

Original Article

The Potential of West Nile Virus Transmission Regarding the Environmental Factors Using Geographic Information System (GIS), West Azerbaijan Province, Iran

Mojtaba Amini¹; Ahmad Ali Hanafi-Bojd²; Sayyad Asghari³; *Ali Reza Chavshin^{1,4}

¹Department of Medical Entomology and Vector Control, School of Public Health, Urmia University of Medical Sciences, Urmia, Iran

²Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

³Department of Geography, School of Humanities, University of Mohaghegh Ardabili, Ardabil, Iran

⁴Social Determinants of Health Research Center, Urmia University of Medical Sciences, Urmia, Iran

(Received 2 Mar 2018; accepted 4 Dec 2018)

Abstract

Background: West Nile fever, as an expanding zoonotic disease, has been reported from different creatures involved in the disease from Iran. In addition to biological mosquito-associated factors, various elements such as their activities, distribution, behavior and vectorial capacity could be affected by environmental factors. We determined the distribution of West Nile virus (WNV) vectors, the environmental factors affecting WNV transmission and the high-risk areas across West Azerbaijan Province (Northwestern Iran), regarding the potential of WNV transmission using Geographical Information System (GIS).

Methods: Mosquitoes' larvae and adults were collected from different habitats of the province in 2015 and identified using standard morphological keys. The data regarding the distribution of mosquitoes across the studied area were organized in ArcMap databases. Inverse Distance Weighted (IDW) interpolation analysis was conducted on the data of synoptic stations to find climatic variables in the collection sites of different mosquito species. Layers of transmission-related environmental factors were categorized and weighed based on their effects on disease transmission.

Results: Overall, 2813 samples of different mosquito species from different regions of the province were collected and identified. According to the GIS analysis, areas in the northeastern province, which have lower altitudes and slopes with higher temperatures and more water bodies, were found to have better condition for the activity of mosquitoes (as high-risk areas: hot spots).

Conclusion: The precision of our results was proven to be in line with previous study results that identified high-risk areas, where WNV-infected vectors were captured from these same areas.

Keywords: Mosquitoes; *Aedes caspius*; *Culex pipiens*; Iran

Introduction

Due to notable problems caused by mosquitoes and mosquito-borne diseases (MBDs), the studies of factors influencing the presence, activities, and distribution of mosquitoes and MBDs are important and form absolute parts of the epidemiology of MBDs, in which environmental conditions and their changes are components of this process (1). In addition to biological factors, mosquitoes' activities, distribution, behavior, and even their vectorial

capacity could be affected by environmental conditions (2-5).

Mosquitoes' feeding rates vary expressively with temperature. Moreover, feeding behavior and host availability are affected by climate. Additionally, length of the gonotrophic cycle as an important factor in diseases transmission could be influenced by precipitation patterns (6). Finally, a positive correlation between temperature and West Nile and West

*Corresponding author: Dr Ali Reza Chavshin, E-mail: chavshin@umsu.ac.ir, chavshin@gmail.com

Nile virus (WNV) incidence in mosquitoes have been reported (7, 8).

Among the different tools used to identify the effects of environmental factors on MBDs and their epidemiology, is Geographical Information System (GIS) (9). In addition, global positioning systems (GPS), remote sensing, and spatial statistics could also play important role in MBDs research, surveillance, and control programs (10).

Among the MBDs, West Nile fever is an emerging zoonosis rapidly spread and caused expansive health threat in different parts of the world (11, 12). The transmission cycle of WNV includes a wide range of migratory birds as reservoirs (13), equines (14) and humans (15) as dead-end hosts, and numerous mosquito species including different species e.g. *Culex pipiens*, *Cx. restuans*, *Cx. salinarius*, *Cx. tarsalis*, *Aedes vexans*, *Ae. albopictus*, and *Coquillettidia perturbans* as biological vectors (16–18). WNV has been isolated and identified in numerous mosquito species including different species of *Culex*, *Aedes*, and *Anopheles* (19–21). Due to the wide use of GIS in the study of vector-borne diseases (including MBDs) (22, 23), several studies have employed GIS across the world to predict, risk assessment (18), surveillance (25, 26) and the environmental factors influencing WNV transmission (27, 28).

Because of the presence of theoretically favorable environments for the establishment of WNV across Iran, the presence of WNV has been investigated and showed the seroprevalence rate of (1.3%) in human (29–33), 23.7% in equines (34), overall (15%) infection in birds (35) and recently among the potential vector species, in which among them WNV has been isolated and reported from *Ae. caspius* (36) and *Culex* spp (37).

