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Vectorial capacity and Age determination of *Anopheles Stephens* Liston (Diptera: Culicidae), during the malaria transmission in Southern Iran

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Abstract
The objective was to determine the population dynamics of *Anopheles stephensi* in relation to malaria transmission. The study was carried out in three villages of Bandar-Abbas's county, south of Iran, from April 2011 to March 2012. Mosquitoes were collected by Total catch, Human and Animal bait collection, Window trap, Pit shelters and CDC light traps. *An. stephensi* play as a dominant vector with endophagic and endophilic behavior. Ovary dissection revealed six dilatations indicate at least 9% of the population can reach to the dangerous age to potentially malaria transmission. Two peaks of blood feeding were observed, 9.00-10.00 P.M, and the 1.00-2.00 A.M. The gonotrophic cycle, survival rate, life expectancy of the species was 4, 0.82 and five days, respectively. Vectorial capacity was measured as 0.028. In conclusion, hot and wet climatic conditions support the persistence, density and longevity of *An. stephensi* could result in more significant indigenous malaria transmission.

Keywords: Malaria, *Anopheles stephensi*, malaria, Age determination, Iran

1. Introduction
Malaria remains a killer disease which causes more than 1 million people death every year. Eighty six per cent of malaria cases occur in Africa South of Sahara; nevertheless, it remains a global problem also affecting countries in large areas of Asia and Latin America [1]. Despite considerable progress in malaria control over the past decades, it is endemic in more than 107 countries of the world and kills 1.1-2.7 million people annually. Furthermore, more than 40% of the world populations live in areas with the risk of malaria transmission [2].
Malaria remains a major public health problem in the southern areas of Iran, particularly in Hormozgan Province [3]. Malaria 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].
A total of indigenous malaria cases were reported in 2011, among which only 70% were Iranian, with the remaining coming from abroad. According to current reports, 90% of cases have been reported from three Provinces in the southeast of Iran, Hormozgan, Kerman and Sistan and Baluchestan [5]. In these three provinces, the major peak of malaria transmission occurs between September and November, with 21% of malaria cases in this region caused by *P. falciparum* [6]. The situation of malaria in Hormozgan is classified as the area with local transmission.
Many different species of *Anopheles* mosquitoes are vectors of malaria in the world. *An. quadrimaculatus* complex has been reported as the main malaria vector in North America [7]. In western North America, *An. freeborni* was reported as the main vector, while *An. albimanus* in Central America and *An. darlingi* in South America. *An. hermsi* was also reported a vector in California. In Africa, *Anopheles gambiae* s.l. and *An. funestusin* reported the main vectors in Africa [8]. *Anopheles stephensi*, *An culicifacies s.l* and *An. dirus* were reported the main vectors in Asia [8].
Anopheles stephensi has very wide distribution extending westwards Ethiopia, Yemen, Oman, the United Arab Emirates, Iran and Afghanistan, eastwards through Pakistan, India, Bangladesh, Myanmar, Thailand, Laos and Vietnam, northwards Nepal and southern China and southwards to Sri-Lanka [9].

Entomological research revealed the presence of five proven malaria vectors including; *Anopheles stephensi* Liston, *An. culicifacies s.l.* Giles, *An. fluviatilis* James, *An. dthali* Patton, and *An. superpictus* Grassi [4]. Residual spraying (two rounds per year) was practiced for many years in malaria vector control. Anti malaria drugs, particularly Chloroquine has been widely used for treatment [4, 10, 11].

Anopheles stephensi lives in near association with humans dwelling, intent 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 [12].

Control programs against anopheline vectors, such as large-scale use of insecticide-treated nets (ITNs), and indoor residual spraying (IRS) reduce mosquito density and survival rate [2, 13, 14]. The susceptibility test in the Hormozgan Province showed that this species was susceptible to Bendiocarb, Propoxur, Malathion, Fenitrothion, Deltamethrin, Permethrin, Cyfluthrin and Lambdasyhalotrin while, resistance to DDT and tolerant to Dieldrin [14]. Resistance to DDT and Dielderin reported for the first time from many regions of Iran and littoral of the Persian Gulf and Oman Sea [15].

The entomological impact of such programs can be evaluated by age structure and life expectancy of anopheline vectors [16, 17, 18]. It suggested that mosquito vectors mainly die of predation or environmental factors rather than old age. Clemens and Patterson were described the mortality of mosquitoes increases with age [19, 20]. When mortality increases with age, the parous rate can be used directly for the estimation of survival per feeding cycle [21]. In the second model, mortality is estimated by mark-release-recapture experiments or laboratory multiple age-grading studies [22, 23].

