Original Article

Investigation on Mosquitoes Fauna (Diptera: Culicidae) and Probable Vector of West Nile Virus in Lorestan Province, Western Iran

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Abstract

Background: Fauna and larval habitat characteristics studies on mosquitoes are important tools to identify the breeding places of the vectors and management of the control strategies. This study was done to provide data on Culicidae fauna, larval habitat characteristics and identifying potential vectors of West Nile virus in Lorestan Province, west of Iran.

Methods: Culicidae mosquitoes were collected at three counties and nine site stations from Lorestan Province, west of Iran in 2017. Adult mosquitoes were collected using human and animal bite collection methods, New Jersey and CDC light traps and pit shelters by aspirator. Larva were collected by dipping method. RT-PCR technique was employed for detection of the West Nile virus among mosquito's samples.

Results: 4805 mosquitoes were collected from three counties and nine sites in Lorestan Province during June–October 2017, including 4363 adults and 442 larvae. The most abundant species collected from all counties in both adult and larval stages were *Culex pipiens* (49.10%), *Cx. theileri* (31.82%), *Anopheles maculipennis* (11.09%), *An. superpictus* (2.66%), *An. stephensi* (2.12%), *Cx. perexiguus* (1.89%), *An. dthali* (1.17%) and *An. sacharovi* (0.15%) respectively. West Nile virus was detected in none of mosquitoes examined.

Conclusion: The results of this study revealed that arbovirus vectors such as *Cx. pipiens* along with *Cx. theileri* and *Cx. perexiguus* are well adapted to a broad range of habitats and different climatic conditions in Lorestan Province. That necessitates further routine surveillance of arboviral infections.

Keywords: *Anopheles*; Arboviruses; *Culex*; West Nile; Habitat

Introduction

Mosquitoes (Diptera: Culicidae) are the medically-important species for malaria, filariasis, encephalitis and some other arboviral diseases transmission (1-3). The Culicidae family include 2 subfamilies, 41 genera and 3584 species (4). The most

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important genera of the family are *Anopheles*, *Culex* and *Aedes*. Mosquitoes are vectors of important vector-borne diseases malaria, filariasis, dengue fever, yellow fever, chikungunya, west Nile Virus and Zika Virus which are among the greatest health problems in the world (5-7).

Considering the fact that in Lorestan Province, Iran, some vector mosquitoes of diseases such as West Nile, dirofilariasisand malaria are present; study on thecomposition, distribution and ecology of mosquitoes have great impacts in vector control programs. These findings guide us for better planning of disease control strategies. Regards to new human activities and weather change in the research area, the outcomes of our investigation should helps health services to management vectors increasing and establishment in the area, therefore, the risk of transmission of diseases by mosquitoes gets minimized. The aim of the present study has been determining of the composition, distribution and some ecological aspects of mosquitoes in Lorestan Province, Iran, which is of high medical importance from the point of view of the arboviral vectors.

Materials and Methods

Study areas

Lorestan Province is located in western Iran, between 32º 40´-32 º 34´ N latitudes and 46º50´-46º 51´ E longitudes. The province covers an area of approximately 28,308 km² with a population of around 1,800,000 people (Fig. 1, Table 1). This province contains 11 counties with one million people called metropolitan. Out of them, Khorramabad, with a population of 800,000, is ranked amongst the 40 most populous metropolitan cities of Iran. The hottest months of the year are from mid- July to mid-September when temperatures range from 28 °C to 40 °C and the coldest month's experience 1 °C around December– January, but at certain times in winter it can reach −15 °C. Khorramabad County has moderate winters and hot summers. Average annual rainfall is approximately 516 millimeters, the maximum rainfall has been recorded during the winter season. On the whole, the province has a semi-arid, steppe climate in the south and Mediterranean climate in the north. (Fig.1, Table 1).

