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Anopheles stephensi Biological Forms; Geographical Distribution and Malaria Transmission in Malarious Regions of Iran

M.A. Oshaghi, F. Yaaghoobi, H. Vatandoost, M.R. Abaei and K. Akbarzadeh
Department of Medical Entomology and Vector Control,
Institute of Public Health Researches, School of Public Health,
Tehran University of Medical Science, P.O. Box 6446-14155, Tehran, Iran

Abstract: The species biological form composition and their spatial distribution was studied in south of Iran using egg morphological characters. All three known biological forms of the species; *An. stephensi stephensi* (type), intermediate and *Mysorensis* occur in the country; however their distribution and roles in malaria transmission are varied in each province. In Hormozgan province, all three forms were present. The type and intermediate forms with respectively 90 and 10% frequency occurred in urban-coastal or semi urban/rural-plain areas where *An. stephensi* is highly dominant and is the sole vector of disease. *Mysorensis* was found only in rural-mountainous areas where malaria is transmitted by other vectors. This is in agreement with the conventional idea that type form is important only in urban settings. However, in Sistan and Baluchistan which is the most malarious region of the country, in various human settings (urban/rural) and different climates only *Mysorensis* form was found. In Fars (Kazeron) which was previously a malaria endemic area and *An. stephensi* was the major vector, only intermediate was found. The biological differences in feeding behaviour and the differing vectorial capacity that have been observed in south of Iran can be explained in terms of the availability or limitation of animals' hosts. The results of this study emphasize on independent studies in each region, precaution in generalization and on reconsidering the conventional view about *An. stephensi* biological forms in western outreaches of the Indo-Pakistan subcontinent.

Key words: *Anopheles stephensi*, type, intermediate, *Mysorensis*, malaria, Iran

INTRODUCTION

Malaria is still one of the main health problems in south of Iran. In last decade 10,000-50,000 of malaria cases were reported in the country which most of them occurs in south-east corner of Iran (MHME, 2002). According to recent information, a total of 18,000 cases were reported from these areas. Anopheles stephensi Liston 1901 is known to be an important urban malaria vector in the middle-east and Indian subcontinent (Krishnan, 1961; Manouchehri *et al*., 1976). This species is the most prevalent anopheline species in the malarious areas of Iran and is considered as a main malaria vector in the southern Iran (Manouchehri *et al*., 1976).

Based on egg morphological characters, three biological forms: *An. stephensi stephensi* (type), intermediate and *Mysorensis* have been reported in this species. The type form is known to be an efficient vector of urban malaria, whereas the *Mysorensis* form is considered rural species with poor vectorial capacity due to high zoophilic behaviour (Subbarao *et al*., 1987). Instead of variation in habitat and vectorial capacity of the forms, crossing experiments between geographical (Coluzzi *et al*., 1973) or biological forms (Subbarao *et al*., 1987) have shown that different strains of *An. stephensi* mated readily and produced viable offspring without any post-capulatory barriers or male sterility.

Except for a few studies on the composition of *An. stephensi* biological forms in some south parts of Iran (Mesghali 1961), the composition of biological forms of this important species in Iran particularly in the malarious regions of the country is not known yet. This study was conducted to determine the composition of *An. stephensi* biological forms in three southern provinces of the country; Sistan and Baluchistan, Hormozgan and Fars which the first two ones are the most important malarious regions in the country (Edrissian, 2002).

MATERIALS AND METHODS

Study area: Study area comprised three provinces of Sistan and Baluchistan, Hormozgan and Fars. Details of selected location for sample collection is shown

Corresponding Author: M.A. Oshaghi, Department of Medical Entomology and Vector Control, Institute of Health Research, School of Public Health, Tehran University of Medical Sciences, P.O. Box 6446-14155, Tehran, Iran
Tel: 0912 3878837, 02-8951393 Fax: 021-6462267

Table 1: Details of sample collection sites including province, kind of settings and climate region for *Anopheles stephensi* in this study

Province	Settings	Climate	Location Name
Hormozgan	Urban	Coastal	Bandar-abbas
	Rural	Mountainous	Kooveh
	Rural	Mountainous	Siahoo
	Semi	Plain	Geno
	urban/rural	Plain	Roodeshore
Sistan and	Urban	Coastal	Chahbahar
Baluchistan	Urban	Mountainous	Rask
	Rural	Coastal	Garkendi
	Rural	Coastal	Oraki
	Rural	Plain	Hossein-abad
	Rural	Hilly	Zeinoldini
	Rural	Mountainous	Piridin
	Semi-	Hilly	Gasre-ghand
	urban/rural	Plain	Bampour
Fars	Rural	Plain	Dadin sofla (Kazeron)

