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POPULATION DYNAMICS OF *ANOPHELES CULICIFACIES* AND MALARIA IN THE TRIBAL AREA OF CENTRAL INDIA

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ABSTRACT. A longitudinal study (1993–94) on malaria was conducted in Dungaria, a typical forest fringe tribal village in Mandla district of central India (Madhya Pradesh). Our initial objective was to obtain in-depth baseline data on malaria transmission in the tribal village to elucidate the factors responsible for persistent malaria in the area and thereby to help in formulating an improved malaria control program. *Anopheles culicifacies* Giles was the predominant vector of malaria, although *Anopheles fluviatilis* James were recorded in small numbers. The transmission season was from May to November. Analysis of the malaria cases revealed hyperendemic malaria, with *Plasmodium falciparum* the predominant species. The prevalence of *Plasmodium vivax* was mainly in the summer and that of *P. falciparum* in autumn. The study suggested that a number of factors were responsible for the continuation of malaria transmission in the village.

KEY WORDS *Plasmodium falciparum, Plasmodium vivax,* malaria, *Anopheles, Anopheles fluviatilis,* tribal village, transmission

INTRODUCTION

Several investigators have analyzed the impact of various ecological (e.g., construction of dams and clearing of forests) and socioeconomic factors (e.g., urbanization, industrialization, and population migration) on the resurgence of malaria in India (Pattanayak et al. 1994). Considering these multiple factors, 5 distinct epidemiological types of malaria have been recognized: tribal malaria, urban malaria, rural malaria, industrial malaria, and border malaria (Sharma 1996). Malaria control requires specific approaches and control strategies for each epidemiological type of malaria. According to the statistics of the National Malaria Eradication Programme (NMEP), tribal people constitute about 7.8% of the total population of India but suffer 30% of the total malaria cases, 60% of the total *Plasmodium falciparum* cases, and 50% of the total malaria deaths (NMEP 1990–94). Tribal malaria is hypoendemic and perennial in a population of about 54 million living in the tribal areas. This malaria is not responsive to existing control measures, which emphasizes the need for better understanding of its epidemiological features and transmission dynamics (Sharma 1996). However, almost nothing is known about these aspects of malaria in the tribal area. Therefore, a longitudinal study was carried out to study the dynamics of malaria transmission and epidemiology in Dungaria, a typical small tribal village on the forest fringe. Because Dungaria is representative of tribal villages in Madhya Pradesh, this study may serve as a model for planning malaria control for tribal malaria.

MATERIALS AND METHODS

Study area: Mandla, the center of Madhya Pradesh, is a region of deep valleys, hills, and hillocks with thick dense forest (23°N latitude, 80°10′E longitude). The study village, Dungaria, is located on the slopes of the hillocks adjoining a perennial stream (Fig. 1). The latter with several tributaries criss-crosses the village and provides numerous breeding sites for mosquitoes. The village was unapproachable during rains (July to October). The village consists of 125 houses in 4 hamlets with a population of 650 and is under DDT residual spray twice a year during June and August (1 g/m^2/round).

A typical tribal house consists of a living room and a kitchen almost combined. About 90% of the houses have tiled roofs and mud walls; the remaining 10% have a thatched roof with mud walls. Tribal people spend most of their time outside the dwellings and sleep on the floor of the verandah (walled on 3 sides and open on 1 side) or outdoors, and they are poorly clothed. The doors are low and small and windows are seldom present. Often domestic animals are sheltered in the house. The family size varied from 2 to 17 with an average of 6 members. Most of the men and women work as laborers in forest nurseries, road construction, and other casual jobs away from their homes (Singh et al. 1996).

The climate is characterized by a hot summer (March–June), a monsoon/rainy season (July–October), and a cool autumn season (November–February). The mean annual rainfall for 1993–94 was 1,400 mm. Rainfall records for this village were not available because of the remoteness of the area; thus, data were obtained from the Agriculture Engineering College in Jabalpur. The mean maximum temperature ranges between 20.5 and 44°C, with May being the hottest month and the mean minimum temperature ranges between 3.5 and 25°C, with December being the coldest month (Fig. 2).

