ROLE OF *ANOPHELES CULICIFACIES S.L.* AND *AN. PULCHERRIMUS* IN MALARIA TRANSMISSION IN GHASSREGHAND (BALUCHISTAN), IRAN[1]

M. ZAIM,[2] S. K. SUBBARAO,[3] A. V. MANOUCHEHRI[2] AND A. H. COCHRANE[4]

ABSTRACT. A 2-site immunoradiometric assay (IRMA) was performed on the head and thorax of *Anopheles culicifacies s.l.* and *An. pulcherrimus* females, the 2 most common anopheline species in the District of Ghassreghand (Baluchistan, Iran), collected during the 2 peak malaria transmission seasons (May and September–October 1991). Positive IRMA results revealed the 2 species as potential vectors of malaria in this highly endemic district. This finding serves as the first report on natural infection of *An. pulcherrimus* in Iran and is the second on natural infection of *An. culicifacies* since the previous report of 1959.

INTRODUCTION

Malaria is one of the major health problems in Baluchistan, southeastern Iran from which, in recent years more than 50% of the country's total cases have been reported. In 1991 with about 41,000 reported cases of malaria (annual parasite incidence of 28 in 1,000 population), this area has been confronted with the highest number of malaria cases in the past 16 years (Centre for Disease Control 1991).

In recent years, more than 80% of the cases of malaria in Baluchistan has been reported from the "Shahrestans" of Iranshahr and Chabahar where *Anopheles culicifacies sensu lato* is the most common anopheline species. This species has been incriminated as a vector of malaria in Iran during an epidemic outbreak in Zabol (Sistan and Baluchistan Province) in 1959 (Manouchehri and Ghiassedin 1959). Also in these 2 named shahrestans, four other known malaria vectors of Iran exist; however, their role in transmission in this part of the country has not been studied. These latter species are *An. dthali* Patton, *An. fluviatilis* James, *An. stephensi* Liston and *An. superpictus* Grassi (Zaim 1987). *Anopheles pulcherrimus* Theobald, which has been incriminated as a vector of malaria in Iraq on epidemiological grounds (Rishikesh 1972) and in central Asia (Christophers 1933) and Kunduz area, northeast Afghanistan through natural infection (M. S. Badaway, in Zahar 1974), is also present in relatively high numbers in the rice growing regions of Chabahar (districts of Ghassreghand and Nikshahr, 300–900 m above sea level) (Zaim et al. 1992).

A highly sensitive immunodiagnostic technique, a 2-site immunoradiometric assay (IRMA) based on species-specific monoclonal antibodies (mAbs) has been developed by Zavala et al. (1982) that can detect, identify and quantify sporozoites in individual or pools of mosquitoes. The assay has been successfully used for the determination of the *Plasmodium vivax* and *P. falciparum* sporozoite rate and load in fresh and dried field collected mosquitoes (Collins et al. 1984, Subbarao et al. 1988).

This study uses the IRMA to examine the potential role of *An. culicifacies s.l.* and *An. pulcherrimus*, the 2 most common anophelines in the Ghassreghand (Baluchistan) where the annual parasite incidence of malaria averages about 60/1,000 population.

MATERIALS AND METHODS

Study area: The investigation was carried out over a period of 11 months (February–December 1991) in the District of Ghassreghand, Shahrestan of Chabahar. Streams and stream fed pools, rice fields and palm irrigation plots are the main sources of mosquito breeding in the area. The average maximum and minimum temperatures in summer are 39.7° and 26.2°C and in winter 24.1° and 11.8°C, respectively. The average annual rainfall is about 100 mm. The district has been under an indoor residual spraying program for malaria control using malathion (50% wp, 2 g/m^2) in February and primiphos-methyl (Actellic) (40% wp, 2 g/m^2) in September 1991.

Mosquito collections: Pyrethrum space spray catches were performed, once a month in 4 villages (Bocan, Hit, Homeiry and Zeineddini). In each village 8 fixed shelters (4 human and 4 animal shelters) were sampled. Mosquitoes were identified to species and the collections during the 2 main peaks of malaria transmission, i.e.,

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[2] Department of Medical Entomology, School of Public Health, P.O. Box 6446, Teheran 14155, Iran.

