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Original Article

Mosquito Surveillance and the First Record of the Invasive Mosquito Species *Aedes (Stegomyia) albopictus* (Skuse) (Diptera: Culicidae) in Southern Iran

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Abstract

Background: Epidemics of mosquito-borne viral infections such as dengue, chikungunya, West Nile and Rift Valley fevers in neighbouring countries and risk of introduction of exotic vectors into Iran have placed this country at a significant risk for these mosquito-borne diseases.

Methods: After the first dengue case reported in Iran in 2008, active entomological surveillance of *Aedes albopictus* (Skuse) and *Ae. aegypti* (Linnaeus) were conducted in May/Jun, Sep, and Oct/Nov, 2008-2014. Based on occurrence of dengue cases and the presence of potential entry sides including ports and boarder gates, 121 sites in eight provinces were monitored for mosquito vectors. Larval collections were carried out using droppers or dippers and adult collections with CDC light traps, human landing catches, aspirator and Pyrethrum spray space catches.

Results: A total of 8,186 larvae and 3,734 adult mosquitoes were collected belonging to 23 Culicinae species, including 13 of the genus *Culex*, 1 *Culiseta*, 1 *Uranotaenia*, and 8 of the genus *Aedes*. Five *Aedes albopictus* larvae were identified from the Sistan & Baluchestan province bordering Pakistan in 2009. In 2013, seven *Ae. albopictus* adult mosquitoes were also collected in a coastal locality near the city of Chabahar in the same province.

Conclusion: The detection of larvae and adults of this species in different parts of this province reveal its probable establishment in southeast Iran, which has implications for public health and requires active entomological surveillance as well as the implementation of vector control to prevent the further spread of this critical vector.

Keywords: *Aedes albopictus*, Dengue, Introduction, DNA barcoding, Iran

Introduction

Recent epidemics of mosquito-borne viral infections in countries neighboring Iran i.e. dengue, chikungunya and West Nile infections in Pakistan, dengue and Rift Valley fever in Saudi Arabia, and West Nile infection in Iraq (1, 2, 3) have placed this country at a serious risk for mosquito-borne diseases. Our interconnected world favors the movement of insect vectors of pathogens, particularly some mosquito species such as *Aedes albopictus* (=*Stegomyia albopicta*) (4) and *Ae. aegypti* (=*St. aegypti*) (4) which may facilitate transmission of tropical and subtropical pathogens in new areas. Climate change also may affect the Middle

Eastern region, favoring transmission of mosquito-borne diseases (5).

Sixty one mosquito species, 40 in the subfamily Culicinae and 21 in the subfamily Anophelinae are currently known to be occurring in Iran (School of Public Health, Tehran University of Medical Sciences, unpublished data). Among these species are several potential vectors of pathogens, e.g. *Anopheles stephensi*, *An. culicifacies*, *An. fluviatilis*, *An. superpictus*, *An. sacharovi*, *An. maculipennis s.l.,* and *An. dthali,* the main vectors of malaria parasites (6, 7), *Culex theileri* and *An. maculipennis s.l.*, the principal vectors of dirofilarial worms (8), *Cx. pipiens pipiens*, *Cx. modestus*, *An. maculipennis s.l., Cx. univittatus, Cx. quinquefasciatus*, and *Ae. vexans*, probable vectors of West Nile virus (9, 10), *Cx. pipiens pipiens, Cx. torrentium, Cx. univittatus, Cx. tritaeniorhynchus*, and *Cx. bitaeniorhynchus*, probable vectors of sindbis virus complex and *Ae. vexans* and *Cx. pipiens* complex, vectors of Rift Valley fever virus (11).

The introduction of an exotic mosquito species into a new environment is highly significant. The most notable example is the worldwide expansion of the mosquito *Ae. albopictus* (the Asian tiger mosquito). This mosquito of Asian origin has spread worldwide during the last three decades, now being established in North, Central and South America, Africa, Oceania, southern Europe and the Middle East. *Aedes albopictus* has established in Pakistan, where it has become a serious pest mosquito species in the city of Karachi (3). It has also been recorded from Lebanon, Syria, and Turkey (12-14).

Aedes albopictus is a competent vector of 26 arboviruses (15, 13) including dengue, chikungunya, Japanese encephalitis, and West Nile viruses, and thus is a threat to public health. It can also transmit filarial worms to dogs (16, 17).

