Original Article

Age Determination and Vectorial Capacity of Anopheles Maculipennis Sensu Lato (Diptera: Culicidae), in the Central Plateau of Iran

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(Received: 30 Dec 2015; Revised: 8 Mar 2016; Accepted: 13 May 2016)

Abstract

Background and Purpose: Islamic Republic of Iran has greatly reduced its malaria burden and has a national goal to eliminate malaria by 2025. The aim of this study was to determine the population dynamics of Anopheles maculipennis sensu lato, in relation to probable malaria transmission. For this purpose, the study was conducted in three villages in Isfahan Province of Iran, from April to March 2014.

Materials and Methods: Two mosquitoes sampling methods were conducted, comprises human landing catch and human bed net collection. The results of this investigation were subjected to one-way ANOVA using SPSS.

Results: A. maculipennis s.l. was found as a dominant vector with exophagic and endophilic behavior. Two peaks of blood feeding were observed, 9.00-10.00 p.m and 1.00-2.00 a.m. The gonotrophic cycle, survival rate, and life expectancy of the species were 4, 0.82, and 5 days, respectively. Malaria vectorial capacity of A. maculipennis was measured 0.0128 and 0.059 for Plasmodium vivax and Plasmodium Falciparum, respectively.

Conclusion: The findings indicate that there is a negative correlation between the temperature and daily age of A. maculipennis s.l. The method described can be used as a standard method to determine the daily age of Anopheles, as well as of other mosquito species since it is fast and precise and needs small samples. Survey on the age structure of vectors is very important as it is useful in monitoring the success of large-scale vector control measures.

*[Edalat H, Nikookar SH, *Moosa-Kazemi SH, Sepahvand F, Zolfi R.* **Age Determination and Vectorial Capacity of Anopheles Maculipennis Sensu Lato (Diptera: Culicidae), in the Central Plateau of Iran**. *Iran J Health Sci 2016; 4(2): 31-42] http://jhs.mazums.ac.ir*

***Keywords*:** Malaria, Anopheles Maculipennis, Vectorial Capacity, Age Determination, Iran

1. Introduction

Malaria is a disease which 214 million cases estimated worldwide in 2015. Global malaria incidence and mortality rate decreased to 37% and 60% between 2000 and 2015, respectively (1). Malaria occurs mostly in poor tropical and subtropical areas of the world, and the most vulnerable groups are young children and pregnant women (2).

Malaria is a major public health problem in the southern areas of Iran (3). The disease in this area has been classified as indigenous with unstable transmission. Malaria is most often associated with Plasmodium vivax infection in endemic area. It is characterized by a high incidence in disease infection. Vectors tend to be zoophagic. There is seasonal variation in population densities and also low detectable field infection rates (4).

According to current reports of the Iranian Ministry of Health and Medical Education, 90% of malaria cases have been reported from three provinces in the southeast of Iran including: Hormozgan, Kerman and Sistan and Baluchestan (5) At the present, the situation of malaria in Iran is classified as the elimination phase (6,7). In these three provinces, the major peak of malaria transmission occurs between September and November, with 21% of malaria cases in these regions caused by Plasmodium falciparum (8).

Anopheles quadrimaculatus complex has been reported as the main malaria vector in North America (9). In western North America, Anopheles freeborni was reported as the main vector, while Anopheles albimanus in Central America, and Anopheles darlingi in South America. Anopheles hermsi was also reported a vector in California. In Africa, both species of Anopheles gambiae s.l. and Anopheles funestus were reported the main vectors (10). Three Anopheles stephensi, Anopheles culicifacies s.l, and Anopheles dirus were reported the main vectors in Asia (10). Anopheles maculipennis complex is the main vector of malaria in Europe and the Middle East (11). By now, nine Palearctic members of Anopheles species includes Anopheles atroparvus, Anopheles beklemishevi, Anopheles labranchiae, A. maculipennis, Anopheles martinus, Anopheles melanoon, Anopheles messeae, Anopheles sacharovi, Anopheles Persiensis, and A. maculipennis have been identified as the major vectors of malaria in the north and central plateau of Iran. Some ecological, morphological, and physiological aspects of these species were carried out during the recent studies (12). Among these species, A. maculipennis sensu lato has a very wide distribution from westwards of the Palearctic region (13).

