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STUDIES ON *ANOPHELES FLUVIATILIS* AND *ANOPHELES CULICIFACIES* SIBLING SPECIES IN RELATION TO MALARIA IN FORESTED HILLY AND DEFORESTED RIVERINE ECOSYSTEMS IN NORTHERN ORISSA, INDIA

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ABSTRACT. In a malaria-endemic region in northwestern Orissa, India, a longitudinal study was undertaken to delineate information on the sibling species of *Anopheles fluviatilis* and *Anopheles culicifacies* and their bionomics and role in malaria transmission in forested and deforested ecosystems. In forested villages, *An. fluviatilis* sibling species S (97.97%) and T (2.02%) were present. The former was highly anthropophagic (human blood index 0.88). Among the sibling species of *An. culicifacies,* species B (27.96%), C (71.1%), and B/C heterozygotes (0.94%) were present and were highly zoophagic. In deforested riverine villages *An. fluviatilis* was nearly absent and *An. culicifacies* sibling species A (0.48%), B (21.1%), C (77.94%), and B/C heterozygotes (0.48%) were present. In forested villages, the annual parasite incidence (269 cases/1,000) and the slide positivity rate (45%) were significantly higher than those in deforested areas, which had values of 39 cases/1,000 and 27%, respectively. The study showed that the high endemicity of malaria in the forested villages was due primarily to 2 vectors, the high rate of anthropophagy of *An. fluviatilis* species S, and also the more favorable ecological conditions for this vector.

KEY WORDS *Anopheles fluviatilis, Anopheles culicifacies,* sibling species, bionomics, malaria transmission, ecosystem

INTRODUCTION

A large part of Orissa State in eastern India is hilly and forested, with a meso- to hyperendemicity of malaria. More than one third of all *Plasmodium falciparum* cases and one half of the number of malaria deaths in India during 1990 (Yadav 1991) occurred in Orissa. A study in the northwestern part of the state showed high transmission of malaria in this area (Yadav et al. 1990). Although the area has undulating forested hills (elevation 400–500 m), the forest cover in flat lands and river basin areas has been denuded, and these areas are being used for rice cultivation during the monsoon season (June–October). Large and exposed deforested areas are now interspersed with hill streams and criss-crossing rivers amid the deciduous wet forest, thereby presenting 2 distinct ecosystems.

From earlier records (Senior White 1943) and our present knowledge, *Anopheles fluviatilis* James and *Anopheles culicifacies* Giles are the 2 major vectors of malaria in the eastern plateau covering northwestern Orissa. In India, *An. culicifacies* has been cytotaxonomically identified as a complex of 5 sibling species provisionally designated as species A, B, C, D, and E, which vary in their biological characteristics and role in malaria transmission (Subbarao 1988; Subbarao et al. 1988a, 1988b; Kar et al. 1999). *Anopheles fluviatilis* is also a complex of 3 sibling species, namely S, T, and U, which also vary in biological characteristics (Subbarao et al. 1994, Sharma et al. 1995, Nanda et al. 1996).

In order to delineate the role of the *An. culicifacies* and *An. fluviatilis* sibling species in malaria transmission in this area, a longitudinal study was carried out during 1992 in villages of the forested hilly and deforested riverine ecosystems. The present study reports on the bionomics of these vectors in relation to malaria incidence in the study villages.

MATERIALS AND METHODS

Study area: The study was conducted during January to December 1992 in Sundargarh District, which lies between 21°35′ and 22°32′N, and 83°32′ and 85°22′E in northwestern Orissa. The area has a tropical monsoon-type climate, and one third of the geographical area has deciduous wet forest (Anonymous 1991). The population is mainly tribal and people live in small villages situated generally along watercourses.

