbauer C, *et al.* Increase in human West Nile and Usutu virus infections, Austria, 2018. *Euro Surveill* 2018; 23(43):1800545 [http://dx.doi.org/10.2807/1560-7917.ES.2018.23.43.1800545] [PMID: 30376913]
- [3] Mannasse B, Mendelson E, Orshan L, *et al.* Usutu virus RNA in mosquitoes, Israel, 2014-2015. *Emerg Infect Dis* 2017; 23(10): 1699-702. [http://dx.doi.org/10.3201/eid2310.171017] [PMID: 28930008]
- [4] Grottola A, Marcacci M, Tagliacucchi S, *et al.* Usutu virus infections in humans: a retrospective analysis in the municipality of Modena, Italy. *Clin Microbiol Infect* 2017; 23(1): 33-7. [http://dx.doi.org/10.1016/j.cmi.2016.09.019] [PMID: 27677699]
- [5] Nikolay B. A review of West Nile and Usutu virus co-circulation in Europe: how much do transmission cycles overlap? *Trans R Soc Trop Med Hyg* 2015; 109(10): 609-18. [http://dx.doi.org/10.1093/trstmh/trv066] [PMID: 26286946]
- [6] Nikolay B, Diallo M, Faye O, Boye CS, Sall AA. Vector competence of *Culex neavei* (Diptera: Culicidae) for Usutu virus. *Am J Trop Med Hyg* 2012; 86(6): 993-6. [http://dx.doi.org/10.4269/ajtmh.2012.11-0509] [PMID: 22665607]
- [7] Vázquez A, Ruiz S, Herrero L, *et al.* West Nile and Usutu viruses in mosquitoes in Spain, 2008-2009. *Am J Trop Med Hyg* 2011; 85(1): 178-81. [http://dx.doi.org/10.4269/ajtmh.2011.11-0042] [PMID: 21734145]
- [8] Jöst H, Bialonski A, Maus D, *et al.* Isolation of usutu virus in Germany. *Am J Trop Med Hyg* 2011; 85(3): 551-3. [http://dx.doi.org/10.4269/ajtmh.2011.11-0248] [PMID: 21896821]
- [9] Keshavarzi D, Soltani Z, Ebrahimi M, *et al.* Monthly prevalence and diversity of mosquitoes (Diptera&58; Culicidae) in Fars Province, Southern Iran. *Asian Pac J Trop Dis* 2017; 7(2): 112-20. [http://dx.doi.org/10.12980/apjtd.7.2017D6-369] [PMID: 28504431]
- [10] Khaleghizadehi A, Sehhatisabet ME. Avifaunal surveys of the Larian Dam and Lashgarak Area, an important IBA for migrants in Iran. *Sandgrouse* 2007; 29(1): 53.
- [11] Ahmadnejad F, Otarod V, Fallah MH, *et al.* Spread of West Nile virus in Iran: a cross-sectional serosurvey in equines, 2008-2009. *Epidemiol Infect* 2011; 139(10): 1587-93. [http://dx.doi.org/10.1017/S0950268811000173] [PMID: 21396143]
- [12] Fereidouni SR, Ziegler U, Linke S, *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. [http://dx.doi.org/10.1089/vbz.2010.0244] [PMID: 21923253]
- [13] Rogers DJ, Randolph SE. Climate change and vector-borne diseases. *Adv Parasitol* 2006; 62: 345-81. [http://dx.doi.org/10.1016/S0065-308X(05)62010-6] [PMID: 16647975]
- [14] Chinikar S, Shah-Hosseini N, Mostafavi E, *et al.* Seroprevalence of West Nile virus in Iran. *Vector Borne Zoonotic Dis* 2013; 13(8): 586-9. [http://dx.doi.org/10.1089/vbz.2012.1207] [PMID: 23697768]
- [15] Naficy K, Saidi S. Serological survey on viral antibodies in Iran. *Trop Geogr Med* 1970; 22(2): 183-8. [PMID: 4317129]
- [16] WHO. Integrated Vector Management. Cairo: WHO Regional Officer for Eastern Mediterranean 2004.
