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BLOOD PREFERENCES OF MALARIA VECTORS IN TEHRAN

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Abstract This study was conducted with aim of investigation of malaria vector hematophagic tendencies base on seasonal activities as well as geographical region in a malaria endemic area, Kahnouj, south of Iran. It was conducted for 14 months from Apr. 2002 up to the end of May 2003. It was based on collection of female anopheline from different shelters and exam their blood meal via ELISA test methods. Five vectors active in the study area were: *Anopheles fluviatilis, An. stephensi, An. dthali, An. culicifacies,* and *An. superpictos*. The dominant anopheline in hilly area was *An. fluviatilis* sibling species T. This species was active during whole year and reached to a peak in December. In contrast, the dominant species spread in flat region was *An. stephensi*, which shows strongly endophilic behavior with two seasonal activity peaks. Anthropophilic index for *An. fluviatilis* and *An. stephensi* was estimated 2.68% and 0.5%, respectively. Population of other species was too low and did not show propensity for human blood. The most malaria cases occurred in the hilly area whereas *An. fluviatilis* is an actively dominant species. It seems that *An. fluviatilis* is responsible for transmission of malaria in hilly districts of Kahnouj. Malaria transmission in the study area is much influenced by residents rest habits while a wild vector, *An. fluviatilis* shows exophilic behavior and uses microclimate shelters to obtain human blood. Use of personal protection such as bed nets instead of residual spraying may be considered as an effective measure in malaria control in hilly regions.
Key Words *Anopheles*, mosquitoes, malaria, ELISA, vectors

INTRODUCTION

The ability of an *Anopheles* species to feed on human blood generally defined as anthropophily, represents success of species and in their capacity to transmit malaria. Generally, feeding behavior of *Anopheles* and *Culex* were influenced by season, human host behavior, and also availability of alternative hosts (Kumar et al., 2002). Vythilingam et al. (2003) 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 therefore high anthropophily behavior of an anopheline vector can be closely related to sporozoite infection. (Nandi et al., 2000). The innate factors such as the number of olfactory cells may effect on propensity of an *Anopheles* in seeking a host (Van denBroek and den Otter, 1999).

In Iran, about 12% of the population live under the risk of both falciparum and vivax malaria (Beljeav, 2000) and 62% of cases were reported from south eastern provinces including Sistan and Baluchestan, Hormozgan and Kerman (Masoumi, 2001). Kahnouj district has the greatest number of malaria cases in Kerman province (Health Center of Kahnouj, 2002). Of the 21 anopheline mosquitoes in Iran, 7 are involved in transmission of malaria and five of them are present in south of Kerman province. They are: *Anopheles fluviatilis, An. stephensi, An. culicifacies, An. superpictus* and *An. dthali* (Zahar, 1974). *An. fluviatilis* and *An. stephensi*, were dominant species in slopes and flat areas of Iran (Eshghi et al., 1976; Manouchehri et al., 1976). The unique feature that helps to explain the malaria transmission in this area is the extremely anthropophilic behavior of some *Anopheles* vectors.

Previous studies showed that *An. fluviatilis* complex is one of the major vector of malaria in India (Singh et al., 2004) which sibling species S showed high propensity to human blood while species T and U were recorded mainly zoophagic (Nandi, 2000). Recently, only species T of this vector was found in Iran (Nadaf, 2002), which was identified as a main vector of malaria in hilly areas of south of Iran (Eshghy et al., 1976). *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 depended on seasons and population density of vectors (Sharma et al., 2004). Also, a study carried out in the desert and non-desert region of Rajestan demonstrated that *An. fluviatilis* has more activity in the non-desert region (Verma, 1999). This vector also contributes in malaria transmission in Afghanistan (Rowland et al., 2002).

Surveys indicate that in Iran or other countries of Middle-east or Asia, *An. fluviatilis* manifests its maximal activity in hilly regions. Studies performed in Bagh-Boueih and Quiz regions of Jiroft district, Kerman province, South of Iran, revealed the maximal activities of this species occurs in March, September and October, with a long activity peak frequency through months of winter (Eshghy et al., 1966). Edrisian et al. (1985) studied on human blood index of malaria vector in 19 provinces of Iran, between years 1982-1984, and estimated that 5.1% of collected *An. fluviatilis* fed on human blood.

An. stephensi is the commonest 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 (Manouchehri et al., 1976). This species is considered to be endophilic, but a small proportion of its population has been caught outdoors (Manouchehri et al., 1976; Zaim et al., 1986). The population of *An. stephensi* was affected by temperatures and low rainfall during activity season in South Punjab, Pakistan (Herrel et al., 2004). However, this species showed high potential for transmission of human malaria parasites in Afghanistan particularly in urban areas (Rowland et al., 2002). Investigations by Eshghi and Motabar (1975) revealed that *An. stephansi* has great tendency of hematophagy from animals, and its anthropophily indices ranged between 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 hill area) were determined.