Four villages were selected for the study. Villages Birkera and Manko represented a hilly forested area. Birkera is situated about 10 km from Rourkela City in Bisra Block. The human and cattle populations of the village were 1,045 and 694, respectively. Village Manko belongs to Lathikata Block and is 15 km away from Rourkela, with human and cattle populations of 1,228 and 303, respectively. The major vector breeding habitats in these villages were perennial streams, stream channels, seepages, and a few terraced paddy fields during the monsoon season. Villages Jamsera and Jabaghat are situated on the Koel-Brahmani River in Bisra Block, about 6–7 km from Rourkela. They represented a deforested riverine ecosystem. The human and cattle

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Table 1. Man-hour densities of malaria vectors in the forested villages Birkera and Manko during 1992.[1]

	Anopheles fluviatilis					*Anopheles culicifacies*				
	Birkera		Manko			Birkera		Manko		
Month	HD	CS	HD	CS	Mean	HD	CS	HD	CS	Mean
Jan.	8.0	12.0	4.0	4.0	7.0	1.0	9.0	0.0	9.5	4.9
Feb.	5.5	4.5	7.0	1.5	4.6	0.5	19.0	3.0	23.0	11.4
March	4.0	0.0	6.5	0.5	2.7	2.5	46.0	5.5	50.5	26.1
April	0.5	0.0	5.0	0.5	0.4	15.5	47.0	7.5	57.5	31.9
May	0.0	0.0	0.0	0.0	0.0	7.0	32.5	10.0	64.5	28.5
June	0.0	0.0	0.5	0.0	0.1	10.0	63.0	12.5	77.5	40.8
July	1.0	1.5	0.0	0.0	0.6	22.5	49.5	5.0	33.0	27.5
Aug.	0.0	0.5	0.5	1.5	0.6	9.5	52.0	4.0	28.0	23.4
Sept.	1.0	0.0	1.5	0.0	0.6	2.5	39.5	1.0	10.5	13.4
Oct.	17.5	1.5	5.0	1.0	6.3	1.5	42.0	2.5	13.5	14.9
Nov.	35.5	0.5	14.5	2.0	13.1	1.5	32.5	4.5	23.0	15.4
Dec.	9.5	0.0	1.5	0.5	2.9	3.5	27.5	0.5	4.0	8.9

[1] HD, human dwellings; CS, cattle sheds.

populations were 476 and 152 in Jamsera and 985 and 309 in Jabaghat, respectively. The breeding sites in these 2 villages were mainly riverbed pools, paddy fields, rain pools, and so on. In the study villages most of the dwelling rooms had thatched or tiled roofs and mud-plastered walls and floors.

Entomological surveys: Indoor resting mosquito collections were made on a fortnightly basis in the morning between 0600 and 0800 h. In each village, mosquitoes were collected for 15 min each from 4 cattle sheds and 4 human dwellings using an aspirator and torch light. Mosquitoes were anesthetized with ether, *An. culicifacies* and *An. fluviatilis* were identified, and their man-hour densities were recorded. From the collections, half-gravid females of *An. culicifacies* and *An. fluviatilis* were utilized for identification of sibling species and blood-meal source. Ovaries of half-gravid females were removed and fixed in modified Carnoy's fixative (1: 3 acetic acid: methanol). From the same mosquito blood from the gut was smeared on Whatman No. 1 filter paper (Whatman International Inc., Maidstone, England) for a blood-meal assay.

Fixed ovaries were processed in 50% propionic acid and stained in 2% lacto-aceto orcein according to the method of Green and Hunt (1980) for making polytene chromosome preparations. Polytene chromosomes were examined with a Zeiss Axioplan Universal microscope (Carl Zeiss, Oberkochen, Germany), and the sibling species of *An. culicifacies* and *An. fluviatilis* complexes were identified according to the diagnostic paracentric inversions on the polytene chromosomes (Subbarao et al. 1988b, Subbarao et al. 1994). Blood-meal samples were tested by countercurrent immunoelectrophoresis using agarose gel (Bray et al. 1984). The number of mosquitoes of each species found fed on human as well as mixed (human + bovine) blood were added to calculate the human blood index (HBI).