Iranian West Azerbaijan Province in the northwestern part of the country, could be considered as one of the most suspicious areas in Iran regarding the possibility of establishment and transmission of WNV, because

of its abundant water resources and wetlands for migratory birds from different parts around the world, which serve as reservoirs of WNV (38, 39) and also, the presence of potential vector species of mosquitoes in this region e.g. *Culex pipiens* s.l., *Ae. caspius*, *Anopheles maculipennis* s.l., *Culiseta longiareolata* (40–42). As the first isolation of WNV from its potential vectors was reported from this region (36), and this region borders several countries such as Turkey, Iraq, Armenia, and the Republic of Azerbaijan, more attention is needed at this area.

Due to the special circumstances mentioned above about West Azerbaijan Province, we aimed to determine: 1) the distribution of probable WNV vectors, 2) the environmental factors affecting WNV transmission and 3) the high-risk areas across the province regarding the potential of WNV transmission using GIS.

Materials and Methods

Study area

West Azerbaijan Province is located in the northwest of Iran between latitudes 35° 58′–39° 46′ N and longitudes 44° 3′–47° 23′ E. This province formally includes 17 counties. It is bordered by Turkey, Iraq, Armenia, and the Republic of Azerbaijan. In addition, it is also bordered by Iranian provinces such as East Azerbaijan, Zanjan and Kurdistan (Fig. 1). According to information obtained from Forests, Range and Watershed management organization, West Azerbaijan Province have five types of Micro-climates, including Highly semi-arid (HSA), Moderate semi-arid (MSA), Slight semi-arid (SSA), semi-wet (SW) and wet (W).

Distribution of potential West Nile Vectors in West Azerbaijan Province

Mosquitoes were collected during May–Nov 2015 from 24 localities (wetlands) across the province (Table 1). Adults and larvae were

collected from different habitats using standard methods (43). Collected samples were quickly identified using a stereo microscope and a standard morphological key at the collection sites (44).

Climatic and environmental data

As distribution of mosquitoes and their transmitted diseases are notably dependent on environmental and climatic factors, the effective climatic data, including maximum monthly temperature, minimum monthly temperature, mean monthly temperature, mean relative humidity and rainfall data of decade (2004–2014) were obtained from 16 stations of West Azerbaijan Meteorological Organization (Table 2). Datasets at district level were created in Excel sheets for further analysis by ArcGIS 10.3. The pattern of recent decade of Maximum, Mean and Minimum temperature of different areas in West Azerbaijan Province was analyzed and mapped in Fig. 2 and summarized in Table 2.

Spatial Analysis

For proper analysis, the data regarding distribution of mosquitoes across West Azerbaijan Province were acquired from previous studies (30, 33) and added to the findings of this survey in database created in ArcMap. Inverse distance weighted (IDW) interpolation analysis was conducted on the data of synoptic stations to find the climatic variables in the collection sites of different mosquito species. This analysis interpolates a raster surface from points and estimates cell values by averaging the values of nearby sample data points. The closer a point is to the center of the cell which is being estimated, the more weight it is given. The equation for IDW analysis is:

$$\hat{v} = \frac{\sum_{i=1}^n \frac{1}{d_i} v_i}{\sum_{i=1}^n \frac{1}{d_i}}$$

where

\hat{v} = value to be estimated

v_i = known value

d_1, \dots, d_n = distances from the n data points to the point estimated n

Layers of environmental factors that are important in transmitting the WNV were categorized and weighted based on their effects on disease transmission, and important impact on vector-borne diseases (3, 45–47) (Table 3). The categorized and weighted environmental factors were overlaid with the vectors distribution across the West Azerbaijan Province for determination of high-risk areas for the establishment of the transmission cycle of WNV based on the mentioned environmental factors. As the five species (*Ae. caspius*, *An. maculipennis*, *Cs. longiareolata*, *Cx. pipiens* and *Cx. theileri*) reported from study area, also are known proven and suspected vectors of WNV in different parts of the world, they have been included in final analysis (Fig. 3).

Ethics approval and consent to participate

Prior to the approval of all projects by the Urmia University of Medical Sciences (UMSU), they are reviewed and endorsed by the Ethics Committee of the UMSU. Sample collection was carried out from private human and animal dwellings. At least one day prior to any sample collection, the owners were informed by the Local Health System officers. The whole process was coordinated, managed and documented by the “Local Health System officer” in the study areas.

Results

Overall, 2813 mosquito specimens from different regions of the province were collected and identified (Table 1). Analysis of some climatic variables across the collection sites for different mosquito species is summarized in Table 4. The most frequent species in our study was *Cx. pipiens* (22 out of 24 collection sites), while *Ae. caspius* was found only in 10

localities.

The altitudinal activity of suspected vectors was determined across the areas with an average of 1214m for *Ae. caspius* to 1364m for *Cs. longiareolata* (Table 4). Considering the altitude of the study area which ranges from 605m to 3600m, the values of the altitude were classified into five categories, by overlying the presence of potential vectors and their distribution map on altitude values of the province, height less than 1615m (605–1500m), are more

prepared and favored places for mosquitoes in the study areas. Finally using the scale of the most important environmental variables (Table 3), determination of high risk areas for WNV and distribution of potential vectors across the studied areas was analyzed and revealed in Fig. 3. The map of the mosquitoes including proven and suspected vectors of WNV, ie *Cx. pipiens*, *Cx. theileri* and *Ae. caspius* were collected mostly in hot spots, determined by the model (Fig. 3).