Entomological research revealed the presence of two proven malaria vectors including Anopheles superpictus Grassi and A. maculipennis s.l. in the central plateau of Iran (14). A. maculipennis s.l. lives in near association with humans dwelling. This species tends to blood feed on both human and animals and can complete a gonotrophic cycle in short time. Larvae grow and develop in a wide variety of sunlight to shadow surface pools. Larvae found in artificial breeding places associated with human activity such as roadside ditches, borrow pits, and the hoof prints of domestic animals (14).

In the same study, the physiological age

composition of A. stephensi was carried out in southern Iran (15). Ovary dissection of A. stephensi revealed at least 9% of the population can reach to the dangerous age to potentially malaria transmission. Two peaks of blood feeding were reported as 9.00-10.00 p.m, and 1.00-2.00 a.m. The gonotrophic cycle, survival rate and life expectancy of A. stephensi were reported 4, 0.82 and 5 days, respectively. Vectorial capacity was measured as 0.028.

Control programs against anopheline vectors - such as large-scale use of insecticide-treated nets and indoor residual spraying - were reduce mosquito density (15). The susceptibility test in the Hormozgan Province showed that A. stephensi was susceptible to bendiocarb, propoxur, malathion, fenitrothion, deltamethrin, permethrin, cyfluthrin, and lambdasyhalotrin while resistance to dichlorodiphenyltrichloroethane (DDT) and tolerant to dieldrin. Resistance to DDT and dieldrin reported for the first time from many regions of Iran and littoral of the Persian Gulf and Oman Sea (16,17).

The analysis of survival data for wild populations of some tropical species of malaria vectors revealed that adult female mortality rates were increased with age. The patterns of mortality of most of the populations were increased with by logarithm retime (18-23). In this situation, the parous rate can be used directly for the estimation of survival rate in life cycle (24). Mark-release-recapture experiments or laboratory multiple age-grading studies as a second model could be used for mortality estimation (12,25).

Many of travelers make travel to Isfahan Province. This part of Iran holds specific location due to access to the Zayande-Rood River. This area was endemic for malaria, and so the study on the age structure of malaria vector is very important. The results of this will be valuable to develop programs for improving the planning of malaria control in Isfahan Province, the last endemic area of the central plateau of Iran.

2. Materials and Methods

Isfahan is located in the center of the Iran (32.6577° N and 51.6692° E). This area is limited to Qom, Markazi, and Semnan provinces in the north, to Fars in the south, to Charmahal-Bakhtaran in the west and to Yazd in the East (Figure 1).

Figure 1. Map of Iran indicating the location of the study area in Isfahan province, 2014

The current study areas have moderate climate and constitute the former malarious areas of Iran. The maximum and minimum mean monthly temperature of Isfahan was 34°C and 14.5°C in August and January, respectively. The mean temperature and relative humidity were reported up to 27%

and 30-40% at Isfahan in July, respectively. The average annual rainfall was about 120 mm.

Three villages, Vinicheh, Dizicheh, and Karkavand were selected randomly with similar ecological habitats in plateau, slope and mountains areas based on the WHO criteria (21). The houses are on flat and slopes surrounded by rice land vegetation. Domestic animals found around the houses include cattle, sheep, and dogs. The rainy season is from December to May, and the dry season is from June to October. The village had not been under the vector control program during the study period. Age structure of A. maculipennis was studied in three villages in Isfahan from June 2014 to July 2014. Mosquitoes were collected biweekly using human landing catch as well as human bed net collection (26).

The traps set up overnight 18.00-06.00 hours, examined hourly and mosquitoes were collected using an aspirator. Human landing catches were taken outdoors and indoors from 18.00 to 06.00 hours. One team worked from 18:00 to 24:00 hours and the other from 24:00 to 06:00 hours. All mosquitoes landing on human bait were caught using small tubes, which were subsequently plugged with cotton wool and labeled according to time and site.

All collected mosquitoes were transferred into the plastic jars. The house and trap number and date of collection were recorded. The samples were transferred into the cool box with ice packs, and then, identified morphologically under dissecting microscope (at ×40) using Shahgudian's specific systematic keys (26). In the laboratory frequency of female and male of A. maculipennis in each sample were recorded, and females were classified according to the blood digestion stages (abdominal conditions). Unfed and freshly fed of A. maculipennis s.l. were dissected for parity and classified as nulliparous and parous based on the tracheal skeins of the ovaries (27). A random sample of Anopheles mosquitoes was dissected to extract gut and glands for oocysts and sporozoites examination.