MATERIALS AND METHODS

Study Area. The study took place for over a period of 14 months from April 2002 up to May 2003 in Kahnouj district, Kerman province. The study area is located at southern side of Zagross mountain range, south of Iran near Oman Sea. Kahnouj district is separated into two distinguishable areas, hilly region with altitude average 940 m above sea level and the flat with altitude average about 150 m above sea level. This district is officially divided into five sections, three in hill 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) with geographical situation of hilly area and Heidar Abaad (Roudbar section) and Borjak-Hassan (Ghaleh-Ganj section) in flat area with desert type geographical situation. Total population living in the five villages was 2,350 people. The average temperature in summer is between 27º C and 40º C in the hill and 29º C and 45º C in the flat area whilst the average temperature in winter is between 8º C and 20º C in the hill and 10º C and 25º C in flat area respectively.

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

Collection techniques. All of those villages underwent entomological studies and mosquito collection every 15 days using both space spraying collection and hand catch collection. 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 collection was made twice a month by space-spraying of indoor shelters with non-persistent pyrethroid insecticide while attempts were made to insure that the sheets covered the floor space and all horizontal surfaces as accurately as possible.

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

ELISA Tests. Tests were performed as described by Edrissian et al. (1985) as follows: the dried spots of blood on the papers were cut in circular with puncture to make small discs, 2-3 mm in diameter. Each disc was put in a well of the Micro ELISA plate (NUNC Co., Denmark). The dried blood on 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 50 µl 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 dark chamber at room temperature for 30 min. As control, two wells blood free (blank) and two wells with human blood (positive control) were used. The results were assessed by examination with the naked eye and also absorbance was measured with ELISA reader at 405 nm about 30 min after the addition of substrate solution. The test well was considered positive if it gives a visible yellow color.

Malaria Surveillance. Kahnouj district is served by Health Centers, Ministry of Health. Microscopy is performed on out patients with fever or suspected malaria. Malaria cases detection are mostly performed passively and rarely actively. The positive cases are treated with chlorquine/primaquen.

RESULTS

In this study, total *Anopheles* collected from both hilly and flat regions was 1552 mosquitoes (966 *Anopheles* from the hilly area and 586 from the flat area) during 14 months. The species consisted of *Anopheles fluviatilis, An. stephensi, An. culicifacies, An. dthali,* and *An. superpictus*, with dominancy of *An. fluviatilis* population in the hilly area, and *An. stephensi* population in the flat area with intensification to indoor shelters. In the hilly area, 66.6% of total females anophelines were fed and eligible for ELISA test whereas 71.2% of collected females mosquitoes were fed in flat area. The majority of fed females of *An. fluviatilis* were collected from outdoor shelters (459 mosquitoes) in both areas follow by *An. dthali* (25 mosquitoes). In contrast, most of fed females of *An. stephensi* (395 mosquitoes) were found indoors followed by a few *An. fluviatilis* (32 mosquitoes). The population of other species in indoor as well as outdoor places was low in both areas.

The highest Human Blood Index was found in *An. fluviatilis* population which was 2.82% of fed females of this species while 0.50% of *An. stephensi* fed on human blood. Generally, the mosquitoes particularly *An. fluviatilis* in hilly area showed more propensity for human blood rather than those in the flat area. Two peaks of human blood feeding were found in *An. fluviatilis*, one was observed from September to November and the next peak from February to April. 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 against human blood.

An. fluviatilis was active throughout year in both indoor and outdoor resting place places, reaching a peak in December and gradually decreased since April. *An. stephensi* was absent during winter months. However, its population increased at the beginning of March, reached a peak in May and then gradually decreased during summer. The second peak of this species, which was higher, appeared in August and September. Total malaria cases were 993 during 2001 while 1048 malaria cases were recorded in 2002. The incidence of malaria gradually increased from May reached to a peak in November and December in both years. Over the study period, *Plasmodium vivax* accounted for 94%, *Pl. faciparum* 5.3% and mixed 0.2% of malaria cases. Transmission occurred mainly in October-December.

DISCUSSION

Kahnouj is one of the malaria regions in southeast of Iran. The anopheline fauna does not appear to have changed much over several decades. Five vector species of malaria were found in this study, which had been previously recorded by Eshghy et al. (1976). This district, like other malaria endemic areas in Iran has been under pressure of anti-malaria programs, including residual spraying insecticide against the vectors since 1958 (Zaim, 1987). However, no evidence shows the effect of residual spraying on changing behavior of anopheline with respect to blood preferences.