[3] Malaria Research Centre, 22 Sham Nath Marg, Delhi 110 054, India.

[4] Department of Medical and Molecular Parasitology, New York University School of Medicine, 550 First Ave., New York, NY, USA.

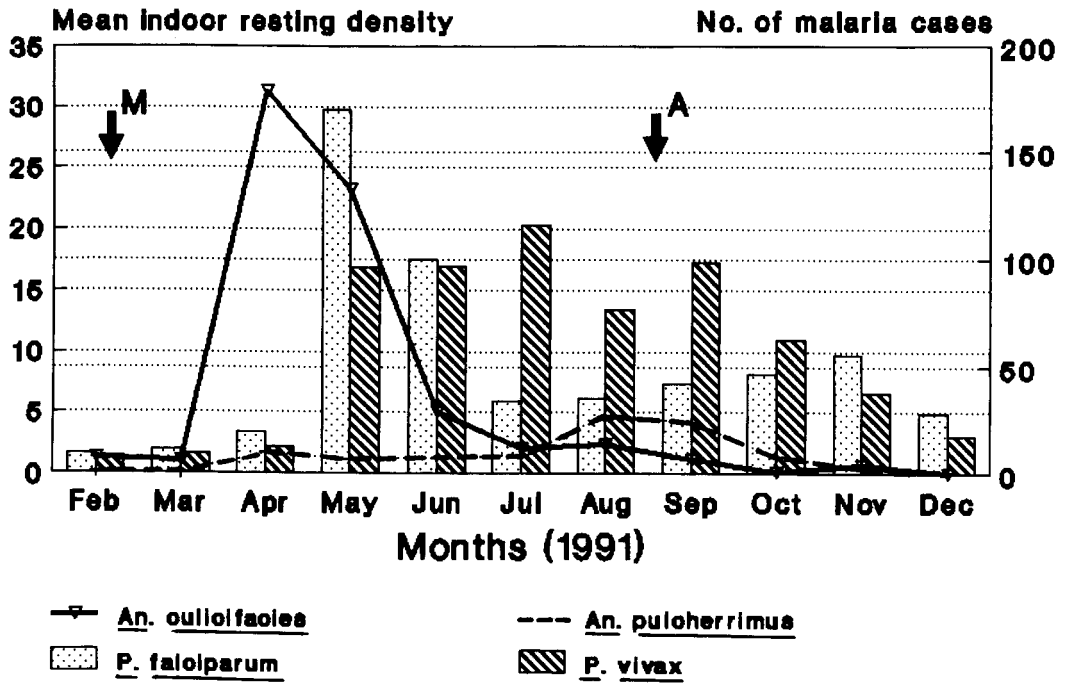

Fig. 1. Monthly indoor resting densities of *Anopheles culicifacies s.l.* and *An. pulcherrimus* and the number of malaria cases reported in Ghassreghand (Baluchistan, Iran), February–December 1991. M and A indicate the months the district was sprayed with 2 g/m^2 malathion and primiphos-methyl (Actellic), respectively.

May and September-October, were dried at room temperature and stored in a refrigerator, for use in IRMA, after their abdomens were cut off.

Two-site IRMA: Mosquitoes were assayed for *Plasmodium* infections using a previously described 2-site IRMA (Zavala et al. 1982, 1983), with the following modifications: pools of 10 mosquitoes were homogenized in 100 μl grinding buffer and 300 μl phosphate buffer saline-10% bovine serum albumin was added to make a final volume of 400 μl. Thirty μl of this homogenate was used as antigen source. The remaining procedure was the same as described by Subbarao et al. (1988).

Positive controls consisting of extracts of *P. vivax* and *P. falciparum* sporozoites and negative controls consisting of extracts of mosquitoes reared in the laboratory, were included in each microtiter plate. Unknown preparations giving readings at least twice as high as the maximum negative control value were considered positive. Suspected positive extracts were reassayed to confirm their positivity.