Mosquito sampling: Indoor resting collections (per man hour) were carried out twice a month from January 1993 to December 1994 (48 collections). *Anopheles* resting inside 4 designated houses lo-

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Fig. 1. Typical tribal village Dungaria showing undulating terrain and scattered houses.

cated in different parts of the village (2 human dwellings and 2 cattle sheds) were sampled in the early morning (0600 h) for 15 min each by a team of 2 insect collectors with flashlights and mouth aspirators. All houses were treated with DDT in June and August (June 1st, 1993 and June 5th, 1994; August 28th, 1993 and August 30th, 1994) each year (with about 50% coverage). Simultaneously, anophelines resting outdoors were collected with mouth aspirators (Buttiker 1958, Breeland 1972) by another team of 2 collectors from stone quarries, bushes, small plants near streams, on fences, firewood stored outside the houses, and tree holes, etc. Outdoor collections were made only during the 1st year of the study. Anopheline mosquitoes were identified using taxonomic keys (Christophers 1933, Nagpal and Sharma 1995) and sorted according to abdominal condition (World Health Organization 1975). Polodovova's technique of counting dilatations on the ovariole was used to determine physiological age (Detinova 1962). Ovarioles with 2 and more dilatations are combined as more than 3 dilatations are rare. Only female *An. culicifacies* and *An. fluviatilis* were examined for salivary gland infections and parity because the remaining species were abundant only during limited periods of the year. These examinations were made in the field laboratory of the Malaria Research Centre about 25 km from the village. Both *Anopheles culicifacies* and *Anopheles fluviatilis* belong to complexes of species that can be distinguished only by cytogenetic or DNA probe techniques, and we did not attempt to identify them further under primitive field conditions. Within the geographical range of the present study, *An. culicifacies* species A, B, C, and D have been found of which C and D (90 to 95% of the population) were incriminated as vectors (Subbarao et al. 1992). Members of the *An. fluviatilis* species complex are not known for this area.

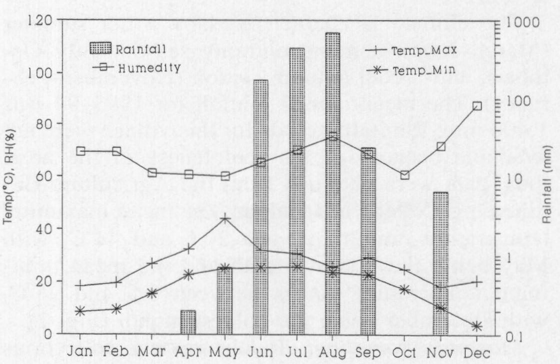

Fig. 2. Meteorological data of study area during the year 1993–94.

Bait catches: Human-bait collections (indoor and outdoor) were made from 1800 to 0600 h for 70 nights (3 nights per month; but only 2 nights per month every August due to heavy rainfall). Catches were made by 2 collectors equipped with flashlights and aspirators seated facing each other on stools on verandahs where people sleep during the night. Catches on verandahs were considered as indoor catches, and a 2nd pair of collectors similarly equipped was seated outdoors about 5 m away from the houses. The feet, legs, arms, and heads of the collectors were bare. A night biting catch consisted of 12 episodes of 50 min of uninterrupted catching. Each episode began on the hour, and all mosquitoes landing on or biting either member of a pair were collected and stored in a paper cup. Each of the 24 paper cups collected nightly was labeled by hour and station. During the 10-min hourly rest period, weather conditions were taken on the site. Indoor and outdoor teams exchanged positions on the hour to avoid collector bias.

During the catches, a trained Research Assistant identified the mosquitoes morphologically and examined them immediately for sporozoites and parity.

