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Anthropophily of Malaria Vectors in Kahnouj District, South of Kerman, Iran

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Abstract

This study was conducted to investigate hematophagic tendencies of the malaria vector based on a seasonal basis as well as by geographical region in a malaria endemic area in Kahnouj, southern Iran. This study was carried out for 14 months from Apr/ 2002 up to the end of May 2003. Female anophelines were collected from different shelters in hilly and plain regions of Kahnouj district and their blood meal was tested using ELISA test methods. The five vectors that were active in the study area consisted of *An. fluviatilis*, *An. stephensi, An. dthali, An. culicifacies,* and *An. superpictus*. The dominant Anopheline in hilly areas was *An. fluviatilis* sibling species T. This species was active during whole year and reached a peak in December. In contrast the dominant species in flat regions was *An. stephensi* which showed strongly endophilic behavior with two seasonal activity peaks. The anthropophilic index for *An. fluviatilis* and *An. stephensi* was estimated at 2.68% and 0.5%, respectively. The population of other species was too low and they did not show a propensity for human blood. The most malaria cases occurred in the hilly area where *An. fluviatilis* is the active dominant species. It seems that *An. fluviatilis* is responsible for transmission of malaria in hilly districts of Kahnouj. Thus malaria transmission in this study area is much influenced by resident's rest habits while a wild vector, *An. fluviatilis* shows exophilic behavior and uses microclimate shelters with high incidence of human blood feeding. Thus, adapting people to use personal protection such as a bed net instead of residual spraying may be considered as an effective measure in malaria control in hilly regions.

Keywords: *Anthropophily,* Anopheles*, Seasonal activity, Malaria, Iran*

Introduction

The ability of an *Anopheles* species to feed on human blood is generally defined as anthropophily and represents success of a species and its capacity to transmit malaria. Generally, feeding behavior of *Anopheles* and *Culex* are influenced highly by season, human host behavior and also the availability of alternative hosts (1). Vythilingam *et al.* (2) during a longitudinal study in Sekong province, Loa PDR, revealed that the inoculation rate of *Anopheles* vectors were strongly correlated to vectorial capacity in the wet season and therefore the high anthropophilous behavior of an anopheline vector can be closely related to sporozoite infection (3). However, innate factors such as the number of olfactory cells may affect the propensity of an *Anopheles* to find a particular host (4).

In Iran about 12% of the population lives under the risk of both *falciparum* and *vivax* malaria and 62% of cases were reported from south eastern provinces including Sistan and Baluchestan, Hormozgan and Kerman (5). Kahnouj district has the greatest number of malaria cases in Kerman province (Report of the Health Center of Kahnouj 2002, Unpublished). Seven out of the 21 anopheline mosquitoes of Iran are involved in transmission of malaria and five of them are prevalent in the south of Kerman

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province. They consist of *An. fluviatilis, An. stephensi, An. culicifacies, An. superpictus* and *An. dthali* (6). *An. fluviatilis* and *An. stephensi,* were the dominant species in the slopes and flat areas of Iran (7, 8). The unique feature that helps to explain the malaria transmission in this area is the strongly anthropophilic behavior of some *Anopheles* vectors.

However, surveys indicate that in Iran and other countries of the Middle-East or Asia, *An. fluviatilis* is prevalent mostly in hilly regions. Studies performed in Bagh-Boueih and Quiz regions of Jiroft district, Kerman province, South of Iran, revealed that maximal activity of this species occurred in March, September and October, with a long activity peak frequency through the months of winter (7). Later, Edrisian and colleagues studied the human blood index of malaria vectors in 19 provinces of Iran, between the years 1982-1984, and estimated that 5.1% of collected *An. fluviatilis* had fed on human blood.

An. stephensi is the dominant species in most coastal regions of southern Iran as well as Kahnouj district. It was known to be the main vector in malaria endemic areas (9). This species is considered to be endophilic, but a small proportion of its population has been caught outdoors (9, 10). The population of *An. stephensi* was extremely affected by temperatures and low rainfall during the activity season in South Punjab, Pakistan (11). However, this species showed a high potential for transmission of human malaria parasites in Afghanistan, particularly in urban areas (12). Investigation by Eshghi and Motabar (3) revealed that *An. stephansi* had a great tendency to hematophagy from animals, and its anthropophily indices ranged from 5.4% to 20.4% regarding species caught in Kazeroun and Bander-Abbas, South of Iran.

