

## Identification of *Anopheles* fauna in a hyperendemic *falciparum* area of Orissa State, India

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**Background & objectives:** Keonjhar district of Orissa State has been hyperendemic for *falciparum* malaria since many years with alarming deaths due to cerebral malaria. Therefore an entomological investigation to know more about the relative prevalence of *Anopheles* species was done.

**Methods:** Daytime indoor resting and outdoor resting, light trap and double bed net collections were made. Surveys were also made to collect *Anopheles* immature in streams and paddy fields. The *Anopheles* mosquitoes obtained by different catching methods were identified and the known vector species were subjected to gut and salivary gland dissection for vector incrimination. The infected specimens of *An. fluviatilis* and *An. minimus* were subjected to polymerase chain reaction assay for identification of sibling species.

**Results:** Of the anophelines collected, the most abundant was *An. splendidus* (18.2%) and *An. fluviatilis* (17.7%), followed by *An. maculatus* (14.0%) and *An. minimus* (9.0%). The sporozoite rate of *An. fluviatilis* and *An. minimus* was 0.9 and 1.4 respectively. The infected specimens have been identified as sibling species S of the *An. fluviatilis* complex and A of the *An. minimus* complex.

**Interpretation & conclusions:** *An. fluviatilis* and *An. minimus* are the major two species in the transmission of malaria in Keonjhar district in Orissa.

**Key words** *Anopheles* fauna - *An. fluviatilis* - *An. minimus* - India - Orissa - sporozoite rate

Keonjhar district of Orissa State, India is afflicted with high incidence of malaria since many decades<sup>1-3</sup>. During 2001 - 2005, the annual parasite incidence (API) in the district ranged from 13.9 to 25.8. *Plasmodium falciparum* has been the predominant malaria parasite (>95%) in the district<sup>3</sup>. Death due to cerebral malaria is alarmingly high<sup>3</sup> and during the last five years (2001 - 2005) there were 208 reported deaths due to malaria,

which accounted for about 5.0 per cent of the total number of malaria deaths recorded in India during this period (*Source:* Data from the Office of the Chief District Medical Officer, Keonjhar). The indoor residual spraying initiated using DDT in 1953 under the National Malaria Control Programme was continued in this area till 2000. From 2001 onwards, synthetic pyrethroids are being used for indoor residual spraying in place of DDT

in 11 of the 13 primary health centres (PHCs) of the district under the Enhanced Malaria Control Programme (EMCP)<sup>3</sup>. In spite of these intervention measures, malaria continues to persist in the district. Information on *Anopheles* species composition and vectors of malaria in the district during the post-DDT era is very limited<sup>2</sup>. We carried out an entomological investigation in one of the malaria endemic PHCs of Keonjhar district for a period of 18 months from August 2005 to January 2007 in order to elucidate the relative prevalence of *Anopheles* species with the specific objective of vector incrimination.

### Material & Methods

**Study area:** Keonjhar district, situated in northern Orissa, is part of the extension of the Chhotanagpur plateau (Singhbhum hills) of Bihar that comprises Mayurbhanj, Keonjhar and Sundargarh districts of Orissa State<sup>1,2</sup>. This district covers an area of 8303 sq. km between 20° 11" to 22° 10" N latitude and 85° 11" to 86° 22" E longitude and has a population of 1.5 million distributed in 2118 villages. Tribes constitute about 44.5 per cent of the total population<sup>3</sup>. Topography and climatological features of the district have been reported elsewhere<sup>3</sup>. Majority of the houses (>90%) are made up of mud wall and tiled roof. The houses have generally two rooms. Majority of the households have a cattle shed very close to their houses.

Among the 13 PHCs of the district, which recorded the higher API, Baunspal PHC was selected randomly for the study. This PHC reports death due to cerebral malaria every year. During 2001 to 2005, the API of the PHC ranged from 7.5 to 15.8 with 18 reported malarial deaths (*Source*: Data from the CDMO's office, Keonjhar). *Anopheles* mosquitoes were collected from 7 randomly selected villages *viz.*, Mamulipusi, Puradihi, Dhankuniasahi, Boitarani, Natokotha, Bayakumutia, and Mundla where the population is mostly tribes. The selected villages are located in hilly terrain with an altitude ranging from 573.4 to 601.2 meter from mean sea level, and are covered by deep forest traversed by rivulets and hill streams.