Aedes albopictus has adapted to rural, suburban, and urban environments and adult females are particularly aggressive to humans. When biting, females can rapidly fly away when they are disturbed, and then complete their blood meal on another host. The phase of aquatic development varies between 7-20 d depending on temperature and food abundance, and adults prefer to bite outdoors during the day. In temperate regions with high humidity, adult longevity has been recorded to reach 30-40 d. In various laboratory experiments, females lived up to 117 d, which is unrealistic in a natural environment (18, 19).

Therefore, entomological surveillance of *Culicinae* mosquitoes has been performed throughout southern parts of Iran to check whether exotic mosquitoes have been introduced. Here we report the first findings of *Ae. albopictus* in Iran.

Methods

Study area

One hundred and twenty one sites from eight provinces (Sistan & Baluchestan, Hormozgan, Bushehr, Fars, Kerman, Khuzestan, Ilam, and Khorasan-Jonobi) were selected for active entomological surveillance, based on reports of some cases of dengue fever from the province of Sistan & Baluchestan and the risk level for dengue transmission (20), as well as the presence of potential entry sides for exotic mosquitoes including ports and boarder gates (Table 1).

Table 1: Ranges of altitudes and biomes of the studied provinces of Iran, and mean annual precipitation during the study period, 2008-2014

Province	Altitude range (m.a.s.l.)		Biome	Mean annual precipitation (mm)
	Max	Min		
Sistan & Baluchestan	1,394	8	Subtropical desert, warm temperate desert scrub	89.3
Hormozgan	30	2	Subtropical desert	176.1
Bushehr	940	0	Subtropical desert	268.0
Khuzestan	1,457	3	Subtropical desert scrub	209.2
Fars	2,251	410	Warm temperature thorn step	334.7

Kerman	2,660	560	Warm temperature desert scrub	198.0
Ilam	1,404	150	Subtropical desert scrub	583.7
Khorasan-e-Jonobi	1,846	810	Warm temperature desert scrub	168.5

The southern coastal line of Iran, including the Persian Gulf and Oman Sea, is approximately 1,770 km long and the most significant river is Karun, in the southwest. The eastern part is arid with less than 200 mm of rainfall and consists of desert areas. The southern coastal plains of Iran have mild winters, but very hot and extremely humid summer days. The temperature can exceed 49 °C during July in the interior part of southern Iran. The total annual precipitation ranges from 90-600 mm and the relative humidity from 30% to 90% in the studied provinces.

Mosquito collection

Field collections were conducted three times (May/Jun, Sep, Oct/Nov) during 2008-2014, coincident with mosquito activity in the provinces of Sistan & Baluchestan, Hormzgan, Bushehr, Kerman, Khuzestan and Korasan-e-Jonobi and 1 or 2 times only in the other provinces (Fig. 1).

Fig. 1: Map showing the study areas in Iran, 2008-2014 http://www.freeworldmaps.net/asia/iran/map.html

Ten one-floor houses and 35 sampling stations were utilised for the installation of S-Ovt (Sentinel-ovitrap) in Chabahar and Sarbaz counties, province of Sistan & Baluchestan (from second half of May until end of Aug 2013). Ovitraps were made of a black conical plastic cup (height 15 cm, diameter at base 14 cm, diameter at top 18 cm). One wooden paddle (5×15 cm) was attached with a paper clip to the inner wall of each cup to serve as oviposition substrate (21). A grass infusion of *Orayza sative* or *Cynodon dactylon* in concentration of 10% and 30% was used to attract female mosquitoes. The grass stock infusion was prepared by adding 40 g of *Cynodon dactylon* to 5 L and 125 g of *Orayza sative* to 15 L of tap water and stagnated for seven days (22, 23). The ovitraps were checked twice a week for the presence of eggs and larvae of mosquitoes. The collected paddles were labelled and put to dry at room temperature for 25 h. The paddles were examined under a stereo-microscopy and the eggs were transferred to the insectary for rearing.

