EPIDEMIOLOGY OF MALARIA TRANSMISSION AND DEVELOPMENT OF NATURAL IMMUNITY IN A MALARIA-ENDEMIC VILLAGE, SAN DULAKUDAR, IN ORISSA STATE, INDIA

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Abstract. We describe the epidemiology of malaria in San Dulakudar, a village in Sundargarh District in the state of Orissa in eastern India. Malaria transmission is perennial with Plasmodium falciparum, accounting for greater than 80% of malaria cases. Transmission intensity varies with season with high transmission after the monsoon rains in autumn and winter, low transmission in summer, and intermediate transmission in spring. The anthropophagic mosquito Anopheles fluviatilis was identified as the main vector for malaria transmission. Based on observations of spleen rates and supported by data on malaria parasite prevalence and malaria incidence, San Dulakudar can be classified as a hyperendemic area for P. falciparum malaria. Parasite prevalence and malaria incidence rates decrease with age, suggesting that residents of San Dulakudar develop immunity to malaria. The study demonstrates the presence of regions in the Indian subcontinent such as Sundargarh District where P. falciparum is the primary cause of malaria and where malaria transmission rates are comparable to those found in many parts of Africa.

INTRODUCTION

The severity of malaria caused by infection with Plasmodium falciparum depends on the complex interplay of the infecting parasite with the immune status and genetic background of the host. Epidemiologic studies conducted in areas of high malaria transmission in Africa and southeast Asia indicate an age-dependent development of protective immunity against P. falciparum malaria.1–3 The intensity of malaria transmission is a crucial factor that determines the epidemiology and rate of development of immunity.4 In areas with high transmission rates, malaria-related mortality is highest during the first few years of life. By the age of five, most children develop a considerable degree of immunity, which reduces the risk of death from P. falciparum malaria, provides protection against severe disease, and reduces the frequency of clinical malaria episodes.5–7 Naturally acquired immunity builds up with repeated exposure to malaria, which is manifested by lower parasite densities and fewer clinical malaria episodes in older children and adults. However, sterile immunity is never acquired. In areas with lower transmission intensity, the age at which clinical immunity develops shifts upwards. In areas with very low malaria transmission rates, immunity develops so slowly that individuals in all age groups are equally susceptible to clinical malaria and severe disease.8–9 A number of epidemiologic studies of malaria have been carried out in areas of moderate to high malaria transmission in Africa. Literature on epidemiologic descriptions of malaria from southeast Asia and the Indian subcontinent, where malaria transmission rates can vary considerably over short geographical distances, is scanty. Although transmission is generally both low and seasonal, foci of intense malaria transmission do exist.10–16 Naturally acquired immunity against P. falciparum is reported from a malaria hyperendemic region in Myanmar13 and from endemic regions of eastern Indonesia.16 The present study uses longitudinal and cross-sectional surveys to investigate the epidemiology of malaria in the village of San Dulakudar in Sundargarh District, which is situated in an area of high malaria transmission in the state of Orissa in eastern India (Figure 1). The epidemiology of malaria transmission and development of clinical immunity to P. falciparum malaria in San Dulakudar is similar to that reported from areas of Africa with high malaria transmission rates.

MATERIAL AND METHODS

Study area. The Sundargarh District, which is located between 21°35’ and 22°35’N and 83°32’ and 85°22’E, presents ideal ecologic conditions for malaria transmission with undulating uplands intersected by forested hills, rocky streams, and paddy fields. The climate is tropical with an annual rainfall of 160–200 cm. The year can be roughly divided into the following seasons: a hot dry summer from April to mid-June, a monsoon from mid-June to September, autumn from October to November, winter from December to January, and spring from February to March. The maximum temperature during the summer is 40–45°C and the minimum temperature during winter is 5–10°C.

Industrial development in the Sundargarh District based on its mineral resources has led to extensive deforestation and development of new settlements with modern civic infrastructure, resulting in changes in malaria transmission patterns. Variations in transmission patterns are seen over short geographic distances. For example, Rourkela Steel Township, which has an excellent urban infrastructure with approximately 20,000 houses of different types built for employees of the local steel plant, roads, electricity, safe drinking water, an underground sewage system, and effective malaria control programs, has very low malaria transmission. In contrast, malaria transmission is very high in villages situated in forests with perennial streams and surrounded by paddy fields just 25–30 km outside Rourkela. The present study was conducted in one of these small villages, San Dulakudar, in the Sundargarh District of Orissa, located approximately 30 km from the Rourkela Steel Township. The village is comprised of four hamlets on patches of cleared forest, and is surrounded on
three sides by a slow flowing rocky stream, in which water flows throughout the year except for summer months.