**Entomological collections:** The entomological collections were conducted bimonthly from August 2005 to March 2006 and monthly from May 2006 to January 2007. Daytime indoor resting adult *Anopheles* mosquitoes were collected using a mouth aspirator and torchlight, between 06.00 and 07.00 h, spending a total of 90 man-hours in human dwellings and 45 man-hours in cattle sheds. The artificially dug pit shelters and pot

shelters were searched for outdoor resting *Anopheles* mosquitoes<sup>4</sup> spending a total of 24 man-hours. In three villages, the battery operated modified version of CDC miniature light traps<sup>5</sup> were hung from dusk to dawn in human dwellings at a height of 1 foot from the ground and in cattle sheds at a height of 4 feet from the ground (2 traps each in human dwellings and in cattle sheds per night per village). A total of 82 trap night collections each in human dwellings and cattle sheds were made during the study period.

Double bed net (consisting of an inner protective net and a larger outer net) collections (DBN)<sup>6</sup> were made from dusk to dawn in three villages (2 DBN collections per night in human dwellings) and in total 104 DBN collections were made. To engage human volunteers for sleeping under double bed nets, clearance was obtained from the ethical committee of Vector Control Research Centre (VCRC), Puducherry. The *Anopheles* mosquitoes collected by different methods were identified and recorded. All suitable specimens of known and potential vector species were dissected and examined for malaria parasite infection in gut and salivary gland. For oocyst infections, midguts of only unfed, semi-gravid and gravid females were examined.

After dissection and microscopic examination, the body parts of the individual sporozoite infected specimen of *An. fluviatilis* and *An. minimus* were kept in Eppendorf tube, dried for 4-5 h at 90°C and sent to the VCRC laboratory for polymerase chain reaction (PCR) assay. The molecular identification of *An. fluviatilis* and *An. minimus* was carried out following the methodology described by Singh *et al*<sup>7</sup> and Garros *et al*<sup>8</sup> respectively.

On the same day of adult collection, surveys were made from streams and paddy fields, which are the major breeding habitats in the area, to collect immature of *Anopheles*. The collected immature were reared to adults and identified.

### Results & Discussion

A total of 4471 *Anopheles* mosquitoes of 19 species were obtained from adult collections and from immature collections another 1327 anophelines belonging to 19 species were recorded during the study period. One species which was not obtained from adult collections was recorded from immature collections, thus increasing the number of *Anopheles* species to 20 (Table I). The species recorded included *Anopheles aconitus*, *An. annularis*, *An. culicifacies*, *An. fluviatilis*, *An. jeyporiensis*, *An. maculatus*, *An. minimus*, and *An.*

**Table I.** Number of *Anopheles* species collected by various methods in the study area

Species	From adult collections						Total	From immature collections
	Hand catch		DBN	Light trap				
	Indoor resting	Outdoor resting		HD	CS			
	HD	CS		HD	CS			
<i>An. aconitus</i>	0	1	0	0	0	2	3	8
<i>An. annularis</i>	5	20	0	0	0	101	126	14
<i>An. barbirostris</i>	0	2	0	0	1	22	25	45
<i>An. culicifacies</i>	150	143	0	0	5	44	342	39
<i>An. fluviatilis</i>	587	2	1	122	20	5	737	291
<i>An. jamesii</i>	1	14	0	0	1	155	171	17
<i>An. jeyporiensis</i>	39	69	0	1	2	145	256	34
<i>An. karwari</i>	0	1	0	0	0	9	10	0
<i>An. maculatus</i>	6	22	0	3	4	333	368	445
<i>An. majidi</i>	0	0	0	0	0	0	0	1
<i>An. minimus</i>	401	0	1	93	6	1	502	19
<i>An. nigerrimus</i>	0	15	0	1	2	122	140	43
<i>An. pallidus</i>	2	2	0	0	1	17	22	2
<i>An. ramsayi</i>	0	0	0	0	0	23	23	2
<i>An. splendidus</i>	4	43	0	0	1	911	959	94
<i>An. subpictus</i>	109	120	0	1	9	40	279	49
<i>An. tessellatus</i>	0	1	0	0	0	5	6	2
<i>An. theobaldi</i>	1	5	0	0	3	207	216	131
<i>An. vagus</i>	58	63	0	4	1	13	139	15
<i>An. varuna</i>	25	10	4	10	2	96	147	76
Total	1388	533	6	235	58	2251	4471	1327

HD, human dwelling; CS, cattle shed; DBN, double bed net

*varuna*, which have been recognized as malaria vectors in India. Of the total *Anopheles* species obtained from adult collections, the most abundant ones were *An. splendidus* (21.4%) and *An. fluviatilis* (16.5%), followed by *An. minimus* (11.2%), *An. maculatus* (8.2%) and *An. culicifacies* (7.6%). Among the known primary vector species, *An. fluviatilis* was the predominant one (16.5%) followed by *An. minimus* (11.2%) and *An. culicifacies* (7.6%). From the immature collections, *An. maculatus* (33.5%) was the predominant species followed by *An. fluviatilis* (21.9%) and *An. theobaldi* (9.9%) (Table I).

