Original Article

Investigation on the Occurrence of Aedes Species in Borderline of Iran and Azerbaijan for Control of Arboviral Diseases

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Abstract
Background: To investigate the diversity of the genus Aedes present in the natural areas of Ardabil Province, north-west of Iran.
Methods: This cross-sectional study was carried out from Apr to Oct 2016 in North-western of Iran. Thirty-three areas of 10 cities which are border areas were selected randomly. The larvae were collected 2 times in each month during the seasonal activities of mosquitoes and the larvae were identified morphologically according to the appropriate identification keys.
Results: Overall, 694 larvae were collected from four counties, from which only 7.2% were Aedes larvae. Three species of Aedes were identified which include Ae. caspius, Ae. vexans and Ae. flavescens. Aedes flavescens is reported from Ardabil Province for the first time.
Conclusion: Aedes species were a high density in borderline of Iran and Azerbaijan. Therefore, the north parts of Ardabil Province are a suitable habitat for Aedes species mosquitoes. Care should be taken for vector control in the case of occurrence of any arboviruses transmitted by Aedes mosquitoes.

Keywords: Larval habitat; Aedes; Borderline; Iran

Introduction

Mosquito-borne arboviruses are health threat in the worldwide. For instance, more than 2.5 billion people live in high-risk areas of dengue fever (DF) in the world and over 100 million people are infected with this disease (1, 2).

Currently, there is no vaccine and no specific treatment for DF (3). Recently, outbreaks of DF have happened in Malaysia, Taiwan, and India (4). Endemic DF always occurred in Southeast Asia (5). However, some outbreaks recently have been occurred in parts of the Middle East, South Asian countries including Pakistan (6, 7).

Some factors affecting the distribution of DF in different parts of the world are as follows: Increasing urban population density, increased travel, and unsystematically urbanization (8-10). DF has been reported in 120 countries (11). The agent of DF is a Flaviviridae family and the main vectors are Aedes aegypti and Ae. albopictus (12). Thus mosquitoes are an invasive species in world widespread in tropical and temperate regions of the world. The ability to lay eggs and grow in dishes cultural artifacts. In the last two decades and facilitate the movement in the world are impacts factors in distribution of this mosquitoes (13).
**Aedes aegypti** and *Ae. albopictus* are vectors of important diseases such as DF, yellow fever and Chikungunya (14-16). *Aedes aegypti* is a mainly urban vector and is feeding exclusively from human (17). *Aedes albopictus* are mostly found in suburban and rural environments and are feeding of the different species of mammals, including humans, as well as the different species of birds (18).

Currently, vector control is the best method to control of the DF (19). In Iran, studies regarding the biodiversity and distribution of *Aedes* are limited. However, there is no information on *Aedes* mosquito’s diversity components in north-western of Iran. The aim of this study was to investigate the diversity of *Aedes* genus present in the natural areas, as well as the differences on the faunistic composition of *Aedes* species in function of the climatic and ecological features of Ardabil Province borderline.

Various *Aedes* mosquito species are considered as potential vectors of Zika virus including *Ae. africanus, Ae. albopictus, Ae. polynesiensis, Ae. unilineatus, Ae. vitates, Ae. apicoargentus, Ae. leuteocephalus, Ae. aegypti, Ae. vitattus, Ae. furcifer, and Ae. hensilli*.

**Materials and Methods**

**Study area**

This cross-sectional study was carried out from Apr to Oct 2016 in north-western of Iran. Ardabil Province is located in northwestern Iran 37.45° to 39.42° N and 47.30° to 48.55° E. The province has an area of 17 953km². This province is bordered to the north with the Republic of Azerbaijan and along the border is 282.5km (Fig. 1). In 159km from the border, flowing Aras and Balha rivers. During the border, Iran linked to the Republic of Azerbaijan for two areas Bilehsavar and Aslanduz. Ardabil Province in the longitudinal axis of the expansion (1°35’) and high extent to the north-south latitudes (2°31’) have a large variety of climates. About 2/3 textured mountainous with large variation in height and the rest are flat areas and posts. North province (Mugan plain) with low altitude has relatively warm weather and central and southern regions have a cool mountain climate (20-22).