The epidemiology of malaria in San Dulakudar was followed over three years from January 1998 to December 2000 through longitudinal and cross-sectional studies in a collaborative effort undertaken by the Malaria Research Center (MRC) Field Station in Rourkela, Ispat General Hospital (IGH) in Rourkela, and the International Center for Genetic Engineering and Biotechnology (ICGEB) in New Delhi. San Dulakudar had a population of 264 in January 1998 at the start of the study. The age distribution of the population from January 1998 to December 2000 is shown in Table 1. During the study period, there were 14 births and 10 deaths in the village. None of the deaths was attributed to malaria or its complications. Six deaths were due to old age–related diseases and one each due to snake bite, post-partum hemorrhage, alcoholic hepatitis, and suicide. In addition, 14 persons left the village for various reasons, while 12 persons came to the village during this period.

Informed consent was obtained from all human adult participants and from parents or legal guardians of minors. This study was reviewed and approved by the Ethical Review Committee of the MRC of the Indian Council of Medical Research in New Delhi.

Malaria surveillance. Both active and passive surveillance were used for identification of malaria cases. A voluntary health worker, who was a resident of San Dulakudar, was used for malaria surveillance in the village. The health worker was trained at the MRC Field Station in Rourkela in methods for recording body temperature, making thick and thin blood smears with blood collected from finger pricks, and administration of anti-malarial therapy. The health worker visited each household once a week. He measured body temperature and collected finger prick blood from all individuals who reported that they had a fever in the past 24 hours. The finger prick blood was used to make thick and thin blood smears. The village worker brought the smears to the MRC Field Station in Rourkela for detection and identification of malaria parasites. Blood smears were stained with Jaswant Singh Bhattacharjee (JSB) stain,17 which is comprised of two solutions, one containing methylene blue in phosphate buffer and one containing eosin yellow in distilled water. Staining with JSB stain is a rapid method that takes only 60 seconds per smear. Stained blood films were examined at 100× magnification under an oil immersion lens of a compound microscope by trained microscopists. At least 100 microscopic fields of a thick blood film were examined to declare a slide negative. Asexual parasites were counted against 200 leukocytes and parasite density was calculated as number of asexual parasites per microliter of blood assuming a mean normal leukocyte count of 8,000/μL. All slide-positive cases were provided anti-malarial treatment as per the guidelines of the National Anti-Malaria Program of the Government of India. *Plasmodium vivax* and *P. malariae* cases were treated with chloroquine with a single dose of 10 mg/kg of body weight, followed by primaquine at a dose of 1.25 mg/kg of body weight in equally divided doses over a five-day period. *Plasmodium falciparum* cases were treated with chloroquine at a dose of 25 mg/kg in three divided doses of 10 mg/kg on days 0 and 1 and 5 mg/kg on day 2, and a single dose of primaquine (0.75 mg/kg on day 0). Primaquine was not given to infants and pregnant women. The health worker followed-up each patient until he or she no longer had a fever, and reported to MRC Rourkela Field Station if the patient developed any complications including persistence of fever or if the patient was admitted to nearby hospitals for any reason. In addition to this method of active surveillance, residents of the village reported to the health worker in case of fever. The health worker provided presumptive anti-malarial treatment with chloroquine (10 mg/kg of body weight) to all such cases and collected finger prick blood for thick and thin blood smears. Smears collected through such passive surveillance were brought to MRC Field Station twice a week for detection of parasites. The patients found positive for *P. vivax* and *P. malariae* were administered radical treatment with primaquine, whereas patients positive for *P. falciparum* were given chloroquine and primaquine so as to complete the recommended doses of anti-malaria drugs as per the national drug policy described earlier.