The aim of this study was to determine important hematophagic tendencies of malaria vectors in an endemic area, Kahnouj district. In addition, the effects of seasonal changes on human blood preferences of the mosquitoes in two areas with different heights (flat and hilly area) were determined.

Materials and Methods

Study area The study took place over a period of 14 months from April 2002 up to May 2003 in Kahnouj district, Kerman province. The study area is located on the southern side of the Zagross mountain range, in the south of Iran near the Oman Sea. Kahnouj district is separated into two distinguishable areas, a hilly region with altitude averaging 940 meters above sea level and a flat region with altitude averaging about 150 meters above sea level. This district is officially divided into five sections, three in hilly and two in flat areas. One village from each section was chosen for collecting mosquitoes. These villages include Darreh-Shoor (Fariab section), Garmami (Bechegan section) and Bergah (central section) within the hilly area and Heidar Abaad (Roudbar section) and Borjak-Hassan (Ghaleh-Ganj section) in the flat area with a desert type geographical situation. The total population in the five villages was 2350 (Local Health Center of Kahnouj District, 2002). The average temperature in summer is between 27º C and 40º C in the hilly and 29º and 45º C in the flat area whilst the average temperature in winter is between 8º and 20º C in the hill and 10º and 25º C in the flat area respectively (Local geometric station in Khanouj district 2002).

The hilly region often has numerous small, narrow rivers which are suitable breeding places for mosquito larvae particularly *An. fluviatilis* while most water sources in the flat area consist of wells, pools and pounds which serve as the breeding sites of *An. stephansi*. Three other malaria vectors, *An. superpictus* Grassi, *An. Dthali* Patton and *An. culicifacies* are present in Kahnouj area in relatively low numbers.

Collection techniques All the selected villages underwent entomological studies and mosquito collection was done every 15 d using

both space spraying collection and hand catch methods. Indoor places were randomly selected from each village and consisted of 4 human and 4 animal shelters located in different parts of the village. Mosquito collections were made twice a month by space-spraying of indoor shelters with non-persistent pyrethroid insecticide while attempts were made to ensure that the sheets fully covered the floor space and all horizontal surfaces as accurately as possible.

Hand catch collection was performed from outdoor resting places in each village including natural shelters such as wild animal holes or cracks and hollows particularly around rivers and/or from 2 pit shelters,(120×90 cm and 150 cm deep). All collected anophelines were identified to species and blood meals of freshly fed females smeared on Whatman filter paper which was allowed to dry. These were packed inside plastic bags and kept at -20°C until used.

ELISA tests The tests were performed as described by Edrissian et al. (1985) as follows: the dried spots of blood on the papers were cut to make small discs, 2-3 mm in diameter. Each disc was put in a well of a Micro ELISA plate (NUNC Co. Denmark). The dried blood on the filter paper was eluted with 50 µl of distilled water in each well for 2 hr at room temperature. Then 50 µl of coating buffer (carbonate bicarbonate, pH 9.6) was added to each well. The filter papers were stirred inside the wells and removed and then the plates were left overnight at +4°C inside a humid box. The plates were washed with phosphate buffered saline-Tween 20 (pH 7.2) three times. Then amounts of 50µl diluted goat anti-human IgG conjugated to alkaline phosphatase were added onto each well, incubated at 37 °C for 2 hr and washed as before. Then 100 µl of substrate solution(1 mg/ml P-nitrophenyl phosphate, Sigma, in 10% diethanolamine buffer pH 9.8 containing 0.5 mmol $MgCl_2$ and 0.02% Na N_3) was added to each well and left in a dark chamber at room temperature for 30 min. As controls, two wells were left blood free (blank) and two wells were treated with human blood (positive control). The results were assessed by examination with the naked eyes and also absorbance was measured with an ELISA reader at 405 nm about 30 min after the addition of the substrate solution. The test well was considered positive if it gave a visible yellow color.