Many of travelers make their way to visit to Hormozgan Province and Qeshm Island when visiting Iran. This part of Iran holds specific location due to access to the free waters of Oman Sea, Indian Ocean and to other aspects such as trade, commerce and navigation. Iran is in the malaria pre-elimination stage and this is because of the importance of Hormozgan Province for malaria transmission in Iran, it was decided to evaluate age structure of *An. stephensi* due to malaria transmission. In addition, the current study can be useful for the impact of mosquito control strategies that rely principally on IRs and ITNs. New data of this research which will be valuable to develop programs for improving the planning of malaria control in this malaria endemic area of southern Iran.

2. Materials and methods

Hormozgan Province, located in south between 25° 24’-28° 57’ N latitudes and 52° 41’-59° 15’ E longitudes, area 181,471 km2; population 1,518,000) is located in southeastern Iran, bordering with Sistan and Baluchistan in the east, Kerman and Fars to the north and Oman Sea and the Persian Gulf, in the south (Fig.1). The Province is composed of counties: Rodan, Bandar-lengeh, Haji-Abad, Qeshm, Abu-Moosa, Jask, Minab and Banda-Abbas. The last three counties, with tropical climate, constitute the main malarious areas of Hormozgan. Therefore, anti-malaria measures, including house-spraying with residual insecticides and larviciding with *Bacillus thuringiensis* H14 is applied annually in these areas. In 2011, the maximum and minimum mean monthly temperature was 34 °C and 14.5 °C in August and January respectively. The average yearly rainfall is about 120 mm.