**Sample collection**

Overall, 33 areas of 10 cities of priority border areas were selected randomly. During the seasonal activity, the larvae were collected in each month 2 times. Using a ladle handling, and the standard ladle of 350mL was collected from natural and artificial larval habitats. In each habitat, sampling was collected from different parts and the ladle was made 10 times on each side. In the case of well water used from the bucket and the limited larval habitats such as cavity trees were used from droppers. The larval stages III and were stored in lactophenol solution and after about a week and transparent larvae, using Berlese’s Fluid were prepared microscopic slides and identified morphologically using appropriate identification keys.

**Results**

Overall, 2000 mosquito larvae were collected, from which only *Aedes* larvae were selected and identified. From 33 areas, six (18%) were positive for the presence of *Aedes* larvae. In four counties, (40%) *Aedes* larvae were collected. Totally, 694 larvae were collected from four counties that 7.2% were *Aedes* larvae. Three species of *Aedes* genus were identified which included *Ae. caspius, Ae. vexans,* and *Ae. flavescens.* These species were reported from thee Ardabil Province for the first time. All three species were collected from 78–2114 meter above sea level altitude. *Aedes* larvae were collected from two different climate zones. The first zone: the northern part of the province where the climate is hot and humid and low altitude (60–78km)
that includes Pars Abad and Bilehsavar and the second zone: southern part of the province with mountainous climate and high altitude (2114-2110m), which includes the Khalkhal and Sareyn (Table 1).

In the first zone the larvae were collected in Jun and Sep months but in the second zone in Jul and Aug. All three species Aedes more in border areas The maximum Aedes larvae from three species were collected in border-line of Iran and Azerbaijan but in other areas in this study, only two species for low abundance were collected (Table 2). The survey of larvae habitat characteristics showed that most of larvae were collected (66%) in areas without trees and shrubs and sunny. Totally 80% of larvae habitats were temporary that including holes and marshes around rivers and craters were caused by the overflow waters and 86% were natural habitat. About 50% of the larvae collected in the afternoon and at cooler temperatures and 33% at mid-day and 17% at AM. The water of larval habitat more was mainly stagnant and 50% transparent and type of bed habitat was 80% clay and 20% sand.

Table 1. Total larvae collected from Ardabil Province, north-western of Iran, 2016

| location    | Village | Total larvae | Aedes larvae | Genus | Species | Elevation | Y       | X       |
|-------------|---------|--------------|--------------|-------|---------|-----------|---------|---------|
| Sareyn      | Alvars  | 40           | 1            | Aedes | caspius | 2110m     | 38.14985| 47.96122|
| Bilehsavar  | Jafarabad | 68         | 10           | Aedes | caspius | 176m     | 39.50238| 48.04068|
|             |         |              | 2            | Aedes | flavescens |         |         |         |
| Khalkhal    | khangahe | 213         | 1            | Aedes | caspius | 2114m    | 37.53637| 48.5755 |
|             |         |              | 1            | Aedes | flavescens |         |         |         |
| Parsabad    | Oltan   | 251          | 3            | Aedes | caspius | 74m      | 39.60545| 47.76123|
|             | Mahmoudab | 70        | 10           | Aedes | caspius | 87m      | 39.54975| 47.97872|
|             |         |              | 3            | Aedes | flavescens |         |         |         |
|             |         |              | 3            | Aedes | vexans |          |         |         |
| Normohamadkandi | 52    | 10           | 1            | Aedes | caspius | 165m     | 39.4721 | 47.49537|
|             |         |              | 5            | Aedes | flavescens |         |         |         |
|             |         |              | 1            | Aedes | vexans |          |         |         |
| Total       |         | 694          | 50           |       |         |           |         |         |