Malaria surveillance Khanouj district is served by Health Centers and the Ministry of Health. Microscopy is performed on out patients with fever or suspected malaria. Malaria case detection is mostly performed passively and rarely actively. The positive cases are treated with chorolquin/ primaquen.

Results

In this study, 1552 *Anopheles* mosquitoes were collected from both hilly and flat regions (966 *Anopheles* from the hilly area and 586 from the flat) during 14 months. The species consisted of *Anopheles fluviatilis, An. stephensi, An. culicifacies, An. dthali,* and *An. superpictus*, with dominancy of *An. fluviatilis* in the population of the hilly area, and *An. Stephensi* in the flat area with intensification to indoor shelters (Table 1).

In the hilly area, 66.6% of total female *Anopheles* had fed and were suitable for the ELISA test whereas 71.2% of collected female mosquitoes had fed in the flat area. The majority of fed females of *An. fluviatilis* were collected from outdoor shelters (459 mosquitoes) in both areas followed by *An. dthali* (25 mosquitoes). In contrast, most of the fed females of *An. stephensi* (395 mosquitoes) were found in indoor places followed by a few *An. fluviatilis* (32 mosqitoes). The population of other species in indoor as well as outdoor places was low in both areas (Table 1).

The highest Human Blood Index was found in the *An. fluviatilis* population at 2.82% of fed females while 0.50% of *An. stephensi* had fed on human blood. Generally,the mosquitoes particularly *An. fluviatilis* in the hilly area showed more propensity for human blood rather than those in the flat area (Table 1).

Two peaks of human blood feeding were found in *An. fluviatilis*, one was observed from September to November and the other from February to April (Fig. 1). The females of *An. stephensi* showed low propensity for human blood (only in May). Among other species, only one female of *An. superpictus* was found positive for human blood (Fig. 2).

An. fluviatilis was active throughout the year in both indoor and outdoor resting places and reached a peak in December gradually decreasing until April which showed another peak (Fig1). *An. stephensi* was absent during the winter months but its population increased at the beginning of March and reached a peak in May then gradually decreasing during summer. The second peak of this species, which was higher, appeared in August and September (Fig. 2).

Total malaria cases were 993 during 2001 while 1048 malaria cases were recorded in 2002. The incidence of malaria gradually increased from May and reached a peak in November and December in both years (Fig. 3). Over the study period, *Plasmodium vivax* accounted for 94%, *P. falciparum* 5.3% and mixed infection for 0.2% of all malaria cases. Transmission occurred mainly in October-December.

Table 1: Human blood index of *Anopheles* species collected from indoor and outdoor shelters of the hilly and flat areas in Khahnooj

Area	*Anopheles* Species	Outdoor		Indoor		Total No. of collected mosquitoes
		No. of fed females	ELISA Pos.	No. of fed females	ELISA Pos.	
Hilly area	*An. fluviatilis*	547	16	32	0	892
	An. stephensi	1	0	4	0	16
	An. culicifacies	3	0	4	0	7
	An. dthali	25	0	5	0	40
	An. superpictus	6	1	0	0	11
Flat area	*An. fluviatilis*	2	1	3	0	8
	An. stephensi	6	0	391	2	565
	An. culicifacies	5	0	1	0	8
	An. dthali	2	0	0	0	2
	An. superpictus	3	0	0	0	3
	An. sergenti	1	0	0	0	1

