ANOPHELES CULICIFACIES: SIBLINGS SPECIES COMPOSITION AND ITS RELATIONSHIP TO MALARIA INCIDENCE

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ABSTRACT. Entomological and epidemiological surveys in May, August and November 1985 and March 1986 were conducted in villages in Bulandshahr, a western district in Uttar Pradesh and in three eastern districts, Jaunpur, Ballia and Saran. In Bulandshahr, *Anopheles culicifacies* sibling species A and B were found, with a predominance of species A. Both *Plasmodium vivax* and *P. falciparum* were present and the malaria incidence remained high (SPR, 6–50%) indicating an active transmission. In contrast, in three eastern districts predominance of species B with an occasional occurrence of species A was observed. Malaria cases were almost absent in Ballia and Saran and in Jaunpur 10.3% slide positivity rate was observed in May but in later surveys cases were considerably lower (SPR, 0.5–2.9%) indicating the absence of indigenous transmission. In the eastern districts, malaria parasites are regularly brought in from endemic areas by the migrant labor population. Although *An. culicifacies* s.l. occurs in both the areas, the difference in malaria incidence appears to be due to the difference in the composition of the sibling species which is, the predominant presence of species A in the western district and its absence in eastern districts. This indicates that species A is responsible for active malaria transmission while species B is not.

INTRODUCTION

Anopheles culicifacies Giles is an established vector of malaria in the Indian subcontinent. While discussing the role of this species in the transmission of malaria in different parts of the country, Ramachandra Rao (1984) stated that one of the unresolved paradoxes in the epidemiology of malaria related to the differential vectorial potential of *An. culicifacies* in regions of apparently similar physiogeography. Now that *An. culicifacies* has been identified as a complex of four sibling species, species A, B (Green and Miles 1980), species C (Subbarao et al. 1983) and species D (K. Vasantha et al. unpublished data), a possibility arises of their having different vectorial potentials. These differences may provide an explanation to the epidemiological paradox observed. Among the members of the *An. maculipennis* and *An. gambiae* (White 1982) complexes, distinct differences in vectorial potentials were observed.

The sibling species of *An. culicifacies* have defined distribution patterns (Subbarao 1984), environmental preferences (Subbarao et al. 1980, 1987) and show differential responses to DDT (Subbarao et al., unpublished data). To relate the prevalence of sibling species of *An. culicifacies* with malaria incidence, the district Bulandshahr in western Uttar Pradesh, where incidence of malaria was high (Information, National Malaria Eradication Programme (NMEP) Directorate) and districts Jaunpur and Ballia (U.P.) and Saran (Bihar) in the eastern part of northern India, where incidence of malaria was low or almost absent (NMEP Directorate), were selected. These two regions of northern India were surveyed to study the composition of *An. culicifacies* sibling species and to relate it with the epidemiology of malaria. Results of this study are reported in this paper.

MATERIALS AND METHODS

Entomological and epidemiological surveys were carried out in districts of Bulandshahr, Jaunpur and Ballia (Uttar Pradesh) and Saran (Bihar) in May, August and November, 1985 and March 1986. In each district, two types of villages were selected, villages with rivers or with extensive canal irrigation and non-riverine villages.

Description of study areas. Bulandshahr is situated between the rivers Ganges and Yamuna and has three major canals passing through it. Villages surveyed were in the Kaserkalan and Dhanpur blocks. Most of these villages were riverine and had extensive canal irrigation. In the other villages, tube well irrigation was prevalent.

Areas in the Bulandshahr unit entered into a consolidation phase in 1963 and into a maintenance phase in 1966 under the National Malaria Eradication Programme (NMEP). In these areas, the annual parasite incidence (API) (cases per thousand population per year) was less than 1 until 1970; there was then a resurgence of malaria and the API rose to 7.3 in 1976. The API is used as a parameter to decide whether or not insecticide should be sprayed in an area. Areas which have an API of 2 or more are sprayed with suitable insecticide under the Modified Plan of Operation (MPO) under NMEP (Pattanayak and Roy 1980). Based on this, indoor spraying with residual insecticide

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was resumed in Bulandshahr in 1976. In spite of continuous spraying, the incidence of malaria remained high and the API was 10.1 in 1984.