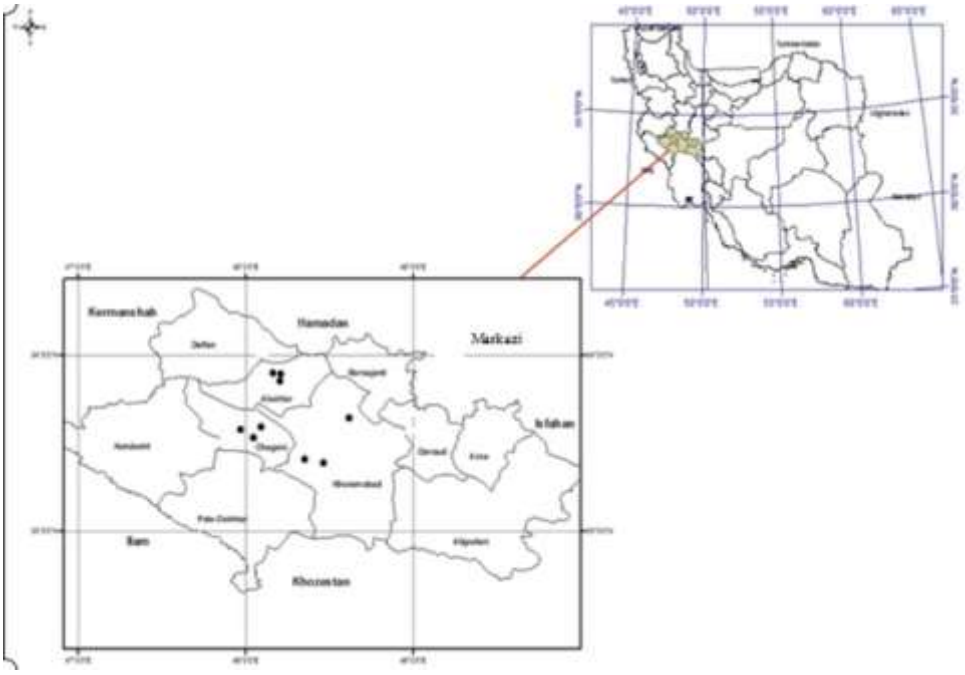

Fig. 1. Map of Iran and locations of study areas in Lorestan Province, Iran

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Mosquito collection and species Identification

For mosquito collection, nine sites with different biotopes in Khorramabad, Selseleh (Alashtar) and Chegeni Counties were selected (Fig. 1, Table 1).

In this study, the collection of mosquito larvae was performed in different habitats using the standard dipping technique (using 350 ml Clark's dippers) and whirl pack bags (8). CDC and New Jersey light traps, animal and human bites and shelter pit methods were used for collecting mosquitoes. Mosquitoes capture was done during June to October 2017. Larvae and adult mosquitoes were identified by morphological keys (9).

Physical and biological characteristics of larval habitats

The ecological characteristics containing geographical data of collecting sites (latitude, longitude and altitude), type of habitat (stagnant, stream, seepage and water container), vegetation situation (presence or lack of vegetation), kind of vegetation (leaf-Null, *Oryza sativa*, *Typha latifolia*, *Carex dipsacea* and *Acorus calamus*), turbidity, exposure to sunlight (full, partial sunlight and covered or shaded), depth, substrate type (muddy, sandy, rocky and concrete), distance from animal and human houses and physicochemical attributes such as water temperature were recorded (7-9, 10).

Statistical analyses

The species richness (R: Margalef index), unified indices (D: Simpson's diversity index and H: Shannon–Wiener index) and evenness (E: distribution of abundances among the species) as measures of diversity, were calculated for Lorestan Province and different study counties.

Below is the formulae and their rationale for our research:

$$R = \frac{S - 1}{\ln N}$$

$$D = 1 - \sum_{i=1}^{S} \frac{n_i (n_i - 1)}{N (N - 1)}$$

$$H\Delta = \sum_{i=1}^{S} (p_i)\left[\ln (p_i)\right]$$

$$E = \frac{H\Delta}{\ln (s)}$$

Results

In total, 4363 adults and 442 larvae were collected from three counties and nine sites in Lorestan Province during June–October 2017. The properties of geographical larval habitats (latitude, longitude and altitude) have been shown in Fig. 1 and Table 1. West Nile virus was not detected in mosquitoes. *An. maculipennis* s.l., *An. superpictus* s.l, *Cx. perexiguus*, *Cx. pipiens*, and *Cx. theileri*, were collected at both adult and larval stages (Table 2).