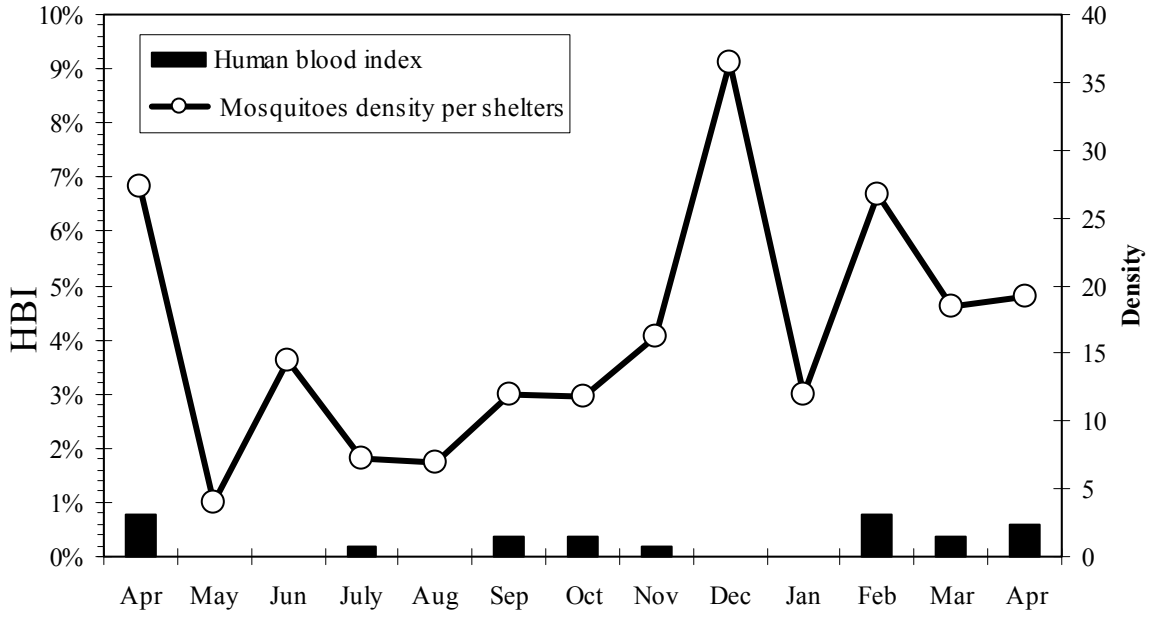

Fig. 1: Human blood index (HBI) and density of *Anopheles fluviatilis* per shelter in the Kahnooj district from April 2002 to April 2003. The mosquitoes were collected and then fed females were tested by direct Enzyme-Linked Immunosorbent Assay (ELISA) for human blood

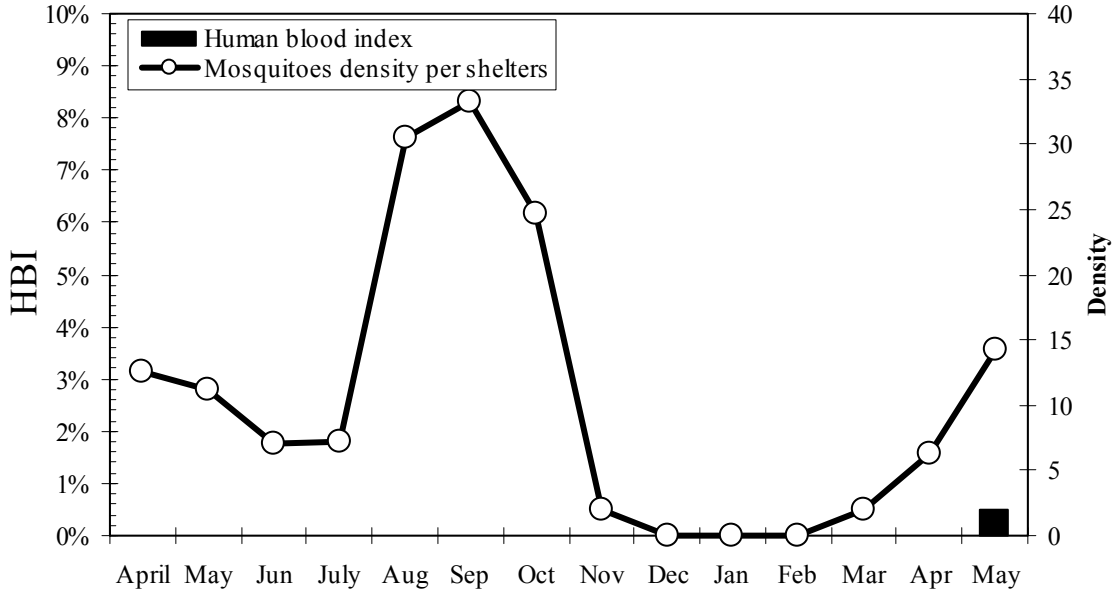

Fig. 2: Human blood index (HBI) and density of *Anopheles stephensi* per indoor shelter in the Kahnooj district from April 2002 to April 2003. The mosquitoes were collected and then fed females were tested by direct Enzyme-Linked Immunosorbent Assay (ELISA) for human blood.