Table 2. Larval abundance of Aedes in Ardabil Province, north-western of Iran, 2016

| Species     | Aedes caspius | Aedes flavescens | Aedes vexans | Total | % |
|-------------|---------------|------------------|--------------|-------|---|
| Kowsar      | 0             | 0                | 0            | 0     | 0 |
| Bilehsavar  | 10            | 2                | 0            | 12    | 24|
| Parsabad    | 23            | 8                | 4            | 35    | 70|
| Germi       | 0             | 0                | 0            | 0     | 0 |
| Khalkhal    | 1             | 1                | 0            | 2     | 4 |
| Nir         | 0             | 0                | 0            | 0     | 0 |
| Namin       | 0             | 0                | 0            | 0     | 0 |
| Meshkinshahr| 0             | 0                | 0            | 0     | 0 |
| Sareyn      | 1             | 0                | 0            | 1     | 2 |
| Ardabil     | 0             | 0                | 0            | 0     | 0 |
Discussion

In this study three species of Aedes larvae (Ae. caspius, Ae. vexans and Ae. flavescens) identified from Ardabil Province, North-western of Iran that one species (Ae. flavescens) was reported for first time. Aedes larvae were dispersed in all regions of Ardabil Province but the frequency of Aedes larvae were maximum in north regions of this province that borderline of Iran and Azerbaijan. Only two adult species was reported (Ae. caspius and Ae. vexans) (22). Aedes caspius is distributed in Palearctic areas (23) and in Iran reported from more than 16 provinces such as Gilan, West Azerbaijan, Khorasan, Zanjan, Lorestan, Isfahan, Yazd, Kerman, Hormozgan, Bushire and Khozestan (24). This species very more frequently collected from six regions (Parsabad, Aslanduz, Bilehsavar, Khalkhal and Sar-eyn) in this study that two areas (Aslanduz and Bilehsavar) were located in borderline of Iran and Azerbaijan and both are customhouse. This species is also collected with extensive distribution from Gilan and Ardabil Province (25-26). Moreover, Ae. caspius reported from Bushire (27), Eastern of Iran (28), Chaharmahal and Bakhtiari (29), East Azerbaijan (30) and Qom Province (31). In 2016, Ae. caspius along with Ae. albopictus and Aedes unilineatus reported from Sistan and Baluchestan (32). Aedes caspius more feeding on mammals and humans (9, 33) and can be transmitted Rift Valley fever, dirofilariasis and tularemia (34).

Aedes vexans is distributed in Holoarctic and Oriental areas (35). This species and Ae. aegypti and Culex quinquefasciatus have the most distribution in world (33). In Iran also reported from Gillan, West Azerbaijan, Mazandaran, Bushire and Hormozgan (23). In this study Ae. vexans larvae were collected 2 times in Jun and Sep from Parsabad and Aslanduz. The number of this species were lower in comparison to other Aedes species but in Gillan Province the most larvae collected were Ae. vexans (25). The results of this study showed
that the larvae of *Ae. vexans* were collected of less than 200m altitude that this result matched to another study (22) and the adults of this species collected from east Azerbaijan in high altitude (30). The feeding preference of this species is on the mammals then birds and reptiles (32). *Ae. vexans* can transmitted various diseases such as eastern and western equine encephalitis, Japanese encephalitis and California encephalitis.

*Aedes flavescens* was reported first time in Ardabil Province and 11 larvae of *Ae. flavescens* collected from three parts of this area (Parsabad, Bilehsavar and Khalkhal). Totally 100% of larvace habitats were temporary and most whit vegetation. Total of these larvae were collected at afternoon. Zaim et al. for first time reported *Ae. flavescens* from West Azerbaijan in 1987 and one time from large pool whit vegetation (24). These results showed that *Ae. flavescens* were dispersed in north-west of Iran.

**Conclusion**

*Aedes* species were a high density in borderline of Iran and Azerbaijan. 40% of Ardabil Province was found *Aedes* mosquitoes. So the north parts of Ardabil Province are a suitable habitat for *Aedes* species mosquitoes. Therefore, more studies need to be done in these areas.