Of the 20 *Anopheles* species recorded in the present study, 18 were those already reported by Dash *et al.*<sup>2</sup>. *An. minimus* and *An. theobaldi* were not reported by Dash *et al.*<sup>2</sup>. Two species recorded by them *viz.*, *An. aitkenii* and *An. turkhudi* could not be collected during the present study. Entomological surveys conducted in other places of Orissa State during the post-DDT era did not record the presence of *An. minimus*<sup>2,9-11</sup>. The reappearance of *An. minimus* in the district was reported recently by Jambulingam *et al.*<sup>3</sup> after a period of about 50 yr of launching the malaria eradication programme.

**Table II.** Number of different *Anopheles* species dissected for gut & gland infection.

Species	Number dissected	Oocyst +ve gut	Sporozoite +ve gland	Sporozoite rate
<i>An. annularis</i>	14	0	0	0
<i>An. culicifacies</i>	221	0	0	0
<i>An. fluviatilis</i>	693	0	6	0.9
<i>An. jeyporiensis</i>	46	0	0	0
<i>An. maculatus</i>	8	0	0	0
<i>An. minimus</i>	485	0	7	1.4
<i>An. varuna</i>	19	0	0	0

The reason for the reappearance has been attributed to the decreased insecticide pressure in recent years<sup>12</sup>. The relative prevalence of this species in this area as recorded from the adult collections was higher when compared to some of its prevalence records in northeastern States<sup>13,14</sup>.

In the present study, seven *Anopheles* species *viz.*, *An. fluviatilis*, *An. minimus*, *An. culicifacies*, *An. jeyporiensis*, *An. varuna*, *An. annularis*, and *An. maculatus* were dissected and examined for gut and gland infection (Table II). Salivary glands of six *An.*

*fluviatilis* and seven *An. minimus* were found with sporozoites of human *Plasmodium* and the sporozoite rates were 0.9 and 1.4 respectively. None of the other species dissected, was found positive for gland or gut infection.

The diagnostic PCR assay of six infected adult specimens of *An. fluviatilis* showed that they were species S which has been identified as an important malaria vector elsewhere in India<sup>12</sup>. Similarly, the PCR assay of seven infected adult specimens of *An. minimus* showed that all were species A. *An. minimus* from Assam, India, with sporozoite rates ranging from 2.3 to 3.3 per cent has also been identified as species A<sup>15</sup>.

*An. minimus* was incriminated as a vector of malaria in this area during pre-DDT era by Senior White and Das<sup>16</sup>. Subsequently, Senior White and Narayana<sup>17</sup> and Senior White<sup>18,19</sup> detected natural infection with malaria parasites in *An. minimus* and *An. fluviatilis* in the area and the reported infection rates of these two species ranged from 3.9 to 15.4 and 2.6 to 5.7, respectively. Thereafter, there has been no study on vectors of malaria in the area. During 1982, Dash *et al*<sup>9</sup> found sporozoites in one specimen out of 174 female *An. annularis* in Jhumpura PHC of Keonjhar district, and considered this species to be the main vector of malaria of this area. In the present study, *An. annularis* was dissected only in small numbers and its role in malaria transmission could not be established. The present study documented the involvement of *An. fluviatilis* and *An. minimus* in malaria transmission in the district. The sporozoite rate of *An. minimus* was found to be higher than that of *An. fluviatilis*. No gut infection was noticed in any of the vector species dissected in the present study, probably because the mid guts of the fully fed specimens were not examined as they were subjected to blood meal analysis.

