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ORIGINAL ARTICLE

Entomological survey of malaria vectors in Dashtestan County, South of Iran

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Abstract Anopheline mosquitoes are responsible for transmission of some diseases such as malaria. This study was carried out in five villages of Dashtestan county, Bushehr province, south of Iran with mountainous and plain areas. *Anopheles* larvae were sampled once a month from May to July 2021 by dipping method using standard dippers. Adults were captured by the total catch technique. In this study, 1062 *Anopheles* mosquitoes were collected including 850 adults and 212 larvae. Samples were *A. superpictus, A. stephensi, A. dthali* and *A. fluviatilis*. The dominant species at all sites (larvae and adults) were *A. dthali* (31.35%), *A. superpictus* (28.93%), *A. stephensi* (27.77%), and *A. fluviatilis* (11.95%), respectively. Among adults, *A. stephensi* was the most frequent species, but among larvae, *A. dthali* was the dominant species. The highest number of *A. dthali* was captured from Dalaki village with 35%, while *A. superpictus* and *A. fluviatilis* were not caught at this station. The minimum collected adults of all species occurred in Bashirabad. This result showed that *A. stephensi* plays an important role in south Iran, which is under the elimination phase. The fauna and niches of *Anopheles* has different patterns depending on ecological, climatic, and topographic features. These items affect host preferences, feeding behaviors, and distribution of these species. A monthly or annual entomological survey is necessary in regions with mobile populations since imported malaria is a problematic issue in the elimination programs of Iran.

Keywords *Anopheles* · Culicidae · Total catch · Iran

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Introduction

Malaria disease is a significant and occasionally fatal effected by a plasmodium that commonly infects a certain type of anopheles mosquito which feeds on humans. Most of these patients were due to *Plasmodium vivax*, predominantly in Afghanistan and Pakistan and some countries were under the elimination phase (WHO 2020). In Iran, malaria disease is transmitted by *Plasmodium falciparum* and *P. vivax* as *P. vivax* is the major protozoa in endemic areas (Moemenbellah-Fard et al. 2012).

According to the ecology and egg morphology, *A. stephensi* belongs to three variants: *mysoriensis*, typical, and intermediate (Oshaghi et al. 2006) These *Anopheles* are the main species involved in circulating malaria in Sistan-Baluchistan, Kerman, and Hormozgan provinces (Alipour et al. 2013). Other species such as *A. culicifacies*, *A. fluviatilis, A. superpictus, A. maculipennis, A. dthali*, and *A. sacharovi* were incriminated as malaria vectors in different parts of Iran. (Edrisian 2006).

In general, mosquitoes in Iran consist of seven genera, 64 species, and three subspecies (Tahghighi 2019). From them, 24 species belong to the subfamily Anophelinae (Azari-Hamidian 2007). *Anopheles* have also a role in the transmission of filarial worm *Wuchereria bancrofti* and *Brugia malayi* in the tropical parts of the world. For example, *A. funestus* is the important vector of *W. bancrofti* in Africa (Bockarie et al. 2008). Moreover, 51 viruses have been recorded in *Anopheles* throughout the world. It sounds that

only the *o'nyong-nyong* arbovirus (arthropod-borne virus) (ONNV) is transmitted to vertebrates by *Anopheles* mosquitoes (Minkeu et al. 2018).

The strategy of the national malaria program shifted from control to elimination in 2010 (Khosravani et al. 2017). In this step, indoor residual spraying (IRS) and long-lasting insecticide-treated nets (LLINs) with proper insecticide classes were two vital interventions (Zandian et al. 2019). On the other hand, many studies have shown that anopheline mosquitoes were resistant or tolerant to some sensitivity levels against certain insecticides by some mechanisms such as increased metabolic detoxification of pesticides and declined sensitivity of the target proteins or genes (Liu 2015). Female *A. stephensi* adults, for instance, are resistant to pyrethroid and carbamate insecticides and larvae are resistant to chlorpyriphos in south Iran (Abbasi et al. 2019).