Point prevalence survey. Cross-sectional prevalence surveys were conducted in San Dulakudar three times a year in April, August, and December from 1998 to 2000. A prevalence survey could not be carried out in August 1999 due to some logistical problems. The schedule for the prevalence surveys was announced in the village a week in advance. All members of the village who volunteered for a medical screening were included in the survey. Volunteers were examined by clinicians for fever, enlargement of spleen, and common ailments in addition to malaria during these cross-sectional surveys. Classification of sizes of spleen and degree of emiccy of malaria were made according to method of Hackett.18 Blood was collected by finger prick from each individual for preparation of thick and thin blood smears for detection and identification of malaria parasites. Samples were also collected for determination of hemoglobin levels and parasite genotyping during some of the surveys. Age, sex, fever, splenic enlargement, and other relevant information were recorded on a patient data sheet for subsequent entry into a

<table>
<thead>
<tr>
<th>Year</th>
<th>&lt;1</th>
<th>1-5</th>
<th>&gt;5-15</th>
<th>&gt;15-30</th>
<th>&gt;30</th>
<th>Total population</th>
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<td>1998</td>
<td>5</td>
<td>19</td>
<td>75</td>
<td>64</td>
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</tr>
<tr>
<td>1999</td>
<td>4</td>
<td>21</td>
<td>75</td>
<td>65</td>
<td>98</td>
<td>263</td>
</tr>
<tr>
<td>2000</td>
<td>7</td>
<td>22</td>
<td>75</td>
<td>66</td>
<td>96</td>
<td>266</td>
</tr>
</tbody>
</table>
computer database. Persons found to be positive for malaria parasites in their peripheral blood during the cross-sectional surveys were treated with standard dosage of anti-malarials as described earlier. Children and adults found to be anemic either on clinical examination or after determination of hemoglobin levels were provided iron supplementation. Other common ailments detected clinically were also treated.

**Determination of hemoglobin levels and electrophoresis.** Finger prick blood samples were collected for determination of hemoglobin levels. The samples were carried to Biochemistry Laboratory of IGH in Rourkela on ice and hemoglobin measurements were made colorimetrically within four hours of collection. Twenty microliters of blood was transferred into plastic tubes containing 5 mL of Drabkin’s reagent for determination of hemoglobin levels. Hemoglobin concentrations were used to classify individuals as severely anemic (< 70 g/L), moderately anemic (70–100 g/L), mildly anemic (> 100–110 g/L in those less than six years and > 100–120 g/L in children more than six years of age and adults) or normal. Heparinized blood (5 mL) was collected from volunteers participating in the point prevalence surveys and transported on ice to the Biochemistry Laboratory of IGH in Rourkela for hemoglobin electrophoresis. The entire adult population (> 15 years old) of both sexes was screened once for sickle cell hemoglobin electrophoresis. The samples were carried to Biochemistry Laboratory of IGH in Rourkela on ice and hemoglobin electrophoresis of the hemolysate. Hemoglobin electrophoresis of the hemolysate was performed using both agar gel (0.05 M citrate buffer, pH 6.2) and a cellulose acetate membrane (0.08 M Tris-acetate, pH 8.6), along with normal hemoglobin (AA) and sickle cell trait hemoglobin (AS) as controls.

**Genotyping P. falciparum field isolates.** Two-three drops of finger prick blood were blotted on 3M Whatman (Maidstone, Kent, United Kingdom) filter paper, air-dried, and stored in individual envelopes under dry conditions for analysis of P. falciparum genotypes at the ICGEB in New Delhi. The DNA was extracted from dried blood spots on filter paper for use as template in polymerase chain reactions (PCRs) as follows. Blood-impregnated filter papers were fixed with methanol in 0.5-mL PCR tubes. After removal of the methanol, the filter papers were air-dried and soaked in 50 μL of distilled water and the tubes were heated at 95–100°C for 15 minutes with intermittent vortexing. Five to 10 microliters of this solution was used for each PCR.

Polymorphic repetitive regions, block 2 of merozoite surface protein-1 (MSP-1) and block 3 of MSP-2, were used for genotyping the P. falciparum isolates by the nested PCR method using primers and conditions previously described. The first PCR was performed using oligonucleotide primer pairs corresponding to conserved sequences of MSP-1 and MSP-2 genes. The product of the first PCR performed with MSP-1 primers was used as template for the nested PCR with primers specific for block 2 allelic variants (K1, MAD20, and RO33). The product of the first PCR performed with MSP-2 primers was used as template for the nested PCR with primers specific for the FC27 and 3D7/IC families of the polymorphic central repetitive region (block 3) of MSP-2. The amplification products were separated by electrophoresis on 2.5% agarose gels and detected by staining with ethidium bromide.