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**References**

1. Shepard DS, Undurraga EA, Betancourt-Charriotto M, Guzmán MG, Halstead SB, Harris E, Mudin RN, Murray KO, Tapia-Conyer R, Gubler DJ (2014) Approaches to refining estimates of global burden and economics of dengue. PLoS Negl Trop Dis. 8(11): e3306.
2. Michael B, Deen J, Buchy P, Gubler D, Harris E, Hombach J (2009) World Health Organization dengue guidelines for diagnosis, treatment, prevention, and control new edition. WHO, Geneva, Switzerland.
3. Wan SW, Lin CF, Wang S, Chen YH, Yeh TM, Liu HS, Anderson R, Lin YS (2013) Current progress in dengue vaccines. J Biomed Sci. 20(1): 37–42.
4. Woodall JP, Yuill TM (2016) Why is the yellow fever outbreak in Angola a ‘threat to the entire world’? Int J Infect Dis. 48: 96–97.
5. World Health Organization (2013) Global strategy for dengue prevention and control 2012–2020. WHO, Geneva.
6. Amarasinghe A, Letson GW (2012) Dengue in the Middle East: A neglected, emerging disease of importance. Trans R Soc Trop Med Hyg. 106(1): 1–2.
7. Rasheed SB, Butlin RK, Boots M (2013) A review of dengue as an emerging disease in Pakistan. Public Health. 127(1): 11–17.
8. Li Y, Kamara F, Zhou G, Puthiyakunnun S, Li C, Liu Y, Zhou Y, Yao L, Yan G, Chen XG (2014) Urbanization increases *Aedes albopictus* larval habitats and accelerates mosquito development and survivorship. PLoS Negl Trop Dis. 8(11): e3301.
9. Machado-Machado EA (2012) Empirical mapping of suitability to dengue fever in Mexico using species distribution modeling. Appl Geogr. 33: 82–93.
10. Vazquez-Prokopec GM, Kitron U, Montgomery B, Horne P, Ritchie SA (2010) Quantifying the spatial dimension of dengue virus epidemic spread within a trop-
ical urban environment. PLoS Negl Trop Dis. 4(12): e920.
11. Brady OJ, Gething PW, Bhatt S, Messina JP, Brownstein JS, Hoen AG, Moyes CL, Farlow AW, Scott TW, Hay SI (2012) Refining the global spatial limits of dengue virus transmission by evidence-based consensus. PLoS Negl Trop Dis. 6(8): e1760.
12. Bennett JE, Dolin R, Blaser MJ (2014) Principles and practice of infectious diseases. Elsevier Health Sciences. Elsevier publication, USA.
13. Lounibos LP (2002) Invasions by insect vectors of human disease. Annu Rev Entomol. 47(1): 233–266.
14. Hay SI, Battle KE, Pigott DM, Smith DL, Moyes CL, Bhatt S, Brownstein JS, Collier N, Myers MF, George DB, Gething PW (2013) Global mapping of infectious disease. Phil Trans R Soc B. 368(1614): 20120250.
15. Juliano SA, Philip Lounibos L (2005) Ecology of invasive mosquitoes: Effects on resident species and on human health. Ecol Lett. 8(5): 558–574.
16. Morrison AC, Zielinski-Gutierrez E, Scott TW, Rosenberg R (2008) Defining challenges and proposing solutions for control of the virus vector Aedes aegypti. PLoS Med. 5(3): e68.
17. Powell JR, Tabachnick WJ (2013) History of domestication and spread of Aedes aegypti-A Review. Memorias do Instituto Oswaldo Cruz. 108: 11–17.
18. Delatte H, Dehecq JS, Thiria J, Domerg C, Paupy C, Fontenille D (2008) Geographic distribution and developmental sites of Aedes albopictus (Diptera: Culicidae) during a Chikungunya epidemic event. Vector Borne Zoonotic Dis. 8(1): 25–34.
19. Halloran ME, Longini IM (2014) Emerging, evolving, and established infectious diseases and interventions. Science. 345 (6202): 1292–1294.
20. Azari-hamidian S, Yaghoobi-ershadi MR, Javadan 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.
21. Moradi-Asl E, Rassi Y, Hanafi-Bojd AA, Vatandoost H, Saghaforou A, Adham D, Aabasgolizadeh N, Omidi Oskouei A, Sadeghi H (2018) The Relationship between Climatic Factors and the Prevalence of Visceral Leishmaniasis in North West of Iran. Int J Pediatr. 6(2): 7169–7178.
22. Moradi-Asl E, Hanafi-Bojd AA, Rassi Y, Vatandoost H, Mohhebal M, Yaghoobi-ershadi MR, Habibzadeh S, Hazrati S, Rafizadeh S (2017) Situational Analysis of Visceral Leishmaniasis in the Most Important Endemic Area of the Disease in Iran. J Arthropod Borne Dis. 11(4): 482–497.
23. Zaim M, Cranston PS (1986) Checklist and keys to the Culicinae of Iran (Diptera: Culicidae). Mosq Syst. 18(18): 233–245.
24. Zaim M (1987) The distribution and larval habitat characteristics of Iranian Culicidae. J Am Mosq Control Assoc. 3(4): 568–573.
25. Azari-Hamidian S (2007) Checklist of Iranian mosquitoes (Diptera: Culicidae). J Vector Ecol. 32(2): 235–242.
26. Moradi-Asl E, Hazrati S, Vatandoost H, Emdadi D, Ghorbani E, Ghaemian A, Rafiee M, Panahi A, Shokri A (2018) Fauna and Larval Habitat Characteristics of Mosquitoes (Diptera: Culicidae) in Ardabil Province, Northwestern Iran. J Health. 9(3): 259–266.
27. Dow RP (1953) Notes on Iranian mosquitoes. Am J Trop Med Hyg. 2(4): 683–695.
28. Jelínek J (1981) Results of the Czechoslovak-Iranian entomological expeditions to Iran 1970 and 1973. Coleoptera: Ni-
tidulidae. Acta Entomol Mus Natl. 40: 105–119.