Table 1. Collection sites of mosquitoes in West Azerbaijan Province, northwestern Iran, 2015

County	Collection site	Longitude	Latitude	Altitude	<i>Ae. caspius</i>	<i>An. maculipennis</i>	<i>Cx. pipiens</i>	<i>Cx. theileri</i>	<i>Cs. longiareolata</i>
Bazargan	Bazargan	44.38944	39.38833	1400	0	25	1	13	0
	Yarim-Ghiye	44.43656	39.44604	1409	175	65	0	420	0
Khoy	Hashiyeh rood	45.06315	38.57117	1058	3	0	6	18	0
Mahabad	Mahabad	45.71667	36.75	1351	0	10	89	25	4
	Khoor-khooreh	45.72341	36.98732	1279	205	2	120	0	30
	Kani barazan-wetland	45.77716	36.98689	1275	0	0	68	1	0
	Hajib khosh	45.79718	36.90788	1288	0	0	136	0	0
	Gapis	45.74795	36.93805	1286	0	0	4	7	0
Makoo	Beytas	45.69427	36.67645	1396	3	0	28	39	0
	Makoo	44.43333	39.33333	1411	6	7	82	23	3
	Sangar	44.43429	39.31578	1355	288	132	100	169	90
	Milan	44.4332	39.34351	1445	37	115	25	22	0
	Keshmesh tappeh	44.40093	39.33351	1385	0	0	150	94	0
	Glik gadim	44.66767	39.71264	807	31	0	26	0	0
Miandoab	Deim-Gheshlagh	45.07119	39.34766	784	0	58	0	0	0
	Deimgeshlag	44.7987	39.62488	797	190	0	20	0	0
	Miandoab	46.06947	36.98592	1291	0	0	98	0	0
Naghadeh	Naghadeh	45.41667	36.95	1338	0	56	0	0	7
	Yadegarloo	45.52839	37.03822	1284	0	10	0	23	19
Plodasht	Poldasht	45.07111	39.34778	788	0	10	55	89	0
	Khol-kholeh	44.74524	39.67784	784	0	3	0	40	0
	Ghooch-Ali	44.74391	39.66296	1401	0	25	1	5	0
Sardasht	Sardasht	45.48333	36.15	1556	0	18	0	0	0
Urmia	Urmia	45.05	37.66667	1328	0	128	151	163	246
	Naz-loo	44.98442	37.65172	1358	0	0	441	67	10
	Ghahraman-loo	45.17485	37.64896	1000	170	0	24	0	0
	Koor-Abad	44.64239	37.72749	1545	0	68	34	0	6
	Silvana	44.85142	37.42867	1577	0	120	0	0	0
	Gojar	44.83373	39.48785	1736	0	7	15	19	7
	Mavana	44.79643	37.56658	1617	0	13	0	32	24
	Shaharchay dam	44.98628	37.4952	1433	0	11	0	10	3
Talebin	44.83398	37.54025	1608	0	0	0	149	0	

Table 2. Average of environmental variables in meteorological stations of West Azerbaijan Province of Iran, 1997–2016

Localities	Geographical properties			Environmental variable				
	Longitude	Latitude	Altitude	Total Rainfall	Mean Relative Humidity	Mean Temp	Min Temp	Max Temp
Bookan	46.21667	36.53333	1386.1	360.5	46	14.5	6.3	19.9
Chaldoran	44.38333	39.06667	1788	449.5	58	9.2	2.7	13.9
Ghareh Ziahadin	45.01667	38.9	1108	357.3	52	13.6	8	18
Khoy	44.96667	38.55	1103	289.2	59	12.1	5.5	18.6
Mahabad	45.71667	36.75	1351.8	403.8	53	13.0	6.9	19.15
Makoo	44.43333	39.33333	1411.3	302.8	57	10.6	5.5	15.6
Miandoab	46.05	36.96667	1300	303.8	53	14.4	5.6	20.0
Naghadeh	45.41667	36.95	1338	339.0	53	13.9	5.8	19.2
Oshnavie	45.13333	37.05	1415.9	437.3	52	13.5	4.5	18.6
Piranshahr	45.15	36.7	1443.5	672.7	52	12.0	6.2	17.9
Poldasht	45.07119	39.34778	787	198.8	51	14.7	4.8	20.2
Salmas	44.85	38.21667	1337	247.5	57	11.5	5.2	17.8
Sardasht	45.48333	36.15	1556.8	841.2	49	13.1	9.2	16.8
Shahindej	46.73333	36.6667	1395	334.8	47	14.9	6.7	20.4
Takab	47.1	36.4	1817.2	338.6	55	9.4	2.5	16.4
Urmia	45.05	37.66667	1328	338.9	61	11.6	5.4	17.7

