Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage:www.elsevier.com/locate/apjtd

Document heading doi:10.1016/S2222-1808(11)60030-3

Anopheline mosquitoes and their role for malaria transmission in an endemic area, southern Iran

Mehravaran Ahmad, Vatandoost Hassan*, Oshaghi Mohamamd Ali, Abai Mohammad Reza

Department of Medical Entomology and Vector Control, School of Public Health and National Institute of Health Research, Tehran University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Article history:
Received 15 April 2011
Received in revised form 10 May 2011
Accepted 28 June 2011
Available online 28 September 2011

Keywords:
Fauna
Mosquito
Anopheles
Iran
Malaria transmission
Endemic area
Malaria

ABSTRACT

Objective: To find the fauna of *Anopheles* mosquitoes for control implementation in Jiroft, a southern city of Iran with subtropical climate and seasonal malaria transmission. **Methods:** *Anopheles* specimens from the various mountainous and plain villages in Jiroft area were collected during malaria transmission from 2006 to 2007. Different methods of collection including hand catch, night biting catch using animal and human bait, total catch and shelter pit and larval collection were performed using WHO methods. **Results:** In total, 365 larvae and 871 adult mosquitoes were colleted and identified using morphological characters and DNA sequence data. Faunistic investigation showed that *Anopheles* species including *Anopheles culicifacies*, *Anopheles stephensi*, *Anopheles superpictus* (*An. superpictus*), *Anopheles fluviatilis*, *Anopheles sergenti* (*An. sergenti*), *Anopheles pulchrrimus* (*An. pulchrrimus*), *Anopheles turkhodi* and *Anopheles dthali* were found in the study area. *An. sergenti*, *An. pulcherrimus* and *An. superpictus* were reported for the first time in Jiroft. **Conclusions:** It can be concluded that several malaria species can be found in this region. In addition to the fauna, further investigation is required on the ecology and bionomics of *Anopheline* mosquitoes for the implementation of vector control as well as malariological survey in the region.

1. Introduction

Malaria is still a public health problem in the southern part of Iran. According to the report of Ministry of Health and Medical Education of Iran, there has been a sharp decline of malaria trend since 15 years ago. Altogether a total of 33 *Anopheles*, including siblings, biological forms and genotypes were recorded, among which the 7 (including 17 siblings, biological forms and genotypes) have been implicated as the main vector. In the southern part of the country, there are six *Anopheles* mosquitoes including *Anopheles stephensi* (*An. stephensi*)[1-4], *Anopheles culicifacies* (*An. culicifacies*)[5], *Anopheles dthali* (*An. dthali*), *Anopheles fluviatilis* (*An. fluviatilis*)[6-10], *Anopheles superpictus* (*An. superpictus*) and *Anopheles pulcherrimus* (*An. pulcherrimus*)[11-14]. *Anopheles sachacrovi* (*An. sachacrovi*)[15,16] and *Anopheles maculipennis* (*An. maculipennis*)[17,18] are considered as malaria vectors in the northern part of the country.

Malaria transmission in Jiroft district occurs throughout the year. There is some old study about the malaria and the *Anopheline* mosquito fauna in Jiroft District[19]. The objective of this study was to find the appropriate information about mosquito fauna for planning of vector control implementation in the study area.

2. Materials and methods

2.1. Study area

Jiroft district is situated in the southern Kerman province. The district is located between 28° 10′- 29° 20′ latitudes and 56° 31′- 58° 45′ longitudes (Figure 1). Jiroft has plains and mountainous areas. The maximum and minimum air temperatures were 48 ℃ and 3 ℃, respectively. The average annual relative humidify was 47% and the mean annual rainfall was 135 mm. This district covers an area of 18 438 km^2 and have a population of almost 208 874. Agriculture and husbandry are the main occupations of people.

*Corresponding author: Dr. Vatandoost H, Department of Medical Entomology and Vector Control School of Public Health and National Institute of Health Research, Tehran University of Medical Sciences, Tehran, Iran, P.O. Box: 14155-6446.
Tel: 0098-21-88951393
Fax: 0098-21-88951393
E-mail: hvatandoost1@yahoo.com; vatando@tums.ac.ir

Foundation Project: Supported by a grant from Tehran University of Medical Sciences (grant No. 5439-27-01-8).

Figure 1. Map of the study area situated in Kerman province, southern part of Iran.