Malaria surveillance: After conducting a baseline demographic survey in the study villages, house-to-house surveillance of fever cases was conducted on weekly basis from January to December 1992 by employing resident surveillance workers. Slides collected were brought to the Field Station laboratory in Rourkela City, stained with JSB stain (Singh et al. 1953), and examined under an oil-immersion lens. Patients having *Plasmodium vivax* or *P. malariae* infections were treated with a single dose of a 600-mg chloroquine base, and in the *P. vivax* cases primaquine at the dose of 15 mg daily for 5 consecutive days was also administered (adult dosage). Children received proportionate doses, but pregnant women and infants were not given primaquine. *Plasmodium falciparum* cases were treated with a 600-mg chloroquine base on day 0, 600 mg of chloroquine plus 45 mg of primaquine on day 1 and 300 mg of chloroquine on day 2 (all adult dosage). Mixed infections of *P. vivax* and *P. falciparum* were treated like the latter cases.

RESULTS

Man-hour densities of *An. fluviatilis* and *An. culicifacies* resting indoors in human dwellings and cattle sheds in the forested villages are given in Table 1. High densities of *An. fluviatilis* were observed during the cooler months (October–January), but very low levels were observed in summer (May–June). Contrarily, the prevalence of *An. culicifacies* in forested villages was relatively lower during winter (November–January) than the remaining months. Densities of *An. fluviatilis* in human dwellings were significantly higher than those in cattle sheds, but the reverse was observed in the case of *An. culicifacies.*

In the riverine villages in the deforested area, high densities of *An. culicifacies,* reaching up to 290 per man-hour were recorded (Table 2). Only 1 specimen of *An. fluviatilis* was found. The density of *An. culicifacies* was higher in cattle sheds.

Table 2. Man-hour densities of the malaria vector *Anopheles culicifacies*[1] in the deforested riverine villages Jamsera and Jabaghat during 1992.[2]

Month	Jabaghat		Jamsera		Mean
	HD	CS	HD	CS	
Jan.	10.0	80.0	14.0	119.0	55.8
Feb.	8.0	112.0	11.5	130.5	65.5
March	29.0	228.0	24.0	173.5	113.6
April	9.5	115.0	22.5	179.5	81.6
May	5.0	34.5	17.0	84.5	35.3
June	9.5	53.5	15.5	127.0	51.4
July	2.0	6.0	2.0	19.5	7.4
Aug.	3.0	34.5	2.0	29.0	17.1
Sept.	2.5	19.0	1.0	44.0	16.6
Oct.	12.5	87.5	20.5	173.5	73.5
Nov.	16.5	166.0	11.5	290.5	121.1
Dec.	7.0	65.5	14.0	171.0	64.4

[1] Only 1 specimen of *Anopheles fluviatilis* was found in March from Jabaghat.
[2] HD, human dwellings; CS, cattle sheds.

In forested villages, *An. fluviatilis* species S constituted about 98% and species T about 2% of the catch (Table 3). No specimen of species U was found. Species B and C of the *An. culicifacies* complex were found sympatric in forested villages, and species C (71.1%) outnumbered species B. A few double-inversion heterozygotes ($2g^1 + h^1/+g^1h^1$) between species B and C (<1%) were also observed. Species A and D were not found.

In deforested riverine villages, only 1 specimen of *An. fluviatilis* species T was found in the month of March. *Anopheles culicifacies* species A was found in low numbers (0.48%); species B comprised 21.1% and species C comprised 77.94%. Species D was not found (Table 4). A small proportion of heterozygotes (0.48%) between species B and C was also found in these villages.