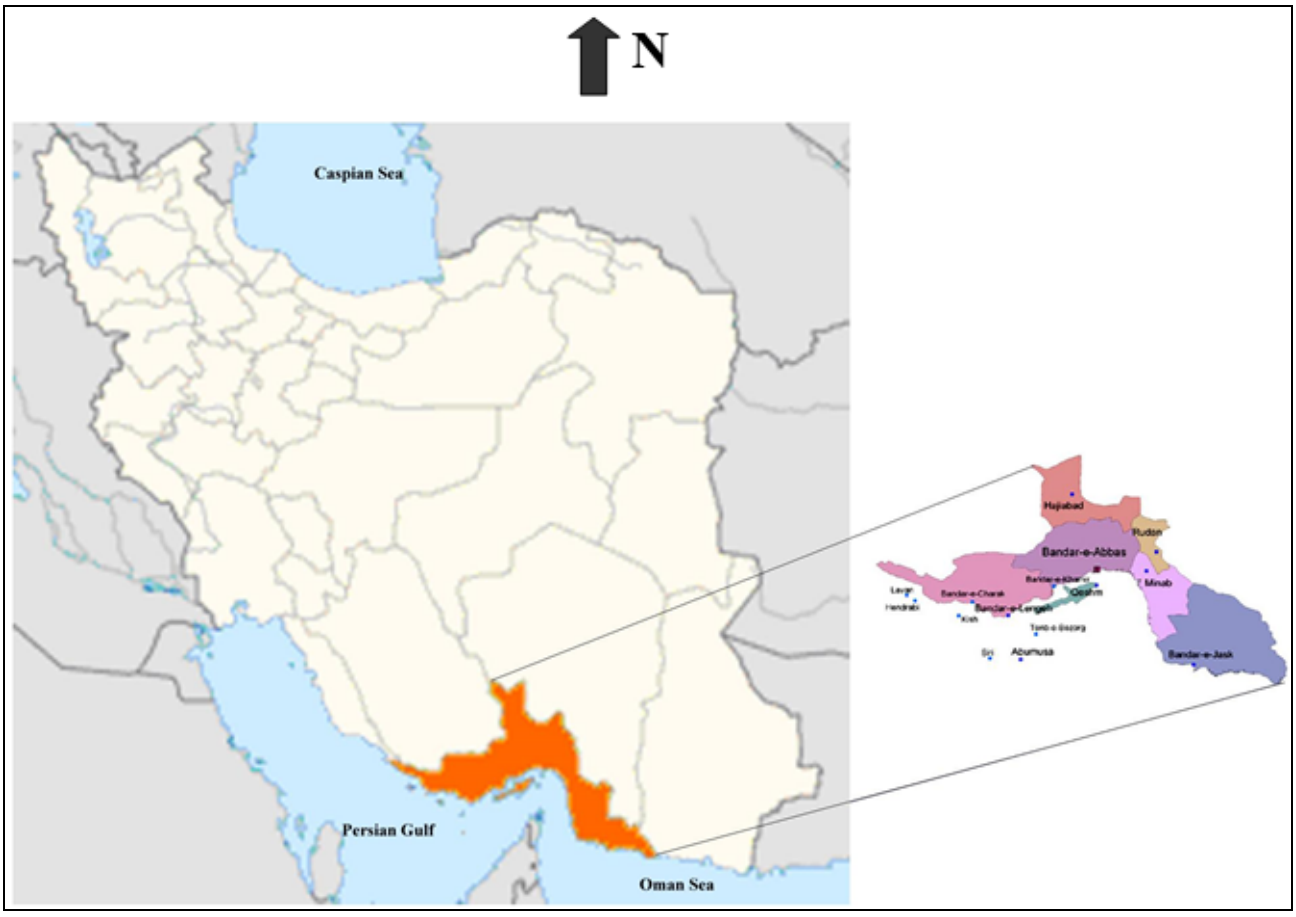

Fig 1: Map of Iran indicating the location of the study area in Bandar -Abbas District situated in the center of Hormozgan Province

Essin is a rural county of Bandar- Abbas located in slop area with a total population of 9790. Tahloo, Upper and Lower Hormodar villages were selected with similar ecological habitats. The villages are adjacent to each other. The houses are on flat land and surrounded by date trees. 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 average annual incidence of malaria is about 30 per thousand population. The villages had not been under the vector control program during the study period. Age structure of *An. stephensi* was studied in three villages, in Essin in the northern areas of Bandar-Abbas. Mosquitoes were collected biweekly in Bandar -Abbas, from April 2011 to March 2012.

Mosquitoes were collected biweekly using six sampling methods: knock-down pyrethrum space-spray, human and animal bait (18.00–05.00 hours), window trap, pit shelters and CDC light traps. CDC light-traps (John W. Hock Company, Gainesville, Florida) were hung adjacent to mosquito nets.

Pyrethrum space spray catches (PSSC) six fixed and two variables (four human and four animal shelters), were used in this program. Every morning indoor rested mosquitoes in five animal shelters were collected by the standard method [24], using 0.2% pyrethrum spray.

Pit shelter (120× 90×150 cm) collection were carried out using an aspirator from the walls of the pits between 05:00 to 06:00 AM [24].

Two inlet and outlet window traps of the Muirhead-Thompson type fitted to houses and animal shelters for endophily/exophily survey [25]. The traps set up overnight 18.00 to 06.00 hours, examined hourly and mosquitoes were collected using an aspirator.

Human and animal landing catches were taken outdoors and indoors from 18.00 to 06.00 hours, because people and domestic animals slept indoors and outdoors during most of the study period. One local used human bait; they exposed their arms, faces and feet. A tethered outside its shelter, about 15m from the human bait, used animal bait. These were fixed stations for collection throughout the study period. One team worked from 18:00 to 24:00 h and the other from 24:00 to 06:00 h. 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. Biting collections and light-trap collections performed the nights. Pit-shelter collections and PSSC (as described above) performed the following day, early in the morning.

Mosquito sampling: All collected mosquitoes were transferred into the plastic jars. The house and trap number and date of collection were recorded on the jar label and then jars were transferred into the cool box with ice packs and then identified morphologically under dissecting microscope (at 40X) using Shahgudian's systematic keys [26].

In the laboratory frequency of female and male of *An. stephensi* in each sample were recorded, and females were classified according to the blood digestion stages (abdominal conditions). Unfed and freshly fed *An.stephensi* were dissected for parity and classified as nulliparous and parous based on the tracheolar skeins of the ovaries [24, 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 sporogony cycle, the life expectancy in days and vectorial capacity in three villages Daily survival rates were computed using the method of Davidson (Davidson 1954). p = G×P (where p = probability of survival, G = gonotrophic cycle and p = parous Proportion). Life expectancy was determined using the formula 1/–loge p, as described by Garrett-Jones and Grab [17]. Vectorial capacity was calculated using the formula of VC = (ma) a pn/–loge 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 *P. 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* [18].