Determination of probability of daily survival, the duration of progeny cycle, the life expectancy in days and vectorial capacity in three villages' daily survival rates were computed using the method of Davidson (28). Probability of survival was calculated using the formula, $p = G \times P$ (where, p = probability of survival, G = gonotrophic cycle and P = parous (28). Life expectancy was determined using the formula $1/-\log e\ p$, as described by Garrett-Jones and Grab (28). Vectorial capacity was calculated using the formula of $VC = (ma)\ a\ pn/-\log e\ p$, where ma was the man biting rate, and a considered the daily rate of blood feeding on man, p was the daily rate of survival, and n indicated as the length of the sporogonic cycle (28). The rate ma was calculated from the biting collections and p from the proportion parous as described above. The duration of the sporogony cycle as a function of temperature can be calculated by the formula $n = T/(t-tmin)$, where, n = Duration of sporogony cycle; T = 111, 105 and 144 for P. falciparum, P. vivax, and Plasmodium malariae, respectively; t = Actual average temperature in degrees centigrade and t min = 16 for P. falciparum and P. malariae and 14.5 for P. vivax (21).

The duration of blood digestion and

ovarian development of A. messeae was reported previously (29). The sum of degree-hours at different humilities is composed of the differences between the actual temperature at each hour and the threshold temperature, which at a humidity of 30-40% is 4.5° C; at 70-80%, 9.9° C; and at 90-100%, 7.7° C. The duration of blood digestion as a function of temperature and humidity can be calculated by the formula S = K/C-N, where, S = Duration of blood digestion; C = The actual temperature at each hour, N = The minimum temperature to ovaries development and K = Fix blunk index, which at a humidity of 30-40% is 46.5; at 70-80%, is 36.5; and at 90-100%, 37.5, respectively (21).

The duration of gonotrophic cycle as a function of temperature and humidity calculated by the formula:

GC = S+ 12 or 24 hours

The dangerous age can be calculated by the formula, sporogony cycle/gonotrophic cycle (21).

Host preference [human blood index (HBI)] is mainly calculated by this formula" HBI = Anthropophilic index × Gonotrophic cycle − 1. The difference between the mean diurnal temperature and the threshold temperature was calculated for each 24-hour period (21). These differences were added until the sum of the effective temperatures at 30-40% humidity is 65.4; at 70-80%, 36.5 and 90-100%, 37.1 (21).

The analysis was conducted using STATA (version 11.0; Stata, College Station, TX, USA). The confidence interval (CI) for sporozoite and the parous rate was 95% CI and calculated using the Fleiss quadratic (29). Chi-square analysis was performed to test for significance between parous rates between villages. To test the efficiency of mosquito male frequency in estimating nulliparous females, graphical and parametric methods were utilized to examine bias and error in methods (30).

3. Results

Out of the 3139 collected A. maculipennis females, 32.3% and 67.7% were collected in human landing catch and human bed net collection, respectively (Table 1).

About 82.84% and 76.6% of A. maculipennis unfed female was collected using human landing catch and human bed net collection in Isfahan, respectively (Table 2).

In total, 3139 females of A. maculipennis were dissected for parity. Most of them were found parous as range 54-57%. A significant differences observed due to mean of the parous rate of the samples collected by human landing catch and human bed net collection ($P < 0.050$).

Table 1. Composition of A. maculipennis; males and females sampled by different methods in Isfahan province April to March 2014

	Sampling method	
	Human landing catch	Human bed net collection
Number of bait	3	3
Number of mosquitoes	1014	2125
Average	338	708.3
Percentage	32.3	67.7

A. maculipennis: Anopheles maculipennis

Table 2. Abdominal conditions (%) of A. maculipennis females captured by different techniques in Isfahan province April to March 2014

	Sampling method	
	Human landing catch	Human bed net collection
	N (%)	N (%)
Un fed	840 (82.84)	1628 (76.6)
Freshly fed	174 (17.15)	497 (23.4)
Semi gravid	0 (0)	0 (0)
Gravid	0 (0)	0 (0)
Total	1014 (100)	2125 (100)

A. maculipennis: Anopheles maculipennis

In this study, experiments were performed on the duration of blood digestion and ovarian development of A. maculipennis. The mean temperature and Humidity were recorded as 27° C and 30-40% at Isfahan, in July, so the duration of blood digestion was calculated as 3.72 days (46.5/27-14.5). Therefore, the gonotrophic cycle was found 4 days (3.7 days +12 hours).

Probability of daily survival found as 0.860 and 0.870 in human landing catch and human bed net collection, respectively (Table 3).