However, imported malaria is a persistent challenge for health care management (Nodez et al. 2018; Ghahremani et al. 2019). This study aimed to survey the fauna of anopheline mosquitoes in Dashtestan County, south of Iran. Our study provides useful information about the diversity of *Anopheles* species distribution at sampling stations to combat malaria in the elimination phase.

Materials and methods

Study area

This descriptive cross-sectional study was performed in five villages of Dashtestan County. This area lies east of Bushehr province with an area of 6327 square kilometers. According to the latest census in 2016, the population of Dashtestan was 252,047. Dates are the most important agricultural crop of this region and play an important role in the economy of this county and the whole province. Samplings were performed from the five villages of Rud-e-Faryab (29° 15′ 14″ N 51° 28′ 36″ E), Sarqanat (29° 27′ 54″ N 51° 15′ 54″ E), Dalaki (29° 25′ 46″ N 51° 17′ 35″ E), Zirrah (29° 24′ 51″ N 51° 09′ 01″ E), and Bashirabad (29° 24′ 14″ N 51° 11′ 24″ E). The Rud-e-Faryab village is located in a mountainous area, while other places are on the plains (Fig. 1). The temperature of these five villages is approximately 35, 38, and 40 °C in May, June, and July, respectively. The relative humidity is more than 45% for all of them.

Sample collection

Samplings were carried out once a month from May to July 2019 at the larval habitats and internal places on the four sides of each village. Larvae were sampled by immersion using standard dippers (350 ml). The average number of larvae/10 dippers was calculated. Sampling in each breeding place was done randomly for 15–30 min. depending on the size of the larval places. The collected samples were stored in containers filled with lactophenol and then transferred to an insectarium. To identify the fourth instar larvae of Anophelinae, they were removed from lactophenol and mounted onto Canada-Balsam medium. The samples were identified using valid taxonomic keys (Shahgudian 1960, Zaim et al. 1986, Azari-Hamidian et al. 2009). To catch adult *Anopheles* mosquitoes, the total catch technique was used. After installing a front curtain at the room entry and covering all openings, the floor was covered with a white sheet of cloth. The room ceiling and walls were fully sprayed with pyrethroid insecticides. After 15 min, collected mosquitoes were transferred to the laboratory in small cardboard boxes.

