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RESEARCH ARTICLE

Evaluation of the VecTest™ Malaria Antigen Panel Assay using *Anopheles sacharovi* Specimens in an Endemic Area, Sanliurfa Province, Turkey

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Summary

In recent years, malaria is located in southeastern Anatolia of Turkey. Because of no information is available about sporozoite rates in the mosquitoes in Turkey, *Anopheles (An.) sacharovi* were collected in Sanliurfa province and examined by the VecTest™ Malaria Antigen Panel Assay , a rapid immunochromatographic assay intended for the qualitative determination of three *Plasmodium* circumsporozoite antigens (*P. falciparum, P. vivax* 210, *P. vivax* 247) in infected Anopheline mosquitoes. *Anopheles sacharovi* specimens were collected using CDC light traps in 5 villages belonging to Sanliurfa province in July 2004. The pools containing 10 mosquitoes were prepared and totally 390 *Anopheles sacharovi* females were used for the Vectest™. Test was performed in the field conditions just after collection. The positivity was observed only in one pool and *P.vivax* 247 antigen line. Infection rate was detected as 0.25% in mosquitoes. The detection of *P.vivax* 247 antigen in An. sacharovi is an important natural evidence of vector capacity of this species for *P. vivax* variant 247 in Turkey. Results were suggestive of most likely involvement of *An. sacharovi* in malaria transmission in Sanliurfa province.

Keywords: *Anopheles, Plasmodium vivax, Variant 247, Turkey*

VecTest™ Sıtma Antijen Panel Yönteminin Sıtmanın Endemik Olduğu Şanlıurfa'dan Toplanan *Anopheles sacharovi* Örnekleri Kullanılarak Değerlendirilmesi

Özet

Son yıllarda sıtma genelde Güneydoğu Anadolu'da lokalize olmaya başlamıştır. Türkiye'deki sivrisineklerde sporozoit oranı hakkında veri bulunmadığı için Şanlıurfa'da *Anopheles (An.) sacharovi* örnekleri toplanmış ve enfekte Anopheline sivrisineklerde üç Plasmodium sirkumsporozoit antijenini (*P. falciparum, P. vivax* 210, *P. vivax* 247) kalitatif olarak saptayan bir test olan VecTest™ Sıtma Antijen Panel Yöntemi ile incelenmiştir. *Anopheles sacharovi* örnekleri CDC ışıklı tuzaklarla Şanlıurfa iline bağlı 5 köyden Temmuz 2004'de toplanmıştır. Yakalanan toplam 390 *An. sacharovi* dişisi herbiri 10 örnekten oluşan havuz yapılarak teste tabi tutulmuştur. Test, toplamanın hemen sonrasında saha koşullarında yapılmıştır. Pozitiflik sadece bir havuzda ve *P. vivax* 247 antijen çizgisinde gözlenmiş ve buna göre de sivrisineklerdeki infeksiyon oranı %0,25 olarak belirlenmiştir. *Plasmodium vivax* 247 antijeninin saptanması, Türkiye'de *P. vivax* varyant 247 antijen için bu türün vektöryal kapasitesinin doğal kanıtı olmasından dolayı önemlidir. Sonuçlar, *An. sacharovi*'nin Şanlıurfa ilinde sıtma bulaşımındaki rolünü göstermektedir.

Anahtar sözcükler: *Anopheles, Plasmodium vivax, Varyant 247, Türkiye*

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INTRODUCTION

More than 2 billion people are at risk of malaria, which primarily affects poor populations in tropical and subtropical areas, where the temperature and rainfall are most suitable for the development of the malaria-causing *Plasmodium* parasites in *Anopheles* mosquitoes. Four *Plasmodium* species, *Plasmodium falciparum, P. vivax, P. malariae* and *P. ovale* cause malaria in humans. Recently, a fifth species, *P. knowlesi* which was originally described as a malaria parasite of long-tailed macaque monkeys, was also reported as a causative agent of natural human malaria infection [1].

Malaria parasites are transmitted by more than 70 species of *Anopheles* mosquitoes world-wide but, in each malarious area, only a few species serve as vectors while the majority of *Anopheles* females are not infected [2]. The climatic conditions in Turkey are suitable for malaria vectors to proliferate. Agricultural infrastructural changes, Southeastern Anatolian Dams and Irrigation Project, insufficient environmental conditions, urbanization, national and international population moves are thought to be key factors that can contribute to malaria control. In recent years, malaria is located in south-eastern Anatolia. Sanliurfa, Batman, Diyarbakir, Siirt, and Mardin provinces are the most affected areas. In western provinces of Anatolia, like Aydin and Manisa, an increase in the number of autochthonous cases might be observed from time to time. This is due to workers moving from malaria districts to western parts in search of job opportunities.