Larval collections were performed using dippers or droppers in artificial and natural breeding sites. Several adult collections were also carried out using CDC miniature light traps and human landing catches conducted outdoors (in gardens, stables and animal shelters) under bed nets baited with carbon dioxide cylinder and with one or more sides tacked up about 30 cm, from 14 to 20 p.m. (during the period of maximum biting activity for *Aedes* species, with two local men hired as baits). Three standard CDC light traps baited with carbon dioxide cylinders were suspended in different sites including animal shelters, trees near breeding sites in the middle of the village. Traps were operated from sunset to sunrise. Animal baits included a calf, a young goat or a camel placed near traps from 14 to 20 p.m. All mosquito collections were carried out two consecutive nights during the field activity.

Several mosquito collections were conducted with aspirators during the day. Adult mosquitoes were also collected indoors with aspirators and pyrethrum spray space catches. Additionally, water-holding containers were also checked on ships for breeding sites by dippers or droppers, which arrived from Pakistan, Bangladesh, China and

India at the ports cities of Chabahar, Bandar-e-Mahshahr, Bushehr and Bandar Abbas. The ships were selected randomly. Larvae were preserved in Lactophenol and adult mosquitoes collected from different parts of the ships were pinned in the field and kept in collection boxes. A collection form was used to record all pertinent data for larval and adult collections in the field. All specimens were transferred to the laboratory of Medical Entomology at the School of Public Health, Tehran University of Medical Sciences, and microscopic slides of the preserved larvae were prepared using Liquid de Faur's medium (24) manufactured at our laboratory.

Morphological identification

Morphological identification of larvae, females and some male mosquitoes were carried out using the keys of Becker et al. (25) and the interactive CD of Schaffner et al. (26) and Zaim, M (10). Specimens tentatively identified as *Ae. albopictus* including fourth stage larvae and adults were sent to the National Centre for Vector Entomology, Institute of Parasitology, at the University of Zurich, Switzerland, for morphological and molecular confirmation. Some specimens of *Ae. albopictus* were deposited in the museum of Medical Entomology, Department of Medical Entomology & Vector Control, Tehran University of Medical Sciences (TUMS).

Genetic identification

Part (460 bp) of the mitochondrial cytochrome oxidase subunit I gene (mt COI) was amplified using primers C1-J-1718_mod (5-GWGGRTTTGGWAAYTGAYTAG-3) and C1-N-2191_mod (5-GTAAAATTAAAATATAAACTTC-TGG-3) at a concentration of 1 µM each. Amplifications were performed in 50µl volumes containing master mix of the multiplex PCR kit with the following reaction conditions: Initial HotStarTaq DNA polymerase activation at 95 ºC for 15 min, and 40 cycles at 95 ºC for 30 sec, annealing at 50 ºC for 30 sec and extension at 72 ºC for 60 sec. A final elongation step at 72 ºC for 10 min was included. Sequencing of the purified amplicons (minelute PCR kit, Qiagen) was done by a private company (Synergene, Switzerland).

Results

A total of 437 potential aquatic habitats were checked in 8 provinces of which 271 (69.1%) were positive for mosquito larvae, and 8,186 larvae and 3,734 adult mosquitoes were collected belonging to 23 *Culicinae* species, including 13 of the genus *Culex*, 1 *Culiseta,* 1 *Uranotaenia*, and 8 *Aedes* (Table 2).

Table 2: Number of adult, larvae and species of Culicinae collected in the study area (2008-2014)

Province	Species	No. of specimen		No. Species	Collection date
		larvae	Adult		
Sistan & Baluchestan	*Cx. perexiguus*	121	0	17	Mar, July, 2008
	Cx. pipiens	55	96		Mar, Jun, 2009
	Cx. theileri	146	0		Apr, May, Nov, Feb, 2013
	Cx. mimeticus	75	0		Apr, May, Jun, Sep, 2014
	Cx. sinaiticus	12	4		
	Cx. laticinctus	0	29		
	Cx. sitiens	438	0		
	Cx. tritaeniorhynchus	311	7		
	Cx. quinquefasciatus	1145	0		
	Cx. bitaeniorhynchus	18	0		
	Ur. unguiculata	0	0		
	Cs. longiareolata	159	3		
	Ae. caspius	113	199		
	Ae. caballus	1	0		
	Ae. flavescens	2	0		
	Ae. vexans	2	1465		
	Ae. albopictus	5	6		
Hor-mozgan	*Cx. arbieeni*	2	0		Sep, 2008
	Cx. perexiguus	4	0		Jan, Mar, Sep- Nov, 2009
	Cx. pipiens	235	19		Jany, Mar-Jun,
	Cx. theileri	155	0		Aug-Nov, 2010
	Cx. mimeticus	81	0		Jan, 2012