Duration of blood digestion and ovarian development of *Anopheles 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; [18]. The duration of Gonotrophic cycle as a function of temperature and humidity can be calculated by the formula GC = S+ 12 or 24 hrs. The dangerous age can be calculated by the formula sporogony cycle/Gonotrophic cycle [18]. Host preference (HBI) is mainly calculated by this formula" HBI=Antropophilic index ×Gonotrophic cycle-1. The difference between the mean diurnal temperature and the threshold temperature is calculated for each 24-hour period. These differences are added until the sum of the effective temperatures at 30-40% humidity is 65.4; at 70-80%, 36.5 and at 90-100%, 37.1 [18].

3. Statistical analysis

Analysis was performed using Stata 8.0 and Epi-Info. The confidence interval (CI) for sporozoite and parous rate was 95% CI, calculated using the Fleiss quadratic [29]. Chi-square analysis was carried out 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].

4. Results

Out of the 1095 collected *Anopheles stephensi* females, 82.46% were obtained by PSCC, 7.4% in the animal bait collection, 5.48% in the human bait collection, 2.1% by light trap, 1.47% in pit shelter and 1.1% in widow trap collections (Table 1).

Table 1: Composition of *Anopheles stephensi* male and females sampled by different methods at Essin, Bandar- Abbas County, Hormozgan Province, 2011- 2012.

	Sampling Method					
	PSSC	**Animal bait collection**	**Human bait collection**	**CDC Light trap**	**Pit shelter collection**	**Out let and inlet window trap**
No. of bait, trap, shelter	8	1	2	3	3	6
No. of mosquitoes	903	81	60	23	16	12
Average	112.87	81	30	7.6	5.3	2
Percentage	82.46	7.4	5.48	2.1	1.46	1.1

Table 2. Presented abdominal condition (%) of *An. stephensi* female captured by different collection methods at Essin (Bandar-Abbas County), Hormozgan Province (2011-2012). About 73.7% of *An. stephensi* female collected using PSSC were semi gravid and gravid, where the rest (27.15%) of the females captured were unfed and freshly fed.

Table 2: Abdominal conditions (%) of *Anopheles stephensi* females captured by different techniques at Essin, Bandar- Abbas County, Hormozgan Province, 2010 – 2011

	Sampling Method									
	PSSC		**CDC Light trap**		**Pit shelter collection**		**Inlet window trap**		**Out let Window trap**	
	No	**(%)**	**No**	**(%)**	**No**	**(%)**	**No**	**(%)**	**No**	**(%)**
Un fed	92	10.19	14	60.87	8	50	2	66.6	0	0
Freshly fed	154	17.05	4	17.39	2	12.5	1	33.4	2	22.2
Semi gravid	523	57.92	3	13.04	4	25	0	0	1	11.1
Gravid	134	14.84	2	8.70	2	12.5	0	0	6	66.7
Total	903	100	23	100	16	100	3	100	9	100

In light traps also, about 78.2% of the captured females were unfed and freshly fed. In outlet traps yielding almost three times than the inlet traps, where the ratio of gravid/ freshly fed were 3(6/2=3) in outlet traps.

A total of 479 samples were dissected for parity. Of 903, Anopheles female captured by PSSC, 41.2% were parous. Most of *Anopheles* collected in animal bait collection and human bait collection were found parous as 51%, 67% respectively. Significant differences observed due to mean of parous rate of the samples collected by PSSC method compared by human bait catches ($P<0.05$).

In this study performed experiments on the duration of blood digestion and ovarian development of *An. stephensi*. Mean temperature at Essin area was 23 °C in July, so the duration of blood digestion was calculated as 2.8 days (36.5/23-14.5). Therefore, the Gonotrophic cycle was found four days (2.8 days +24 hours). Probability of daily survival found as 0.75, 0.84 and 0.90 in PSSC, animal and human bait collection respectively. (Table 3). The duration of the sporogony cycle for *P. vivax* and *P. falciparum* were 12.35 days (105/23-14.5), and 27.7 days (111/23-19) respectively.