- [17] 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): 1-33. [http://dx.doi.org/10.11646/zootaxa.2078.1.1]
- [18] Jochmann R, Lipkow E, Blanckenhorn WU. A field test of the effect of spiked ivermectin concentrations on the biodiversity of coprophagous dung insects in Switzerland. *Environ Toxicol Chem* 2016; 35(8): 1947-52. [http://dx.doi.org/10.1002/etc.3081] [PMID: 26013817]
- [19] Reusken C, De Vries A, Ceelen E, *et al.* A study of the circulation of West Nile virus, Sindbis virus, Batai virus and Usutu virus in mosquitoes in a potential high-risk area for arbovirus circulation in the Netherlands, "De Oostvaardersplassen. *Eur Mosq Bull* 2011; 29: 66-81.
- [20] Martínez-de la Puente J, Ferraguti M, Ruiz S, Roiz D, Soriguer RC, Figuerola J. *Culex pipiens* forms and urbanization: effects on blood feeding sources and transmission of avian Plasmodium. *Malar J* 2016; 15(1): 589. [http://dx.doi.org/10.1186/s12936-016-1643-5] [PMID: 27931226]
- [21] Amirkhanian JD. The salivary gland chromosomes of *Culex pipiens molestus*. *Iranian J Publ Hlth* 1974; 3: 40-54.
- [22] Fritz ML, Walker ED, Miller JR, Severson DW, Dworkin I. Divergent host preferences of above- and below-ground *Culex pipiens* mosquitoes and their hybrid offspring. *Med Vet Entomol* 2015; 29(2): 115-23. [http://dx.doi.org/10.1111/mve.12096] [PMID: 25600086]
- [23] Hamer GL, Kitron UD, Brawn JD, *et al.* *Culex pipiens* (Diptera: Culicidae): a bridge vector of West Nile virus to humans. *J Med Entomol* 2008; 45(1): 125-8. [http://dx.doi.org/10.1093/jmedent/45.1.125] [PMID: 18283952]
- [24] Bravo-Barriga D, Gomes B, Almeida APG, *et al.* The mosquito fauna of the western region of Spain with emphasis on ecological factors and the characterization of *Culex pipiens* forms. *J Vector Ecol* 2017; 42(1): 136-47. [http://dx.doi.org/10.1111/jvec.12248] [PMID: 28504431]
- [25] Roiz D, Vazquez A, Rosà R, *et al.* Blood meal analysis, flavivirus screening, and influence of meteorological variables on the dynamics of potential mosquito vectors of West Nile virus in northern Italy. *J Vector Ecol* 2012; 37(1): 20-8. [http://dx.doi.org/10.1111/j.1948-7134.2012.00196.x] [PMID: 22548533]
- [26] Bagheri M, Terenius O, Oshaghi MA, *et al.* West Nile virus in mosquitoes of Iranian wetlands. *Vector Borne Zoonotic Dis* 2015; 15(12): 750-4. [http://dx.doi.org/10.1089/vbz.2015.1778] [PMID: 26565610]
- [27] Balenghien T, Vazeille M, Grandadam M, *et al.* Vector competence of some French *Culex* and *Aedes* mosquitoes for West Nile virus. *Vector Borne Zoonotic Dis* 2008; 8(5): 589-95. [http://dx.doi.org/10.1089/vbz.2007.0266] [PMID: 18447623]
- [28] Weitzel T, Jawieñ P, Rydzanicz K, Lonc E, Becker N. *Culex pipiens* s.l. and *Culex torrentium* (Culicidae) in Wrocław area (Poland): occurrence and breeding site preferences of mosquito vectors. *Parasitol Res* 2015; 114(1): 289-95. [http://dx.doi.org/10.1007/s00436-014-4193-1] [PMID: 25339516]
- [29] Lühken R, Steinke S, Leggewie M, *et al.* Physico-chemical characteristics of *Culex pipiens sensu lato* and *Culex torrentium* (Diptera: Culicidae) breeding sites in Germany. *J Med Entomol* 2015; 52(5): 932-6. [http://dx.doi.org/10.1093/jme/tjv070] [PMID: 26336210]