Ecology of *Anopheles stephensi* in a Malarious Area, Southeast of Iran

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Abstract- District of Jiroft is situated in south-east of Iran which is one of the malarious regions. *Anopheles stephensi is* considered as one of the main malaria vector in this region. Ecology of this species was studied in the area to understand its vector behavior for implementation of effective vector control measures. Different methods like total catch, pit shelter, night bite collection on human and animal, larval dipping methods were used for species identification, seasonal activity, anthropophilic index and egg morphological characteristics. Anthropophilicity index was assessed by ELISA test. Activity of *Anopheles* species started at the beginning of April, and its peak occurs in late spring. The larvae were found in the river bed with pools, stagnant streams, slow foothill streams, temporary pools, and slowly moving water with and without vegetation, drainage containers of air conditioner and palm irrigation canals. From different methods of adult collection, it was found that spray sheet collection is the appropriate method. ELISA testing of 144 blood meals of females revealed the anthropophilicity of 11.8% indicating host preference on animal, mainly cow. Ridge length and their number on the egg floats confirmed *Anopheles stephensi mysorensis* form. This study showed that *Anopheles stephensi* is the main vector of malaria in the region, although some other species may play a role. Our findings could provide a valuable clue for epidemiology and control of malaria in the southeast of Iran.

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Introduction

Malaria is one of the most important vector-borne diseases in the world, especially in the developing countries. But control of malaria has been encountered with many problems such as insecticide resistance in vectors and drug resistance. Recently, Iran has been classified in to four different strata according to the epidemiology of malaria (1). During past two years, around 15000 malaria cases have been reported. The disease is a major health problem in south-east of Iran. It is unstable with two seasonal peaks mainly in spring and autumn. Outbreaks usually occur after the rainy season. South-eastern of Iran includes the provinces of Sistan-Baluchistan, Hormozgan and the tropical areas of Kerman provinces are characterized by "refractory malaria" (2).

In this part of the country, six anopheline mosquitoes including *Anopheles culicifacies*, *Anopheles stephensi*, *Anopheles dthali*, *Anopheles fluviatilis*, *Anopheles superpictus*, and *Anopheles pulcherrimus* are known to be the malaria vectors (3-21). *Anopheles sacharovi* and *Anopheles maculipennis* are considered as malaria vector in the northern part of the country (22-28). *Anopheles stephensi* is an important vector of human malaria throughout the Middle East and South Asian, including the Indo-Pakistan subcontinent (29) with a westward expansion through Iran and Iraq into the Middle East and Arabian Peninsula (30). This species is considered to be the main malaria vector in the Persian Gulf region (31). Based on morphological characteristics of the egg, namely egg length, egg breadth, and number of ridges on the egg floats, three biological forms have been reported in this species: *Anopheles stephensi*: type form, *mysorensis* and intermediate form. The type form is known to be an efficient vector of urban malaria, whereas the *mysorensis* form is considered to be a rural species with

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poor vectorial capacity due to its highly zoophilic behavior (32).

This region, Jiroft, is a malaria prone area with *Plasmodium vivax* as the dominant species, whereas *Plasmodium falciparum* parasite also exists. Annual parasite incidence (API) was almost 3/1000 in 2010. Around 95% cases are Iranian and remaining are Afghan refugees. About 89% of cases are indigenous imported, introduced and relapse cases are also recorded. Factors involved in malaria transmission in the study area are: 1) Socioeconomic conditions, 2) Dam construction, 3) Immigration from neighboring countries, 4) Urbanization, 5) Irrigation projects, lack of vector control measures, 6) Geographical and climatic conditions.

The species of anophelines in this region are: *Anopheles stephensi, Anopheles dthali, Anopheles fluviatilis, Anopheles superpictus, Anopheles culicifacies, Anopheles turkhudi, Anopheles sergenti,* and *Anopheles pulcherrimus.* The first five species are familiar to the malaria vectors in Iran. The main objectives of this study were to find some aspects of malaria vector for implementation a of any vector control measures.

Materials and Methods

Study area

District of Jiroft is situated at south of Kerman province with the surface area of 18438 km^2. Its coordinates are 28°40′13″N and 57°44′13″E. The region is subtropical the climate is congenial for malaria (Figure l).