**Entomologic surveys.** Adult mosquito densities were monitored in San Dulakudar at monthly intervals by manual catching using a suction tube method. Indoor resting collections were made once each month in the morning between 6:00 AM and 8:00 AM from four human dwellings and four cattle sheds. Mosquitoes from each dwelling were kept separately in test tubes and species were identified based on morphology. Densities per person-hour searching (person hour density or PHD) of total anophelines and vector species were determined. A human blood index (HBI) or proportion of mosquitoes that had fed on a human host was determined for each vector species by blood meal analysis. Stomach blood collected from freshly fed and semi-gravid female mosquitoes was smeared on Whatman No. 1 filter paper and assayed by gel diffusion technique to determine if the mosquitoes had fed on human blood.

Adult mosquito collections were made on human baits from 6:00 PM to 6:00 AM once a month during low (April to June 2000), intermediate (July to September 2000 and February to March 2000), and high transmission (October to January 2000) seasons. One human volunteer was used each for indoor and outdoor collections. Mosquitoes landing on the bait were caught with the help of suction tube and flash-light. The hourly collections of mosquitoes caught on human baits were kept separately in test tubes and the species were identified. The human biting rate (HBR) for each vector species was calculated. The mosquitoes collected on human baits were preserved under dry conditions and assayed for presence of sporozoites by an enzyme-linked immunosorbent assay using monoclonal antibodies against the P. falciparum and P. vivax circumsporozoite protein. Monoclonal antibodies against P. falciparum and P. vivax (Pv210 and Pv247) circumsporozoite protein along with appropriate positive controls were kindly provided by Dr. Robert A. Wirtz (Centers for Disease Control and Prevention, Atlanta, GA). The entomologic inoculation rate (EIR) was calculated from the HBR and sporozoite rate for different transmission seasons.

**Statistical analysis.** The Students t-test was used to assess the significance of differences between two groups for normally distributed data. The chi-square test for trend was used to assess the significance of differences in the proportions.

**RESULTS**

**Pattern of malaria incidence in San Dulakudar.** Data on malaria incidence in San Dulakudar was collected through active and passive surveillance as described in the Materials and Methods. A malaria episode was defined as a case where an individual had an axillary temperature greater than 37.5°C and P. falciparum, P. vivax, or P. malariae asexual forms were detected in a thick blood smear. A second episode of fever occurring within 28 days of the first episode was considered a recrudescence or short-term relapse and treated as a single episode. A total of 573 malaria cases were reported from San Dulakudar during the study period from January 1998 to December 2000. An average of 56.3% of the malaria cases were detected through active surveillance (range = 54.3–59.8%) and the remaining through passive surveillance each year. Of the total malaria cases reported from San Dulakudar, 89.4% were attributed to P. falciparum infection, 10.1% to P. vivax infection, 0.4% to mixed P. falciparum and P. vivax infections, and 0.1% to P. malariae infection. The incidence of P. falciparum malaria in San Dulakudar during the study period is shown in Figure 2. Malaria transmission is perennial and
fresh *P. falciparum* malaria cases are reported throughout the year (Figure 2). *Plasmodium falciparum* malaria incidence peaks during autumn and winter between October and January after the monsoon season ends in September and is lowest in the hot dry summer months from April to June when temperatures rise above 40°C (Figure 2). Malaria incidence rates are at intermediate levels at other times of the year (Figure 2). Depending on the pattern of temperature and rainfall, short-lived upsurges in *P. falciparum* malaria transmission are seen during the spring as in March 1999, the early summer as in April 1998 and 2000, or during the monsoon as in July 1999 and 2000 and September 1998. The overall annual malaria transmission pattern was similar during the three years covered by this study indicating that malaria transmission in San Dulakudar is stable. Subtler variations in the transmission pattern were seen in the three years of the study and may relate to alterations in climatic conditions such as the intensity and duration of the monsoon rains and timing of onset of winter. However, peak transmission always occurs during autumn and winter season, between October and January, after the end of the monsoon season.