	Cx. sinaiticus	52	3	14	Jul-Sep, Nov, 2013 Jul, Oct-Nov, 2014
	Cx. laticinctus	0	2		
	Cx. tritaeniorhynchus	142	9		
	Cx. quinquefasciatus	135	0		
	Ur. unguiculata	0	8		
	Cs. longiareolata	648	0		
	Ae. caballus	1	23		
	Ae. vexans	26	3		
	Ae. vittatus	0	4		
Bushehr	*Cx. pusillus*	63	0	16	Aug, 2008 Feb, 2013 Mar-Jun, Aug-Sep, Nov, 2014
	Cx. hortensis	1	0		
	Cx. perexiguus	51	0		
	Cx. pipiens	522	34		
	Cx. theileri	16	0		
	Cx. mimeticus	22	0		
	Cx. sinaiticus	1	0		
	Cx. laticinctus	2	0		
	Cx. sitiens	56	0		
	Cx. tritaeniorhynchus	5	0		
	Cx. quinquefasciatus	80	0		
	Ur. unguiculata	8	0		
	Cs. longiareolata	62	0		
	Ae. caspius	165	227		
	Ae. detritus	0	18		
	Ae. vexans	7	2		
Khuzestan	*Cx. pusillus*	28	0	9	Feb, 2013 May, July-Sep, 2014
	Cx. perexiguus	1	0		
	Cx. pipiens	187	0		
	Cx. theileri	9	0		
	Cx. sitiens	6	0		
	Cx. tritaeniorhynchus	157	0		
	Cx. quinquefasciatus	58	0		
	Ur. unguiculata	6	0		
	Ae. caspius	101	1347		
Khorasan-e-Jonobi	*Cx. hortensis*	40	1	10	July,Oct, 2008 Jun-July, 2009 May-Jun, Aug, 2014
	Cx. impudicus	0	3		
	Cx. arbieeni	0	4		
	Cx. perexiguus	2	0		
	Cx. pipiens	1	2		
	Cx. theileri	27	0		
	Cx. mimeticus	1	0		
	Cx. tritaeniorhynchus	2	0		
	Cs. longiareolata	825	59		
	Ae. vexans	0	2		
Kerman	*Cx. perexiguus*	56	0	9	July, 2008 and 2009
	Cx. mimeticus	19	0		
	Cx. laticinctus	4	0		
	Cx. sitiens	71	0		
	Cx. tritaeniorhynchus	203	0		
	Cx. quinquefasciatus	23	0		
	Cx. bitaeniorhynchus	35	0		
	Cs. longiareolata	45	0		
	Ae. caspius	56	0		
Fars	*Cx. pusillus*	1	0	8	Oct, 2008
	Cx. perexiguus	180	0		
	Cx. pipiens	19	0		
	Cx. theileri	64	0		
	Cx. mimeticus	105	0		
	Cx. tritaeniorhynchus	9	0		
	Cx. quinquefasciatus	60	0		
	Cx. bitaeniorhynchus	42	0		
Ilam	*Cx. hortensis*	0	1	12	July, 2009
	Cx. impudicus	0	4		
	Cx. arbieeni	0	4		
	Cx. perexiguus	5	18		
	Cx. pipiens	39	39		
	Cx. theileri	21	26		
	Cx. sinaiticus	0	17		
	Cx. laticinctus	0	32		
	Cx. tritaeniorhynchus	0	3		

	Cx. quinquefasciatus	509	0	
	Cx. bitaeniorhynchus	0	1	
	Cs. longiareolata	24	10	
Total		8186	3734	11920

Five *Ae. albopictus* larva were detected among the specimens from the counties of Nikshahr and arbaz (Fig. 2) situated on the southeast of Sistan & Baluchestan Province, close to the international border of Pakistan in 2009 (Table 3).
COI sequence (>462 bp) of one specimen was 100% identical to *Ae. albopictus* GenBank and BOLD entries. This sequence is deposited on Gen Bank under the accession number KU351083. Two *Ae. albopictus* larvae were collected in a small and shallow ditch with sandy bottom and a lot of plants and three of them in two plastic bottles and earthenware.

