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

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Received March 12, 2007

**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
in 11 of the 13 primary health centres (PHCs) of the district under the Enhanced Malaria Control Programme (EMCP). 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. 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. 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. Topography and climatological features of the district have been reported elsewhere. 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

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 spending a total of 24 man-hours. In three villages, the battery operated modified version of CDC miniature light traps 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) 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. and Garros et al., 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.
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*. Two species recorded by them *viz.*, *An. aikenii* 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*\(^{2,8,11}\). The reappearance of *An. minimus* in the district was reported recently by Jambulingam *et al*\(^{12}\) after a period of about 50 yr of launching the malaria eradication programme. The reason for the reappearance has been attributed to the decreased insecticide pressure in recent years\(^{12}\). 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\(^{13,14}\).

In the present study, seven Anopheles species *viz.*, *An. flaviatilis*, *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.
Anopheles 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\(^1\). 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\(^15\).

An. minimus was incriminated as a vector of malaria in this area during pre-DDT era by Senior White and Das\(^16\). Subsequently, Senior White and Narayana\(^17\) and Senior White\(^8,10\) 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\(^8\) found sporozoites in one specimen out of 174 female An. annularis in Jhumura 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\(^1,20\). 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\(^9,20\). The finding of this study is in corroboration with the statement made by Rao\(^20\) 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.

**Acknowledgment**

Authors thank Dr P. K. Das, former Director, Vector Control Research Centre, Puducherry, for his interest and encouragement. The co-operation extended by the technical staff of VCRC field station, Malkangiri, is gratefully acknowledged.

**References**


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