Table 3. Categorizing the environmental factors based on their effects on disease transmission, and important impact on vector-borne diseases (3, 45–47)

Environmental Factor	Ratio	Standard weight	Category	Group	Effect on diseases Transmission
Mean Temperature (°Celsius)	55	0.55	26–30	4	Very High
			20–26	3	High
			18–20	2	Medium
			14–18	1	Low
Relative Humidity (%)	35	0.35	60<	4	Very High
			50–60	3	High
			40–50	2	Medium
			<40	1	Low
Altitude (m)	5	0.05	0–800	4	Very High
			800–1200	3	High
			1200–2000	2	Medium
			<2000	1	Low
Slope (%)	5	0.05	<8	3	Very High
			8–15	2	Medium
			15<	1	Low

Table 4. Average of some environmental and climatic variables in the collection sites of mosquitoes, West Azerbaijan Province, northwestern Iran, 2015

Species	No. of collection sites	Precipitation (mm)	Average of Altitude (M)	Average of Relative Humidity (%)	Average of Maximum Temperature (°C)	Average of Minimum Temperature (°C)	Average of Mean Temperature (°C)
<i>Ae. caspius</i>	10	326.3	1214.7	56	17.21	5.61	11.74
<i>An. maculipennis</i>	20	347.3	1273.9	54.91	17.75	5.66	12.4
<i>Cx. pipiens</i>	22	328.6	1227.5	55.33	17.89	5.6	12.3
<i>Cx. theileri</i>	21	330.65	1275.1	55.78	17.65	5.6	12.1
<i>Cs. longiareolata</i>	12	345.1	1364.25	56.14	17.95	5.66	12.3



Fig. 1. Study area in northwestern Iran

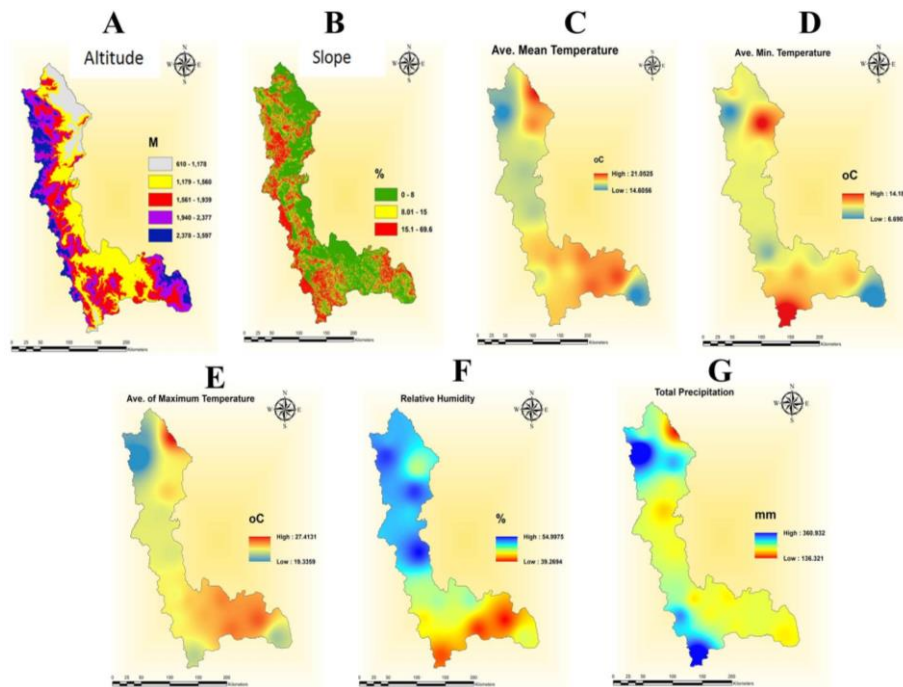


Fig. 2. Environmental and meteorological variables affecting mosquito distribution in West Azerbaijan Province, northwestern Iran, (A): Altitude, (B): Slope, (C): Average of Mean Temperature, (D): Average of Minimum Temperature, (E): Average of Maximum Temperature, (F): Relative Humidity and (G): Total Precipitation.