After identification of the sibling species, their respective blood meals were also analyzed for host preferences. *Anopheles fluviatilis* species S in hilly forested villages was found to be highly anthropophagic with an HBI of 0.88 (Table 5). Two specimens of species T found in these villages had mixed blood (fed on both human and bovine blood), and the only specimen of species T collected from the deforested riverine villages had bovine blood. Analysis of blood meals of *An. culicifacies* sibling species showed that both species B and C were predominantly zoophagic. A few of species B and C had fed on human blood in forested villages and the HBI was 0.033 and 0.046, respectively. In deforested riverine villages, species A and B were found to be exclusively zoophagic and species C was also almost zoophagic with an HBI of 0.003.

Malaria incidences in the forested hilly villages and deforested riverine villages are given in Tables 6 and 7, respectively. In the former, fever prevalence in the population, as indicated by annual blood examination rate (ABER, calculated as total blood smears examined in a year/total population), was 59.7% (1,357/2,273), whereas in the latter, the ABER was only 14.4% (211/1,461). Annual slide positivity rate (SPR) in forested villages was 45.1%

Table 3. Sibling species composition in the forested villages Birkera and Manko (pooled data) during 1992.

Month	*Anopheles fluviatilis* sibling species			*Anopheles culicifacies* sibling species			
	S	T	Total	B	C	B/C[1]	Total
Jan.	15	0	15	0	3	0	3
Feb.	33	2	35	10	10	0	20
March	13	0	13	8	20	0	28
April	1	0	1	7	53	0	60
May	0	0	0	2	11	0	13
June	0	0	0	4	2	0	6
July	1	0	1	6	9	0	15
Aug.	13	0	13	0	4	0	4
Sept.	14	0	14	12	14	2	28
Oct.	2	0	2	6	16	0	22
Nov.	5	0	5	4	8	0	12
Total	97	2	99	59	150	2	211
	(97.97%)	(2.02%)	(100%)	(27.96%)	(71.1%)	(0.94%)	(100%)

[1] Double-inversion heterozygotes ($2g^1 + h^1/+g^1h^1$).

Table 4. *Anopheles culicifacies* sibling species composition in the deforested riverine villages Jabaghat and Jamsera (pooled data) during 1992.

Month	A	B	C	B/C[1]	Total
Jan.	1	4	35	0	40
Feb.	0	16	55	0	71
March	0	15	32	0	47
April	1	8	46	0	55
May	0	0	7	0	7
June	0	1	4	1	6
July	0	0	1	0	1
Aug.	0	11	17	1	29
Sept.	0	10	21	0	31
Oct.	0	7	56	0	63
Nov.	0	16	51	0	67
Total	2	88	325	2	417
	(0.48%)	(21.1%)	(77.94%)	(0.48%)	(100%)

[1] Double-inversion heterozygotes.

(612/1,357) and the annual parasite incidence (API) was 269 cases/1,000 with a predominance of *P. falciparum* cases (83.5%) followed by *P. vivax* (13.7%), *P. malariae* (1.5%), and mixed infections of *P. vivax* and *P. falciparum* (1.3%). In the deforested riverine villages, the SPR and API were 27% and 39/1,000, respectively. *Plasmodium falciparum* cases accounted for 57.9%, *P. vivax* cases accounted for 38.6%, mixed infections of *P. vivax* and *P. falciparum* accounted for 3.5%, and no *P. malariae* case was found.

DISCUSSION

Analysis of the results of our investigation revealed distinct differences in the vector species prevalence and malaria incidence in the forested hilly and deforested riverine villages in Sundargarh District. In the forested villages, both *An. fluviatilis* and *An. culicifacies* were prevalent, and low densities of *An. fluviatilis* during April–September were compensated for by an abundance of *An. culicifacies* during that period. Hence, these 2 vectors were responsible for maintaining high malaria transmission in the forested area. The preference of species S for resting in human dwellings and feeding on human blood makes it a highly efficient vector. Although vector incrimination was not done during this study, during May–December 1992 an average sporozoite rate of 2.1% (5/236) was observed in *An. fluviatilis* in the forested hills in the southern part of Sundargarh District at 800- to 900-m elevation, where species S constituted almost the total *An. fluviatilis* population (Yadav et al., unpublished data). Studies carried out in different parts of India have shown that other members of the *An. fluviatilis* complex are primarily zoophagic and play a minor role in malaria transmission (Sharma et al. 1995, Nanda et al. 1996). Therefore, species S, which constitutes most of the *An. fluviatilis* population in the forested area of Orissa, can be concluded to play a major role in malaria transmission. In Koraput District in southern Orissa, previous studies also indicated that *An. fluviatilis* was a prin-