**Malaria transmission vectors in San Dulakudar.** Indoor mosquito collections carried out every month beginning March 1998 in San Dulakudar demonstrate the presence of 18 anopheline species (Table 2). Two of the identified species, *Anopheles culicifacies* and *An. fluviatilis*, have been previously shown to have potential for malaria transmission. Blood meal analysis indicates that *An. fluviatilis*, with an HBI of 0.70, is predominantly anthropophagic, whereas *An. culicifacies*, with an HBI of 0.03, is predominantly zoophagic. Vector densities of *An. culicifacies* and *An. fluviatilis* were measured once a month in San Dulakudar during the study period. Representative data on vector densities and malaria incidence are shown for the year 2000 in Figure 3. The peak of malaria incidence coincides with the peak vector density of *An. fluviatilis*, suggesting that it is the predominant vector for malaria transmission in San Dulakudar (Figure 3B). *Anopheles culicifacies* may play a complementary role in the intermediate season, especially the secondary peaks seen following occasional rains in March and during the monsoon season from June to September.

Volunteers were used to determine the HBRs for *An. culicifacies* and *An. fluviatilis* during different times of the year. The HBR for *An. fluviatilis* was estimated to be 0.3, 2.0, and 15.8 bites/person/night during the low, intermediate, and high transmission seasons, respectively. *Anopheles culicifacies* had an HBR of 1.5, 2.8, and 0.2 bites/person/night during the low, intermediate, and high transmission seasons, respectively. Mosquitoes collected from the village during different transmission periods were tested for presence of *P. falciparum* and *P. vivax* sporozoites using monoclonal antibodies against the circumsporozoite protein. The sporozoite rate and the HBR were used to estimate the EIR. The EIR varied significantly during different seasons of the year. The EIR was estimated to be 0.23 infective bites/person/day during the high transmission period and 0.02 infective bites/person/day during intermediate transmission period. No infected mosquitoes were detected in the collections made during the low transmission season.

**Malaria parasite prevalence and spleen rates in San Dulakudar.** Cross-sectional malaria parasite prevalence surveys were conducted in San Dulakudar during April, August, and December every year from 1998 to 2000, except for August 1999 due to some logistical problems. Results of the cross-sectional parasite prevalence are shown in Figure 4. Blood samples were collected from residents of San Dulakudar who voluntarily participated in the mass blood surveys conducted in the village. A total of 769 blood samples were collected during these cross-sectional surveys, of which 160 samples were positive for malaria (*P. falciparum* = 133, *P. vivax* = 20, *P. malariae* = 4, and mixed infections [*P. falciparum* plus *P. vivax*] = 3). The average parasite rate or slide positivity rate from all surveys was 20.8%, with *P. falciparum* accounting for 83.1%, *P. vivax* accounting for 12.5% and *P. malariae* accounting for 2.5% of the infections. Mixed infections with *P. falciparum* and *P. vivax* accounted for 1.9% of the positive slides. Average parasite rates for 1998, 1999, and 2000 were 23.5%, 24.0%, and 16.4%, respectively. The highest parasite rates were observed during cross-sectional surveys carried out in December in each of the three years of the study.

Spleen rates of all age groups were also measured during the cross-sectional surveys. Classification of sizes of spleen and the degree of endemicity of malaria were made according

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**Table 2**

Percent composition of anopheline species in San Dulakudar, India

<table>
<thead>
<tr>
<th>No.</th>
<th>Anophele species</th>
<th>No of mosquitoes collected*</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>An. culicifacies</em></td>
<td>5,378</td>
<td>43.2</td>
</tr>
<tr>
<td>2.</td>
<td><em>An. fluviatilis</em></td>
<td>665</td>
<td>5.3</td>
</tr>
<tr>
<td>3.</td>
<td><em>An. subpictus</em></td>
<td>2,031</td>
<td>16.3</td>
</tr>
<tr>
<td>4.</td>
<td><em>An. annularis</em></td>
<td>1,784</td>
<td>14.3</td>
</tr>
<tr>
<td>5.</td>
<td><em>An. vagus</em></td>
<td>1,112</td>
<td>8.9</td>
</tr>
<tr>
<td>6.</td>
<td><em>An. pallidus</em></td>
<td>907</td>
<td>7.3</td>
</tr>
<tr>
<td>7.</td>
<td><em>An. nigerimus</em></td>
<td>159</td>
<td>1.27</td>
</tr>
<tr>
<td>8.</td>
<td><em>An. acconitus</em></td>
<td>161</td>
<td>1.29</td>
</tr>
<tr>
<td>9.</td>
<td><em>An. splendidus</em></td>
<td>100</td>
<td>0.80</td>
</tr>
<tr>
<td>10.</td>
<td><em>An. theobaldi</em></td>
<td>24</td>
<td>0.19</td>
</tr>
<tr>
<td>11.</td>
<td><em>An. ramsayi</em></td>
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<td>0.19</td>
</tr>
<tr>
<td>12.</td>
<td><em>An. tesellatus</em></td>
<td>26</td>
<td>0.22</td>
</tr>
<tr>
<td>13.</td>
<td>*An. barbirostris</td>
<td>37</td>
<td>0.29</td>
</tr>
<tr>
<td>14.</td>
<td><em>An. jeyporiensis</em></td>
<td>15</td>
<td>0.12</td>
</tr>
<tr>
<td>15.</td>
<td><em>An. varuna</em></td>
<td>20</td>
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</tr>
<tr>
<td>16.</td>
<td><em>An. maculatus</em></td>
<td>5</td>
<td>0.04</td>
</tr>
<tr>
<td>17.</td>
<td><em>An. jamesi</em></td>
<td>7</td>
<td>0.06</td>
</tr>
<tr>
<td>18.</td>
<td><em>An. karwari</em></td>
<td>1</td>
<td>0.008</td>
</tr>
</tbody>
</table>