The number of malaria cases is lowest in winter and reaches its peak in summer and autumn. In the past years, the comprehensive malaria prevention program has started bear fruits. The program was very successful and malaria cases decreased from 84.345 in 1994 to 796 cases in 2006.

Almost all malaria cases are caused by *P. vivax* in Turkey. There is also imported *P. vivax* and *P. falciparum* cases. The districts where malaria cases occur are the places where there is high population movement, agriculture is the main occupation, the increase in the population is high and the education/cultural level is low. The conventional method for the diagnosis of malaria has relied on the microscopical examination of Giemsa stained blood smears in local malaria centers.

Environmental factors, behavioral patterns of vectors and movement of human populations combine to provide favorable conditions for malaria transmission. The most important vector species is *Anopheles sacharovi* Favre, 1903 in Turkey. This species has wide distribution throughout Turkey. Nevertheless, Kasap et al.[3] in 1987 and De Sousa et al.[4] proved under laboratory conditions that *Anopheles superpictus* Grassi, 1899 is also a vector of the human malarias *P. vivax* and *P. falciparum*.

Detection of advance-stage sporozoites in mosquitoes provides evidence incriminating particular vector species. Traditional methods of determining sporozoite rates by direct dissection of fresh mosquito salivary glands [5] or by using a parasite antigen capture enzyme linked immunosorbent assay (ELISA) procedure [6] is relatively time consuming, requires specialized equipment and trained personnel.

Bangs et al.[7] evaluated a dipstick assay for the determination of specific circumsporozoite protein (CSP) of *P. falciparum, P. vivax* 210, or *P.vivax* 247 .

The VecTest™ Malaria Panel Assay is a rapid immuno-chromatographic assasy intended for the qualitative determination of three Plasmodium circumsporozoite antigens (*P. falciparum, P. vivax* 210, *P. vivax* 247) in infected Anopheline mosquitoes. In this study, we used the VecTest™ Malaria Panel Assay in a preliminary study for the detection of the risk of malaria transmission in Sanliurfa province, most endemic malaria site in Turkey.

MATERIAL and METHODS

Mosquitoes were collected using CDC light traps in 5 localities belonging to Sanliurfa province (villages of Sekerli, Mezra, Sandi and Pamuklu; Birecik town) in July 2004. In total, 390 *An. sacharovi* females were used for the VecTest™ (Medical analysis Systems, Inc., CA, USA, MAS™). Test was performed in the field conditions just after collection.

The mosquitoes were identified to species and pooled into groups of 10 mosquitoes and placed in eppendorf tubes. In total, 20 pools from Sekerli village, 9 pools from Birecik village, 5 pools from Birecik town, 4 pools from Pamuklu village and 1 pool from Sandi village were prepared. Following the kit's instructions, 13 drops (250 µL) of the grinding solution provided in the dropper bottle were added to the sample. The mosquitoes were ground by hand using the grinding pestle provided in the kit. The pestles were washed twice with phosphate buffered saline - Tween 20 (PBST) and wiped clean with a tissue between samples. Strips were placed into the mosquito suspensions in the grinding tube and results were read after 15 min by two people and the photographs were taken in 15 min. Results were not accepted as true after 30 min.

RESULTS

In this study, we found a *P. vivax* 247 antigen positivity *(Fig. 1)* in one pool of *An. sacharovi* mosquitoes collected from Birecik town (37° 1' 46 N; 37° 59' 25E; alt. 411 m) located in east part of Sanliurfa province. The minimum sporoziote rate among An. sacharovi was found to be 0.25% (1/390).

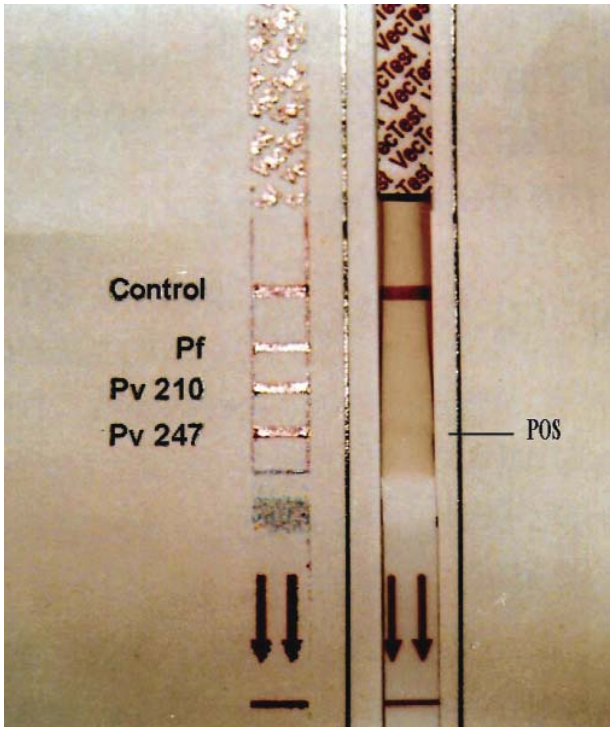

Fig 1. The VecTest showing positivity in the *An. sacharovi* mosquitoes collected from Birecik village, Sanliurfa