Table 3: Collections of *Aedes albopictus* in Sistan & Baluchestan province, southeast Iran (2009-2014)

County	Year	Village	Specimens	Coordinates (WGS84) N	Coordinates (WGS84) E	Collection date	Collection method	Habitat
Nikshahr	2009	Lashar	2 larvae	27.36001^{0}	62.31015^{0}	8.7.2009	Dipper	A small and shallow ditch with sandy bottom and a lot of plants
Sarbaz	2009	Rask	3 larvae	26.63002^{0}	61.25007^{0}	17.7.2009	Dropper	Plastic bottle and earthenware
Chabahar	2013	Voshname-dorri	4♀♀	25.41010^{0}	60.75012^{0}	29.11.2013	Human bait	Close to a solid dam under the shade of a tree
		Paroomi	2♀♀, 1♂	25.45011^{0}	60.93016^{0}	3.12.2013	Human bait	Close to a solid dam under the shade of a tree

Ae. albopictus larvae were found together with those of other mosquito species, including *Cx. perexiguus*, *Cx. quinquefasciatus*, *Cx. sinaiticus*, *Cx. theileri*, *Cx. tritaeniorhynchus*, and *Culiseta longiareolata*. COI sequences (>462 bp) of 3 specimens (2 females, 1 male) were >99.7% identical to *Ae. albopictus* GenBank (e.g. KC690940) and BOLD (e.g. NIBGE MOS-01832) entries. These sequences are deposited on GenBank under the accession numbers KU351080, KU351082, KU351081. The other two specimens were deposited at the museum of the Department of Medical Entomology (TUMS).
During Nov/Dec 2013, seven *Ae. albopictus* adult mosquitoes were collected, including 4 females from the village of Voshname-doori, 2 females and 1 male from Paroomi Village, at about 25 km from the shoreline of Chabahar (Table 3, Fig. 2). All were collected using human bait under bed nets between 12-19 p.m. in the shade and identified morphologically.
Attempts to trap further material using active ovitrap surveillance in 45 sampling stations (10 indoors and 35 outdoors) were unsuccessful in the other counties of Sistan & Baluchestan. Three species of *Culicinae* including considerable numbers of *Ae. vexans,* and few numbers of *Ae. detritus* and *Ae. caspius* were collected along with *Ae. albopictus* with human bait and CO_2. All these 3 species were also collected by CDC light traps baited with CO_2 in villages around the port city of Chabahar.

Discussion

An entomological survey was implemented in southern of Iran from 2008 to 2014, in order to assess the presence and the distribution of the invasive mosquito species *Ae. albopictus*. Its presence was detected for the first time in 2009 in Nikshahr and Sarbaz counties, Sistan & Baluchestan province. The area has an Indian climate, hot, humid in summer, and moderate in winter. There are several long valleys and rivers with a large amount of water. Heavy rain and hail sometimes cause the overflowing of rivers, flood and damage in the surrounding areas.

The counties of Sarbaz and Nikshahr have 121 Km joint border with Baluchestan of Pakistan and *Ae. albopictus* has also been caught from Kohat-Hangu valley in the northwest of this country. The occurrence of this species in the village, with a mean annual precipitation of 450 mm is of interest since over most of its range it is associated with very heavy rainfall (27).