*Total number of different anopheline species collected by suction tube method from four human dwellings and cattle sheds once each month from March 1998 to December 2000.
to the method of Hackett. An average of 77.6% of the children 2–9 years old (range 68.4–88.9%) had an enlarged spleen, whereas an average of 13.1% of the adults more than 20 years old (range 9.1–18.8%) had an enlarged spleen, indicating hyperendemic malaria in San Dulakudar. Since splenic enlargement may also be associated with hemoglobinopathies and thalassemias, hemoglobin electrophoresis and determination of fetal hemoglobin levels for the entire population more than 15 years of age was done. None of the blood samples examined demonstrated any abnormal hemoglobin or increased fetal hemoglobin concentrations. However, the presence of α-thalassemia or β-thalassemia traits in the population could not be excluded with these investigations.

*Plasmodium falciparum* malaria incidence, parasite prevalence, and anemia as a function of age. Malaria attack rates, which reflect the number of clinical malaria episodes per person per year in different age groups, were determined by dividing the total number of *P. falciparum* malaria episodes recorded during a year for individuals in an age group by the population size of that age group. The malaria attack rate varied with age (Figure 5A). The attack rate was found to be highest in children 1–5 years of age with a gradual decrease in the attack rate in higher age groups, suggesting the development of immunity to clinical malaria with age. No age group was completely immune from attacks of clinical malaria. The average attack rate for the entire population was highest (0.75 episodes/person/year) in 1998 when the study was started. During the following years, the average attack rate decreased to 0.59 episodes/person/year in 1999 and to 0.60 episodes/person/year in 2000.

Parasite rates determined during the cross-sectional mass surveys were also analyzed as a function of age (Figure 5B). Parasite rates were highest in the 1–5-year-old age group and decreased with age, with lowest parasite rates in the > 30-year-old age group. Throughout the period of the survey, the highest parasite prevalence was observed in the 1–5-year-old

![Figure 4](image_url)

**Figure 4.** Slide positivity rate determined in cross-sectional surveys carried out in San Dulakudar. Malaria parasites were detected by microscopy of Jaswant Singh Bhattacharjee–stained thick and thin blood smears made with finger prick blood collected during the high (December), intermediate (INT) (August), and low (April) transmission seasons. The percentage of individuals with detectable malaria parasites (slide positivity rate) in each cross-sectional survey is reported. A cross-sectional survey was not carried out in August 1999.

![Figure 5](image_url)

**Figure 5.** Malaria attack rate and slide positivity rate as a function of age in San Dulakudar. **A**. Malaria attack rate in different age groups. The number of clinical episodes of *Plasmodium falciparum* (*Pf*) malaria per person per year (attack rate) in different age groups (< 1 year of age, 1–5 years old, > 5–15 years old, > 15–30 years old, and > 30 years old) is shown based on data on malaria incidence collected in 1998, 1999, and 2000. **B**. Slide positivity rate in different age groups. The percentage of individuals in different age groups (< 1 year of age, 1–5 years old, > 5–15 years old, > 15–30 years old, and > 30 years old) with detectable malaria parasites in finger prick blood is shown. Malaria parasites were detected by microscopy of Jaswant Singh Bhattacharjee–stained thick and thin blood smears made with finger prick blood collected during cross-sectional surveys conducted in San Dulakudar in 1998, 1999, and 2000.
age group and lowest prevalence was observed in the > 30-year-old age group. Children in the 1–5-year-old age group were 5.7 times more likely to be parasitemic than persons > 30 years of age ($\chi^2 = 63.7, P < 0.001$).