Table 3: Parous rate, probability of daily survival, life expectancy (days) Infective life expectancy, and vectorial capacity of the *Anopheles stephensi* females at Essin, Bandar- Abbas County, Hormozgan Province, 2010-2011

	Collection method		
	PSSC	**Animal bait collection**	**Human bait collection**
No. of mosquito	903	132	60
No. of dissected	327	67	40
Parous (95 CI) %	41.2	51	67
Nulli Parous (95 CI) %	58.8	49	33
Probability of daily survival	0.75	0.84	0.90
life expectancy (days)	3.5	5.7	9.5
Infective life expectancy(days) *P. vivax*	0.1	0.6	2.5
Infective life expectancy(days) *P. falciparum*	0.04	0.4	1.8

The dangerous age was 3.08, and 6.92 for *P. vivax* and *P. falciparum* respectively. The life infective expectancies were ranged from 0.1 to 2.5 days for *P. vivax* and from 0.04 to 1.8 days for *P. falciparum* (Table 3). The direct man biting rate (ma) in three villages presented in Table 4, where were 11(32/3 =11). Anthropophagic Index reported previously as 2.5%, and not calculated during this study [31].

Table 4: Man biting rate (bit/man/night) of the *Anopheles stephensi* females catches at Essin, Bandar-Abbas County, Hormozgan Province, Jun 2010

		Village		
		Upper Hormodar	**Tahloo**	**Lower Hormodar**
Number of mosquito catches	Indoor	10	5	17
	Outdoor	6	1	21
	Total	16	6	38

Table 5: Age composition of *Anopheles stephensi* females catches by different techniques at Essin, Bandar-Abbas County, Hormozgan Province, June 2010

Sampling period	Dissected No	*Number of females in age classes							
		*NP	1p	2p	3p	4p	5p	6p	sac
1	NO	*NO	NO	NO	NO	NO	NO	NO	NO
2	NO	NO	NO	NO	NO	NO	NO	NO	NO
3	NO	NO	NO	NO	NO	NO	NO	NO	NO
4	41	21	13	6	NO	NO	NO	NO	2
5	9	5	1	3	NO	NO	NO	NO	NO
6	66	33	30	1	1	NO	NO	NO	1
7	27	19	4	3	NO	NO	NO	NO	NO
8	NO	NO	NO	NO	NO	NO	NO	NO	NO
9	NO	NO	NO	NO	NO	NO	NO	NO	NO
10	NO	NO	NO	NO	NO	NO	NO	NO	NO
11	9	3	6	NO	NO	NO	NO	NO	NO
12	NO	NO	NO	NO	NO	NO	NO	NO	NO
13	NO	NO	NO	NO	NO	NO	NO	NO	NO
14	61	29	25	3	2	NO	NO	2	NO
15	9	5	1	NO	NO	NO	NO	NO	NO
16	NO	NO	NO	NO	NO	NO	NO	NO	NO
17	42	15	10	6	10	1	NO	NO	3
18	NO	NO	NO	NO	NO	NO	NO	NO	NO
19	17	3	1	2	5	3	3	NO	NO
20	NO	NO	NO	NO	NO	NO	NO	NO	NO
21	21	12	9	NO	NO	NO	NO	NO	NO
Total	302 (100%)	145 (48.01%)	100 (33.11%)	24 (7.95%)	18 (5.96%)	4 (1.32%)	3 (0.99%)	2 (0.66%)	6 (1.99%)

*NO = Not operative *NP = Nulli parous *1p = 1- Parous
*2p = 2- Parous *3p = 3-Parous *4p = 4-parous
*5p = 5-Parous *6p = 6-Parous * sac = Sac dilatation

Host preference (HBI) was 6.25×10^{-3}(0.025×1/4). The expectation of infective life of *An. stephensi* female catches by human bait collection was 0.4, and 0.2 for *P. vivax* and *P. falciparum* respectively. The vectorial capacity of this species catches by human bait collection were 2.8×10^{-2}, and 1.4×10^{-2} for *P. vivax*, and *P. falciparum* respectively.

During the 21 round of the sampling at Essin area, 302 females were collected at human bait catches. Of total of the sample dissected, 145 (48%) were nulliparous, 100 (33%) were 1-parous, 24 (8%) were 2-parous, 18 (6%) were 3-parous, 4(1.3%) were 4-parous, 3(1%) were 5-parous, 2(0.7%) were 6 parous, and 6(2%) observed sac dilatation.

5. Discussion

This is the first report of the age determination of *An. stephensi* in southern Iran. The previous investigation was carried out on *An. maculipennis s.l*, in central Iran [13]. In spite of present anophelism without malaria in many parts of Iran since 1975, malaria is a major public health problem in Hormozgan Province. There are two distinct peaks of malaria transmission in this area, one in April/May and the other in September/October.