Malaria surveillance: Active case detection was done by conducting door-to-door searches twice a month. Blood smears were prepared from all current fever cases and people who reported fever during the preceding 14 days. All fever cases were given a 600-mg chloroquine prophylactic treatment. Blood smears were stained with JSB stain (Singh and Bhattacharji 1944), and thick smears were examined under oil immersion in the laboratory. All parasite-positive cases were given radical treatment as per NMEP.

Radical treatment (NMEP)

Plasmodium vivax: A single dose of 600 mg chloroquine and 15 mg primaquine on the 1st day followed by 15 mg primaquine daily for the next 4 days (adult dose).

Plasmodium falciparum: A total dose of 1,500 mg chloroquine (600 mg on day 1 + 600 mg on day 2 + 300 mg on day 3) and 45 mg primaquine (adult dose). For younger age groups the drugs should be proportionately reduced.

Data analysis

Per man hour density (PMH): The number of mosquitoes collected by 2 insect collectors in 1 h in 4 dwellings (15 min in each dwelling by each collector).

Parity rate: The number of parous mosquitoes in the total number of mosquitoes dissected.

Slide positivity rate (SPR): Total number of blood smears found positive for malaria parasites in total number of blood smears examined. Statistical significance of the data was determined by Z-test.

Slide falciparum *rate (SFR):* Total number of blood smears found positive for *P. falciparum* in total number of blood smears examined.

Annual blood examination rate (ABER): Number of blood smears examined in a year in the total population under malaria surveillance.

Annual parasite incidence (API): The proportion of the total number of blood smears found positive for malaria parasite in a year per thousand population.

RESULTS

Mosquito resting catches: The anopheline fauna of the village consisted of 8 species, of which *An. culicifacies* Giles, *Anopheles subpictus* Grassi, *An. fluviatilis* James, *Anopheles splendidus* Koidzumi, and *Anopheles annularis* Van Der Wulp were the most abundant species in indoor resting collections (Table 1). The relative abundance of *An. culicifacies* was high throughout the year (78–97%) with a main peak in August to September and a 2nd small peak in February to March. About 35% of the *An. culicifacies* were semigravid or gravid. The parity rate of *An. culicifacies* varied among months (Table 1), ranging from 27% in April to 69% in July (95% confidence interval [CI]; 31–53%). *Anopheles fluviatilis* was collected mainly from November to February and was completely absent during the hot dry months of May to July. The parity rate varied from 25% in April to 62% in October to November (95% CI; 9–84%).

In outdoor resting collections, the number per man hour of collecting effort of *An. culicifacies* and *An. fluviatilis* was 3.4 and 2.0, respectively (data not shown). Overall, 59% of *An. culicifacies* were blood fed and 20% were semigravid or gravid, whereas 15% of *An. fluviatilis* were fed and 39% were gravid. Other species collected in small numbers were *An. subpictus, An. annularis,* and *Anopheles vagus* Donitz, mainly from fire wood stored outside the houses and fences, etc.

Biting behavior: During the course of 70 all-night biting collections (300 man-nights; indoor 239, outdoor 61: outdoor collections were not made during extreme winter and during excessive rains), 400 anophelines were captured, of which 86.5% were *An. culicifacies,* 4.5% *An. subpictus,* 3.5% *An. fluviatilis,* and small numbers of 7 other species. Figure 3 shows that during the autumn season (November–February) almost all feeding occurred before midnight with the peak at dusk in indoor collections. During summer (March–June) and monsoon season (July–October), feeding occurred all night in both indoor and outdoor collections with some fluctuations (Figs. 4 and 5). Over the whole period of the study the catches per man per night were 1.15 and 1.16 in indoor and outdoor collections, respectively.