Four hundred fifty-two blood samples were collected from San Dulakudar residents during the point prevalence surveys for determination of hemoglobin levels. Severity of anemia was classified based on the hemoglobin concentration in the blood as severe anemia (< 70 g/L), moderate anemia (70–100 g/L), and mild anemia (> 100–110 g/L in those less than six years old and > 100–120 g/L in children more than six years of age and adults). Anemia was absent in only 6.9% of the samples examined, while 31.4% had mild anemia, 52.0% had moderate anemia, and 9.7% had severe anemia. The lowest level of hemoglobin recorded in the severe anemia category was 40 g/L in a 3.5-year-old girl. The distribution of the incidence of anemia in the population screened based on age is shown in Figure 6. Due to the small number of infants (< 1 year of age) in the village, data on anemia in this age group is not considered for the following analysis. There were no non-anemic children in the 1–5-year-old age group. The highest prevalence of severe anemia was found in children 1–5 years of age (30%), followed by those > 5–15 years old (9.5%), those > 30 years old (6.8%), and those > 15–30 years old (4.4%). The differences in prevalence of severe anemia in different age groups were statistically significant ($\chi^2 = 41.9, \text{degrees of freedom} = 12, P < 0.001$). Mild-to-moderate anemia was present in a significant proportion of persons in all age groups. The inverse correlation between parasite prevalence rates, malaria attack rates, and incidence of anemia with age suggest the development of immunity to clinical malaria with age in San Dulakudar.

**Multiplicity of infection and diversity of P. falciparum field isolates in San Dulakudar.** Blood samples collected on filter papers during point prevalence surveys and those found to be positive microscopically for *P. falciparum* were analyzed for presence of diverse MSP-1 and MSP-2 alleles. The presence of multiple alleles reflects co-infection by multiple *P. falciparum* strains. Samples collected during the high transmission season in 1998 and 1999 and during the intermediate transmission season in 2000 were analyzed. The number of MSP-1 and MSP-2 alleles detected, the average number of alleles per patient, and the proportion of cases having multiple infections are shown in Table 3. At least 10 different MSP-1 alleles and 12 different MSP-2 alleles were detected. The number of K1 and MAD20 alleles detected was higher than number of RO33 alleles. In case of MSP-2, more FC27 alleles were detected than 3D7 alleles. Multiple MSP-1 and MSP-2 alleles are commonly found in blood samples from the same patient, indicating frequent co-infection by multiple *P. falciparum* strains (Table 3).

**DISCUSSION**

Development of immunity to clinical malaria in endemic areas is largely influenced by the intensity of malaria transmission. In high transmission areas in Tanzania, the prevalence of *P. falciparum* parasitemia decreased gradually over the years, while mean parasite density and risk of malaria fever decreased abruptly and significantly with age. The rate of development of immunity to malaria also depends on age, so that naïve adults who move to a malaria-endemic area develop immunity more rapidly than naïve children. In areas of low and seasonal transmission, parasite prevalence is very low during the dry summer season and microscopically detectable parasitemia is practically absent. Even in these low transmission areas, the risk of getting a clinical attack decreases with age due to development of immunity, albeit at a slower rate, and adults have fewer clinical malaria attacks than children. In Daraweesh, Sudan, clinical malaria cases are seen only during the September–November malaria season and parasitemia is rarely seen in anyone other than the clinical cases. All age groups are affected, but the risk of getting a clinical attack is approximately twice as high in those 5–20 years old than in adults more than 30 years old.