2.2. Mosquito collections

The study was taken for a period of 12 months from September 2006 to September 2007. This district is divided into two ecological parts, plain and mountainous. From each section two villages were chosen for collecting mosquitoes. These plain villages include Daryache, Dobane, and Sephid Baz, Dalphard in the mountain. To study the mosquito fauna, mosquito collection was made twice a season (8 occasions) using the methods suggested by WHO[20]. Adult mosquitoes were collected by night biting catch on human and cow, hand catch (manual aspirators), total catch (spray sheet collections), shelter pit trap and dipping method for collecting larva. In total catch method, indoor places were randomly selected from each village. Indoor places include 4 human and 4 animal shelters that located in different parts of the village. Hand catch collection was performed from outdoor resting places such as natural shelters, hollows and 3 pit shelters. The adult mosquitoes were identified using key identification[21].

2.3. Biological forms of An. stephensi

Some blood-fed females of the *An. stephensi* were allowed to lay eggs on wet paper in the laboratory to identify the species based on egg patterns[22].

2.4. Sibling species of An. fluviatilis

To identify species of *An. fluviatilis*, molecular identifications were carried out on adult mosquitoes based on sequences of the second internal transcribed spacer (ITS2) and D3 of ribosomal DNA. PCR reaction mixes and thermocycler parameters were described previously by Mehravaran *et al*[23].

3. Results

In this study, 365 larvae and 871 adult *Anopheles* were colleted from both plain and mountainous areas. The numbers of adult mosquitoes acquired at the plain region were 716 and 155 from the mountainous region. Faunistic investigation showed that *Anopheles* species including *An. culicifacies*, *An. stephensi*, *An. superpictus*, *An. fluviatilis*, *Anopheles sergenti* (*An. sergenti*), *An. pulcherrimus*, *Anopheles turkhodi* (*An. turkhodi*) and *An. dthali* were found in the study area. In plain region, *An. stephensi*, *An. dthali* were collected by hand catch method, *An. stephensi*, *An. culicifacies*, *An. dthali*, *An. sergenti*, *An. turkhodi* by total catch method, *An. stephensi*, *An. fluviatilis*, *An. dthali* by shelter pit, *An. stephensi*, *An. fluviatilis*, *An. sergenti*, *An.culicifacies*. *An. pulcherrimus*, *An. dthali* by night biting. In mountainous region, *An. stephensi*, *An. dthali* were found by hand catch method, *An. stephensi*, *An. dthali*, *An. turkhodi*, *An. culicifacies*, *An. fluviatilis*, *An. sergenti*, *An. superpictus* by total catch method, and *An. stephensi*, *An. dthali*, *An. turkhodi*, *An. fluviatilis*, *An. superpictus* by night biting method. This study showed that *An. sergenti*, *An. superpictus* and *An. pulcherrimus* were recorded for the first time in Jiroft district. Egg morphological characteristics of *An. stephensi* specimens showed that only mysorensis biological form existed in the area study. Sequence variation of ITS2-rDNA and D3-rDNA fragments of *An. fluviatilis* were analyzed using standard PCR-direct sequencing assay. Totally one haplotype was seen between populations in 514 bp of ITS2 gene and 316 bp of D3. Phylogenetic analysis of the sequences of this study in combination with other available sequence data of *An. fluviatilis* complex in GenBank with accession number GQ857439-49 for ITS2 and GQ864403-13 for D3 showed that all of Jiroft specimens are sister taxa of *An. fluviatilis* T of the complex. Totally 365 larva were collected among which six genera were identified including: *An. stephensi*, *An. culicifacies*, *An. superpictus*, *An. pulcherrimus*, *An. dthali* and *An. sergenti*.

4. Discussion

In Iran, malaria is a main health problem in the south and south-east provinces. Jiroft district is one of the endemic malaria regions in Kerman province, southeast of Iran. It was shown in the study that the *Anopheline* fauna does not appear to have changed much over several decades[24]. Eight genera of *Anopheline* mosquitoes were found from Jiroft district including the first record of *An. pulcherrimus*, *An. superpictus* and *An. sergenti* in this study area. Investigation showed that six genera including *An. stephensi*, *An. culicifacies*, *An. dthali*, *An. turkhodi*, *Anopheles multicolor* (*An. multicolor*) and *An. fluviatilis* were existed in Jiroft[19], whereas in this study species of *An. multicolor* was not found. The present study demonstrates that *An. stephensi* is the predominant species in plain and mountainous region. The result is similar to the previous studies carried out by Manouchehri *et al*[25-27]. In this area, it has a peak activity on animals in the second quarter of the night and on human in the first half of the night and again at 3-4 am. It is assumed that *An. stephensi* plays an important role in malaria transmission and *An. culicifacies*, *An. dthali* and *An. fluviatilis* can be considered as secondary vectors. *An. stephensi* has three egg phenotypes: mysornsis, intermediate and type form. All of them are recorded in Iran, mysorensis form[28], as well as all three forms[29]. The results of egg morphological characteristics of *An. stephensi* specimens showed that only mysorensis biological form existed in this area. Investigation previously showed an occurrence of *An. fluviatilis* species T in Iran[6]. It was shown in the study that all of Jiroft specimens are *An. fluviatilis* T of the complex, but new study in Chabahar county, Sistan and Baluchestan province indicates that U species is exist in Iran[23]. Totally 365 *Anopheline* larvae were colleted and identified including 6 species as follows: *An. culicifacies*, *An. stephensi*, *An. superpictus*, *An. pulcherrimus*, *An. dthali*, *An. sergenti*. All larvae were collected from natural habitats such as streams, drying river beds, seepages, swamps, grassland and pools.