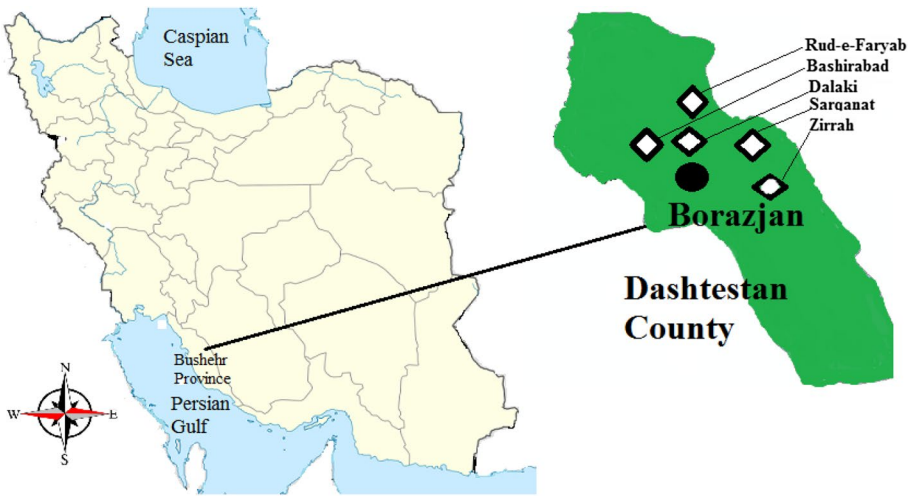

Fig. 1 Map of *Anopheles* sampling site locations, Dashtestan city, Iran

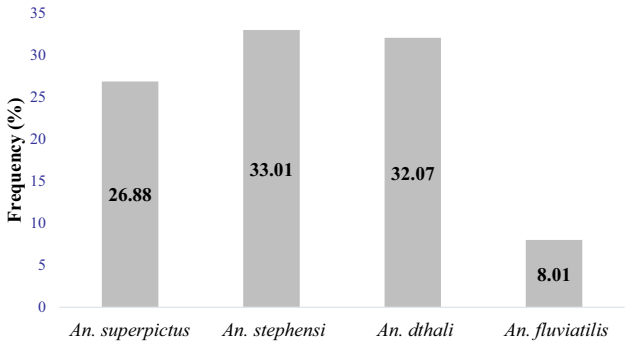

Fig. 2 Adult *Anopheles* captured by total catch in Dashtestan County

Fig. 3 Frequency of *Anopheles* larvae sampled in five villages of Dashtestan County

Results

In this study, 1062 *Anopheles* mosquitoes were collected, including 850 adults and 212 larvae. The four collected species included *Anopheles superpictus*, *Anopheles stephensi*, *Anopheles dthali* and *Anopheles fluviatilis*. The dominant species (both larvae and adults) at all sites were *A. dthali* (31.35%), *A. superpictus* (28.93%), *A. stephensi* (27.77%), and *A. fluviatilis* (11.95%), respectively. *A. stephensi* adults were the most frequent species (33.01%) (Fig. 2), whereas *A. dthali* larvae were the abundant species (31.17%) (Fig. 3). The highest number (#45) of *A. dthali* adults was captured from Dalaki village, while *A. superpictus* and *A. fluviatilis* were not caught at this station (Table 1). The minimal (9%) collected adults of all species occurred in Bashirabad. The least (7.6%) *Anopheles* mosquitoes among all adults belonged to *A. fluviatilis*. This species also had the lowest number of larvae. *A. dthali* larvae had the largest proportion in Rud-e-Faryab and Dalaki village, but *A. superpictus* had a high frequency of the larval population in Sarqanat (80) and Dalaki (37) villages. In Zirrah, *A. stephensi* larvae were collected more than all other species. The total numbers of captured *Anopheles* in May, June, and July were 422, 350, and 290, respectively.

Discussion

The preceding results showed that adult *Anopheles stephensi* was the predominant species among all mosquitoes caught at sampling sites in our study. This was consistent with other previous research (Abbasi et al. 2022). This mosquito species is the most important species of *Anopheles* in the south, and the main vector of malaria in Iran (Azizi et al. 2011). Besides Bushehr province, this species has been recorded from Fars, Hormozgan, Ilam, Kerman, Kermanshah, Khuzestan, Kohgiluye-Buyerahmad, Lorestan, and Sistan-Baluchistan provinces (Hanafi-Bojd et al. 2011; Soltani et al. 2017).

In southern Iran within Bushehr province, *Anopheles* species are active during June-October. *A. dthail* is considered as a secondary malaria vector in mountainous areas of the neighbouring province of Hormozgan. *A. fluviatilis* is also spread on the southern slopes of the Zagros chain, from southwest of Kermanshah to Sistan-Baluchistan zones including Bushehr province with an altitude range between 50 and 1100 m above sea level (Hanafi-Bojd et al. 2012). In the case of *A. stephensi* adults, there is no difference between the densities of this species in the plain or mountainous areas in our view. Moreover, some studies showed that the number of captured *A. stephensi* from upland places was higher than those on plateaus in the spring season in south Iran (Mehravaran et al. 2012).

Table 1 The total numbers of *Anopheles* species by sites in the Dashtestan County

Species	Sampling locations										Total	
	Rud-e-Faryab		Sarqanat		Dalaki		Zirrah		Bashirabad			
	Larva	Adult	Larva	Adult	Larva	Adult	Larva	Adult	Larva	Adult	Larva	Adult
A. superpictus	30	20	80	15	37	0	68	15	35	7	250	57
A. stephensi	20	10	55	20	53	35	72	2	25	3	225	70
A. dthali	60	10	45	15	100	45	40	3	20	5	265	78
A. fluviatilis	50	6	15	1	20	0	5	5	20	5	110	17
	160	46	195	51	210	70	185	25	100	20	850	222

Since *A. stephensi* is an indoor-resting mosquito (Thomas et al. 2017), it was more frequently sampled in the human dwellings than other species in this survey. This species has an endophagic (feeding indoors) activity during March–June, and September–November in the Bashagard district (Hanafi-Bojd et al. 2012). *A. stephensi*, *A. culicifacies* and *A. dthali* in Jask County had two maximum activities in March and November (Yeryan et al. 2016).