Though during pre-DDT era *An. minimus* was one of the primary vectors of malaria all along the foothills of the Himalayas extending from the Terai region of Uttar Pradesh to Assam and the neighbouring eastern region, during post-DDT era this species has been considered as a major malaria vector only in the areas of Northeastern region of the country<sup>3,20</sup>. The present study documented the role of *An. minimus* in malaria transmission in Orissa State (East-Central India) during post-DDT era. The role of *An. fluviatilis* in malaria transmission in many areas of Orissa State has been reported earlier<sup>9,20</sup>. The finding of this study is in corroboration with the statement made by Rao<sup>20</sup> that *An. minimus* and *An. fluviatilis* are vectors in all areas

of India where they are prevalent. Therefore, the malaria vector control in the district should target these two species and since the malaria incidence in this area is high further studies are needed to understand the bionomics of these two vector species.

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### References

1. Watts RC. Report No. II. Malaria survey of the mining settlement of Singhbhum, Orissa. (Superintendent. Govt. Printing, Bihar and Orissa, Patna) 1924.
2. Dash AP, Behura DK, Roy JR. On the distribution of Anopheline mosquitoes in Orissa, India. *J Zool Soc India* 1984; 36 : 1-14.
3. Jambulingam P, Sahu SS, Manonmani A. Reappearance of *Anopheles minimus* in Singhbhum hills of East-Central India. *Acta Trop* 2005; 96 : 31-5.
4. Das PK, Gunasekaran K, Sahu SS, Sadanandane C, Jambulingam P. Seasonal prevalence and resting behaviour of malaria vectors in Koraput District, Orissa. *Indian J Malariol* 1990; 27 : 173-81.
5. Gunasekaran K, Jambulingam P, Sadanandane C, Sahu SS, Das PK. Reliability of light trap sampling for *Anopheles fluviatilis*, a vector of malaria. *Acta Trop* 1994; 58 : 1-11.
6. World Health Organization. Manual on Practical Entomology in Malaria, Part II. Methods and Techniques. Geneva; 1975.
7. Singh OP, Chandra D, Nanda N, Raghavendra K, Sunil S, Sharma SK, *et al.* Differentiation of members of the *Anopheles fluviatilis* species complex by an allele-specific polymerase chain reaction based on 28S ribosomal DNA sequences. *Am J Trop Med Hyg* 2004; 70 : 27-32.
8. Garros C, Koekemoer LL, Coetzee M, Coosemans M, Manguin S. A single multiplex assay to identify major malaria vectors within the African *Anopheles funestus* and the Oriental *An. minimus* Groups. *Am J Trop Med Hyg* 2004; 70 : 583-90.
9. Dash AP, Bendle MS, Das AK, Das M, Dwivedi SR. Role of *An. annularis* as a vector of malaria in inland of Orissa. *J Com Dis* 1982; 14 : 224.
10. Collins RT, Beljaev AE, Pattanayak S, Agarwal RS. Studies on malaria transmission in Orissa State, India 1981 through 1986. Part II: observation on the *Anopheles* fauna. *J Com Dis* 1990; 22 : 191-204.
11. Gunasekaran K, Sahu SS, Parida SK, Sadanandane C, Jambulingam P. Anopheline fauna of Koraput district, Orissa State, with particular reference to transmission of malaria. *Indian J Med Res* 1989; 89 : 340-3.
12. Sharma VP. DDT: The fallen angel. *Curr Sci* 2003; 85 : 1532-7.
13. Prakash A, Bhattacharyya DR, Mohapatra PK, Mahanta J. Mosquito fauna and malaria vectors in Jairampur, district Changlang, Arunachal Pradesh. *Indian J Malariol* 2000; 37 : 74-81.

14. Prakash A, Bhattacharyya DR, Mohapatra PK, Mahanta J. Investigation on malaria vectors and mosquito fauna in South Tripura District, Tripura State. *Indian J Malariol* 1998; 35 : 151-9.
15. Subbarao SK. Anopheline species complexes in South-East Asia. Technical Publication. New Delhi: Regional Office for South-East Asia (SEARO), World Health Organization; 1998. Report No.: 18.
16. Senior White R, Das BK. On malaria transmission in the Singhbhum hills. *J Mal Inst Ind* 1938; 1 : 169-84.
17. Senior White R, Narayana PA. On malaria transmission in the Singhbhum Hills Part II. An experiment with trap nets. *J Mal Inst Ind* 1940; 3 : 413-25.
18. Senior White R. House spraying with DDT and with pyrethrum extract compared: first results. *J Mal Inst Ind* 1945; 6 : 83-96.
19. Senior White R. On the anthropophilic indices of some *Anopheles* found in east-central India. *Indian J Malariol* 1947; 1 : 111-22.
20. Rao TR. *The Anophelines of India*. Revised edition. New Delhi: Malaria Research Centre, Indian Council of Medical Research; 1984.

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