Mosquito collection

Entomological evaluations were carried out from those villages in which malaria transmission occurs because of the presence of *Anopheles stephensi* which had been reported previously. Based on these criteria, several localities in each region were selected and from these the following localities were randomly selected for the survey: Dalphard, Sefidbaz and Dehrud from the mountainous region, Daryache, Saghari and Dobone from the plateau. These sites have been the main malarious zones in this district in the last 3 years. Pyrethrum space spray catches were performed seasonally in eight shelters (four human and four animal shelters) located in different parts of the villages in each region by standard methods (33,34), using 0.2% pyrethrum spray. The density was calculated as the number of mosquitoes per shelter, attempts were also made to catch anophelines outdoors using pit shelters, night catch and suction tube. Rural districts of the area have been checked seasonally for their anopheline fauna during the season of their activity and continued for just over 12 months. The larvae were captured using standard dippers (500ml) from breeding places close to adult collection sites. The mean number of larvae was calculated per 10 dippers.

Blood meal identification

For identification of host preference pattern, blood fed mosquitoes were collected by Pyrethrum space spray catches, pit shelter, night catch on human and animal, suction tube inside human dwellings, stables, storerooms and outdoor resting places around villages. The blood meals of the identified anophelines were smeared on circles of Whatman No.1 filter paper. They were interleaved with non-absorbent 'onionskin' paper and sent with the necessary information to the Department of Parasitology in the Pasteur Institute of Iran for enzyme-linked immunosorbent assay (ELISA) testing (35).

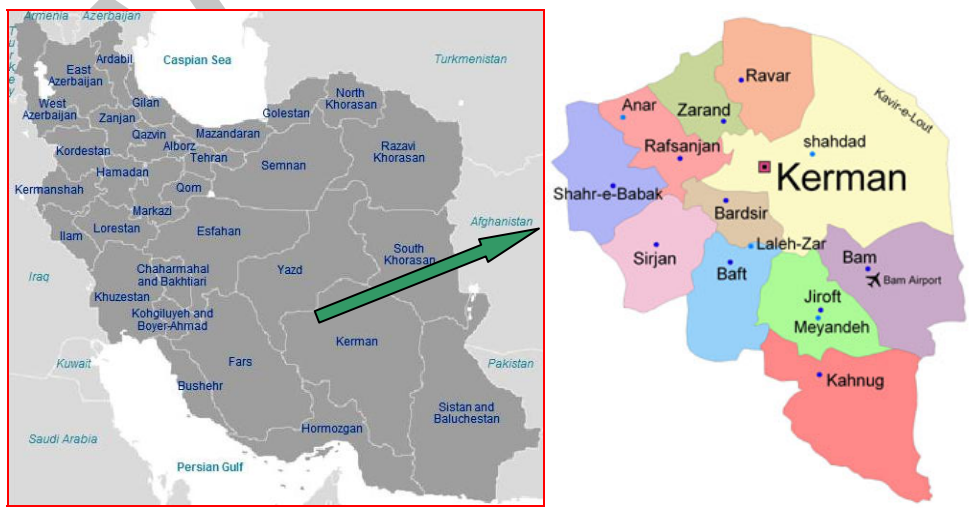

Figure 1. Study area in Kerman province, Iran.

Biological forms of *Anopheles stephensi*

Blood-fed mosquitoes transported alive to separate tubes including wet filter paper and allowed to lay eggs. The egg characteristics of 5-8 eggs from each female, including their length, breadth, and number of ridges on the egg float were measured under compound microscope. Measurements were carried out using an ocular micrometer in a stereoscopic microscope at 15 × power, and the number of ridges along one side of the egg float was counted at the same time. All the procedures were followed as described previously (36,37).

Results

Anopheles stephensi which has a wide distribution in Jiroft, was collected in all rural districts of the study area. It breeds in various man-made water collections, including wells, cisterns, fountains, ornamental ponds and in the water stored in drums and used for building construction. Larvae could also be collected from pools, stream margins, catch basins and seepage canals. Larvae were also found in sewage water, wells, and the small amounts of water in the drainage containers of air conditioners in houses. In rural areas *Anopheles stephensi* was found to breed in pools, stream beds, palm irrigation canals, at the margins of stream and rivers, in seepage and in marshy areas with a gentle flow of water. It was also observed in water with a high salinity. In the mountainous area, *Anopheles stephensi* was found together with *Anopheles culicifacies, Anopheles pulcherrimus, Anopheles sergenti* and in the plain regions it was found with *Anopheles culicifacies, Anopheles pulcherrimus, Anopheles sergenti, Anopheles superpictus* and *Anopheles dthali*. This specie remains reproductively active throughout 9 months in this region. *Anopheles stephensi* activity starts at the beginning of April, reaches a peak in May and then gradually decreases. The density of *Anopheles stephensi* in mountainous areas is higher in the spring than in the autumn. There is low activity during the cold winter and hot summer periods. Night biting collections were made in rural areas since we could use cows for bite collections. However, in rural areas, more than 262 of specimens were collected on animal bite and 13 on human bait. This showed that *Anopheles stephensi* in the rural mountainous areas and in the rural plains is highly zoophilic.