Table 5. Blood-meal analysis of *Anopheles fluviatilis* and *Anopheles culicifacies* sibling species in the study villages of Sundargarh District, Orissa.

Species		Total tested	Human blood	Bovine blood	Mixed blood[1]	Non reactive	Human blood index
Hilly forested villages							
An. fluviatilis	S	97	80	4	5	8	0.88
	T	2	0	0	2	0	—[2]
An. culicifacies	B	59	2	53	0	4	0.033
	C	150	5	133	2	10	0.046
	B/C[3]	2	0	2	0	0	—
Deforested riverine villages							
An. fluviatilis	T	1	0	1	0	0	—
An. culicifacies	A	2	0	2	0	0	—
	B	98	0	96	0	2	—
	C	325	0	314	1	10	0.003
	B/C[3]	2	0	2	0	0	—

[1] Human + bovine blood.
[2] Not calculated because of low numbers.
[3] Double-inversion heterozygotes.

Table 6. Malaria cases in the hilly forested villages Birkera and Manko (pooled data) of Sundargarh District, Orissa, during 1992.

Month	Blood smears tested	Malaria cases[1]					Cases/1,000 population	Slide positivity rate (%)
		Pv	*Pf*	*Pm*	*Pv* + *Pf*	Total		
Jan.	79	7	36	2	1	46	20.2	58.2
Feb.	90	10	33	1	1	45	19.8	50.0
March	91	7	23	2	0	32	14.1	35.2
April	85	9	27	1	0	37	16.3	43.5
May	99	9	28	0	1	38	16.7	38.4
June	78	9	14	0	0	23	10.1	29.5
July	122	3	53	0	2	58	25.5	47.5
Aug.	146	7	50	0	0	57	25.1	39.0
Sept.	166	5	44	1	2	52	22.9	31.3
Oct.	147	6	69	0	1	76	33.4	51.7
Nov.	129	7	63	0	0	70	30.8	54.3
Dec.	125	5	71	2	0	78	34.3	62.4
Total	1,357	84 (13.7%)	511 (83.5%)	9 (1.5%)	8 (1.3%)	612 (100%)	269.2	45.1

[1] *Pv*, *Plasmodium vivax*; *Pf*, *Plasmodium falciparum*; *Pm*, *Plasmodium malariae*.

cipal vector in the hills and that *An. culicifacies* played a secondary role in malaria transmission (Gunasekaran et al. 1989, 1994).

Among sibling species of *An. culicifacies*, species B and C were found sympatric in the villages in forests, with a predominance of the latter. Studies carried out in different parts of India have established that species A, C, D, and E are vectors of malaria, whereas species B is not an established vector (Subbarao et al. 1988a, 1988b, 1992; Kar et al. 1999). Although species B and C were primarily zoophagic in the study villages, a small proportion of each of the species was found to have fed on human blood and the HBI of species C was higher than that of species B. Thus, it seems that species C may be acting as a secondary vector in the forest villages.

A marked difference in the host-feeding patterns of *An. fluviatilis* and *An. culicifacies* sibling species observed in the forested villages seems to be due to species-specific feeding preferences rather than host availability. The human to cattle ratio in these villages was 2.27:1 and *An. fluviatilis* species S was predominantly anthropophagic, but *An. culicifacies* species B and C coexisting in this area were mostly zoophagic.