An. stephensi is the dominant anopheline and vector of malaria in Hormozgan Province. It is especially abundant in three counties of Bandar-Abbas, Minab, Jask, also Hormuz and Qeshm Islands. Based on morphological characteristics of the egg, *An. stephensi* includes three eggs phenotype: type, intermediate, and mysorensis. They are considered as biological forms [32]. Manouchehri *et al* (1976) reported the naturally infected with the parasite as the Sporozoite rates 0.2 - 1.8% in Hormozgan Province [3, 15].

Anopheles stephensi larvae found mostly in the range of larval habitats with a bed of clay, sand, stony, with or without vegetation, salty, sweet or brackish water, sunny, sunny or shady. The distribution of this species is restricted to the Zagros Mountains in southern Iran [15]. The range of distribution was limited to the Palearectic, Afrotropical and Oriental regions [9].

Behavioural studies of *An. stephensi* in the south of Iran have shown that it is highly zoophilic, although a wide range of anthropophily indices (0.5-47%) has been reported from the different geographical regions of Iran [15, 31, 32, 33, 34], and in India [35]. This species is considered to be endophagous and endophilic and is therefore, more likely to come into contact with the residual insecticides used in antimalaria spraying programs [15].

Taxonomic studies have revealed three biological forms of populations in this taxon, designated type, intermediate, and mysorensis forms. The type form reported the vector of urban malaria, whereas the mysorensis form is zoophilic and considered to be a rural vector species with poor vectorial capacity in India [32]. In India type form is vector of urban area and mysoransis is vector in rural area [14]. Of the three described biological forms, previously only mysorensis has been described from the south of Iran where it was incriminated as the main vector [3, 33]. Some experimental studies showed intra-specific variation in the reproductive capacity of *An. stephensi* but no evidence that this species constituted a species complex [14].

An. stephensi widespread in tropical Asia and the principal vectors of malaria the Middle East and South Asian region, Indian sub-continent and Arabian Peninsula, the Persian Gulf and southern Iran [13, 33, 34, 35].

Despite the regular house-spraying with residual insecticides, and larviciding of breeding places, *An. stephensi* showed two district peaks of population density during March-April and the other peak in September-October. Vatandoost *et al* (2006) reported that the second peak was higher than at the first [14].

Most of the populations of *An. stephensi* found with two main peaks of abundance; during the spring and autumn [36]. For example, in Punjab Province of Pakistan the spring peak from March to mid-May and the post-monsoon peak spans mid-September to mid-November, during the dry season. *An. stephensi* reported the main vector of malaria in southern Iran [14]. The main peak of *An. stephensi* was reported as in April-May, whereas the secondary peak fluctuated from August to November.

In parallel our study showed, *An.stephensi* is the main vector of malaria in general the highest indoor resting density of the anophelines catches, and it is also dominant anophelines species. Previous studies also confirmed the dominant species in the coastal area of Chabahar city [5].

In our study, date irrigation found the main breeding places, along with stream pools. During winter months, from October to April, stream pools the predominant larval habitat of *An. stephensi* in the most areas. In parallel, an agricultural region irrigated by rivers, deep wells, and cement pools, which are the main larval breeding sites of this species [14]. The minimum temperature for growth of larvae of this species reported between 17-26 °C. The duration of egg and larvae growing reported as 3, and 7, days respectively [34], however, the variety range of larval breeding places reported whole the year [5, 37].

In this area, the association of the species found with *An. fluviatilis*, *An. dthali*, *An. culicifacies*, *An. superpictus*, as the vectors of malaria and non-vectors such as *An. turkhudi* and *An. sargentii*. The low activity of *An.stephensi* found during the cold winter and hot summer periods.

The present study revealed considerable numbers of *An. stephensi* resting indoors in and pit shelters, with than 62.5% of females were semi-gravid and gravid blood-fed (Table 2). Also the study showed that the ratio of gravid/ freshly fed females caught in window exit traps, suggestion that the majority of endophagic females remained indoors for several days until becoming gravid. Previous investigations have shown it is considered to be endophagous and endophilic behaviors [32, 33, 36].

Our animal bait collections yielded 1.5 times *An. stephensi* females than human bait collections, whereas the previous studies determined the anthropophilic index of this species range between 0.5 to 47% has been reported from the different geographical regions of Iran [3, 15, 31, 32, 33, 35, 38].