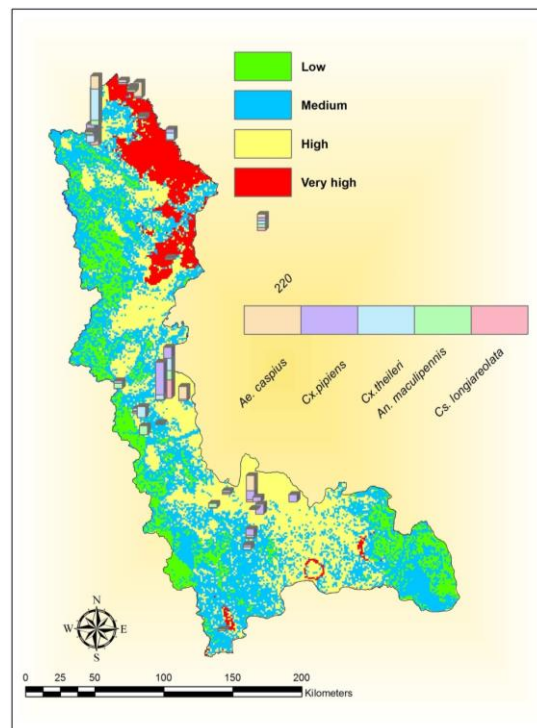


Fig. 3. Spatial distribution of mosquitoes and hot spots for vectors of West Nile virus in West Azerbaijan Province regarding environmental factors affecting the transmission of WNV, northwestern Iran, 2015

Discussion

The study area has a large number of wetlands that host of migratory birds. Although WNV is an enzootic disease among birds (48), however, humans transmission is possible through bites of infected mosquitoes (49). WNV is responsible for disease outbreaks among human in the United States, Europe, and the Middle East (50). It has been detected in recent studies from birds, horse, human and mosquitoes in Iran (31–36).

In the present study, environmental factors affecting the transmission of WNV were studied and high-risk areas were determined (Fig. 3). Earlier study on the impact of climate and environmental variables on WNV in Iran, using data from seropositive horses, found four studied factors that correlated with WNV infection in equine, these factors were temperature, distance to wetlands, and local and regional Normalized Difference Vegetation Index (NDVI) (27). Results from previous studies also indicated the presence of WNV vectors (*Cx. pipiens*, *Cx. theileri*, *Ae. caspius*) in the region (36, 40) and their infection with WNV in Iran (36). In addition, the studied areas have the potential for establishment of WNV transmission. The results of current study regarding the determination of the high-risk areas are interesting because they are in accordance with results from previous study that isolated West Nile virus from vectors (36). The isolation of the virus in the mentioned study has been reported from the high-risk areas identified in current study and this point shows the acceptable accuracy of the results of the current study which analyzed environmental factors (temperature, relative humidity, Altitude and Slope). These areas should be considered in planning of WNV epidemic control programs. Among the probable reasons increasing the risk of establishing transmission cycle of WNV in these areas is the wide range of wetlands in the area and the presence of migratory birds as the reser-

voirs of disease and also the appropriateness of environmental conditions for the presence and abundance of various species of mosquitoes, as potential vectors of WNV. Also it seems northeastern areas of the province, which have lower altitudes and slopes with warmer temperatures and more water bodies, were found to have better condition for the activity of mosquitoes.

Diversity of environmental conditions in West Azerbaijan Province, which provides suitable environment for the establishment of various species of mosquitoes (Table 1) have been found in this current and related studies (36, 40, 42). Other results have shown the effect of temperature in transmission of WNV by *Cx. pipiens*, and the effect of temperature on the replication of the virus within the mosquito's body and the incubation period, as well (51). WNV has the ability to replicate in mosquitoes in wider range of temperatures between 14 °C in mosquitoes (52) to 45 °C in birds (53). By increasing the temperature, WNV propagation rate could be increased.

The suitable temperature for WNV replication is provided in the northeastern and southern parts of the provinces of Iran. Therefore, these areas need more investigation on blood feeding pattern of mosquitoes and their infection with WNV. Recent study on the feeding patterns of potential WNV vectors in South-West Spain showed that *Cx. modestus*, *Cx. perexiguus* and *Cx. pipiens* mainly feed on birds, while *Cx. theileri* and *Ae. caspius* mainly feed on mammals. *Cx. perexiguus* had the highest potential for enzootic virus transmission, followed by *Cx. modestus* and *Cx. pipiens*. According to results of the South-West Spanish study, potential transmission risk to humans was low for *Cx. pipiens*, *Cx. theileri* and *Ae. caspius* (54). The frequency of feeding on humans was only affected by season, while the low number of human blood meals was related to their study site. On the other hand,

the researchers worked in natural areas with very low anthropic presence (54). Most of these mosquito species have been reported from our study area as well (36), therefore, they may have some roles in both avian to avian enzootic cycle and avian-to-mammal transmission.

Certainly, the current study did not cover all important environmental factors affecting the potential of WNV transmission regarding the environmental factors and the effect of other important factors such as wetland, Normalized Difference Vegetation Index (NDVI) and land use should be analyzed and determined in future studies.

Conclusion

The present study serves as a preliminary guide that shows the important effects of environmental conditions on one of the important members (mosquitoes) in the transmission cycle of WNV and should be continued with supplementary studies. Taking into account the vector bio-ecologic conditions, other environmental factors and the interaction of the virus and the vectors as well as other important rings in the transmission of disease, the data obtained from this current study will be very useful and effective in knowing the exact nature of the disease transmission pathways and help in designing its control strategies.