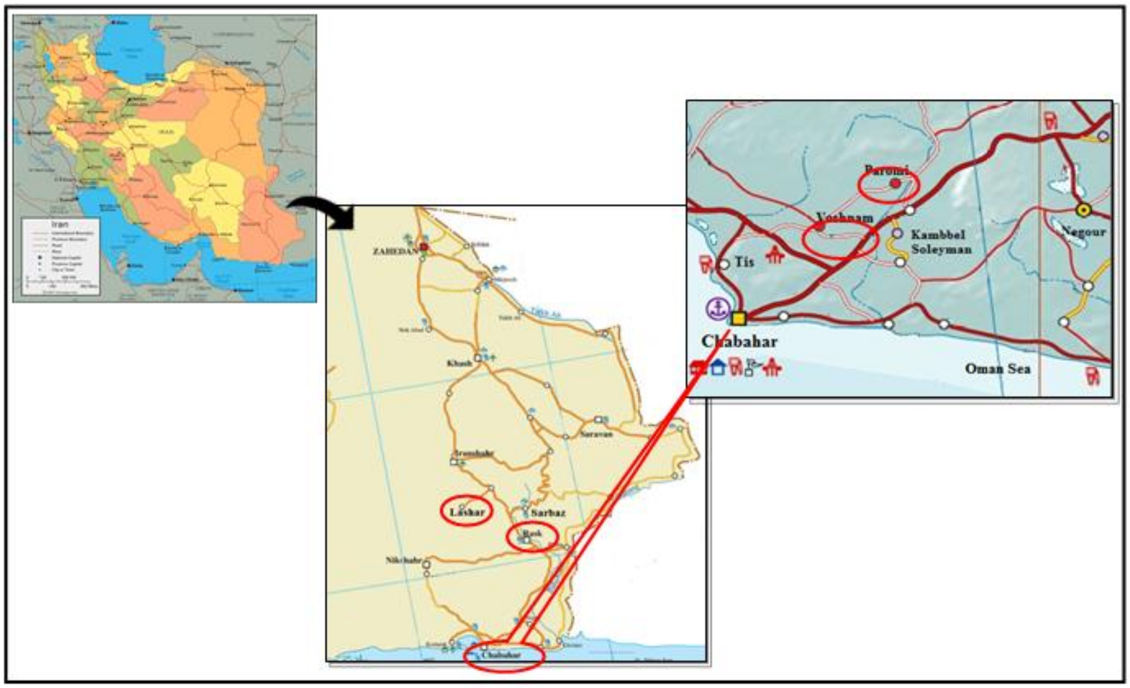

Fig. 2: Localities of collection of *Ae. albopictus* in Sistan & Baluchestan province, 2009 and 2013

Through Nov/Dec 2013, adults of this species were collected in the same province at villages near Chabahar, southeastern of Iran, which lies in the Gulf of Oman (Fig. 2). *Ae. albopictus* species identification was performed both morphologically and molecularly (i.e. sequencing of mt-CO1 gene) on 2 females, 1 male and 1 larva. This species was found near the Oman Sea and Indian Ocean and at a distance of 360 miles from Karachi, Pakistan. By using ovitraps no specimen of *Ae. albopictus* was collected in the area mean that population is at low level.

The discovery of *Ae. albopictus* in south and southeast of Iran is great concern like in other areas of the world. Iran is at risk for the introduction of the species via the ornamental plant Lucky bamboo (*Dracaena sanderiana*) since 2006 and the onset of imports from China and Thailand where *Ae. albopictus* is indigenous. It could also be imported with used tires from China via Dubai into Chabahar of Iran in the southeast. Moreover, Iran is bordered by Pakistan infested with *Ae. albopictus* (28). There is a high possibility the species frequently introduced in southeastern Iran by ships arriving from Karachi.

Dengue fever is endemic in Pakistan with a seasonal rise in cases since 1994, and at least 14 outbreaks have been reported to date. Of these, nine outbreaks have been documented in Baluchestan province (20) which shares 800 km border with Iran. Dengue fever and *Ae. aegypti* is also prevalent in Saudi Arabia (29). Thus, the virus can be introduced into Iran by pilgrims (about 1,500,000 people per year) and the mosquitoes by airplane. This species is the main vector of dengue but was not collected in the present study. Finally, movements of Iranian tourists to China, Thailand, India and Malaysia in summer raise the risk of introduction of dengue or chikungunya viruses when returning. Both *Ae. aegypti* and *Ae. albopictus* (the main vectors of dengue virus) are prevalent in these countries (20, 30, 31).

Furthermore, passive introduction of *Ae. albopictus* in Iran could occur from vehicles traveling from Turkey, Bulgaria and Syria. Following the discovery of *Ae. albopictus* in the south of the country, different training workshops were launched to increase the knowledge about dengue, biology and ecology of the mosquito vector for medical entomologists and physicians from different health centers of the high risk provinces during 2013-2014. In addition, establishment of a network of entomological surveillance is in progress in the health centers of all 31 provinces of Iran especially in the high-risk areas.