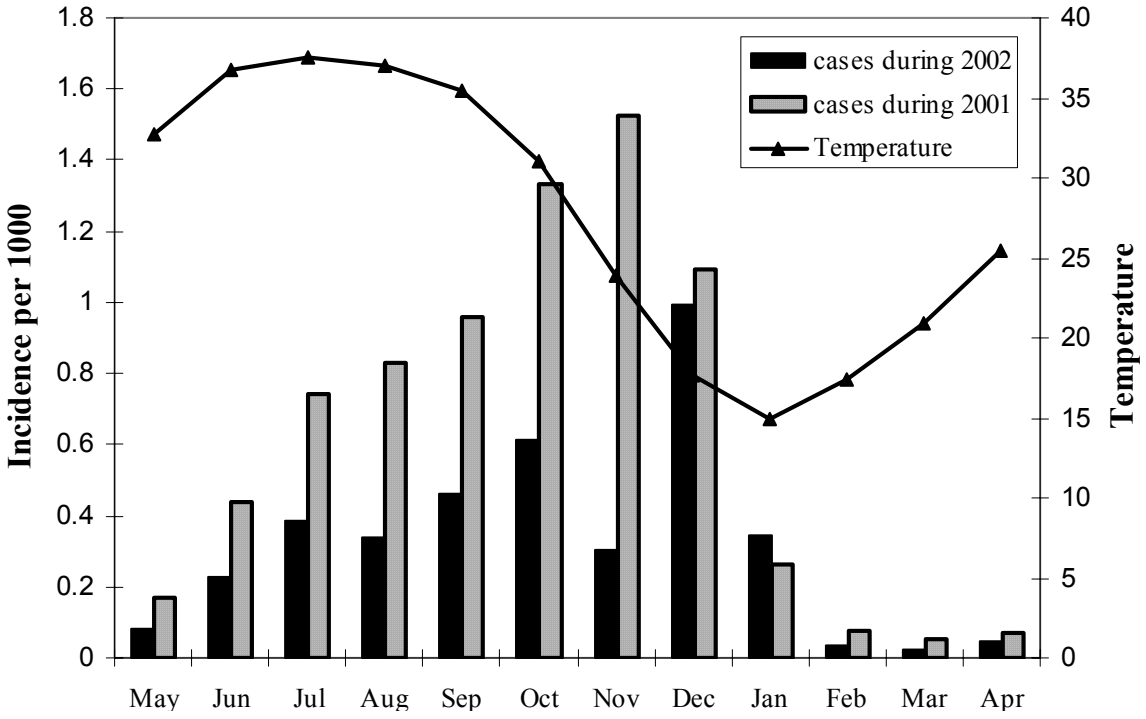

Fig. 3: API of malaria cases in Kahnouj district, during 2001 and 2002. The line indicates mean temperature (ºC) in 2001 and 2002

Discussion

Kahnouj is one of the endemic malaria regions in the southeast of Iran. The anopheline fauna does not appear to have changed much over several decades (13). Five vector species for malaria were found in this study which had been previously recorded by Eshghy et al. (3). This district, like other malaria endemic areas in Iran, has been under pressure from anti malaria programs including residual spraying of insecticides against the vectors since 1958 (14). Despite using different insecticides for along time in Kahnouj district, there is still no evidence to show any effect of residual spraying on changing the behavior of anophelines with respect to blood preferences.

The present study demonstrates that *An. fluviatilis* in the hilly area and *An. stephensi* in the plain area are the predominant species. The results are similar to previous studies carried out by Eshgy et al. (3) and Manouchehri et al. (9) indicating that despite nearly 3 decades of utilization of insecticides in agriculture as well as in antimalaria programs, these vectors are still active in the same area. In contrast, and comparing with previous records, populations of *An. dethali*, *An. superpictus*, and *An. culicifacies* decreased during the same time (15).