In conclusion, we can conclude that several malaria

species can be found in this region. In addition, further investigation is required on the ecology and bionomics of *Anopheline* mosquitoes for implementation of vector control as well as malariological survey in the region.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors thank Mr. Gruhi and Mr. Azad Avar Jiroft Health Center, Jiroft University of Medical Sciences and Ms Mashayekhi, Kerman Health Center, Kerman University of Medical Sciences for their cooperation. This study was financially supported by the Tehran University of Medical Sciences (Grant No. 5439-27-01-86).

References

[1] Abai MR, Mehravaran A, Vatandoost H, Oshaghi MA, Javadian E, Mashayekhi M, et al. Comparative performance of imagicides on *Anopheles stephensi*, main malaria vector in a malarious area, southern Iran. *J Vector Borne Dis* 2008; **45**(4): 307-312.

[2] Davarai B, Vatandoost H, Ladonni H, Shaeghi M, Oshaghi MA, Basseri HR, et al. Comparative efficacy of different imagicides against different strains of *Anopheles stephensi* in the malarious areas of Iran, 2004-2005. *Pak J Biol Sci* 2006; **9**: 885-892.

[3] Vatandoost H, Oshaghi M, Abaie MR, Shahi M, Yaghoobi F, Baghai M, et al. Bionomics of *Anopheles stephensi* Liston in the malarious area of Hormozgan province, southern Iran. *Acta Trop* 2006b; **97**: 196-205.

[4] Davari B, Vatandoost H, Oshaghi MA, Ladonni H, Enayati AA, Shaeghi M, et al. Selection of *Anopheles stephensi* with DDT and dieldrin and cross-resistance spectrum to pyrethroids and fipronil. *Pestic Biochem Physiol* 2007; **89**(2): 97-103.

[5] Emami SN, Vatandoost H, Oshaghi MA, Mohtarami F, Javadian E, Raeisi A. Morphological method for sexing anopheline larvae. *J Vector Borne Dis* 2007; **44**(4): 245-249.

[6] Naddaf SR, Oshaghi MA, Vatandoost H, Asmar M. Molecular characterization of the *Anopheles fluviatilis* species complex in the Islamic Republic of Iran. *East Mediterr Health J* 2003; **9**(3): 257-265.

[7] Hanafi-Bojd AA, Vatandoost H, Jafari R. Susceptibility status of *Anopheles dthali* and *Anopheles fluviatilis* to commonly used larvicides in an endemic focus of malaria, southern Iran. *J Vector Borne Dis* 2006; **43**(1): 34-38.

[8] Dezfouli SR, Oshaghi MA, Vatandoost H, Assmar M. rDNA-ITS2 based species-diagnostic polymerase chain reaction assay for identification of sibling species of *Anopheles fluviatilis* in Iran. *Southeast Asian J Trop Med Public Health* 2003; **34**: 56-60.

[9] Vatandoost H, Moinvaziri VM. Larvicidal activity of a neem tree extract (Neemarin) against mosquito larvae in the Islamic Republic of Iran. *East Mediterr Health J* 2004; **10**: 573-581.

[10] Vatandoost H, Shahi H, Abai MR, Hanafi-Bojd AA, Oshaghi MA, Zamani G. Larval habitats of main malaria vectors in Hormozgan province and their susceptibility to different larvicides. *Southeast Asian J Trop Med Public Health* 2004; **35**: 22-25.

[11] Oshaghi MA, Shemshad Kh, Yaghobi-Ershadi MR, Pedram M, Vatandoost H, Abaie MR, et al. Genetic structure of the malaria vector *Anopheles superpictus* in Iran using mitochondrial cytochrome oxidase (COI and COII) and morphologic markers: a new species complex? *Acta Trop* 2007; **101**(3): 241-248.