One study (32) proposed that *Ae. albopictus* could survive in regions where the annual mean temperature is higher than 11 °C and the mean temperature of the coldest month is not lower than -2 °C, therefore, active surveillance must be implemented not only in Sistan & Baluchestan province but also in other regions of the country including urban areas, the port city of Chabahar, Konarak, and the shore lines of Bandar Abbas and Qeshm island. Areas where mosquito control is implemented against *Anopheles stephensi* and Culicinae mosquitoes by *Bacillus thuringiensis* H-14 toxin (local production) in larval breeding sites should also be monitored. These risks should urge Iranian policymakers to support continuous and regular vector surveillance for *Ae. albopictus* all over the country especially in houses where patients with febrile (dengue or measles) conditions are located. For monitoring the presence of *Ae. albopictus*, sampling of man-made containers for immature stages, capture of adult females using human bait, BG-Sentinel traps and ovitraps should be performed in the study areas and other provinces in the southern part of the country.

Our confirmation of the occurrence of *Ae. albopictus* in Sistan & Baluchestan province, coupled with the detection of dengue virus in some patients, indicate the need for active detection and application of control measures or elimination against this species in Iran. Once *Ae. albopictus* is established in an area, it is difficult to eradicate, and constant surveillance and appropriate control strategies are required.

Conclusion

The present study provides evidence of introduction of *Ae. albopictus* into southeastern Iran. Current data suggest the density of this invasive vector being at low level but there is no obstacle for the species population to grow and spread in near future. It is important to further search for adults and immature stages of *Ae. albopictus* in the coming years throughout the southern provinces of the country and if specimen are found to immediately implement adapted control measures.

Ethical considerations

Ethical issues (including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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References

1. Afzal MF, Naqvi SQ, Sultan MA, Hanif A (2015). Chikungunya fever among children presenting with nonspecific febrile illness during an epidemic of dengue fever in Lahore, Pakistan. *Merit Res J Med Sci*, 3(3): 069-073.
2. Rasheed SB, Butlin RK, Boots M (2013). A review of dengue as an emerging disease in Pakistan. *Public Health*, 127(1): 11-17.
3. Ali-Khan HA, Akram W, Shehzad K, Shaalan EA (2011). First report of filed evolved resistance to agrochemical in dengue mosquito, *Aedes albopictus* (Diptera: Culicidae), from Pakistan. *Parasit Vectors*, 4: 146.
4. Wilkerson RC, Lintom YM, Fonseca DM, Schultz TR, Price DC, Strickam DA (2015). Making mosquito taxonomy useful: a stable classification of tribe Aedini that balances utility with current knowledge of evolutionary relationships. *PLoS One,* 10(7): e0133602.
5. Githeko AK, Lindsay SW, Confalonieri UE, Patz JA (2000). Climate change and vector-borne diseases: a regional analysis. *Bull World Health Organ*, 78(9): 1136-47.
6. Shahgudian ER (1960). A key to the anophelines of Iran. *Acta Med Iran*, 3: 38-48.
7. Yaghoobi-Ershadi MR, Manouchehri AV (1986). Malaria and Hormozgan province. *J School Med*, 3-4: 69-79 (in Persian).
8. 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 north-western Iran, with the first finding of filarial larvae in naturally infected local mosquitoes. *Med Vet Entomol*, 23(2): 111-21.
9. Saidi S, Tesh R, Javadian E, Nadim A (1976). The prevalence of human infection with West Nile virus in Iran. *Iran J Publ Health*, 5(1): 8-13.
10. Zaim M (1987). The distribution and larval habitat characteristics of Iranian Culicinae. *J Am Mosq Control Assoc*, 3(4):568-73.
11. Mweya CN, Kimera SL, Kija JB, Mboera LE (2013). Predicting distribution of *Aedes aegypti* and *Culex pipiens* complex, potential vectors of Rift Vally Fever virus in relation to diseases epidemics in East Aferica. *Infect Ecol Epidemiol*., 3. doi: 10.3402/iee.v3i0.21748.
12. Hadad N, Harbach RE, Chamat S, Bouharoun-Tayoun H (2007). Presence of *Aedes albopictus* in Lebanon and Syria. *J Am Mosq Control Assoc.*, 23(2): 226–228.
13. Bonizzoni M, Gasperi G, Chen X, James AA (2013). The invasive mosquito species *Aedes albopictus*: current knowledge and future perspectives. *Trends Parasitol*, 29(9): 460-468.
14. World Health Organization (2009). *Dengue guidelines for diagnosis, treatment, prevention and control*, pp. 3-21.
15. Paupy C, Delatte H, Bagny L, Corbel V, Fontenille D (2009). *Aedes albopictus*, an arbovirus vector: from darkness to the light. *Microbes In-fect*, 11(14-15):1177-85.
16. Cancrini G, Romi R, Gabrielli S, Toma L, Di Paolo M, Scaramozzino P (2003). First finding of *Dirofilaria repens* in a natural population of *Aedes albopictus*. *Med Vet Entomol*, 17(4):448-51.
17. Gratz NG (2004). Critical review of the vectors status of *Aedes albopictus*. *Med Vet Entomol*, 18(3):215-27.
18. Hawley WA (1988). The biology of *Aedes albopictus*. *J Am Mosq Control Assoc Suppl,* 1: 1-39.
19. Estrada-Franco JG, George B, Craig JR (1995). Biology, disease relationships and control of *Aedes albopictus*, Technical Paper No. 42. Pan American Health Organization, Washington D.C, pp. 61.
20. Chinikar S, Ghiasi SM, Shah-Hosseini N, Mostafavi E, Moradi Khakifirouza SM, et al. (2013). Preliminary study of dengue viruses' infection in Iran. *Travel Med Infect Dis*, 11(3):166-9.
21. Barbosa RMR, Regis L, Vasconcelos R, Leal WS (2010). *Culex* Mosquitoes (Diptera: Culicidae) Egg Laying in Traps Loaded with *Bacillus thuringiensis* variety *israelensis* and Baited with Skatole. *J Med Entomol*, 47(3):345-8.
22. Polson KA, Curtis Ch, Seng CM, Olson JG, Chantha N, Rawlins C (2002). The Use of Ovitraps Baited with Hay Infusion as a Surveillance Tool for *Aedes aegypti* Mosquitoes in Cambodia. *Dengue Bull*, 26: 178-184.
23. Sant'ana A, Roque RA, Eirasa AE (2006). Characteristics of Grass Infusions as Oviposition Attractants to *Aedes (Stegomyia)* (Diptera: Culicidae). *J Med Entomol*, 43(2):214-20.
24. Smart J, Jordan K, Whittick RJ (1965). Insects of medical importance. 4th ed. British Museum, Natural History, Oxford, Adlen Press, pp: 286-8.