Of the *An. culicifacies* on human bait, 58% were either semigravid or gravid at the time of capture and 50% were parous. Further analysis of parity data revealed that during the monsoon season 56% of *An. culicifacies* were parous (46% uniparous; 10% multiparous), 50% during winter (44.4% uniparous; 5.5% multiparous), and 40.5% during summer (38% uniparous; 1.3% multiparous). However, there were no significant differences in parity rates among seasons. Only a few females (0.25%) with

Table 1. Relative abundance of indoor resting anophelines (per man hour) and parity status of major anopheline species in Dungaria (1993–94).

Species	January	February	March	April	May	June	July	August	September	October	November	December
An. culicifacies												
No.	370	422	435	313	307	244	384	892	675	461	350	281
PMH	46.2	52.8	54.3	39.1	38.3	30.5	48.0	111.5	84.3	57.6	43.7	35.1
	(77.89)[1]	(90.17)	(94.36)	(94.85)	(96.85)	(95.69)	(92.09)	(84.39)	(84.27)	(86.49)	(89.06)	(77.41)
Parity												
No. dissected	115	61	117	119	91	56	129	121	162	138	90	93
Parity rate	40.9	37.7	33.3	26.9	37.4	28.6	69.0	59.5	60.5	47.8	42.2	40.9
An. fluviatilis												
No.	52	17	7	4	0	0	0	3	1	8	20	34
PMH	6.5	2.1	0.8	0.5				0.3	0.1	0.9	2.5	4.2
	(10.95)	(3.63)	(1.52)	(1.21)				(0.28)	(0.12)	(1.50)	(5.09)	(9.37)
Parity												
No. dissected	52	17	7	4	0	0	0	3	0	8	8	30
Parity rate	48.1	58.8	62.0	25.0				33.3		62.5	62.5	33.3
An. annularis												
No.	0	8	11	0	0	0	5	16	16	36	0	4
PMH		1.0	1.4				0.6	2.0	2.0	4.5		0.5
		(1.71)	(2.39)				(1.20)	(1.51)	(2.00)	(6.75)		(1.10)
An. subpictus												
No.	0	0	0	13	10	11	28	134	93	12	0	0
PMH				1.6	1.2	1.4	3.5	16.8	11.6	1.5		
				(3.94)	(3.15)	(4.31)	(6.71)	(12.68)	(11.61)	(2.25)		
An. theobaldi												
No.	10	8	4	0	0	0	0	0	0	0	4	4
PMH	1.2	1.0	0.5								0.5	0.5
	(2.11)	(1.71)	(0.87)								(1.02)	(1.10)
An. pallidus												
No.	4	5	0	0	0	0	0	0	8	4	8	4
PMH	0.5	0.6							1.0	0.5	1.0	0.5
	(0.84)	(1.07)							(1.00)	(0.75)	(2.04)	(1.10)
An. splendidus												
No.	39	8	4	0	0	0	0	0	0	8	7	36
PMH	4.9	1.0	0.5							1.0	0.9	4.5
	(8.21)	(1.71)	(0.87)							(1.50)	(1.78)	(9.92)

Table 1. Continued.

Species	January	February	March	April	May	June	July	August	September	October	November	December
An. vagus												
No.	0	0	0	0	0	0	0	12	8	4	4	0
PMH								1.5 (1.14)	1.0 (1.00)	0.5 (0.75)	0.5 (1.02)	
Total												
No.	475	468	461	330	317	255	417	1,057	801	533	393	363
PMH	59.3	58.5	57.6	41.2	39.6	31.8	52.1	132.1	100.1	66.6	49.1	45.3

[1] Figures in parentheses are the percent composition of species.

