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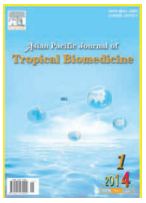

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Molecular characterization, biological forms and sporozoite rate of *Anopheles stephensi* in southern Iran

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PEER REVIEW

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Comments

This is a good study that revealed the biological forms, sporozoite rate, and molecular characteristics of *An. stephensi* populations originated from different parts of the provinces in Iran. The data may represent a useful contribution to our knowledge on biological forms of *An. stephensi* and their sporozoite rate in southern Iran.

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ABSTRACT

Objective: To identify the biological forms, sporozoite rate and molecular characterization of the *Anopheles stephensi* (*An. stephensi*) in Hormozgan and Sistan-Baluchistan provinces, the most important malarious areas in Iran.

Methods: Wild live *An. stephensi* samples were collected from different malarious areas in southern Iran. The biological forms were identified based on number of egg-ridges. Molecular characterization of biological forms was verified by analysis of the mitochondrial cytochrome oxidase subunit I and II (mtDNA-COI/COII). The *Plasmodium* infection was examined in the wild female specimens by species-specific nested-PCR method.

Results: Results showed that all three biological forms including mysorensis, intermediate and type are present in the study areas. Molecular investigations revealed no genetic variation between mtDNA COI/COII sequences of the biological forms and no *Plasmodium* parasites was detected in the collected mosquito samples.

Conclusions: Presence of three biological forms with identical sequences showed that the known biological forms belong to a single taxon and the various vectorial capacities reported for these forms are more likely corresponded to other epidemiological factors than to the morphotype of the populations. Lack of malaria parasite infection in *An. stephensi,* the most important vector of malaria, may be partly due to the success and achievement of ongoing active malaria control program in the region.

KEYWORDS

Anopheles stephensi, Mysorensis, Type, Intermediate, mtDNA markers, Molecular systematic, Iran

1. Introduction

Malaria is the most important vector borne disease in the world with 0.8-1 million deaths annually[1]. Also in Iran, malaria is one of the most important health problems that more than 2 million people of the country live in high risk area and are at risk[2]. In spite of elimination of malaria from most parts of Iran, the disease is still one of the infectious diseases in the country with more than average 15 000 annual cases in the last decade. Most of these cases occurred in south and southeast of the country where malaria control programs are still practiced. Recently the trend of malaria showed a notable decrease in malaria cases, but still cases are reported from Sistan-Baluchistan, Hormozgan and Kerman provinces. Five proven malaria vectors *Anopheles stephensi* (*An. stephensi*), *Anopheles culicifacies s.l.*, *Anopheles fluviatilis s.l.*, *Anopheles dthali* and *Anopheles superpictus s.l.* have been recorded in the southern endemic

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foci of the country including Hormozgan and Sistan-Baluchistan provinces[3]. Among them, *An. stephensi* is the primary vector of malaria in the region and other species act as a secondary vector and significantly prefer human blood[4-6]. The sporozoite rates in India were reported to be 3.6% for *An. stephensi*[7].

Based on egg-float ridge numbers, *An. stephensi* comprises three biological forms including type, intermediate and mysorensis[8]. *An. stephensi* type is an urban form whereas the mysorensis and intermediate forms are rare in urban areas[9-11]. These three forms have shown different vectorial capacities, each one has its own characteristics in malaria transmission in a particular region[4,11]. These facts compel the medical entomologists to study different populations of *An. stephensi* independently in each malaria focus.

Emergence of new powerful tools such as molecular methods enable researches to use molecular markers to solve problems in malaria epidemiology such as identification of closely related species (species complexes, siblings) or populations, detection of pathogens in vectors, and blood feeding behavior of vectors[12-16].

Mitochondrial markers, especially cytochrome oxidase subunit I and II (COI and COII), are amongst the most powerful and reliable molecular markers to distinguish closely related species, or biological forms. Both COI and COII have been used in other arthropods for different purposes[14,17,18].

Direct microscopy method for detection of malaria parasites in *Anopheles* salivary glands was routine. This method needs a high level of technical specialty and quick dissection and test. Because of degeneration of parasites in a short time after the dissection of mosquitoes, direct detection of malaria parasites would be impossible.

During the time, other biochemical-based methods like ELISA were employed[19,20]. Technical difficulties, need for several reagents and instruments, their low sensitivity and specificity lead researchers to develop molecular methods, which have wide range usage in this field and have been employed by several researchers[21,22].

Several malaria control programs such as Indoor residual spraying, Insecticides treated nets, rapid disease detection and treatment, environment management and disruption of mosquitoes larval breeding places have been designed to decrease the burden of the disease in southern provinces in Iran[2,23]. However, the key point for disease control programs is the continuous monitoring and periodically evaluation of mentioned programs. Determination of *Plasmodium* parasite species, sporozoite infection rate, as well as *Anopheles* species composition and distribution are crucial factors for evaluating control programs. This study was conducted to reveal the biological forms, sporozoite rate, and molecular characteristics of *An. stephensi* populations originated from different parts of the provinces. The results of this study will be useful to evaluate the ongoing malaria control programs, and will help decision making persons involved in these programs.

2. Materials and methods

2.1. Study area and mosquito collection

Hormozgan and Sistan-Baluchistan provinces as the main malarious areas are located in southern part of Iran. These provinces are located in northern coast of Persian Gulf and Oman sea (Figure 1), where the weather is warm and humid enough for *Anopheles* species to be active throughout the year. This situation makes *An. stephensi* the main vector responsible for transmission of malaria to human in southern Iran.

Figure 1. Map of Iran and location of Hormozgan and Sistan-Baluchistan Provinces.
Study areas: 1: Bandar-Abbas, 2: Harmoodar, 3: Minab, 4: Bashagard, 5: Iranshahr, 6: Bampoor, 7: Kahiri, 8: Sarbaz.

Wild caught samples were collected form Bandar-Abbas (capital city of Hormozgan Provice), Harmoodar, Minab, Bashagard, Iranshahr, Bampoor, Kahiri and Sarbaz (Figure 1) by hand-catch indoors and pit shelters outdoors. Collected samples were identified using the standard key[24]. For molecular investigations 120 samples (65 from Sistan-Baluchistan and 55 from Hormozgan provinces) were collected twice during the transmission season (May-September 2010).

2.2. Determination of biological forms

Alive collected samples were transferred to Bandar-Abbas and Iranshahr insectariums. The gravid or blood fed females were kept individually in glass tubes with a dump paper at the bottom allowed to lay eggs. The rest of the mosquitoes were colonized using the standard protocol. They fed on guinea pigs and again females were kept individually to lay eggs. At least 15 females from every region were kept to lay eggs. A total of 10 eggs per female were collected and the egg-float ridge numbers were counted using Stereo-Microscope and categorized based on the described criteria for the biological forms[8].