in Table 1. In Sistan and Baluchistan province, Baluchistan includes more than 60% of malaria cases of the country. Baluchistan climatologically has four different areas, coastal in the south, hilly and mountainous in the middle and plain in the north. Four counties of Chahbahar, Sarbaz, Nickshahr and Iranshahr in Baluchistan were selected as coastal, mountainous, hilly and plain areas respectively. In each area an urban, rural and semi-urban/rural location (if existed) were selected to cover various human settings. Hormozgan province includes 20% of malaria cases of the country. This province climatologically has three different areas, coastal in the south, mountainous in north and plain (plateau) in the middle. Bandar Abbas city as an urban coastal, Geno as rural plain and Siahoo and Kooveh as rural mountainous area were selected for this study. In Fars province, samples were collected from Kazeron county, where in the past, malaria was endemic and the disease was transmitted by *An. stephensi*. This county is located in northwest of Hormozgan province. The selected locations in Hormozgan and Sistan and Baluchistan have been the main malarious zones in each province in the last three years and were elected based on the available epidemiological data about the presence of *An. stephensi* in each province.

Sample collection: Sample collections were carried out in two peak of activity in April-June and September-November, 2003 monthly. Larvae were sampled using standard dippers (500 mL) from breeding places close to adult collection sites. Larvae were carefully transported alive to the insectary and were reared to get adult stage. Adult collections were performed using various methods including pit shelter, night biting on animal and human, hand catch using aspirator from indoors of human and animal shelters. All the specimens including blood fed or unfed mosquitoes caught from field were transported alive to the insectary. All the unfed females were kept in cages and fed on Guinea pig.

Egg morphological characteristics: Blood fed female mosquitoes individually were transferred into separate glass tubes (15 cm length and 2 cm diameter) including wet filter paper and let them lay eggs. Morphological characteristics of 10-20 eggs from each female including length, breadth and number of ridges on the egg float were measured under compound microscope. Measurement was carried out using an ocular micrometer in a stereoscopic microscope at 15X power and the number of ridges along 1 side of the egg float was counted at the same time. The ocular micrometer has been calibrated at regular intervals (Garcia, 2001). Based on criteria described by Sweet and Rao (1937) and Subbarao *et al*. (1987), eggs with 10-15, 15-17 and 17-22 ridges designated as *Mysorensis*, intermediate and type form, respectively. The means and standard error of the egg morphological characters for each batch of eggs were computed and based on characters described above were categorized.

RESULTS

***An. stephensi* biological forms in Sistan and Baluchistan:** Eggs of specimens successfully prepared from different sample collection methods, larval breeding places and adult resting sites (indoors/outdoors and human/animal shelters), on human and animals baits and throughout the night. With respect to egg and ridge length and number of ridges on the egg floats only *An. stephensi Mysorensis* was found in Sistan and Baluchistan province. It is the first report on existing of *An. stephensi Mysorensis* form in the province. Interestingly, in all urban, semi-urban/rural, or rural areas and also in all different climatologically regions including coastal, plain, hilly and mountainous regions only *Mysorensis* were found and none of other two biological forms, type and intermediate, were found. The ridge number of eggs ranged from eleven to fifteen with a maximum percentage of eggs falling between 12 and 13 which are similar to the *Mysorensis* form described by Sweet and Rao (1937) and Subbarao *et al*. (1987).

***An. stephensi* biological forms in Hormozgan:** With respect to egg and ridge length and number of ridges on the egg floats all three known biological forms of *An. stephensi*: type, *Mysorensis* and intermediate, were found in Hormozgan province. It is the first report on existing of *An. stephensi* type and intermediate forms in

Table 2: Egg morphological characteristics of *Anopheles stephensi* biological forms from Hormozgan, Sistan and Baluchistan and Fars provinces Iran which are compared with the known forms of India, Pakistan and other southern parts of Iran

Biological form	No. of Ridges Mean±SD * =mode	Ridge length (μ) Mean±SD	Legth (μ) Mean±SD	No. of eggs examined	Region studied (Reference)
Mysorensis	13.6±1.30	225.2±23.70	468.88±19.40	129	Hormozgan, Iran
Intermediate	15.4±0.89	255.8±13.80	542.10±18.50	81	(present study)
Type	18.7±1.70	314.8±24.24	596.52±24.70	513	
Mysorensis	12.55±0.73	198.00±18.2	470.60±27.00	3870	Baluchistan, Iran (present study)
Intermediate	15.11±1.27	248.20±6.74	544.00±8.07	120	Fars (Kazeron),Iran (present study)
Type	17.82±1.55	289.65±22.25	548.06±26.59	4707	India, (Sweet and Rao, 1937)
Mysorensis	13.50±1.25	218.08±20.44	477.12±23.87	6916	
Mysorensis	13.87±1.15	217.58±20.30	482.83±19.13	1472	Pakistan, (Afridi *et al*., 1958)
Mysorensis	12.05	231	448.5	452	South of Iran (Masghali, 1961)
Mysorensis	10-14*	Not indicated	Not indicated	1030	India, (Subbarao *et al*., 1987)
Intermediate	13-16*			989	
Type	16-19*			667	