MAbs 2F2 and 2A10, which recognize a species-specific repetitive epitope on the circumsporozoite protein of *P. vivax* and *P. falciparum*, respectively, were used (Nardin et al. 1982). The mAbs were labeled with I125 (Amsterdam Laboratories, Arlington Heights, IL).

RESULTS AND DISCUSSION

The mean indoor resting densities of *An. culicifacies s.l.* and *An. pulcherrimus* and the number of reported malaria cases in the district of Ghassreghand are presented in Fig. 1. Both species were active throughout most of the year and the peak indoor resting density, under the ongoing malaria control measures, was found to be in April and August. A total of 1,214 malaria cases were reported in this area during the study period, with the highest numbers reported in May and September (Fig. 1).

Table 1 summarizes the results of the IRMA performed on *An. culicifacies s.l.*, *An. pulcherrimus* and the 3 other anophelines (*An. dthali, An. fluviatilis* and *An. stephensi*) collected in indoor resting sites during the peak malaria transmission seasons in Ghassreghand. Out of the 2,305 mosquitoes collected during May 1991 and tested for *Plasmodium* infection, 85% and 10.7% belonged to *An. culicifacies s.l.* and *An. pulcherrimus*, respectively, of which 2 pools of *An. culicifacies s.l.* were found positive for *P. vivax*. Also out of the 1,245 females collected

Table 1. Results of the immunoradiometric assay performed on pools of 10 mosquitoes collected during peak malaria transmission seasons in Ghassreghand, Baluchistan, Iran (1991).

Species	May 1991		Sept.–Oct. 1991	
	No. tested	No. of positive pools*	No. tested	No. of positive pools*
An. culicifacies	1,960	2	276	—
An. dthali	26	—	11	—
An. fluviatilis	56	—	7	—
An. pulcherrimus	246	—	686	2
An. stephensi	17	—	265	—

* Only positive for *P. vivax*.

during September–October 1991, *An. culicifacies s.l.* and *An. pulcherrimus* constituted 22.2% and 55.1% of the collected material, respectively, of which 2 pools of *An. pulcherrimus* were positive for *P. vivax*. Surprisingly no positives were found for *P. falciparum* in this study.

Our recent study on the seasonal activity of *An. culicifacies s.l.* and *An. pulcherrimus* in a village where no anti-mosquito control measures have been in practice, has revealed that *An. culicifacies s.l.* has 2 peaks of activity in the district of Ghassreghand, one in April–May (main peak) and the other in October–November (Zaim et al., unpublished data). *Anopheles pulcherrimus* has also 2 peaks of activity, one in April–May and the other in August–September (Zaim et al. 1992). Because the second peak for malaria cases in the district of Ghassreghand is in September, *An. pulcherrimus* has been suspected as a vector in this area. The present IRMA results correspond well with the observed vector population and malaria cases in the study area and serves as the first report on natural infection of *An. pulcherrimus* in Iran and is the second report on natural infection of *An. culicifacies s.l.* in Sistan and Baluchistan province since the previous report of 1959. It is noteworthy that in preliminary studies carried out in Iran only species A of *An. culicifacies* has been found (Zaim and Javaherian 1991); however, the study is still in progress.

The distribution of *An. pulcherrimus* is mainly restricted to the rice growing areas of the Iranshahr and Chabahar, i.e., districts of Ghassreghand and Nikshahr. Based on epidemiological data, *An. culicifacies s.l.* is considered as the primary vector of malaria in the other areas of the 2 highly endemic shahrestans. *Anopheles stephensi*, which used to be a prevalent species in this part of the country, has responded well to indoor residual spraying programs, and during 1990–91 it comprised less than 4% of the pyrethrum space spray catches of human and animal shelters in the 2 named shahrestans (Zaim, unpublished data). Since positive IRMA results on potential vectors do not prove vector incrimination, the determination of the natural infection rates of *An. culicifacies* and *An. pulcherrimus* based on salivary gland dissections and comparative vector potential studies that are currently underway should bring more light to the relative importance of these 2 species in Baluchistan, Iran.

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