In deforested riverine villages, *An. culicifacies* species B and C were predominant. A few double-inversion heterozygotes, which probably represent interspecific hybrids due to breakdown of premating barriers between species B and C, were also observed. Specimens belonging to species A were found occasionally in a low proportion. As in the forested villages, *An. culicifacies* sibling species were almost zoophagic in spite of the human to cattle ratio of 3.17:1. Except for 1 specimen of *An.*

Table 7. Malaria cases in the deforested riverine villages Jabaghat and Jamsera (pooled data) of Sundargarh District, Orissa, during 1992.

Month	Blood smears tested	Malaria cases[1]				Cases/1,000 population	Slide positivity rate (%)
		Pv	*Pf*	*Pv* + *Pf*	Total		
Jan.	19	5	5	0	10	6.8	52.6
Feb.	13	1	0	1	2	1.4	15.4
March	21	3	2	1	6	4.1	28.6
April	19	3	4	0	7	4.8	36.8
May	13	1	0	0	1	0.7	7.7
June	19	1	3	0	4	2.7	21.0
July	16	2	3	0	5	3.4	31.2
Aug.	22	1	2	0	3	2.0	13.6
Sept.	18	1	1	0	2	1.4	11.1
Oct.	23	1	4	0	5	3.4	21.7
Nov.	13	0	2	0	2	1.4	15.4
Dec.	15	3	7	0	10	6.8	66.7
Total	211	22 (38.6%)	33 (57.9%)	2 (3.5%)	57 (100%)	39.0	27.0

[1] *Pv*, *Plasmodium vivax*; *Pf*, *Plasmodium falciparum*; *Pm*, *Plasmodium malariae*.

fluviatilis recorded from Jabaghat and identified as species T, which is considered to be a poor vector of malaria (Sharma et al. 1995, Shukla et al. 1998), no other species was found. The absence of *An. fluviatilis* in deforested riverine villages may be attributed to the absence of preferred breeding sites such as streams, stream channels, and seepages in these villages. Moreover, *An. fluviatilis* is a hygrophilic sylvatic species with limited flight range; therefore, it was confined within the cool and humid forest zone and could not extend to deforested area with a hot-dry climate. *Anopheles culicifacies,* on the other hand, seems to have a greater adaptability and could withstand the climate of the deforested area as well. *Anopheles culicifacies* species C, which comprised about 78% of the populations in the study villages, was apparently responsible for the local malaria transmission in deforested riverine villages. In central India, species C was the most prevalent species (65–90%) and was incriminated for *P. vivax* and *P. falciparum* sporozoites (Subbarao et al. 1992). Recently, Singh et al. (1996) also found 3 out of 2,921 *An. culicifacies* dissected to be positive for sporozoites in the villages away from the forest in Mandla District in central India.

The vectorial efficiency of the members of the *An. fluviatilis* and *An. culicifacies* complexes was reflected in the malaria incidence in the areas of their prevalence. In forested villages where *An. fluviatilis* species S and *An. culicifacies* species C were predominant, fever prevalence was 4 times more, malaria incidence was 7 times more, SPR was 2 times higher, and *P. falciparum* proportion was 1.5 times greater than those in the deforested riverine villages. Prevalence of *P. malariae* cases only in the forested area further supports the view that the longevity of the vectors was greater in the forest.

In conclusion, the high endemicity of malaria in the villages in the forest was due primarily to perennial transmission maintained by multiple vector species, the presence of a highly anthropophagic vector and its preferred breeding sites, and more favorable climatic conditions for vector multiplication and longevity. In addition, poor accessibility to health services and control operations may contribute to high malaria morbidity in the forested villages. The present study also suggests that knowledge of the bionomics of sibling species of malaria vectors would help in understanding the dynamics of malaria transmission and would facilitate the planning of appropriate and effective control measures.

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