In ELISA testing of 144 blood meals of this species, the proportion giving positive reactions from (+) to (++++) with alkaline phosphates anti-human conjugate varied between collection sites. Of those collected 11.8% had fed on human alone and 59.5% on cow and rest of other animals. The egg morphology was measured as previously described. Based on the egg and ridge length and the number of ridges on the egg floats, only biological forms of *An stephensi*: *mysorensis*, was found in Jiroft district. In mountainous and plain area all of the samples were *mysorensis* form. Ridge number in mosquitoes collected from mountainous and plain regions, ranged from 10 to 14 with a maximum percentage of eggs falling between 11 and 12. This is similar to the ridge counts for the *mysorensis* form.

Discussion

Anopheles stephensi is one of the main malaria vectors in south of Iran. Previous investigations have shown it to be the most prevalent anopheline species in the malarious area of southern Iran. This species is considered to be endophagous and endophilic. *Anopheles stephensi* is the main vector responsible for transmission of malaria to human in Persian Gulf area. Sporozoite rates of samples from the southern parts of Iran were reported to be between 0.2 and 1.8% (2,18).

In this study, both mountainous and plain areas showed only *Anopheles stephensi mysoriensis* and it was highly endophilic. This study have shown that other anopheline species, such as *Anopheles fluviatilis, Anopheles dthali, Anopheles sergenti, Anopheles culicifacies, Anopheles superpictus* and *Anopheles turkhodi* in mountainous area, and *Anopheles culicifacies, Anopheles dthali, Anopheles turkhodi, Anopheles sergenti, Anopheles fluviatilis and Anopheles pulcherrimus* exist in plain areas in Jiroft district. Due to the presence of animals in plain and mountainous areas, the relatively short life span of *Anopheles stephensi mysorensis* and exposure to indoor residual insecticides as well as highly endophilic behavior of *Anopheles stephensi mysorensis,* it is postulated that this species could be considered as the main vector in the plain–mountainous areas of Jiroft. Endophily is an important factor in the poor vectorial capacity of *mysorensis* (32). However, in contrast to published reports from India and the data from Hormozgan, *Anopheles stephensi mysorensis* has been reported to be one of the main malaria vectors in other southern regions of Iran (14) and Afghanistan (38). In most areas *Anopheles stephensi* population with highly frequency has two main seasonal peaks: during the spring and autumn (39). We found the main peak in May, whereas the secondary peak fluctuated from October. In our study the host preference pattern of *Anopheles stephensi* is presented. In ELISA testing of

144 blood meals of this species, the proportion giving positive reactions from (+) to (++++) with alkaline phosphatase anti-human conjugate varied between collection sites. Of those collected (1.8%) had fed on human alone and 59.5% of these were bovid fed. Recently a new method of analyzing of blood meals using RFLP markers has been adopted (14,15). A study was conducted on the comparative performance of imagicides on *Anopheles stephensi,* in this region. Their findings revealed that the main malaria vector species was susceptible to all pyrethroids including deltamethrin, permethrin, cyfluthrin and lambda-cyhalothrin but was tolerant to DDT and dieldrin. The average number of take-offs per min per adult was 2.09±0.13 for DDT, 0.581±0.05 for dieldrin, 1.85±0.08 for permethrin, 1.87±0.21 for lambda-cyhalothrin, 1.53±0.13 for cyfluthrin, and 1.23±0.1 for deltamethrin (Abai *et al*. 2008). In conclusion, the results of current study on the bionomics, seasonal patterns, blood meal identification and egg morphological characteristic of *Anopheles stephensi*, could possibly be used as a guideline for national and regional malaria control programme managers.

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