We have studied the epidemiology of malaria in San Dulakudar, a village endemic for *P. falciparum*, in the Sundargarh District of Orissa, India. Periodic cross-sectional surveys as well as active and passive surveillance were used to study parasite prevalence and incidence of clinical malaria. *Plasmo-

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**TABLE 3**

<table>
<thead>
<tr>
<th>MSP-1 and MSP-2 alleles of <em>Plasmodium falciparum</em> isolates collected in San Dulakudar, India*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. of samples tested</strong></td>
</tr>
<tr>
<td><strong>MSP-1</strong></td>
</tr>
<tr>
<td>Number of alleles</td>
</tr>
<tr>
<td>Average no. of alleles per person</td>
</tr>
<tr>
<td><strong>MSP-2</strong></td>
</tr>
<tr>
<td>Number of alleles</td>
</tr>
<tr>
<td>Average no. of alleles per person</td>
</tr>
</tbody>
</table>

* MSP = merozoite surface protein.
diem falciparum accounted for approximately 89% of all malaria cases observed in San Dulakudar during the study period. The high parasite rates and frequency of clinical malaria episodes in children suggests that San Dulakudar has high malaria transmission rates. The high frequency of enlarged spleens in 2–9-year-old children indicates that San Dulakudar is hyperendemic for malaria.

The highest prevalence of parasitemia, as well as the frequency of clinical malaria episodes, in San Dulakudar was observed in children 1–5 years old. Both prevalence and incidence decreased with age. Adults were less parasitemic and had fewer clinical attacks of malaria. The prevalence and incidence of malaria were found to be lower in infants than in children 1–5 years old. However, the sample size of infants was small and may not represent an accurate picture. The decrease in parasite rates and malaria attack rates with age indicates that following repeated exposure to P. falciparum infection, individuals develop immunity to P. falciparum malaria. In a recent study using sera collected from San Dulakudar residents, adults were shown to agglutinate a wide variety of P. falciparum field isolates, which is consistent with the development of protective immune responses against clinical malaria with age.

More than 92% of the population of San Dulakudar, including all children ≤ 5 years of age, had anemia of various types (Figure 6). The presence of widespread anemia was previously reported in children with malaria in the Sundargarh District. In this study, we report that the severity of anemia is age dependent. Both the prevalence and severity of anemia was higher in children than in adults. The age dependence of severe anemia reflects the development of immunity to malaria over a period of time. Despite an observed decrease in parasite prevalence over the three-year study period in San Dulakudar, no significant decrease in the malaria attack rate or severity of anemia was observed in children. The high prevalence of anemia has been reported from malaria-endemic areas of Africa. Anemia is a major public health problem among children in San Dulakudar as in other P. falciparum-endemic regions of Africa.

Entomologic studies showed that San Dulakudar is under the influence of two major vector species: An. fluviatilis and An. culicifacies (Figure 3). Of these, the role of An. culicifacies in malaria transmission was found to be marginal and restricted to intermediate transmission period during the pre-monsoon and monsoon months. Anopheles culicifacies may be regarded as playing a complementary role in maintaining perennial transmission. Anopheles fluviatilis, a highly anthropophilic vector that breeds in streams, is primarily responsible for malaria transmission in San Dulakudar. The EIR estimated during the high and intermediate transmission seasons is comparable to that reported from many parts of Africa. The high degree of diversity in MSP-1 and MSP-2 alleles and the presence of frequent multiclonal infections observed in San Dulakudar is consistent with the high EIR in San Dulakudar and is similar to that reported from high transmission areas of Africa.

Malaria-related deaths and severe complications of malaria requiring hospitalization were not reported from the village during the study period. However, this observation does not imply that inhabitants of the Sundargarh District are protected from malaria-related mortality or severe disease. Severe malaria and deaths have been reported from other villages in the Sundargarh District that have an apparently similar epidemiologic pattern. The absence of severe complications and malarial deaths in San Dulakudar during the study period may be the result of a low sample size or because of early intervention with anti-malarial drugs. Striking differences in the spectrum of severe and fatal malaria have been reported from areas with similar transmission intensity. The availability of health care facilities and health-seeking behavior in the community may also influence disease outcome to a significant extent.

Literature on epidemiologic description of malaria from the Indian subcontinent is scanty. To our knowledge, the present study is the first detailed epidemiologic report from a P. falciparum-endemic area in India documenting development of partial immunity to clinical malaria in adults. The village of San Dulakudar has uninterrupted malaria transmission year after year resulting from the presence of malaria vectors capable of perennial transmission, with peak transmission in the post-monsoon autumn and winter months between October and January. The decrease in spleen rates, parasite prevalence, frequency of clinical malaria attacks, and incidence of severe anemia with age indicate that following repeated exposure the inhabitants of San Dulakudar progressively develop clinical immunity against P. falciparum malaria. This study demonstrates that there are regions in India that are hyperendemic for P. falciparum malaria with transmission rates comparable to those found in many malaria endemic regions of Africa.

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