Results showed the duration of the sporogony cycle for P. vivax and P. falciparum were 8.4 (105/27-14.5) and 13.9 days (111/27-19), respectively.

The dangerous age was calculated 8.4/4 = 2.1, and 13.9/4 = 3.47 for P. vivax and P. falciparum, respectively. The life infective expectancies were ranged from 1.87-2.23 to 1.68-2.01 days for P. vivax and P. falciparum, respectively (Table 3). The direct man biting rate (ma) presented in table 4.

Table 3. Parous rate, probability of daily survival, life expectancy (days) infective life expectancy, and vectorial capacity of the A. maculipnnis females in Isfahan province; 2014_2014

	Age determination		Pearson chi-square	P value
	Sampling method			
	Human bait collection	Human bed net collection		
Number of mosquitoes dissected	1014	2125		
Parous (95% CI)*	54	57	6.6	0.101
Nulli parous (95% CI)	46	43		
Probability of daily survival	0.86	0.87	1.974	0.373
Life expectancy (days)	6.66	7.19		
Pn P. vivax	0.281	0.31		
Pn P. falciparum	0.122	0.144	1.182	0.554
Infective life expectancy (days) P. vivax	1.87	2.23		
Infective life expectancy (days) P. falciparum	0.81	1.03	3.485	0.175
Gonotrophic cycle	4 days			
Sporogony cycle (n) P. vivax	8.4			
Sprogony cycle (n) P. falciparum	13.9			

*Confidence interval (CI) 95%. CI: Confidence interval, P. vivax: Plasmodium vivax, P. falciparum: Plasmodium falciparum

Table 4. Man biting rate (bit/man/night) of the Anopheles maculipnnis females catches in Isfahan province April to March 2014

Night catch	
Places	**Isfahan**
Indoor	2143
Outdoor	996
Total	3139

The vectorial capacity of this species catches was found as 0.0128, 0.059 for P. vivax and P. falciparum in Isfahan area, respectively. During the 24 round of the sampling at the study area, 3139 females were collected. Of the total dissected samples, 1380 samples were nulliparous in Isfahan area. Of 757 (43%) were 1 - parous, 406 (23.1%) were 2 - parous, 282 (16%) were 3 - parous, 195 (11.2%) were 4 - parous, 71 (4%), and 48 (2.7%) observed sac dilatation In Isfahan area.

4. Discussion

A. maculipennis considered as the most important malaria vector in the palearctic region. A. maculipennis is a species complex so that usually distinguish of these different biotypes by morphological characters is very difficult or impossible. Therefore, many molecular studies on A. maculipennis complex carried out using the ITS2 gene in the world.

The morphological character of this species complex, including egg pattern, has been studied, which could differentiate only between A. maculipennis and A. sacharovi, two members of this complex species (31,32). There are at least six species of this complex had been reported in northern Iran (33-35). In the same study, the survival rate of A. maculipennis s.l. was carried out in Zanjan Province, western Iran (18). Our results agree with prior studies that showed a significant difference between mean parous rate in two sampling method ($P < 0.010$). In addition, in 4 days gonotrophic cycle, daily survival rate of A. maculipennis similarly was had the same range in Isfahan and Zanjan. In our study, seasonal variation was observed in entomological variables. The density of population was more in summer than another season. In Parallel, Ghavami (18) was reported the positive pattern of the occurrences of A. maculipennis in Zanjan area. Several studies were out in Romania, Georgia and northern Iran. In confirmation of our results, this studies demonstrated that the same density pattern of A. maculipennis among the species complex (12,18). A. maculipennis is a widespread species in sub-tropical Asia and the principal vectors of malaria in the Mediterranean region and northern Asia. Larvae were collected in major rivers, urban and rural agricultural lands of northern Iran. In spite of widespread usage of pesticides in breeding places, A. maculipennis showed one district peaks of population density during June-July. Our study revealed that A. maculipennis is the most abundant populations during the summer. Rice field irrigation found as main breeding places, along with stream pools. During October to April, stream pools were found the predominant larval habitat of A. maculipennis in the most areas. In parallel, agricultural regions irrigated by rivers, deep wells, and cement pools are the main larval breeding sites of this species. The minimum temperature for growth of larvae of this species reported between 17° C and 26° C.