Table 1. Geographical characters of the collection sites, Lorestan Province, Iran

Geographical situation					County	Locations	Code
No	Topography	Altitude (M)	Latitude (N)	Longitude (E)			
1	Mountain	1302.76 m	33° 36' 22.79" N	48° 18' 34.67" E	Khorramabad	Robat Namaki	M
2	Plain	1152.29 m	33° 26' 8.29" N	48° 18' 2.10" E	Khorramabad	Sarzangoleh	P
3	Slope	1164.07 m	33° 26' 23.79" N	48° 19' 12.68" E	Khorramabad	Dast Be Zanoo	D
4	Slope	1003.74 m	33° 30' 19.69" N	48° 0' 29.78" E	Chegeni	Cham Divan	D
5	Plain	957.95 m	33° 26' 37.42" N	47° 56' 29.11" E	Chegeni	Sharaf Bostanrood	P
6	Mountain	1110.27 m	33° 33' 15.83" N	48° 1' 3.39" E	Chegeni	Berkeh	M
7	Mountain	1715.29 m	33° 47' 51.83" N	48° 17' 41.56" E	Selseleh	Chartakteh	M
8	Plain	1565.05 m	33° 44' 19.74" N	48° 8' 50.07" E	Selseleh	Varnamad	P
9	Slope	1572.64 m	33° 43' 17.51" N	48° 15' 39.02" E	Selseleh	Kakareza	D

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The most abundant species collected from all counties in both adult and larval stages were *Cx. pipiens*, *Cx. theileri*, *An. maculipennis* s.l., *An. superpictus* s.l. , *An. stephensi*, *Cx. perexiguus*, *An. dthali* and *An. sacharovi* respectively.

Some species such as *Cx. pipiens*, *Cx. theileri*, *An. maculipennis* s.l. have wide distribution in the study areas, but some species such as *An. stephensi* have been collected from limited areas (Table 2).

In larvae and adult stages of mosquitoes, two genera along with 7 species were collected and identified from Khorramabad County. *Culex pipiens*, *Cx. theileri*, *An. superpictus* s.l., *An. maculipennis* s.l., *Cx. perexiguus*, *An. dthali* and *An. sacharovi*were the most abundant species respectively(Table 2). In Chegeni County, two genera including 8 species were identified: *Cx. pipiens*, *Cx. theileri*, *An. maculipennis* s.l., *An. stephensi*, *An. superpictus*, *Cx. perexiguus*, *An. dthali* and *An. sacharovi* (Table 2).

In Selseleh County, five species in two genera were identified: *Cx. theileri*, *Cx. pipiens*, *Cx. perexiguus*, *An. Maculipennis* s.l. and *An. dthali* (Table 2).

Characteristics of mosquito larvalhabitats

Khorramabad and Chegeni larval siteshad stagnant water while Selseleh had seepage water. The characteristics of Chegeni and Selseleh Counties were more similar than Khorramabad County. Both Chegeni and Selseleh Counties had larval sites with turbid water, muddy substrate, and shallow depth. In addition, these sites were covered by sunlight with leaf-Null, *Oryza sativa*, *Typha latifolia*, *Carex dipsacea* and *Acorus calamus*, vegetation.

We found stagnant and clear water without vegetation with exposed to sunlight. The sites were away from human and animal houses, around more than two

Table 2. Composition and abundance percentage of collected species from larval habitats and adult mosquitoes in the Lorestan Province of Iran, June–October 2017

Province	Location	species	Larvae		Adult	
			No.	%	No.	%
	Khorramabad	*An. dthali*	0	0	32	1.91
		An. superpictus s.l.	0	0	57	3.41
		An. sacharovi	0	0	2	0.11
		An. maculipennis s.l.	14	5.90	27	1.61
		Cx. pipiens	124	52.32	988	59.26
		Cx. theileri	89	37.55	535	32.09
		Cx. perexiguus	10	4.21	26	1.55
	Total		237	100	1667	100
Lorestan	Chegeni	*An. dthali*	0	0	23	0.88
		An. superpictus s.l.	9	5.62	62	2.39
		An. stephensi	0	0	102	3.94
		An. sacharovi	0	0	5	0.193
		An. maculipennis s.l.	24	15	466	18
		Cx. pipiens	87	54.37	1090	42.11
		Cx. theileri	34	21.25	795	30.71
		Cx. perexiguus	6	3.75	45	1.73
	Total		160	100	2588	100
	Selseleh	*An. maculipennis* s.l.	1	2.22	1	0.92
		Cx. perexiguus	4	8.88	0	0
		Cx. pipiens	18	40	52	48.14
		Cx. theileri	22	48.88	54	50
		An.dthali	0	0	1	0.92
	Total		45	100	108	100
Total			442	100	4363	100