29. Omrani SM, Azari-Hamidian S, Pour-Shahbazi G, Taghipour S (2015) Fauna and the distribution of mosquitoes (Diptera: Culicidae) in Chahar Mahal and Bakhtiari Province, 2011–2012. J Shahrekord Univ Med Sci. 16(6): 127–138.

30. Abai MR, Azari-Hamidian S, Ladonni H, Hakimi M, Mashhadi-Esmail K, Sheikhzadeh K, Kousha A, Vatandoost H (2007) Fauna and checklist of mosquitoes (Diptera: Culicidae) of East Azerbaijan Province, northwestern Iran. J Arthropod Borne Dis. 1(2): 27–33.

31. Saghafipour A, Abai MR, Farzinnia B, Nafar R, Ladonni H, Azari-Hamidian S (2012) Mosquito (Diptera: Culicidae) fauna of Qom Province, Iran. J Arthropod Borne Dis. 6(1): 54–61.

32. Clements AN (1999) Sensory, reception and behavior. Biol Mosq. 2: 740.

33. Lane RP, Crosskey RW (2012) Medical Insects and Arachnids. Springer Science and Business Media. Springer Publication, UK.

34. Gad AM, Farid HA, Ramzy RR, Riad MB, Presley SM, Cope SE, Hassan MM, Hassan AN (1999) Host feeding of mosquitoes (Diptera: Culicidae) associated with the recurrence of Rift Valley fever in Egypt. J Med Entomol. 36(6): 709–714.

35. Knight KL, Stone A (1977) A Catalogue of the Mosquitoes of the World (Diptera: Culicidae). 2nd edition. Entornological Society of America. The Thomas Say Foundation, USA.