

***ANOPHELES SERGENTI* (THEOBALD) A POTENTIAL MALARIA VECTOR IN EGYPT¹**

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SUMMARY. Two immunoassays for malaria sporozoite detection and identification, the immunoradiometric assay (IRMA) and the enzyme-linked immunosorbent assay (ELISA) using the species-specific monoclonal antibodies are routinely performed in our laboratory. We analyzed (573) anopheline mosquitoes of *A. sergenti* (463), *A. pharoensis* (81) and *A. multicolor* (29) collected from Siwa-oases and Faiyum Governorate (two known active malaria foci in Egypt), for detection of *P. falciparum* and *P. vivax* sporozoites.

P. falciparum sporozoites were detected by both IRMA and ELISA tests in two *A. sergenti* mosquitoes (one from Siwa 1/389 = (0.26 %) and one from Faiyum Governorate 1/74 = (1.35 %)). No *P. vivax* sporozoites were detected. This finding is important in explaining the malaria transmission and provide first incrimination of *An. sergenti* as the responsible vector of malaria in Siwa-oasis, Egypt.

Key-words: *Anopheles sergenti*. Egypt.

***Anopheles sergenti* (Theobald) vecteur potentiel du paludisme en Égypte.**

RÉSUMÉ. La détection et l'identification de sporozoïtes responsables de la transmission du paludisme sont couramment effectuées dans notre laboratoire à l'aide de deux tests immunologiques : le test immunoradiométrique (IRMA) et l'ELISA. Nous avons ainsi testé la présence de sporozoïtes de *P. falciparum* et *P. vivax* chez 573 anophèles, dont 463 *A. sergenti*, 81 *A. pharoensis*, 29 *A. multicolor*. Ces moustiques ont été capturés, soit à l'Oasis de Siwa, soit à celle de Fayoum, ces deux oasis présentant un paludisme endémique.

Les tests immunologiques IRMA et ELISA ont permis de détecter des sporozoïtes de *P. falciparum* chez deux *A. sergenti*, l'un provenant de Siwa 1/389 (= 0,26 %), l'autre de Fayoum 1/74 (= 1,35 %). Des sporozoïtes de *P. vivax* n'ont pas été observés. Ces résultats sont importants car ils permettent d'incriminer *A. sergenti* à Siwa, comme responsable de la transmission du paludisme.

Mots-clés : *Anopheles sergenti*. Égypte.

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Introduction

For a long period of time incrimination of malaria vectors in Egypt was based on salivary glands dissections for sporozoite detection. In spite of several trials, only few were successful in incriminating the responsible vectors and determining their sporozoite rate due to the low efficiency of Egyptian anophelines in transmitting malaria.

Only two malaria vectors have been implicated in Egypt. *A. pharoensis* was only found with sporozoite rates ranging between 0.33 and 1.4 % (Madwar, 1936; Barber and Rice, 1937; El Said *et al.*, 1983 and El Said *et al.*, 1986). *A. sergentii* was found carrying sporozoites on only two occasions; in the Nile Delta (Farid, 1940) and in Faiyum Governorate (El Said *et al.*, 1986). In spite of its abundance in oasis (e. g. Siwa oasis) it has never been caught infected with sporozoites and its role as the major vector of malaria was presumed according to the malaria history in Egypt (Barber and Rice, 1937; Halawani and Shawarby, 1957), entomological and parasitological observations (Kenawy *et al.*, 1986), and its role as a vector in other countries of the Middle East (Farid, 1956).

Recently, two immunoassays, the immunoradiometric assay (IRMA) (Zavala *et al.*, 1982) and the enzyme—linked immunosorbent assay (ELISA) (Burkot *et al.*, 1984 and Writz *et al.*, 1985) using the species-specific monoclonal antibodies against *P. falciparum* and *P. vivax* sporozoites, have made possible efficient processing of large number of mosquitoes to determine sporozoite rates.

This study was planned to determine the sporozoite rate in field collected mosquitoes from Siwa oasis and Faiyum Governorate using these immunoassays.

Materials and methods

MOSQUITOES

Mosquitoes were collected in 1983 and 1984 from Faiyum (Ca. 90 km southwest of Cairo) and Siwa-oasis, Egypt (Ca. 600 km south-east of Cairo). Collection sites and techniques were described in details by El Said *et al.*, 1986 and Kenawy *et al.*, 1986. Indoor collections were made by space spraying with pyrethroid insecticide using the index sheet technique. Outdoor resting mosquitoes were collected from representative habitats by aspiration (Nasci, 1981). Biting mosquitoes were collected by using human baited traps. Mosquitoes were stored at -70°C until tested.

TESTING PROCEDURES

1 — MOSQUITO EXTRACT

To each microfuge tube containing a single dried mosquito, 30 μl of blocking buffer (BB) for (1 liter: 10 g BSA, 5.0 g Casein; 0.1 g Thimersal; 0.01 g Phenol red; 1,000 ml phosphate buffered saline (PBS), pH 7.4) with Nonindent P-40

(NP-40) (5 μ l NP-40/1 ml BB) was added. This mixture was incubated for 1 hour, grinded with a pellet pestle (Knotes scientific glassware/instruments, Vineland, New Jersey 08360) diluted to a final volume of 160 μ l with BB and then kept at -20° C till examination.

2 — MONOCLONAL ANTIBODIES (Mabs)

Both the immunoradiometric (IRMA) and the enzyme-linked immunosorbent (ELISA) assays were performed to analyse the 573 anopheline mosquitoes collected from Siwa oasis and Faiyum Governorate for detection of *P. falciparum* and *P. vivax* sporozoites. Assays were performed during October and November 1986. Positive specimen were retested for confirmation. Positive and negative controls were run on each plate.