Jaunpur lies on the banks of the river Gomati and several tributaries pass through the district. Villages selected were in Dharmapur, Muftiganj and Jalalpur blocks. Ballia district is situated at the confluence of the rivers Ghagra and Ganges. Villages surveyed were in Sonwani, Narahi, Chilakahar and Ratsand blocks. District Saran is situated on the north of the Ganges and adjacent to Ballia district. Villages selected were in Manjhi, Marhaura and Amnaura blocks. In the three districts, almost all the villages were riverine and prone to flooding.

In districts Jaunpur, Ballia and Saran, the API was always low. All three areas entered into the consolidation phase in 1962 and into the maintenance phase in 1964. Since in all three districts the API remained less than one, spraying has not been performed since 1964.

Procedures. Indoor resting *An. culicifacies* were collected before dawn from human dwellings and cattle sheds using suction tubes. Flashlights were used to locate the resting mosquitoes. Only *An. culicifacies,* which can be distinguished from other anopheline species by its sitting posture, were collected. However, presence of other anopheline species was noted during all the surveys. Man hour density (MHD) of *An. culicifacies* was calculated taking into account the total number of *An. culicifacies* (n), time spent in minutes (t) and number of persons involved in the collection (p).

$$\text{MHD} = \frac{n \times 60}{t \times p}.$$

From the total *An. culicifacies* collected, semi-gravid females, which can be identified by the abdominal condition, were separated. Ovaries from these females were removed and fixed in cold 1:3 acetic acid methanol fixative. The proportion of semi-gravid females varies in the field collections and is dependent upon the relative humidity and temperature in an area. Semi-gravid females were selected for the identification of sibling species as it is only in these females that ovaries are found in the Christophers' stage III. This stage has been found to be the most suitable for polytene chromosome preparation.

Human blood smears were collected from persons with fever or those who had had fever up to four days prior to the collection from villages in Bulandshahr. From villages in Ballia, Jaunpur and Saran districts, blood smears were made from voluntary donors who included febrile and afebrile cases. Fever surveys could not be conducted in these three districts since fever cases were very few or absent. Blood smears were collected from all the fixed villages irrespective of the presence of *An. culicifacies* during the surveys.

Glass vials with fixative containing mosquito ovaries were brought to the laboratory in an ice box. Polytene chromosomes prepared from ovaries following Green and Hunt (1980), were examined microscopically to identify the sibling species with the help of the diagnostic inversion karyotypes described by Subbarao et al. (1983). Human blood smears were stained with JSB stain (Jaswant Singh and Bhattacharji (1944) and examined under the microscope for malaria parasites.

RESULTS AND DISCUSSION

Four anopheline species, *An. annularis* Van der Wulp, *An. culicifacies, An. stephensi* Liston and *An. subpictus* Grassi were observed in Bulandshahr villages, while only *An. subpictus* and *An. culicifacies* were found in Jaunpur, Ballia and Saran villages. In district Bulandshahr, *An. culicifacies* was found in riverine and non-riverine villages but in Jaunpur, Ballia and Saran, *An. culicifacies* was almost absent in non-riverine villages. *Anopheles culicifacies* densities, sibling species composition and malaria incidence observed in the districts are presented in Table 1. In Table 1, data from riverine and non-riverine villages in each district were pooled and presented as there was no difference in the composition of sibling species and malaria incidence. In Bulandshahr, species A and B were observed with a predominance of species A in March, May and August while both the sibling species were found in equal proportions in November. In all the four surveys in Jaunpur, Ballia and Saran, mainly species B was found with an occasional occurrence of species A. Thus, the eastern districts Jaunpur, Ballia and Saran differed distinctly from the western district Bulandshahr in their *An. culicifacies* sibling species predominance.

The differences observed in the composition of sibling species populations may be due to the differences in their breeding sites in the two areas. In the riverine villages of Bulandshahr, no mosquito breeding was found on the river banks because there was embankment. Hence, both in riverine and non-riverine villages, *An. culicifacies* breeding was observed only in rain water collections and stagnant water ponds/pools. In contrast, in all the riverine villages in the three eastern districts, *An. culicifacies* breeding was observed on the river banks. In our earlier studies too, where riverine breeding was noticed, a predominance of species B was observed (Subbarao et al. 1987).

Table 1. *Anopheles culicifacies* sibling species composition and malaria incidence in western and eastern regions in northern India.

