VECTORIAL ROLE OF ANOPHELES SUBPICTUS GRASSI AND ANOPHELES CULICIFACIES GILES IN ANGUL DISTRICT, ORISSA, INDIA

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Abstract. Malaria transmission by Anopheles subpictus Grassi, 1899 and Anopheles culicifacies Giles, 1901 was studied from March 2004 to February 2007 in Angul District, Orissa, India, which is highly endemic for malaria. Adult mosquitoes were collected from human dwellings using sucking tubes and a mechanical aspirator. After identification, some An. subpictus and An. culicifacies specimens were subjected to a precipitin test to determine their anthropophilic index and the remaining samples were preserved in isopropyl alcohol for sporozoite detection by nested PCR. An. subpictus was the most prevalent (29.0%) anopheline species detected, followed by An. culicifacies (11.6%). The anthropophilic index for the An. subpictus was higher than An. culicifacies and was highest during the summer season. Malaria sporozoite rates of 0.52% and 1.82% were detected for An. subpictus and An. culicifacies, respectively. Sporozoites were detected during the summer in An. subpictus and during the rainy season and winter in An. culicifacies. The slide positivity rate (SPR) was high during the summer. The high anthropophilic index and presence of sporozoites in An. subpictus during the summer indicate An. subpictus is a contributory factor for the high SPR during the summer, and An. culicifacies is a contributory factor for the high SPR during the rainy and winter seasons, along with other anophelines. In the present study An. subpictus has been incriminated as a vector of malaria for the first time in Orissa.

INTRODUCTION

Anopheles subpictus Grassi is widely distributed and found in abundance in the Oriental region. It is found to the west of India in Afghanistan, Pakistan and Iran and to the east in New Guinea and in the Marinas islands. It is also found in Southern Sri Lanka and northern China. In India it is found throughout the mainland and on Lakshdweep islands, but not on the Andaman and Nicobar Islands (Rao, 1984). Anopheles culicifacies Giles has wide distribution in India and extends up to Ethiopia, Yemen, Iran, Afghanistan and Pakistan in the west and Bangladesh, Myanmar, Thailand, Cambodia and Vietnam in the east. It is also found in Nepal and southern China to the north and extends to Sri Lanka in the south (Rao, 1984).

Among Indian anophelines, An. culicifacies has been regarded as an important vector of malaria (Subbarao, 1988; Surendran et al, 2000). Malaria sporozoites were not detected in An. subpictus until 1981.
in India. Paniker et al (1984) detected malaria sporozoites in *An. subpictus* in a coastal village of Tamil Nadu. Sporozoite positive specimens were also found in Baster District of Madhya Pradesh by Kulkarni (1983). Tyagi and Yadav (2001) in Thar District, Rajasthan found *An. subpictus* amongst eight anopheline species collected from four villages which were endemic for malaria parasites. In Orissa *An. culicifacies* was established as a malaria vector by Nagpal and Sharma (1986). Until now no study has been conducted to evaluate the role of *An. subpictus* in malaria transmission in Orissa. This study has been conducted to determine the role of *An. subpictus* and *An. culicifacies* in malaria transmission in Angul District, Orissa, India.

**MATERIALS AND METHODS**

**Study area**

Orissa is the third most malaria endemic state in India. It has four geographical regions: a northern plateau, a central tableland, a coastal belt, and an eastern step. Malaria is prevalent in all these geophysiological regions of Orissa. The study was conducted in Angul District, Orissa, India, from March 2004 to February 2007. The study area lies between 20º 30' and 21º 30' latitude and between 84º 30' and 85º 15' longitude and is highly endemic for malaria.

**Mosquito collection**

The study villages were visited once a month for entomological collections. Indoor resting mosquitoes were collected from human dwellings in highly malaria endemic villages of Angul District between 6:00 AM and 9:00 AM using a sucking tube and mechanical aspirator. Species identification was done using taxonomic keys of Christophers (1933). Anophelines were collected and per man hour density (PMHD) was determined per the formula given below:

\[
\text{PMHD} = \frac{\text{Number of mosquitoes collected (particular species)}}{\text{(number of persons involved in collection) x (actual time spent in collection)}}
\]

The blood meals were collected on no.1 Whatman filter paper then a gel-diffusion method was carried out followed by a precipitin test (Collins et al, 1983; Parida et al, 2006). Each blood meal was tested against human, cow, bird, dog, pig, goat, rat and snake antisera. Adult unfed specimens and the heads and thoraxes of fully fed malaria specimens of *An. subpictus* and *An. culicifacies* were kept in individual vials for the detection of sporozoites by nested PCR technique. Malaria data were collected from primary health centers (PHCs) and slide positivity rates (SPR) were calculated which can be defined as the number of slides found positive out of one hundred slides examined.

**DNA template preparation**

Total DNA from individual mosquitoes was extracted following a modified method proposed by Collins et al (1987). Briefly, the mosquitoes were homogenized in 100 µl of extraction buffer (pH 9.1) containing 0.1M NaCl, 0.2 M sucrose, 0.1 M Tris-HCl, 0.05 M EDTA, then phenol chloroform isoamyl extraction was carried out. Finally, the DNA was precipitated using ethanol and dissolved in 50 µl of deionized water.