The duration of egg and larvae growing reported 3, and 7 days, respectively. However, the variety range of larval breeding places reported whole the year (36). Our results indicated that, the duration of the blood digestion and gonotrophic cycle of this species was 4 days, that was in accordance with the results of Zanjan study (18). In the same studies, gonotrophic cycle of A. freeborni in Sacramento Valley, Anopheles punctipennis in Maryland and A. quadrimaculatus in Florida, USA was reported 4-6, 4-5, and 5 days, respectively (9,10). The length of the oviposition cycle for A. gambiae and Anopheles merus was calculated 2 days in tropical area. Mean annual indoor temperature and relative humidity in our study were 25° C ± 1° C and 35% ± 5%, respectively. In our study, areas higher temperature and lower humidity were observed in the summer than spring. In fact, the temperature was negative while the relative humidity was positive and various temperature and humidity combinations were effects on the duration of the blood digestion and gonotrophic cycle. In during gonotrophic cycle, probability of daily survival of A. maculipennis in human-baited net trap collection and human landing catch were consistent with the previous study in Iran (18). A significant difference was seen in the mean survival rates per oviposition cycle in the population of A. maculipennis s.l. caught from light traps (0.46) and pyrethrum spray catch (0.50). Probability of daily survival of A. dirus, Anopheles maculatus, Anopheles minimus, and Anopheles jayporiensis in wet season of Laous were reported 0.850, 0.750, 0.770, and 0.860, respectively, whereas in dry, seasons were 0.910, 0.860, 0.770, and 0.890, respectively (37). Malaria vectors at least require a relative humidity of 60% in the constant mean temperature to survive long enough. The mean annual relative humidity recorded in Isfahan was 35% ± 5%. This value is below the threshold required; therefore, this phenomenon can explain the relatively short life span of the A. maculipennis in this area. At the present study, at least duration of the sporogonic cycle of A. maculipennis for P. vivax and P. falciparum in 27° C and 30-40 RH were 8.4, and 13.9 days at in Isfahan, respectively. Our finding was confirmed by previous study in central Iran (18). In contrast, the sporogony cycle of P. vivax in A. stephensi was reported 11 days at 27° C (18). This index for A. gambia s.l. and P. falciparum was reported 10 days at 27° C, and 28 days at 20° C. Indoor temperature was significantly higher during the summer than another season. The metabolic activities increased during the summer which may effects on growth, development and mating of malaria vectors under the low favorable relative humidity during the period, perhaps, explains the significantly lower sporogony cycle of A. maculipennis than other malaria vectors. In fact, sporogony cycle may reflect the influence of mean temperature.

In the present study, the duration of the life expectancy was parallel with Edalat et al. (2014). They reported that there is a significant difference between expectations of life of A. stephensi catches by different techniques (ranged from 3.5 to 9.5 days in southern Iran, $P < 0.050$). This index for A. gambia s.l. was reported 7.4 days while cited 3.2 days for Anopheles pharoensis at 27° C.

In our investigation, the direct man biting rate (ma) was found more than other malaria vectors in the country. Man biting rate was reported various range for A. gambia s.l. and A. pharoensis under distributed regions. According to our findings, antropophilic index and vectorial capacity of A. maculipennis was found the various ranges in Isfahan. In the same study, this index was reported for A. stephensi as 2.8×10^{-2} and 1.4×10^{-2} for P. vivax and P. falciparum, respectively (18). This index for A. dirus, A. maculatus, A. minimus, and A. jayporiensis were reported as the various ranges in wet and dry seasons of Laous. These observations are contrast with the results of our finding because of the differences the malaria vectors as well as use of various anthropophobia indexes.

5. Conclusion

In relation to socio-economic factors including industrial and construction projects, urbanization, large movement of people between the neighboring provinces and countries, presence of two proven vector, progressive ageing among the population of A. maculipennis s.l. central plateau of Iran has potential for malaria transmission. Since different reports revealed that the increasing the large displacement of the people, providing the free and enough health facilities in the central Iran. Our finding indicates that A. maculipennis with 3 and 7 parous ages have potential and may live long enough to transmit the P. vivax and P. falciparum in central Iran. The determining of the age structure and survival rate of anophelines vectors can be used more efficient in public health evaluation plans in different geographical areas of Iran.

Conflict of Interests

The Authors have no conflict of interest.

Acknowledgement

The authors are grateful to H. Vatandoost, Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences for this their cooperation. This study was financially supported by the Institute of Public Health Research, Academic Pivot for Education and Research, Tehran University of Medical Sciences: project No.: T 241/82/64.

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