[12] Oshaghi MA, Chavshin AR, Vatandoost H. Analysis of mosquito bloodmeals using RFLP markers. *Exp Parasitol* 2006; **114**(4): 259-264.

[13] Vatandoost H, Mashayekhi M, Abaie MR, Aflatoonian MR, Hanafi-Bojd AA, Sharifi I. Monitoring of insecticides resistance in main malaria vectors in a malarious area of Kahnooj district, Kerman province, southeastern Iran. *J Vector Borne Dis* 2005a; **42**: 100-108.

[14] Oshaghi MA, Chavshin AR, Vatandoost H, Yaaghoobi F, Mohtarami F, Noorjah N. Effects of postingestion and physical conditions on PCR amplification of host blood meal DNA in mosquitoes. *Exp Parasitol* 2006a; **112**: 232-236.

[15] Sedaghat MM, Linton YM, Nicolescu G, Smith L, Koliopoulos G, Zounos AK, et al. Morphological and molecular characterization of *Anopheles* (*Anopheles*) *sacharovi* Favre, a primary vector of malaria in the Middle East. *Syst Entomol* 2003a; **28**: 241-256.

[16] Doosti S, Azari-Hamidian S, Vatandoost H, Oshaghi MA, Hosseini M. Taxonomic differentiation of *Anopheles sacharovi* and *An. maculipennis* s.l. (Diptera: Culicidae) larvae by seta 2 (antepalmate hair). *Acta Med Iran* 2006; **44**(1): 21-27.

[17] Oshaghi MA, Sedaghat MM, Vatandoost H. Molecular characterization of the *Anopheles maculipennis* complex in the Islamic Republic of Iran. *East Mediterr Health J* 2003; **9**(4): 659-666.

[18] Sedaghat MM, Linton YM, Oshaghi MA, Vatandoost H, Harbach RE. The *Anopheles maculipennis* complex (Diptera: Culicidae) in Iran: molecular characterizations and recognition of a new species. *Bull Entomol Res* 2003b; **93**: 527-535.

[19] Institute of Parasitology and Malariology. *The plan of action of malaria eradication in Iran*. Teheran: Institute of Parasitology and Malariology; 1961, p. 706.

[20] World Health Organization. *Manual on practical entomology in malaria. Part ll. Methods and techniques*. Geneva: WHO; 1975.

[21] Shahgudian ER. A key to the anophelines of Iran. *Acta Med Iran* 1960; **3**(3): 38-48.

[22] Oshaghi MA, Yaaghoobi F, Vatandoost H, Abaei MR, Akbarzadeh K. *Anopheles stephensi* biological forms: geographical distribution and Malaria transmission in malarious regions of Iran. *Pak J Biol Sci* 2006; **9**(2): 294-298.

[23] Mehravaran A, Oshaghi MA, Vatandoost H, Abai MR, Ebrahim Zadeh A, Roodi AM, et al. First report on *Anopheles fluviatilis* U in southeastern Iran. *Acta Trop* 2010; **117**(2): 76-81.

[24] Manouchehri AV, Dganbaksh B, Eshghy N. The biting cycle of *Anopheles dthali*, *An. fluviatilis* and *An. stephensi* in southern Iran. *Trop Geogr Med* 1976a; **28**(3): 224-227.

[25] Manouchehri AV, Javadian E, Eshghy N, Motabor M. Ecology --of *Anopheles stephensi* Liston in southern Iran. *Trop Geogr Med* 1976b; **28**(3): 228-232.

[26] Nisar Ahmad, Hina Fazal, Bilal H Abbasi, Mazhar Iqbal. *In vitro* larvicidal potential against *Anopheles stephensi* and antioxidative enzyme activities of Ginkgo biloba, Stevia rebaudiana and *Parthenium hysterophorous*. *Asian Pac J Trop Med* 2011; **4**(3): 169-175.

[27] Marimuthu Govindarajan. Larvicidal and repellent properties of some essential oils against *Culex tritaeniorhynchus* Giles and *Anopheles subpictus* Grassi (Diptera: Culicidae). *Asian Pac J Trop Med* 2011; **4**(2): 106-111.

[26] Sahabi Z, Amirkhanian JD, Shahgvdian ER. The polytene chromosomal pattern of *Anopheles stephensi* mysorensis of Kazeroon, Iran. *Iran J Public Health* 1973; **2**: 194-199.

[27] Oshaghi MA, Yaaghoobi F, Abaie MR. Pattern of mitochondrial DNA variation between and within *Anopheles stephensi* (Diptera: Culicidae) biological forms suggests extensive gene flow. *Acta Trop* 2006; **99**: 226-233.