Acknowledgements

This article is part of the results of the first author's dissertation for fulfillment of MSc degree in Medical Entomology and Vector Control from the Department of Medical Entomology and Vector Control, School of Public Health, Urmia University of Medical Sciences, Urmia, Iran.

This study was financially supported by Urmia University of Medical Sciences, Urmia, Iran (Project no. 1579). The authors would like to thank the health staff of the studied

regions for their kind support and contributions.

The authors declare that there is no conflict of interests.

References

1. Khormi HM, Kumar L (2011) Examples of using spatial information technologies for mapping and modelling mosquito-borne diseases based on environmental, climatic, socioeconomic factors and different spatial statistics, temporal risk indices and spatial analysis: a review. *J Food Agr Environ*. 9: 41–49.
2. Drakeley CJ, Carneiro I, Reyburn H, Malima R, Lusingu JP, Cox J, Theander TG, Nkya WM, Lemnge MM, Riley EM (2005) Altitude-dependent and independent variations in *Plasmodium falciparum* prevalence in northeastern Tanzania. *J Infect Dis*. 191: 1589–1598.
3. Hanafi-Bojd AA, Vatandoost H, Oshaghi MA, Charrahy Z, Haghdoost A, Zamani G, Abedi F, Sedaghat MM, Soltani M, Shahi M, Raeisi A (2012) Spatial analysis and mapping of malaria risk in an endemic area, south of Iran: a GIS based decision making for planning of control. *Acta Trop*. 122(1): 132–137.
4. Afrane YA, Little TJ, Lawson BW, Githeko AK, Yan G (2008) Deforestation and vectorial capacity of *Anopheles gambiae* Giles mosquitoes in malaria transmission, Kenya. *Emerg Infect Dis*. 14(10): 1533–1538.
5. Paaijmans KP, Blanford S, Chan BH, Thomas MB (2011) Warmer temperatures reduce the vectorial capacity of malaria mosquitoes. *Biol Lett*: 8(3): 465–468.
6. Ciota AT, Mataracchio AC, Kilpatrick AM, Kramer LD (2014) The effect of temperature on life history traits of *Culex* mosquitoes. *J Med Entomol*. 51: 55–62.
7. Ruiz MO, Chaves LF, Hamer GL, Sun T,

- Brown WM, Walker ED, Haramis L, Goldberg TL, Kitron UD (2010) Local impact of temperature and precipitation on West Nile virus infection in *Culex* species mosquitoes in northeast Illinois, USA. *Parasit Vectors*. 3(1): 19.
8. Hahn MB, Monaghan AJ, Hayden MH, Eisen RJ, Delorey MJ, Lindsey N, Nasci RS, Fischer M (2015) Meteorological conditions associated with increased incidence of West Nile virus disease in the United States, 2004–2012. *Am J Trop Med Hyg*. 92(5): 1013–1022.
 9. Eisen L, Eisen RJ (2011) Using geographic information systems and decision support systems for the prediction, prevention, and control of vector-borne diseases. *Annu Rev Entomol*. 56: 41–61.
 10. Kitron U (1998) Landscape ecology and epidemiology of vector-borne diseases: tools for spatial analysis. *J Med Entomol*. 35: 435–445.
 11. Hayes EB, Gubler DJ (2006) West Nile virus: epidemiology and clinical features of an emerging epidemic in the United States. *Annu Rev Med*. 57: 181–194.
 12. Mackenzie JS, Gubler DJ, Petersen LR (2004) Emerging flaviviruses: the spread and resurgence of Japanese encephalitis, West Nile and dengue viruses. *Nat Med*. 10: S98–S109.
 13. Rappole JH, Derrickson SR, Hubálek Z (2000) Migratory birds and spread of West Nile virus in the Western Hemisphere. *Emerg Infect Dis*. 6: 319.
 14. Autorino GL, Battisti A, Deubel V, Ferrari G, Forletta R, Giovannini A, Lelli R, Murri S, Scicluna MT (2002) West Nile virus epidemic in horses, Tuscany region, Italy. *Emerg Infect Dis*. 8(12): 1372–1378.
 15. Lindsey NP, Staples JE, Lehman JA, Fischer M (2010) Surveillance for human West Nile virus disease—United States, 1999–2008. *MMWR Surveill Summ*. 59(2): 1–17.
 16. Hubalek Z, Halouzka J, Juricova Z, Sebesta O (1998) First isolation of mosquito-borne West Nile virus in the Czech Republic. *Acta Virol*. 42: 119–120.
 17. Hubalek Z, Rudolf I, Bakonyi T, Kazdova K, Halouzka J, Sebesta O, Sikutová S, Juricová Z, Nowotny N (2010) Mosquito (Diptera: Culicidae) surveillance for arboviruses in an area endemic for West Nile (Lineage Rabensburg) and Tahyna viruses in Central Europe. *J Med Entomol*. 47(3): 466–472.
 18. Lanciotti RS, Kerst AJ, Nasci RS, Godsey MS, Mitchell CJ, Savage HM, Komar N, Panella NA, Allen BC, Volpe KE, Davis BS, Roehrig JT (2000) Rapid detection of West Nile virus from human clinical specimens, field-collected mosquitoes, and avian samples by a TaqMan reverse transcriptase-PCR assay. *J Clin Microbiol*. 38(11): 4066–4071.
 19. Romi R, Pontuale G, Ciufolini M, Fiorentini G, Marchi A, Nicoletti L, Cocchi M, Tamburro A (2004) Potential vectors of West Nile virus following an equine disease outbreak in Italy. *Med Vet Entomol*. 18(1): 14–19.
 20. Turell MJ, Sardelis MR, Dohm DJ, O'Guinn ML (2001) Potential North American vectors of West Nile virus. *Ann NY Acad Sci*. 951: 317–324.
 21. Ergünay K, Litzba N, Brinkmann A, Günay F, Sarıkaya Y, Kar S, Örsen S, Öter K, Domingo C, Erisoz Kasap Ö, Özkul A, Mitchell L, Nitsche A, Alten B, Linton YM (2017) Co-circulation of West Nile virus and distinct insect-specific flaviviruses in Turkey. *Parasit Vectors*. 10 (1): 149.
 22. Marcantonio M, Rizzoli A, Metz M, Rosà R, Marini G, Chadwick E, Neteler M (2015) Identifying the environmental conditions favouring West Nile Virus outbreaks in Europe. *PLoS One*. 10(3): e0121158.