Areas (districts)	May 1985							August 1985						
	MHD[1]	No.[2] A.C. exam.	% Sp. A	% Sp. B	No.[3] B.S. exam.	SPR[4]	SfR[5]	MHD	No. A.C. exam.	% Sp. A	% Sp. B	No. B.S. exam.	SPR	SfR
Western Region														
Bulandshahr	30.9	147	87.7	12.3	249	25.7	4.0	8.8	187	62	38	472	36.0	25.6
Eastern Region														
Jaunpur	3.9	12	8.3	91.7	184	10.3	0	25.8	124	1.6	98.4	203	2.9	0.5
Ballia	1.3	6	0	100	309	0	0	17.8	71	0	100	263	0.8	0
Saran	0	0	0	0	110	0	0	3.5	12	0	100	89	0	0
	November 1985							March 1986						
Western Region														
Bulandshahr	2.9	64	50	50	213	50.7	46.9	21.7	276	63	37	211	6.6	2.8
Jaunpur	2.2	12	6.3	91.7	689	1.9	0	9.2	33	6.1	93.9	592	0.5	0.2
Eastern Region														
Ballia	22.3	86	1.2	98.8	1027	0.1	0.1	5.9	16	0	100	542	0	0
Saran	0.3	1	0	100	75	0	0	38.6	62	0	100	86	0	0

[1] MHD: Man hour density of *An. culicifacies*.
[2] No. A.C.: Number of *An. culicifacies* sensu lato.
[3] Blood smears were collected from fever cases in Bulandshahr while in other units they were collected from afebrile cases also.
[4] SPR: Slide positivity rate.
[5] SfR: Slide falciparum rate.

In Jaunpur, malaria incidence was low except in May when the slide positivity rate (SPR) was 10.3. All the positive cases were of *Plasmodium vivax* except for two of *P. falciparum,* one each in two surveys. In Ballia, malaria incidence was very low while in Saran, it was absent. It should be mentioned that *P. vivax* and *P. falciparum* parasites are regularly being brought into eastern districts by migrant populations from malaria endemic areas (NMEP Directorate). Migrant labor comes back from endemic areas, viz., Punjab, Rajasthan, Gujarat, Maharashtra etc., to eastern districts in April–May and goes back in August to earn their livelihood. This indicates that, though the parasite was present in the community, it was not being transmitted, i.e., there was no indigenous transmission. Seroepidemiological survey conducted in Ballia district in 1985 also established the lack of indigenous transmission (Dr. Krishna Ray, Asst. Director, National Institute of Communicable Diseases, Delhi, personal communication). This may be due to the poor vectorial capacity of the vector species present in the area. *Anopheles culicifacies* is apparently the only vector species present as *An. subpictus* is not considered a vector of any importance (Ramachandra Rao 1984). The poor vectorial capacity of *An. culicifacies* is more obvious in Jaunpur where increase in densities did not result in the increase in malaria incidence.

In contrast, in the western district, Bulandshahr, the malaria incidence remained high throughout with maximum cases of *P. vivax* in May and August while those of *P. falciparum* were in November. This indicated an active