The present study demonstrates that *An. fluviatilis* in hilly area and *An. stephensi* in plane area are predominat species. Similar results were reported by Eshgy et al. (1976) and Manouchehri et al. (1976). For nearly 3 decades utilization of insecticide in agriculture as well as anti-malaria programmers, still these vectors are active in the same area. In contrast and comparing with previous records, population of *An. dethali*, *An. superpictus*, and *An. culicifacies* reduced at the same time (Zahar, 1974).

Generally, *An. stephenis* is a domestic and endophile species in Iran (Manouchehri et al., 1976b) and therefore, it has been more exposed to sprayed insecticide during last 4 decades but its population still dominates the plane area. During this study, *An. stephenis* was noted to be extremely zoophilic whereas earlier works noted this species to be somehow anthropophile in southwest of Iran (Eshghy et al., 1976; Manouchehri et al., 1976), Saudi Arabia and Tunisia (Washino, 1983). Our results are very comparable to surveys which had

been done in Pakistan and India (Collines et al., 1991). It is most likely *An. stephenis* in Oriental areas have different behavior from those in Euthopian areas with respect to feeding behavior.

An. fluviatilis is a wild species in Iran, which rests both indoors and outdoors but remains exophagic (Eshghy 1976). Our results demonstrated that *An. fluviatilis* has a long period of activity throughout the year and also showed tendency to indoor shelters during winter. This *Anopheles* is a complex of three cryptic species designated as species S, T and U (Singh et al., 2004) which recently only species T has been reported in south of Iran (Nadaf et al., 2003). Nanda et al. (1996) noted that species T is extremely zoophilic while our results showed that 3.1% of *An. fluviatilis* collected from outdoor shelters was positive against human blood. Cow, sheep and goat are the majority of domestic animals in the study areas with ratios of 1:11 cow/human and 1.5:2 sheep or goat/human, respectively. Also the weather of Kahnouj district is hot in summers and mild in winters, and domestic animals are settled outside, thus there is no particular prototype shelter for domestic animals regarding to resting and feeding place for mosquitoes.

Electricity was recently supplied in the study villages. Most houses were equipped with air conditioners and the residents kept the windows closed, so they were secured from mosquito bites during the hot season. In the beginning of spring and autumn when temperature is mild, the residents do not use air conditioners and leave windows opened and have no other protection against mosquitoes. Human blood index in *An. fluviatilis* was higher when human host was accessible for biting. Its population apparently increased in indoor shelters when the outside temperature declined. By falling temperature, this species sheltered indoor instead of in outside microclimates and natural shelters. Zahar (1979) noted that the activity and blood feeding of *An. fluviatilis* is low during winter.

The number of malaria cases in the Kahnouj district increased from beginning of spring and reaches a peak in autumn and declined rapidly in winter when the temperature decreased (Fig 3). Most of malaria cases were recorded from hilly area where the wild anopheline species such as *An. fluviatilis* was active. In contrast and based on Kahnouj Health Center's records, only a few malaria cases were found in flat region which 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 very low propensity to human blood. Transmission of malaria in the hilly area of Kahnouj is dependent on season and temperature where *An. fluviatilis* are active and uses natural microclimate. To control malaria, indoor residual spraying of insecticide would not assist effectively. Informing people to use bed nets may be considered as an effective measure in controlling malaria in Kahnouj district.

An. fluviatilis was recognized as one of the main vector in hilly area while *An. stephensi*, flat area of Kahnouj district (Eshgi et al. 1975). In the present study, *An. fluviatilis* was the most abundant anopheline in hilly area for the whole year while *An. stephensi* showed the highest population in flat area with one major peak from the end of summer to the middle of autumn. These results confirmed the studies by Manouchehri et al. (1976b) which showed *An. stephensi* being active throughout the year in flat area with two peaks, one in April and May and the other, which is higher, during August and September. Manouchehri et al. (1976a) demonstrated that *An. fluviatilis* has a long period of activity throughout the year. This species is extremely exophillic but by declining temperature, it chooses indoor places. *An. stephensi* show high propensity for indoor shelters all year round. In hilly areas, *An. stephensi* was the most predominant species after *An. fluviatilis*, and its density greatly increased in 1976. This species is considered endophagic and exophagic, resting both in houses and outside shelters. Maximum biting activity of this species also occurs before midnight (Manouchehri et al., 1976a). Selection of resting habitat is much influenced by the temperature and humidity of favorable resting places and human habitats.

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