25. Becker N, Petric D, Zgomba M, Boase C, Madon M, Dahl C, Kaiser A (2010). *Mosquitoes and their control*. 2nd Ed, Springer, pp. 577.
26. Schaffner F, Angel G, Geoffroy B, Hervy JP, Rhaiem A, Brunhes J (2001). The mosquitoes of Europe/ Les moustiques d'Europe. Logiciel d'identification et d'enseignement (CD-Rom), Montpellier, France, IRD Editions & EID Méditerranée.
27. Qutubuddin M (1960). The mosquito fauna of Kohat-Hangu valley, West Pakistan. *Mosquito News*, 20(4): 355-361.
28. Akram W, Alikhan HA, Hafeez F, Bilal H, Kim, YK, Lee JJ (2010). Potential of citrus seed extracts against dengue fever mosquitoes, *Aedes albopictus* (Skuse) (Culicidae: Diptera). *Pak J Bot*, 42(4): 3343-3348.
29. Alikhan M, Ghamdi K Al, Mahyoub, JA (2014). *Aedes* mosquito species in Western Saudi Arabia. *J Insect Sci*, 14: 69.
30. Strickman D, Kittayapong P (2003). Dengue and its vectors in Thailand: Calculated transmission risk from total pupal counts of *Aedes aegypti* and association of wing-length measurements with aspects of the larval habitat. *Am J Trop Med Hyg*, 68(2): 209–217.
31. Chinikar S, Ghiasi S, Moradi M, Madihi SR (2010). Laboratory detection facility Dengue Fevers (DF) in Iran: the first imported cases. *Internet J Infect Dis*, 8: 1-3.
32. Kobayashi M, Nihei N, Kurihara T (2002). Analysis of northern distribution of *Aedes albopictus* (Diptera: Culicidae) in Japan by geographical information system. *J Med Entomol*, 39(1): 4-11.