**Nested polymerase chain reaction for the detection of sporozoites**

Amplification of genus and species specific *Plasmodium* was done using the primers: rPLU5 (5’CCTGTTTGTGCCTTAAACTTC3’), PLU6 (5’TATAAATTGTTCGATTTAAAACG3’), rFAL1 (5’TAAACTGGTTTGGAAAACCAAATATATT3’), rFAL2 (5’ACAATGAAACTCAATCATGACTACCCGTC3’), rVIV1 (5’CGCTTCTAGCTTAAATCCACATACTGATAC3’) and rVIV2 (5’ACTTCCAGGCAGGAAAGATCCCTTAAA3’) as described by Snounou et al (1993a,b) and
Mahapatra *et al* (2006). Each 20 µl reaction mixture for nest1 amplifications contained 12 µl of template DNA, 250 nM of each primer (rPLU5 and rPLU6), 4 mM MgCl$_2$, PCR buffer (50 mM KCl, 10mMTris-HCl), 200 mM of each dNTPs and 0.4 units of Taq DNA polymerase. The PCR conditions (nest1) were as follows: 94°C for 4 minutes; 94°C for 30 seconds, 55°C for one minute; extension at 72°C for one minute; 35 cycles and final extension at 72°C for 4 minutes. About 8 µl of the nest 1-amplification products served as the DNA template for each 20 µl of second PCR (nest 2) amplifications. The concentration of the nest 2 primers and other constituents were identical to the nest 1 amplification, except that 0.3 units of Taq DNA polymerase was used. The second PCR (nest 2) amplification conditions were identical to those of first PCR (nest 1) except that the annealing temperature was 58°C for the species-specific primer. The PCR products were analyzed after electrophoresed in 1.5% agarose gel and stained with ethidium bromide.

**Statistical analysis**

Pearson’s correlation coefficient was applied to see the correlations if any between PMHD of *An. subpictus* and *An. culicifacies* with the SPR during different seasons of the study period.

**RESULTS**

A total of 7,700 anopheline specimens comprised of 8 species were caught during the study period. They were *An. subpictus* (29.0%), *An. vagus* Donitz, 1902 (19.7%), *An. nigerrimus* Giles (4.9%), *An. culicifacies* (11.6%), *An. annularis* Van der Wulp, 1884 (9.92%), *An. varuna* Iyengar, 1924 (11.6%) and *An. fluviatilis* James, 1902 (8.4%) of the total number collected. *An. subpictus* was the predominant species among the anophelines. Other known malaria vectors were comparatively fewer in number than *An. culicifacies*. The seasonal prevalences of *An. subpictus* and *An. culicifacies* are depicted in Fig 1. The density of *An. subpictus* was higher than *An. culicifacies* in all three seasons, however, the difference was significant only during summer. These findings led us to screen *An. subpictus* for the presence of malaria sporozoites.
Six of 1,158 *An. subpictus* specimens were found positive for *Plasmodium vivax* sporozoites by nested PCR and showed a sporozoite rate of 0.5%. The sporozoite positive specimens for *An. subpictus* were found during the summer only (Table 1, Fig 2). With *An. culicifacies*, 13 of 716 specimens were positive for malaria sporozoites (10 for *P. falciparum* and 3 for *P. vivax*) and showed sporozoite rate of 1.8%. Malaria sporozoites positive specimens of *An. culicifacies* were found during rainy and winter seasons (Table 1).

Blood meals of *An. subpictus* and *An. culicifacies* were tested against human, bovine, caprine and avian serum by precipitin test. None of the blood meals were found positive for bovine, caprine, or avian serum. The anthropophilic indices were 31.0 % and 25.3% for *An. subpictus* and *An. culicifacies*, respectively. The seasonal anthropophilic indices for *An. subpictus* and *An. culicifacies* are presented in Table 2. Anthropophilic index for *An. subpictus* was comparatively higher than *An. culicifacies* for the three seasons, but was highest during the summer (35.7%).

The seasonal prevalences of SPR are shown in Fig 3. Though the SPR were high throughout the study period, it was significantly higher during the summer. There was a positive correlation between the PMHD for *An. subpictus* and SPR during the summer ($r = 0.846, p < 0.001$) and for *An. culicifacies* during the rainy ($r = 0.789, p < 0.001$) and winter seasons ($r = 0.833, p < 0.001$).

### Table 1

Malaria sporozoites in *An. subpictus* and *An. culicifacies* by nested PCR method.

<table>
<thead>
<tr>
<th>Species</th>
<th>No. tested</th>
<th>No. of positive samples</th>
<th>Parasite species tested</th>
<th>Sporozoite rate (%)</th>
<th>Sporozoite positive in season</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>An. subpictus</em></td>
<td>1,158</td>
<td>6</td>
<td><em>Pf a</em></td>
<td>0.5</td>
<td>Summer</td>
</tr>
<tr>
<td><em>An. culicifacies</em></td>
<td>716</td>
<td>13</td>
<td><em>Pf b</em></td>
<td>1.8</td>
<td>Rainy and Winter</td>
</tr>
</tbody>
</table>

*Pf a, Plasmodium falciparum
Pf b, Plasmodium vivax

### Table 2

Seasonal anthropophilic index of *An. subpictus* and *An. culicifacies*.