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Table 3. Characteristics of mosquito larval collection sites in three counties of Lorestan Province of Iran, June–October 2017

County	Type	Tur	Exp	Veg	DHH	DAH	Depth	Sub	T
Khorramabad	Stagnant	Clear	Exposed	Without	>2km	>2km	<1m	Muddy	23
Chegeni	Stagnant	Turbid	Covered	leaf-Null, *Oryza sativa*, *Typha latifolia*, *Carex dipsacea*	>2km	500m	Shallow	Muddy	9
Selseleh	Seepage water	Turbid	Covered	leaf-Null, *Carex dipsacea* and *Acorus calamus*,	>2km	200m	Shallow	Muddy	13

ND: not determined, Tur: Turbidity, Exp: Sun exposure, Veg: Vegetation type, DHH: Distance from the nearest human houses, DAH: Distance from the nearest animal house, Sub: Substrate type, T: Temperature.

Table 4. The species richness (R), Simpson's diversity index (D), Shannon-Weiner diversity index (H), and evenness (E) of the adult collected mosquitoes in the study areas

County	S	R	D	H´	E
Khorramabad	7	0.809	0.458	1.008	0.519
Chegeni	8	0.763	0.490	1.353	0.650
Selseleh	4	0.640	0.318	0.782	0.564

kilometers. In such a larval breeding site, the water temperature was 23 °C. In this larval habitat, one Anophelinae (*An. maculipennis* s.l.) and three Culicinae species of *Cx. theileri*, *Cx. perexiguus*, and *Cx. pipiens* were collected (Tables 2, 3).

According to the results, presented in tables 2 and 3, larvae of mosquitoes were observed in all different types of the habitats. In addition, our results showed that *Cx. pipiens*, and *Cx. theileri*, had the most distribution and adaptation to different types of larval habitats, respectively. These mosquitoes larvae were captured in most sites but, *Cx. perexiguus* was found only in the shallow, stagnant, and turbid water, covered with plants and with a muddy substrate. In addition, the distribution of *An. sacharovi* was limited and only collected in the habitats with seepage, stagnant and turbid water along with muddy substrate and shallow depth at Sharaf Boostanrood of Chegeni. *An. sacharovi was* found only in paddy fields, having stagnant and clean water with a muddy substrate exposed to sunlight.

We found differences in the species diversity, due to Simpson's diversity index, Shannon-Wiener index (H'), and species richness of the mosquitoes in the study areas of Lorestasn Province (Table 4). The species richness and the three indices were found to be minimum in Selseleh County (R= 0.640; D= 0.318; H'= 0.782; E= 0.564), whereas the estimated diversity (D= 0.458; H'= 1.008), and richness (R= 0.809) were the highest in Khorramabad County.

Discussion

The present research is the first study on distribution, diversity and ecology of mosquitoes, with emphasis on *Cx. pipiens* as potential arbovirus vectors in Lorestan Province, western Iran. Lorestan Province contains diverse geographical areas with different climates. These diverse conditions can provide a suitable environment for the establishment of different species of mosquitoes that can justify the variety of mosquito species in this region. This study showed many mosquito species had ecological adaptations in this area. In spite of these eco-biological characteristics, the

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ecology of mosquitoes present in Lorestan Province is largely unknown. In the present investigation we tried to study distribution and ecology of mosquitoes in three northeastern, northwest and central regions of Lorestan Province. Although some studies had been conducted on fauna and checklist of mosquitoes in parts of this region based on our best knowledge, no studies have been done earlier on the ecology of mosquitoes in these regions (11, 12).