Şekil 1. Şanlıurfa Birecik köyünden toplanan *An. sacharovi* sivrisineklerinde VecTest ile saptanan pozitifliğin gösterilmesi

DISCUSSION

Plasmodium vivax is the most geographically widespread and prevalent malaria parasite in some regions, which accounts annually for 70-80 million clinical cases across much of the tropics and subtropics of the world. The *Csp* sequences of *P. vivax* are of two types, VK210 and VK247, which differ in the amino acid composition of the central repetitive region of the gene, but also by three diagnostic amino acid replacements, one in each of the 5-and 3- terminal regions of the gene and the third in an insertion region (IR) between the central repeat (CR) and the 3- terminal region. Both *P. vivax* variants are present in the New and Old World [8]. On this subject, there is only one study showing the presence of *P. vivax* variant 210 and *P. vivax* variant 247 variants in human malaria patients in Turkey. In Sanliurfa province, 88 malaria patients were diagnosed as *P. vivax* by microscopy and PCR. Among these patients, variant 210 and variant 247 genes were determined in 65.5% and 34.5% of the samples by Restriction Fragment Lenght Polymorphism (RFLP) analysis after PCR, respectively. There was no mixed variant type in any patients [9].

It is known that the CS ELISA is considered to be reference method of choice or gold standard for malaria parasite detection in mosquitoes. It is specific for *P. falciparum* and *P. vivax* variant 210 and variant 247. In our study, CS ELISA could not be performed because of some logistic reasons. But, previous studies [2,7,10,11] showing detailed results about comparison between CS ELISA and the VecTest™ were demonstrated that VecTest™ is sensitive, specific, simple to use, rapid, can identify mixed infection (v210 and v247) and can be used in the field. The 100% positive correlation between CS ELISA and VecTest™ was also shown in different studies [2,7,10]. We are agree with Ryan et al.[2] that the quick and easy dipstick assay offers practical advantages for field workers and performs at an acceptable level for testing the mosquitoes in the field.

In most malaria endemic situations more than one species of *Anopheles* may transmit *P. falciparum* and *P. vivax,* although the relative status and bionomics of each species of vector remains poorly known in many localities [2]. In order to understand the epidemiology of the disease, there is clearly a need for a field assay capable of rapidly detecting *Plasmodium* infected mosquitoes. Malaria rapid panel (MRP) assays were developed to be capable of detecting several different species and variants of human malaria sporozoites in mosquitoes. Sensitivity of the MRP was demonstrated as roughly equivalent to the original CSP ELISA assays that it is based upon. MRP assays can detect Pf, Pv210 and Pv247 from the same Anopheles specimen with a sensitivity of at least 1ng/mL (200 pg) of antigen of any of the three CS proteins. A complete correlation was found between VecTest™ and CS protein ELISA for detection of Pf, Pv210 and Pv247 malaria sporozoites in field-caught Anopheles mosquitoes of several species [10].

In a previous study, three species of genus *Anopheles*, four species of genus *Culex* and two each of genus *Culiseta* and genus *Ochleratatus* were collected in Sanliurfa Province. Of the total *Anopheles* collected, *An. sacharovi* was found to be the most abundant (72%) species [12]. The vectorial importance of *An. sacharovi* was highlighted in the previous papers [13,14]. In the present preliminary study, the detection of *P. vivax* 247 antigen in *An. sacharovi* specimens collected in Birecik town is an important natural evidence of vector capacity of this

species for *P. vivax* variant 247 in Turkey. If we accept that only one mosquito among 10 mosquitoes in the pool is infected by sporozoites, the mosquito infection rate will be calculated as at least 0.25%. There is a need to perform VecTest™ dipstick analysis, CS-ELISA or molecular studies using more *An. sacharovi* specimens as well as other *Anopheles* species from different endemic areas in Turkey in order to better understand; (i) the infection rate in the mosquitoes, (ii) the role in transmission of the other *Anopheles* species present in this region for both variants, VK210 and VK247 and (iii) epidemiology of the disease.

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