<table>
<thead>
<tr>
<th>Season</th>
<th>Species</th>
<th>No. of blood meals studied</th>
<th>No. of blood meals positive in humans</th>
<th>Anthropophilic index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summer</td>
<td><em>An. subpictus</em></td>
<td>56</td>
<td>20</td>
<td>35.7</td>
</tr>
<tr>
<td>Rainy</td>
<td><em>An. subpictus</em></td>
<td>58</td>
<td>17</td>
<td>29.3</td>
</tr>
<tr>
<td>Winter</td>
<td><em>An. subpictus</em></td>
<td>54</td>
<td>15</td>
<td>27.8</td>
</tr>
<tr>
<td>Winter</td>
<td><em>An. culicifacies</em></td>
<td>57</td>
<td>26.8</td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

The results of the present study clearly showed the predominance of *An. subpictus* (29.0% among anophelines). *An. culicifacies* comprised 11.6% of the total species collected. *An. subpictus* was the most abundant species in the study (Paniker et al., 1981; Kulkarni et al., 1987; Premasiri et al., 2005). In a study carried out by Singh et al. (2003) in central India, *An. culicifacies* was the predominant species, accounting for more than 75% of the total mosquitoes, followed by *An. subpictus* (11.4%). In the present study, both *An. subpictus* and *An. culicifacies* showed the highest density during the rainy season (July to October). In Madhya Pradesh, Kulkarni et al. (1987) found both species to be prevalent from July to September. Feeding habits and seasonal prevalence of *An. culicifacies* vary greatly in different parts of India, but *An. subpictus* is predominant during the irrigation season (Rao, 1984).

Malaria sporozoite detection by nested PCR showed sporozoite rates of 0.5% and 1.8% in *An. subpictus* and *An. culicifacies*, respectively. In Sri Lanka, Yapabandra and Curtis (2004) found malaria sporozoite rates in *An. subpictus* and *An. culicifacies* of 3.8% and 1.9%, respectively. *An. subpictus* was established as a primary vector of malaria for the first time in West Bengal with a sporozoite rate (*P. vivax*) of 0.3% (Chatterjee and Chandra, 2000). In the present study, *An. subpictus* samples positive for malaria sporozoite were found during the summer and *An. culicifacies* samples positive for malaria sporozoites were found during rainy and winter seasons. Kulkarni (1987) observed malaria sporozoites in *An. subpictus* in February and July and in *An. culicifacies* in March and September. Paniker et al. (1984) found malaria sporozoites in the gut and salivary glands of *An. subpictus* during the months where more malaria cases occurred in Pudukuppam village, Pondicherry.

*An. subpictus* was found to be the predominant mosquito species biting man (Paniker and Rao, 1984). The same was also found in the present study with an anthropophilic index of 31.0%. Kulkarni (1987) reported an anthropophilic index for *An. subpictus* of 35.6% in Madhya Pradesh. An anthropophilic index of 41.0% was found for *An. subpictus* in Tarakeswar, West Bengal (Chatterjee and Chandra, 2000). It has also been reported to be highly anthropophilic in Western Orissa (Collins et al., 1991). The anthropophilic index for *An. culicifacies* was found to be 25.3% in this study and 22.7% and 29.4% in 1991-1991 and 2000-2001 respectively, in Gujarat (Bhatt et al., 2008).

A high anthropophilic index (35.7%) and presence of malaria sporozoites were observed during the summer season in *An. subpictus*. *An. culicifacies* was found to have an anthropophilic index of 21.1% during the summer. During the same period the SPR was also reported to be very high in the study areas (Fig 3). The density of *An. subpictus* was higher than *An. culicifacies* during the summer. Hence, the higher SPR during the summer in the study area is probably due to *An. subpictus* since other vector species, such as *An. culicifacies*, were low in number, though *An. culicifacies* contributes...
to malaria transmission during the rainy and winter seasons along with other vector species. A positive correlation between PMHD and the SPR during summer was found for *An. subpictus* ($p<0.001$) and for the rainy ($p<0.001$) and winter seasons ($p<0.001$) for *An. culicifacies* were found in the present study.

In the present study *An. subpictus* was incriminated as a vector for malaria for the first time in Orissa. Due to ecological changes some species of mosquitoes though previously reported as a non-vectors are changing their vectorial capacity and becoming vectors thus contributing to the high malaria incidence. Thus, prevention planning must take into consideration the local vectors. Further study of *An. subpictus* species complex and their prevalence by barcoding method is being carried out, which should give greater insight into *An. subpictus* biology.

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**REFERENCES**


Collins RT, Narasimham MVVL, Dhal KB, Mukherjee BP. Gel diffusion analysis of Anophelines blood meals from 12 malarious study villages of Orissa state, India. *J Am Mosq Control Assoc* 1991; 7: 595-603.