3 — IRMA

Aliquots of 30 μ l from each sample were placed into the wells of a polyvinyl chloride microtiter U shaped plate (Dynatech laboratories Inc., Alexandria, Virginia) and incubated for 2 hours at room temperature. The plates were previously coated with the anti-*P. falciparum* Mab, 2A10 (0.1 μ g/50 μ l/well) or anti-*P. vivax* Mab, 2F2 (0.025 μ g/50 μ l/well), incubated overnight at room temperature and washed 3 times by PBS/BSA before use. The same monoclonal antibodies (2A10 and 2F2) labelled with 125 I at a final count of (10,000 cpm for *P. falciparum* and 24,000 cpm for *P. vivax* in PBS/BSA with 10 % human serum) was then added and allowed to incubate. After an hour of incubation and washing cycle of three times, wells were cut and counted using Mini-assay type 6-20 gamme counter.

4 — ELISA

The ELISA technique was performed according to the method described by Burkot *et al.*, 1984 for *P. falciparum* sporozoites detection and modified by Wirtz *et al.*, 1985, for *P. vivax* sporozoite detection. Briefly, wells of polyvinyl chloride, U shaped plates were coated with 30 μ l of a solution of Mab (2A10 = 0.1 μ g/well and 2F2 = 0.025 μ g/well) diluted with PBS and held overnight at room temperature. Plates were washed three times with PBS-TW20 (0.5 ml Tween 20/1 liter PBS, pH 7.4) and the wells were then filled with (BB). After one hour of incubation at room temperature, plates were washed three times again with PBS-TW20 and then emptied. Aliquotes consisted of 30 μ l of each mosquito extract were placed in the respective well in the coated plate. After 2 hours of incubation at room temperature, the plates were washed three times with (BB), and 30 μ l of horse raddish peroxidase (HRPO), conjugated monoclonal antibody (0.05 μ g/50 μ l/well) in case of *P. falciparum* (2A10) and (0.05 μ g/50 μ l/well) in case of *P. vivax* (2F2) was added to each respective well and after 1 hour incubation, plates were washed three times with PBS-TW20. Finally, 150 μ l of peroxidase substrate (Kirkegaard and Perry) was added per well. The substrate for the HRPO-antibody conjugate consisted of 1 mg of 2,2-azino-di-3-ethyl Benzythazoline sulfuric acid (ABTS) per ml of 0.1 μ l citrate phosphate buffer, pH 4.0 with 0.003 % hydrogen peroxide added immediately before use. The reaction was stopped

after 1 hour by adding 50 μ l of 1 N sodium fluoride to each well. Colorimetric measurements were made in the Micro-ELISA auto reader (Dynatech, 580) with 405 nm wavelength filter.

Results and discussion

A total of 573 mosquitoes; 463 *A. sergenti*; 29 *A. multicolor* and 81 *A. pharoensis* were collected from Siwa and Faiyum governorate during the study period (table I). *P. falciparum* sporozoites were detected by both IRMA and ELISA assay in 2 out of 463 indoor collected *A. sergenti*. This finding is important in explaining the malaria transmission in Siwa-Oasis and Faiyum Governorate where it documents for the first time allow the incrimination of this species as a vector in Siwa-Oasis, and support the early suggestions reported by (Barber and Rice, 1937; Halawani and Shawarby, 1957). The finding in Faiyum supports the previous record by El Said *et al.* (1986) as well as that in the Nile Delta by Farid (1940).

TABLE I. — Results of IRMA and ELISA tests for mosquitoes collected in Siwa-Oasis and Faiyum (1983-1984).

	<i>A. sergenti</i>		<i>A. multicolor</i>		<i>A. pharoensis</i>
	Siwa	Faiyum	Siwa	Faiyum	Faiyum
No. mosquitoes tested	389	74	19	10	81
% from indoors	93.6	93.2	78.9	100.0	23.5
% other collections*	6.4	6.8	21.1	0	76.5
No positive	1	1	0	0	0

* From outdoors biting human and using animal traps.

The negative results obtained with the 81 *A. pharoensis* collected from Faiyum Governorate and the 29 *A. multicolor* collected from Faiyum Governorate and Siwa-oasis are not conclusive and larger numbers are needed to be tested. Natural malaria infection was previously detected in *A. pharoensis* from Faiyum (El Said *et al.*, 1986). *A. multicolor* however, cannot be ruled out as a suspected vector since it is a common species in the two study areas. Also, *A. multicolor* is found to be susceptible to malaria infection under laboratory conditions (El Said and Farid, 1982) although it has never been found naturally infected in Egypt. No cross reactions was found between *P. falciparum* and *P. vivax* sporozoites when the same mosquito extracts were used for both the immunoassays (IRMA and ELISA).

The *P. falciparum* sporozoites detected by IRMA and ELISA tests in this investigation were not necessarily present inside the salivary glands of the tested specimens, because the extract of the whole mosquito (head, thorax and abdomen) was used. Also, the circumsporozoite antigens are usually developed in the oocysts

about two days before mature sporozoites appear in the salivary glands. This early detection can give an inflated sporozoite rate and erroneous epidemiological information. However, this factor can be eliminated by processing only the thorax of the mosquito. In the case of *A. sergenti* where it was incriminated as the main vector in the Middle East (Farid, 1940), the present finding identifying this species as a potential malaria vector in Siwa-oasis and Faiyum Governorate.

We concluded that the use of *P. vivax* and *P. falciparum* ELISAs or/and IRMAs for field studies in Egypt could overcome problems associated with microscopic determination and to enhance epidemiological studies of human malaria, particularly in determining the relative importance of different anophelines in the transmission of human malaria in areas with low endemicity.

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