In the current study, two genera and eight species were collected and identified. For the first time, *Cx. perexiguus* species was reported from Chegeni and Selseleh Counties. Some studies had reported *An. dthali*, *An. sacharovi*, *An. maculipennis* s.l., and *An. superpictus* s.l. in Lorestan Province (13, 14). In our study, for the first time, *An. stephensi* and *An. sacharovi* were found in Chegeni, however, in previous studies the existence of these species was not reported in such areas (11-13). Some investigations reported *Culiseata annulata* and *Cs. subochrea* in Lorestan Province and *Cs. subochrea* in Khuzestan Province (11-13, 15). *Culiseta longiareolata* species was reported as the most abundant mosquito in Kermanshah, Kurdistan and Sistan and Baluchistan Provinces (16, 17).

Comparing the results of our study with a recent study carried out in western Iran (10) showed that four species (*An. maculipennis*, *An. superpictus*, *Cx. pipiens* and *Cx. theileri*) were common between Lorestan and west Azerbaijan Provinces. The results of our study compared with the results of research conducted in Zanjan Province (18), showed that four species (*An. maculipennis* s.l., *An. superpictus* s.l., *Cx. pipiens*, and *Cx. theileri*, were common between these two provinces. The comparison of the results of our study with a recent study conducted in Kurdistan Province (19) showed that four species (*An. maculipennis* s.l., *An. superpictus* s.l., *Cx. theileri* and *Cx. pipiens*) are common between two provinces. In Turkey (20), and in the provinces of Ardabil, Kurdistan and West Azarbaijan of Iran, *Cx. theileri* and *Cx. pipiens* were dominant and most abundant species in the area (21) and our study is in line with such results.

The climate changes and biotic and abiotic environment factors, includingplants, temperature and rainfall ranges,significantly affect the type and frequency of larval mosquito habitats. The mentioned factors have impacts on the longevity, larval stages, number of mosquitoes, behavior and adult development of mosquitoes. As a result, the transmission of diseases through mosquitoes is directly affected by environmental factors (22, 23).

The presence of plants as a source of sugar for mosquitoes is very important that influence both larval and adult stage development (24, 25). Plants provide energy for mosquitoes. As a result, the survival rate will increase and the longevity of mosquitoes will be longer than the extrinsic incubation period of the parasite, therefore the incidence of disease increases (26, 27). In our study, four species of plants such as Leaf-Null, *Oryza sativa*, *Typha latifolia*, *Carex dipsacea* and *Acorus calamus* were found in relation to mosquito larval habitats. Few studies have been carried out on plant species in collaboration with mosquito species in Iran. In our study, various genera of mosquitoes such as *Anopheles* and *Culex* were found in relation with various plants. *Aedes vexans* and *Ae. caspius* have been reported earlier in these areas (11-13). The results of our research show that climate changes in association with human activities effect on the species distribution in the area. Abundance of species of mosquitoes related to physio-chemical characteristics of larval sites. For example, *Culex* species were captured from different larval sites, determining that *Culex* species survived in a range of water habitats. Larval habitats in this study were stagnant, stream and seepage, water container, turbid and clear water, sun exposed or covered from sunlight, with a rocky or muddy substrate and shallow depth. Studies showed that some of *Culex* larval species were foundalone or along with other mosquitoes, such as *Anopheles* and *Aedes* (29-31), which has been observed in

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our study.

The results of some studies showed that there is a significant relationship between the distance of larval habitats of *Anopheles* mosquitoes and human and animal sites. These studies suggested that *Anopheles* mosquitoes are found more often near human and animal houses (32). Our study showed that there is a significant correlation between the distance of larval habitats of *Anopheles* and *Cx theileri* with animal and human dwellings due to larval sites of both species are more near to animal and human dwellings. This association was not found between the larval sites of *Cx. pipiens* and with human and animal houses because the habitats of these mosquitoes were found at various intervals from human and animal houses.

Conclusion

The results of this study revealed that arbovirus vectors such as *Cx. pipiens* along with *Cx. theileri* and *Cx. perexiguus* are well adapted to a broad range of habitats and different climatic conditions in Lorestan Province. Determining of distribution and full description of ecology of arboviral vectors under local eco-demographic conditions in Lorestan Province have provided important ecological information on establishment of important mosquito borne diseases and help with minimizing the risk of transmission of disease by mosquitoes.

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Competing interest

The authors declare no conflict of interest.

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