23. Valiakos G, Papaspyropoulos K, Giannakopoulos A, Birtsas P, Tsiodras S, Hutchings MR, Spyrou V, Pervanidou D, Athanasiou LV, Papadopoulos N, Tsokana C, Baka A, Manolakou K, Chatzopoulos D, Artois M, Yon L, Hannant D, Petrovska L, Hadjichristodoulou C, Billinis C (2014) Use of wild bird surveillance, human case data and GIS spatial analysis for predicting spatial distributions of West Nile virus in Greece. *PloS One*. 9(5): e96935.
24. Sánchez-Gómez A, Amela C, Fernández-Carrión E, Martínez-Avilés M, Sánchez-Vizcaíno JM, Sierra-Moros MJ (2017) Risk mapping of West Nile virus circulation in Spain, 2015. *Acta Trop*. 169: 163–169.
25. Eisen RJ, Eisen L (2014) Use of geographic information systems in infectious disease surveillance. In: M'ikanatha NM, Iskander J (Eds) *Concepts and Methods in Infectious Disease Surveillance*, Wiley-Blackwell, pp. 219–229.
26. Moore M (2014) *Geographic Analysis of West Nile Virus in the Upper Minnesota River Valley: A GIS and Multi-temporal Remote Sensing Approach*. [MSc dissertation]. Minnesota State University, Mankato Mankato, Minnesota, USA.
27. Ahmadnejad F, Otarod V, Fathnia A, Ahmadabadi A, Fallah MH, Zavareh A, Miandehi N, Durand B, Sabatier P (2016) Impact of climate and environmental factors on West Nile virus circulation in Iran. *J Arthropod Borne Dis*. 10(3): 315–327.
28. Roiz D, Ruiz S, Soriguer R, Figuerola J (2015) Landscape effects on the presence, abundance and diversity of mosquitoes in Mediterranean Wetlands. *PloS One*. 10(6): e0128112.
29. Naficy K, Saidi S (1970) Serological survey on viral antibodies in Iran. *Trop Geogr Med*. 22: 183–188.
30. Saidi S, Tesh R, Javadian E, Nadim A (1976) The prevalence of human infection with West Nile virus in Iran. *Iran J Public Health*. 5: 8–13.
31. Talebian A (2010) A study of West Nile virus infection in Iranian blood donors. *Arch Iran Med*. 13(1): 1–4
32. Chinikar S, Javadi A, Ataei B, Shakeri H, Moradi M, Mostafavi E, Ghiasi SM (2012) Detection of West Nile virus genome and specific antibodies in Iranian encephalitis patients. *Epidemiol Infect*. 140(8): 1525–1529.
33. Meshkat Z, Chinikar S, Shakeri M, Manavifar L, Moradi M, Mirshahabi H, Jalali T, Khakifirouz S, Shahhosseini N (2015) Prevalence of West Nile virus in Mashhad, Iran: A population-based study. *Asian Pac J Trop Med*. 8(3): 203–205.
34. Ahmadnejad F, Otarod V, Fallah M, Lowenski S, Sedighi-Moghaddam R, Zavareh A, Durand B, Lecollinet S, Sabatier P (2011) Spread of West Nile virus in Iran: A cross-sectional serosurvey in equines, 2008–2009. *Epidemiol Infect*. 139(10): 1587–1593.
35. Fereidouni SR, Ziegler U, Linke S, Niedrig M, Modirrousta H, Hoffmann B, Groschup MH (2011) 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*. 11(10): 1377–1381.
36. Bagheri M, Terenius O, Oshaghi MA, Motazakker M, Asgari S, Dabiri F, Vattandoost H, Mohammadi Bavani M, Chavshin AR (2015) West Nile virus in mosquitoes of Iranian wetlands. *Vector Borne Zoonotic Dis*. 15(12): 750–754.
37. Shahhosseini N, Chinikar S, Moosa-Kazemi SH, Sedaghat MM, Kayedi MH, Lühken R, Schmidt-Chanasit J (2017) West Nile Virus lineage-2 in *Culex* specimens from Iran. *Trop Med Int Health*. 22(10): 1343–1349.