The findings showed that the duration of the blood digestion and gonotrophic cycle of *An. stephensi* was 2.8, and almost four days, respectively. These observations are parallel with the results of previous study in southern Iran [33]. The gonotrophic cycle has been reported for *An. maculipennis* as four days [13], and the same for *An. arabiensis* in Mwea. Overestimate on gonotrophic cycle of *An. freeborni* in Sacramento Valley, *An. punctipennis* in Maryland and *An. quadrimaculatus* in Florida reported to be 4-6, 4-5, and five days respectively [39, 41].

The length of the oviposition cycle for *An. gambiae* and *An. merus* in the tropical area was two days. In fact, various temperature and humidity combinations were effects on the duration of the blood feeding and gonotrophic cycle. The additional time required for oviposition in Essin area may reflect the influence of environmental conditions especially mean temperature.

According to findings, Probability of daily survival of *Anopheles stephensi* in PSSC, animal, and human bait collection during gonotrophic cycle were 0.75, 0.84 and 0.9 respectively. These observations are consistent with the results of previous study in Iran and Laous (Vythilingam *et al*. 2003; 13Ghavami 2005). A significant difference was seen in the mean survival rates per oviposition cycle in the population of *An. maculipennis* s.l. caught from light traps (0.46) and pyrethrum spray catch (0.50) [13]. Probability of daily survival of *An. dirus*, *An. maculatus*, *An. minimus*, and *An jayporiensis* in wet season of Laous reported to be 0.85, 0.75, 0.77, and 0.86 respectively, whereas in dray, seasons were 0.91, 0.86, 0.77, and 0.89 respectively [42].

In the present study, the duration of the sporogonic cycle of *An. stephensi* for *P. vivax* and *P. falciparum* were 12.35, and 27.7 days at 23 °C respectively. Our finding was parallel by previous study in central Iran [13]. In parallel, the sprogony cycle of *P. vivax* in *An stephensi* reported in 16 °C, 21 °C, 27 °C, 33 °C as 18, 15, 11, 9 days respectively [43]. Sporozoite rates of this species reported to be between 0.2 and 1.8% in south of Iran [3, 33].

The duration of sporogonic cycle of *An. maculipennis* s.l. for *P. vivax* was 10 day at 25 °C [13]. This index for *An. Gambia s.l.* and *P. falciparum* reported 10 days at 27 °C, and 28 days at 20 °C [44]. In this study, the additional time required for Sporogony cycle may reflect the influence of mean temperature.

The findings showed the significant differences between expectation of life of *An. stephensi* catches by different techniques ranged from 3.5 to 9.5 days in Essin area ($P<0.05$). This index for *An. Gambia s.l.* was reported 7.4 days and 3.2 days for *An. pharoensis* at 27 °C [44]. According to findings, life infective expectancies of these species catches by the different method were ranged from 0.1 to 2.5 days (mean 0.4) for *P. vivax* and from 0.04 to 1.8 days (mean 0.2) for *P. falciparum*. In our investigation, the direct man biting rate (ma) was 11. Man biting rate for *An. Gambia s.l.* was reported 8.1 and 11.9 for *An. pharoensis* at Awasa area [18]. According to findings, the vectorial capacity of *An stephensi* catches by human bait collection were 2.8×10^{-2} and 1.4×10^{-2} for *P. vivax* and *P. falciparum* respectively. This index for *An. dirus*, *An. maculatus*, *An. minimus*, and *An jayporiensis* in wet season of Laous reported as 6.5, 0.91, 0.37, and 1.58 respectively, whereas in dray seasons were 2.37, 0.56, 0.05, and 1.33 respectively [42]. These observations are contrast with the results of our finding, because of the use of various anthropophobia indexes.

Our finding indicate that *An. stephensi* with 3 and 7 parous ages have potential and may live long enough to transmit the *P. vivax* and *P. falciparum* in Essin area respectively, this means that only 9% of female mosquitoes may live long enough to transmit malaria due to *P. vivax*.

In conclusion, malaria cases classified as imported and local transmission and there are no data about the other mosquito borne diseases, however, due to socioeconomic factors including, industrial and construction projects, urbanization, large movement of people between the neighboring provinces and countries, presence of five proven vector, progressive ageing among the population of *An. stephensi*, there is potential for outbreak of malaria. Since different reports revealed increasing the large displacement of the people providing the free and enough health facilities in the borderline between neighboring provinces and countries is necessary. Based on our findings, 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.

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