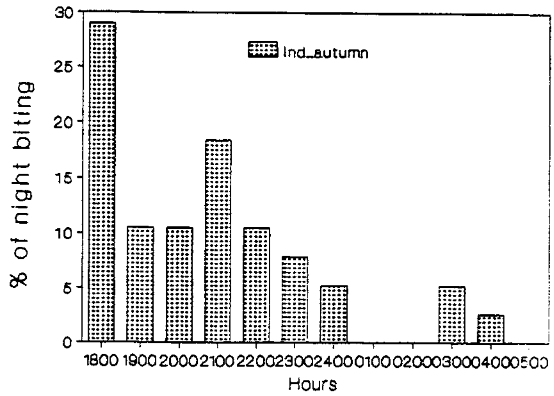

Fig. 3. Indoor and outdoor biting behavior of *Anopheles culicifacies* on human bait, autumn.

up to 4 dilatations in the ovariole were recorded during the monsoon season. Most *An. fluviatilis* feeding was before midnight with a peak between 2000 and 2100 h. Mean catch per man per night averaged 0.046 of which 60% were parous.

Vector infection: A total of 1,350 *An. culicifacies* and 65 *An. fluviatilis* collected resting in human dwellings and 222 *An. culicifacies* collected on human bait were dissected. Of these, 3 *An. culicifacies* were positive for sporozoites in the months of May, August, and November. *An. fluviatilis* were negative for sporozoites from both resting and bait collections.

Malaria prevalence: Malaria prevalence in children (<14 years) and adults (>14 years) are shown in Table 2. Though *P. falciparum* was the predominant species, *Plasmodium vivax* cases were also found in small numbers. Seasonal patterns in both children and adults in each year of the study were similar. *Plasmodium vivax* malaria cases started from March to April and continued up to September and October. *Plasmodium falciparum* cases were found mainly from July to November, and very few cases were recorded during March to June

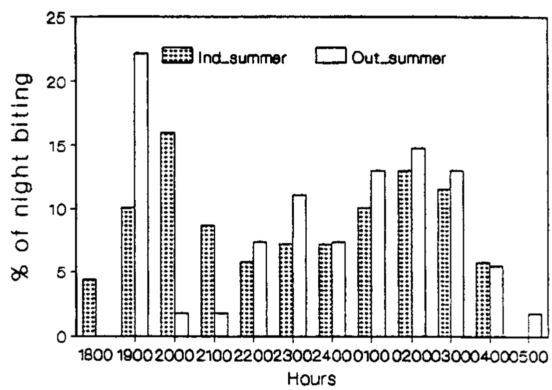

Fig. 4. Indoor and outdoor biting behavior of *Anopheles culicifacies* on human bait, summer.

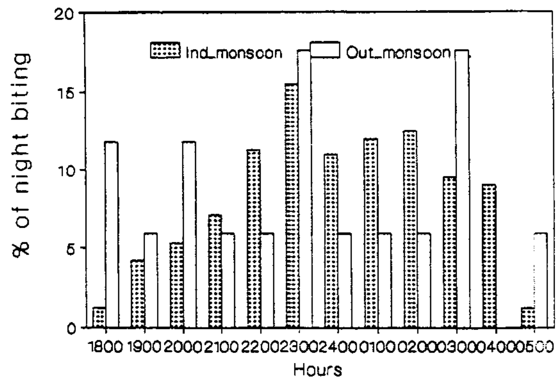

Fig. 5. Indoor and outdoor biting behavior of *Anopheles culicifacies* on human bait, monsoon season.

in both children and adults. However, the %*P. falciparum* was higher in adults when compared to children ($P < 0.0001$). *Plasmodium falciparum* gametocytes were present in 11% of children during both years, while in adults it was 25% (1993) and 23% (1994), which is significantly higher ($P < 0.0001$) than children. Overall ABER and API were 81.4 and 405, respectively, for 1993. However, during 1994 both ABER (67) and API (167) declined, which may be related to the surveillance and drug distribution by project staff.