38. Salmanzadeh R, Majidi A, Jabbari H, Abbasnejad H, Saket A (2011) The Study of Mahabad's Kanibarazan Wetland Biodiversity Indexes, Iran. *Int Proc Chem Biol Environ.* 24: 73–77.
39. John R (2005) "Birds of Azerbaijan" by Michael Patrikeev (book review). *Can fieldnat.* 119: 299.
40. Khoshdel-Nezamiha F, Vatandoost H, Azari-Hamidian S, Bavani MM, Dabiri F, Entezar-Mahdi M, Chavshin AR (2014) Fauna and larval habitats of mosquitoes (Diptera: culicidae) of west Azerbaijan Province, northwestern Iran. *J Arthropod Borne Dis.* 8(2): 163–173.
41. Azari-Hamidian S, Yaghoobi-Ershadi MR, Javadian E, Abai MR, Mobedi I, Linton YM, Harbach RE (2009) 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.* 23(2): 111–121.
42. Khoshdel-Nezamiha F, Vatandoost H, Oshaghi MA, Azari-Hamidian S, Mianroodi RA, Dabiri F, Bagheri M, Terenius O, Chavshin AR (2016) Molecular characterization of mosquitoes (Diptera: Culicidae) in Northwestern Iran by using rDNA-ITS2. *Jpn J Infect Dis.* 69(4): 319–322.
43. Silver JB (2008) *Mosquito ecology: field sampling methods*: Springer.
44. Azari-Hamidian S, Harbach RE (2009) Keys to the adult females and fourth-instar larvae of the mosquitoes of Iran (Diptera: Culicidae). *Zootaxa.* 2078: 1–33.
45. Dohm DJ, O'Guinn ML, Turell MJ (2002) Effect of environmental temperature on the ability of *Culex pipiens* (Diptera: Culicidae) to transmit West Nile virus. *J Med Entomol.* 39: 221–225.
46. Dohm DJ, Turell MJ (2001) Effect of incubation at overwintering temperatures on the replication of West Nile virus in New York *Culex pipiens* (Diptera: Culicidae). *J Med Entomol.* 38(3): 462–464.
47. Kilpatrick AM, Meola MA, Moudy RM, Kramer LD (2008) Temperature, viral genetics, and the transmission of West Nile virus by *Culex pipiens* mosquitoes. *PLoS Pathog.* 4: e1000092.
48. Malkinson M, Banet C (2002) The role of birds in the ecology of West Nile virus in Europe and Africa. In: Mackenzie JS, Barrett ADT, Deubel V (Eds) *Japanese Encephalitis and West Nile Viruses*, Springer. pp. 309–322.
49. Murgue B, Murri S, Zientara S, Durand B, Durand JP, Zeller H (2001) West Nile outbreak in horses in southern France, 2000: the return after 35 years. *Emerg Infect Dis.* 7: 692–696.
50. Kramer LD, Styer LM, Ebel GD (2008) A global perspective on the epidemiology of West Nile virus. *Annu Rev Entomol.* 53: 61–81.
51. Hess A, Cherubin C, LaMotte L (1963) Relation of temperature to activity of western and St. Louis encephalitis viruses. *Am J Trop Med Hyg.* 12: 657–667.
52. Cornel AJ, Jupp PG, Blackburn NK (1993) Environmental temperature on the vector competence of *Culex univittatus* (Diptera: Culicidae) for West Nile virus. *J Med Entomol.* 30: 449–456.
53. Kinney RM, Huang CY-H, Whiteman MC, Bowen RA, Langevin SA, Miller BR, Brault AC (2006) Avian virulence and thermostable replication of the North American strain of West Nile virus. *J Gen Virol.* 87(Pt 12): 3611–3622.
54. Muñoz J, Ruiz S, Soriguer R, Alcaide M, Viana DS, Roiz D, Vázquez A, Figuerola J (2012) Feeding patterns of potential West Nile virus vectors in south-west Spain. *PLoS One.* 7: e39549.