the region and Iran. In urban-coastal and rural-plain areas two biological forms, type and intermediate, were found sympatrically, but no *Mysorensis* was found. The ridge number of eggs ranged from fourteen to twenty-one in which most of eggs (90%) falling between 17 and 21 and the rests falling between 14 and 17. These measures fit the descriptions of respectively type and intermediate forms as reported by Sweet and Rao (1937) and Subbarao *et al.* (1987) (Table 2). The type form was highly predominant in both urban-coastal and rural-plain areas and constitutes 88-89% of samples.

In mountainous area all of the samples were *Mysorensis* form. Samples collected from mountainous regions, ridge number ranged from eleven to fifteen with a maximum percentage of eggs falling between 12 and 13 which is similar to the *Mysorensis* form described by Sweet and Rao (1937) and Subbarao *et al.* (1987).

***An. stephensi* biological forms in Fars:** Morphological characters of eggs collected from specimens showed that only *An. stephensi* intermediate was found in Kazeron. It is also the first report on presence of *An. stephensi* intermediate form in the region. Mosquito samples were caught from a rural plain area. Interestingly, neither type nor *Mysorensis* were found in the region. The ridge number of eggs ranged from fourteen to seventeen with a maximum percentage of eggs falling between 15 and 16 which are similar to the intermediate form described by Subbarao *et al.* (1987).

DISCUSSION

This study showed that the composition and the role of *An. stephensi* biological forms in malaria transmission were quite different in each province. In southeastern part of the country, Sistan and Baluchistan province which is close to Pakistan borderline, different climates or human settings has no effect on the composition of *An. stephensi* biological forms and only *Mysorensis* form was found. This is not in agreement with the conventional idea described by Indian counterparts indicating that *Mysorensis* is a rural species with poor vectorial capacity (Subbarao *et al*., 1987). This study showed that *Mysorensis* could breed and inhabit in urban and semi-urban as well as rural areas. Of course different climates could affect on the prevalence of the species and in coastal and plain areas *An. stephensi* was more abundant than in hilly and mountainous areas but had no effect on its composition. More importantly, in contrast to the Indian counterparts, the available epidemiological data in Sistan and Baluchistan province showed that *An. stephensi* is a malaria vector (Gavami and Zaim, 1996; Moradi, 2002; Oshaghi *et al*., 2004). Also previous studies (Manouchehri *et al*., 1974; Eshghy and Janbakhsh, 1976; Eshghy, 1978) have shown that *Mysorensis* is one of the main malaria vector in some southwest parts of Iran. Similar situation has been found in neighboring country Afghanistan (Rowland *et al*., 2002), where the authors suggested that the conventional view of *An. culicifacies* being the main rural vector and *An. stephensi* important only in urban settings needs to be reconsidered in western outreaches of the Indo-Pakistan subcontinent.

This study reveals that in urban and semi-urban areas of Hormozgan with coastal and plain features *An. stephensi* type form is predominant which is in agreement with the known idea indicating that *An. stephensi* type is an efficient malaria vector in urban regions. The high prevalence of type form in those regions is in concordance with the available

epidemiological data indicating that this species is the most important and the sole vector of malaria in urban and semi-urban areas of Hormozgan province (Manouchehri *et al*., 1979).

Previous studies have shown that *An. fluviatilis*, *An. culicifacies*, *An. dthali* and *An. superpictus* play the main role in malaria transmission in mountainous areas of Hormozgan (Motabar *et al*., 1975; Manouchehri *et al*., 1976). Having found only *Mysorensis* in mountainous areas and its high zoophillic rate is in agreement with the idea that *Mysorensis* is a rural species with poor vectorial capacity (Subbarao *et al*., 1987). Availability of animals in rural areas, relatively short life span of *An. stephensi* in mountainous areas and highly prone to expose to indoor residual insecticides due to it's highly endophilic behavior are the main factors for poor vectorial capacity of *Mysorensis* in the rural-mountainous region.

In Fars province, only intermediate form was found. In past *An. stephensi* was incriminated as the main vector of malaria in Kazeron (Eshghy and Janbakhsh, 1976; Eshghy, 1978). Therefore, again in contrast to the conventional view, intermediate form also could act as a malaria vector.

As a conclusion, having found variation in role of malaria transmission of *An. stephensi* biological forms in south of Iran can be explained in terms of the availability or not of animals hosts in each locality and again highlights the need for independent study in each region and precaution before generalization. Also it needs to reconsider the conventional view about geographical distribution and role of *An. stephensi* biological forms in malaria transmission in western outreaches of the Indo-Pakistan subcontinent.

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