DISCUSSION

Anopheles culicifacies is an endophilic resting species (Rao 1984). Indoor resting densities of *An. culicifacies* remained high at Mandla throughout the year. Further, in tribal houses there are many gaps near eaves and walls and on the roof, which facilitated mosquito ingress and egress and was thus an added factor for maintaining high densities in spite of DDT spraying. However, spraying may not control even the fully endophilic mosquitoes, as earlier studies carried out in Mandla showed only 19% mortality of *An. culicifacies* to 4% DDT susceptibility test papers (Singh et al. 1989). This is further confirmed by high parity rates recorded throughout the year in this study.

Anopheles culicifacies was found resting outdoors in the study village in agreement with other studies in Madhya Pradesh (Saxena et al. 1992). The outdoor resting of *An. culicifacies* is of significance from a malaria control standpoint, because the vector may avoid contact even with an effective insecticide sprayed inside the houses. Moreover, there were numerous sites for malaria mosquitoes to breed uninterrupted in streambed pools, pits, and seepages, where larval control may not be possible.

Anopheles culicifacies is known to maintain malaria transmission from July to October (Vaid et al. 1974), and accordingly, 2 rounds of DDT spray were recommended under the NMEP for interruption of malaria transmission. However, late winter transmission in February and early summer transmission in March by *An. culicifacies* has already been recorded in Madhya Pradesh (Vaid et al. 1974, Kulkarni 1987). In this study, infective *An. culicifacies* were found in the months of May, August, and November. All *An. culicifacies* found to be infective were from human dwelling collections, which indicates that malaria was transmitted within the village. This also was substantiated by the fact that during this study 8 of our 20 team members were infected with *P. vivax* or *P. falciparum* during the summer or monsoon months following the seasonal pattern of Dungaria. Although no sporozoite-positive *An. culicifacies* could be found outdoors in the present study, physiologically aged females were represented in the outdoor catches. Thus, some epidemiologically dangerous female *An. culicifacies* apparently rest out of doors, and outdoor resting could be one of the causes of continued transmission. However, there is no published evidence based on any systematic study from Madhya Pradesh to substantiate these views.

One epidemiologically important finding was that more than 50% of host-seeking female *An. culicifacies* had already fed once. Refeeding during the same gonotrophic cycle by about 7% and 30% of females has been reported (Viswanathan et al. 1955, Reisen et al. 1976, Reuben et al. 1984), but the proportion in this area appears to be considerably higher. However, collections at bait do not necessarily mean that all the females would have fed, as some of these mosquitoes were captured immediately after alighting, but the majority of *An. culicifacies* were found to contain fresh blood in the stomach. Further, the high parity of *An. culicifacies* showed high vector potential for transmitting malaria in this village especially in the absence of other vectors. Lines et al. (1991) also found a strong linear relationship between the higher dilatation categories and the probability of carrying sporozoites. The situation therefore warranted an extensive study because a higher frequency of refeeding would further increase the risk of malaria transmission.

It is noteworthy that no infective specimen of *An. fluviatilis* was found, though this is the originally established vector in central India (Senior White and Adhikari 1940, Kulkarni and Wattal 1982, Subbarao et al. 1992). The presence of *An. fluviatilis* in human dwellings, though in small numbers, raises the question of whether it plays any role in malaria transmission.

The new malaria control strategy of the World Health Organization demands rapid diagnosis of malaria at the village and district level so that effective treatment can be administered quickly (WHO 1992). This requires ample resources, community support, managerial skill, and a team of dedicated workers. Several factors make malaria control operations in the tribal villages ineffective. First, the area is vast and with poor communica-

Table 2. Agewise malaria prevalence in Dungaria (1993 and 1994).