The mean ambient temperature was 37.66 °C in the recent study. Temperature and humidity are pivotal metrological factors affecting malaria incidence significantly (Ghanbarnejad et al. 2021). In this project, *Anopheline* larvae were collected from breeding places in both plain and mountainous regions. The difference between the number of larvae and adults caught is due to several reasons, one of which is the time of the catch, since the weather gets hot in the Dashtestan region in July, so more adults were caught. Among other factors is the catching method, since the adult mosquitoes have previously laid eggs, therefore, in breeding places the frequency of larvae was higher than adults. Another implicated parameter was that due to the onset of hot winds in July, the abundance of adult mosquitoes becomes less due to unfavorable environmental conditions. This consequence was also corroborated in the previous research conducted in Lorestan in the west (Amani et al. 2014). Hormozgan in the south (Shahandeh et al. 2010). Kurdistan (Vahabi 2001). and Hamedan (Dehghan et al. 2011) provinces.

Among all collected samples (larvae and adults), *A. dthali* was the most frequent species. *A. dthali* larvae were more frequently sampled in Rud-e-Faryab village than other suburban areas since this village was located at a high altitude. The same outcome has been observed in a study carried out in Minab County (south of Iran) (Soleimani-Ahmadi et al. 2015). *A. dthali, A. culicifacies* and *A. superpictus* are indicated as malaria vectors in Hormozgan province beside *A. stephensi* (Azizi et al. 2011).

A. superpictus larvae were the second most abundant mosquito in this survey. There were no adults of this and *A. fluviatilis* species in Dalaki village. The former species has been identified as a malaria vector in central Asia (Oshaghi et al. 2011). In Iran, this species is a malaria vector and is widely distributed in all plateaus of Iran as well as mountainous slopes of the Alborz range, south of Zagros mountain range, and along the coastal plains of the Caspian Sea and the Persian Gulf (Shemshad et al. 2007).

According to a study carried out in Farsan County, Chaharmahal-Bakhtiari province, *A. superpictus* was a predominant species, so 67.2% of all *Anopheles* population belonged to this mentioned species (Salehi et al. 2000). *A. culicifacies*, and *A. fluviatilis* were found in some parts of Baluchistan and Hormozgan provinces (Basseri et al. 2012; Majnoonpour et al. 2015), southeast of Iran. *A. fluviatilis* had less density in our study. An investigation conducted in Jiroft County demonstrated that this species was more abundant during the late spring and autumn seasons. Furthermore, this *Anopheles* had exophilic behavior (Kamyabi et al. 2002). *A. fluviatilis* (T species) is zoophilic in India but it is more important in the transmission or maintenance of malaria parasites in downhill flood valleys of Iran (Naddaf et al. 2003; Moemenbellah-Fard 2008; Hoosh-Deghati et al. 2017).

Conclusion

The fauna of *Anopheles* had different patterns depending on ecological, climatic, and topographic features. These items affect host preference, feeding behavior, and distribution of these species. A monthly or annual entomological survey is necessary for regions with mobile populations since imported malaria is a problematic issue for the elimination program in Iran. Since Iran is in the elimination phase, having knowledge about the distribution of *Anopheles* and their foci could be beneficial in preventing the occurrence of malaria in new areas. Knowledge about the habitat and ecology of *Anopheles* also helps to plan in time of emergencies in the form of spraying.

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Declarations

Conflict of interest The authors declare that there is no conflict of interest.

Human and animals rights No human or animal research activities are involved.

Informed consent There was no need.

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