Generally *An. Stephensi* is a domestic and endophile species in Iran (9) and therefore, has been more exposed to sprayed insecticide over 4 decades however its population still remains dominant in the plain area. During this study, *An. stephensi* was noted to be extremely zoophilic whereas earlier workers noted this species to be somehow anthropophilous in the southwest of Iran (7, 9) and Saudi Arabia and Tunisia (15). In fact, our result is very comparable to surveys which have been done in Pakistan and India (16). It is most likely that *An. stephensi* in oriental areas has different behavior from those in European areas with

respect to feeding behavior and zoophily. In Kahnouj these behaviours are similar to those of oriental areas.

An. fluviatilis is a fairly wild species in Iran which rests both indoors and out but remains exophagic (7). Our result demonstrated that *An. fluviatilis* had a long period of activity throughout the year and also showed a tendency to indoor shelters during winter. This *Anopheles* is a complex of three cryptic species designated as species S, T and U (17) of which, recently, only species T has been reported from the south of Iran (3). Nanda and his colleagues (18) noted that species T is extremely zoophile while our results showed that 3.1% of *An. fluviatilis* collected from outdoor shelters were positive against human blood (table 1). Cow, sheep and goat are the majority of domestic animals in the study areas with ratios of 1:2.1 cow/human and 4.5:1 sheep or goat/human, respectively. Also the weather of Kahnouj district is hot in summer and mild in winter, and domestic animals are settled outside, thus there is no particular prototype shelter for domestic animals which would make a resting and feeding place for mosquitoes. Therefore, mosquitoes have more opportunity to bite animals rather than humans but despite this *An. fluviatilis* still shows anthropophagic behavior which could result in transmission of malaria.

Previous studies showed that the *An. fluviatilis* complex is one of the major vectors of malaria in India (17) in which sibling species S showed high propensity to human blood while Species T and U were recorded as mainly zoophagic (19). Recently only species T of this vector was found in Iran (3) and identified as the main vector of malaria in hilly areas of the south of Iran (7). *An. fluviatilis* was known as an anthropophagic mosquito as well as the main vector for malaria in San Dulakudar, Orissa state in eastern India, where the transmission of malaria was very dependant on season and the population density of vectors (20). Also, a study carried out in desert and non-desert regions of Rajestan demonstrated that *An. fluviatilis* has more activity in the non-desert region (21). This vector also contributes to malaria transmission in Afghanistan (12).

Furthermore, electricity was recently supplied to the study villages. Most houses are now equipped with air conditioners and the residents keep windows closed, so they are secured from mosquito bites during the hot season. In contrast, at the beginning of spring and autumn when the temperature is mild enough, to allow them to economize on electricity costs, the residents do not use air conditioners and so they leave windows open with no other protection against mosquito bites provided. Accordingly, the human blood index in *An. fluviatilis* was higher when the human host was accessible for biting (Fig 1). In contrast, its population apparently increased in indoor shelters when the outside temperature declined (Fig. 1). In reality, when temperature falls, this species shelters indoors instead of in the outside microclimate and natural shelters. Zahar (6) noted that the activity and blood feeding of *An. fluviatilis* was very low during winter.

The number of malaria cases in the Kahnouj district increases from the beginning of spring and reaches a peak in autumn and declines rapidly in winter when the temperature decreases (Fig. 3). Most malaria cases were recorded from hilly areas where wild anopheline species such as *An. fluviatilis* were active. In contrast, and based on Kahnouj Health Center's records, only a few malaria cases were found in the flat region which was all induced malaria. This observation indicates that no transmission of malaria occurred, at least during our study, in the flat region where *An. stephensi* was the dominant species with a very low propensity to human blood. In conclusion, transmission of malaria in the hilly area of Kahnouj is dependent on season and temperature where anophelines such as the *An. fluviatilis* vector are active and using natural microclimates. Therefore, in order to control malaria, indoor residual spraying of insecticide would not assist effectively. Teaching people to use bed nets, particularly in

the season people are more exposed to mosquito bites, may be considered as an effective measure in controlling malaria in Khanouj district.

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