Months/ years	Children (<14 years)					Adults (>14 years)				
	BSE[1]	Pv[2]	Pf[3]	SPR[4]	SFR[5]	BSE[1]	Pv[2]	Pf[3]	SPR[4]	SFR[5]
January										
1993	8	0	2	25.00	25.00	2	0	0	0.00	0.00
1994	4	0	4	100.00	100.00	13	0	6	46.15	46.15
February										
1993	8	0	1	12.50	12.50	11	1	2	27.27	18.18
1994	4	0	1	25.00	25.00	5	0	0	0.00	0.00
March										
1993	7	1	1	28.57	14.28	7	0	0	0.00	0.00
1994	2	1	1	100.00	50.00	14	0	0	0.00	0.00
April										
1993	4	1	0	25.00	0.00	13	2	2	30.77	15.38
1994	7	1	0	14.28	0.00	12	1	0	8.33	0.00
May										
1993	15	2	0	13.33	0.00	18	2	2	22.22	11.11
1994	11	3	0	27.27	0.00	20	7	2	45.00	10.00
June										
1993	7	0	0	0.00	0.00	18	2	0	11.11	0.00
1994	20	5	0	25.00	0.00	42	3	2	11.90	4.76
July										
1993	19	0	2	10.53	10.53	47	8	9	36.17	19.15
1994	17	2	1	17.65	5.88	37	1	4	13.51	10.81
August										
1993	14	2	5	50.00	35.71	59	5	22	45.76	37.29
1994	38	6	4	26.31	10.53	62	1	19	32.26	30.65
September										
1993	27	2	15	62.96	55.55	70	4	52	80.00	74.29
1994	39	0	15	38.46	38.46	37	1	5	16.22	13.51
October[6]										
1993	14	2	7	64.29	50.00	21	4	12	76.19	57.14
1994	3	0	1	33.33	33.33	5	0	3	60.00	60.00
November										
1993	34	6	20	76.47	58.82	85	1	45	54.12	52.94
1994	4	1	1	50.00	25.00	11	0	1	9.09	9.09
December										
1993	2	0	2	100.00	100.00	27	0	18	66.67	66.67
1994	15	1	3	26.67	20.00	14	0	2	14.29	14.29
Total										
1993	159	16	55	44.65	34.59	378	29	164	51.06	43.39
1994	164	20	31	31.10	18.90	272	14	44	21.32	16.18

[1] BSE = blood slide examined.
[2] Pv = *Plasmodium vivax*.
[3] Pf = *Plasmodium falciparum*.
[4] SPR = slide positivity rate.
[5] SFR = slide falciparum rate.
[6] Two festivals (Dassera and Diwali) that fall in October are important events in the village, in which everyone indulges in heavy drinking and dancing to please God, to ensure better harvest, and to take away disease from the village. Feast and fun continued throughout the month and people did not like to be disturbed unless they were very seriously ill.

tions. Second, many patients with malaria are not available for blood smears because people even with fever prefer to work in the field during rains. This is further supported by the fact that the percentage of *P. falciparum* cases in total positive cases for malaria parasite was higher in adults as compared to children during both years of this study and so is the presence of gametocyte carriers. The villagers frequently spent the night in the open, presumably providing a source of infection to the anophelines prevalent outdoors. Though efforts were made to document quantitatively the movement of men and women to and from work sites and timings of these work-related movements, the site of anopheline infection remains obscure, as human bait catches could not be done in fields and forests due to many operational problems. Therefore, further indepth studies are required on the site of anopheline infection and the role of potential vectors, especially the taxonomic status of *An. culicifacies* and *An. fluviatilis* in relation to malaria transmission. With these drawbacks and the failure of the insecticides to interrupt transmission, unbridled transmission has been occurring. Taking all these factors into consideration, it is felt that an approach other than spraying with DDT is necessary for the control of malaria in this area. Personal protection from host-seeking *Anopheles* females by sleeping under insecticide-impregnated bed nets is an alternative that has shown promise recently in most parts of the world (WHO 1989). Effectiveness depends on the widespread use of nets during the time when most anophelines blood feed. In this study, about 57% of *An. culicifacies* and 64% of *An. fluviatilis* bite during hours when people are active outdoors throughout the year. Therefore, more carefully monitored trials are necessary before bed nets are recommended for malaria control in the tribal belt.

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