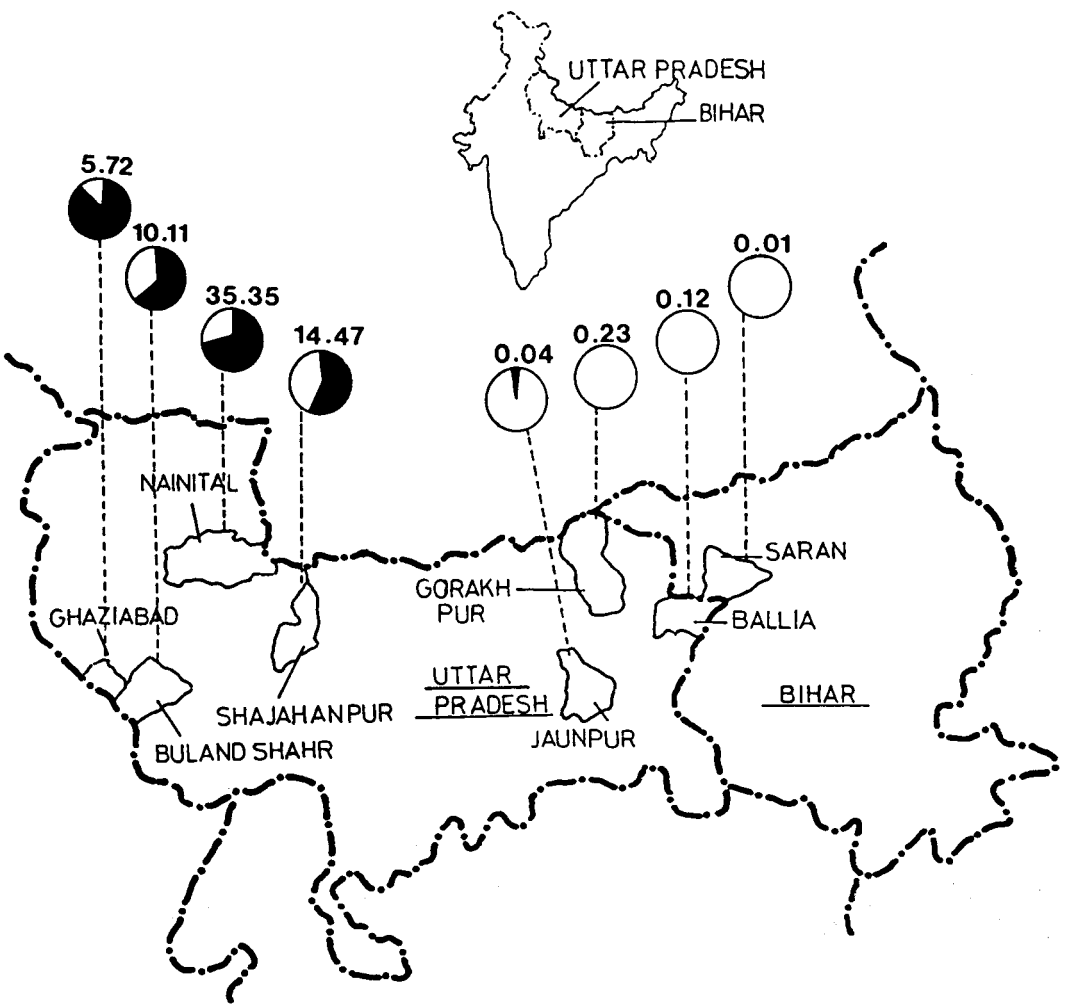

Fig. 1. Proportions of *Anopheles culicifacies* sibling species A and B and the API recorded in certain districts of Uttar Pradesh and Bihar. Shaded areas in pie graph represent proportion of species A. Figures denote API.

transmission. *Anopheles culicifacies* is considered to be the main vector species in this area as the role of the other two suspected vector species, *An. stephensi* and *An. annularis*, may be negligible, if at all, since they were always found in low numbers. Further, in this part of the Indian subcontinent *An. stephensi* is a vector of importance in urban areas (Pattanayak and Roy 1980, Ramachandra Rao 1984) and *An. annularis* is of only secondary importance in some areas (Ramachandra Rao 1984).

In Ballia and Saran where malaria is almost absent, *An. culicifacies* was found to comprise only of sibling species B (Table 1), while in Jaunpur, where malaria was not totally absent, the sibling species composition was constituted predominantly of species B, with species A ranging between 2–8%. The sibling species composition was drastically different in Bulandshahr from that in eastern districts in having species A as the predominant species. Thus, species A seems to be responsible for maintaining the high transmission of malaria in Bulandshahr.

This is substantiated by our observations in other districts in Uttar Pradesh as shown in Fig. 1. In this map, sibling species prevalence of a few more districts other than the ones examined in this study have also been included. The data for these districts have been obtained from other studies (unpublished). To show the relationship between sibling species and malaria incidence, API was considered as a parameter for indicating malaria incidence. Since this study was initiated in 1985, the latest API, i.e., of 1984 was chosen (data obtained from NMEP Directorate).

In districts Ghaziabad, Shahjahanpur and Nainital where both species A and B were found with the predominance of species A, there was a high malaria incidence. In Gorakhpur, Saran and Ballia only species B was observed and these areas had a low incidence of malaria. These findings gain further support from studies carried out in northern India where *Plasmodium* sporozoites were found only in species A, though species A and B were present (Subbarao et al. 1980, 1987). This study for the first time provides evidence of the relationship between the sibling species A and B of *An. culicifacies* and the malaria incidence.

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