

Malaria outbreak in a non endemic tribal block of Balasore district, Orissa, India during summer season

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Abstract. A focal outbreak of malaria at Sialimal sub-centre of Balasore district of Orissa was reported during the month of March, 2010. Three villages of the above block were affected. Regional Medical Research Centre, Bhubaneswar has conducted an entomological survey and a central clinic simultaneously, with door to door household survey to identify the fever cases. Within a span of 18 days around 172 fever cases were reported with Slide Positivity Rate (SPR) of 24.4% and Pf % of 81%. The malaria epidemiological data of the sub-centre area for last three years indicates that the area is non endemic for malaria (API was 0.81). Entomological survey revealed the presence of three known vectors of malaria i.e. *Anopheles culicifacies*, *Anopheles annularis* and *Anopheles subpictus* (local vector). Per Man Hour Density (PMHD) of these three species were 4.2, 2.8 and 10.8 respectively. *Plasmodium falciparum* sporozoites were detected in two *An. culicifacies*, in one *An. annularis* and in one *An. subpictus*. Larval density of Anopheline mosquitoes per dip ranged between 12 to 20. The vectors were found to be resistant to DDT but susceptible to synthetic pyrethroid. With this finding necessary remedial measures were taken by the government to curtail the transmission.

INTRODUCTION

Malaria is mostly present as an endemic disease, but in low transmission areas it may occur as an outbreak (Zoller *et al.*, 2009). Malaria outbreaks are often complex and multi-factorial and have natural and unnatural determinants (Najera *et al.*, 1998). Several reports are available on small malaria epidemics with continued localized transmission (Zoller *et al.*, 2009). Factors responsible for the malaria outbreaks include, population migration to an endemic area or vice-versa, increase in breeding sites and potential vector population, presence of new efficient vectors, drug or insecticide resistance by the parasite and vectors and break down in the control measures (Khera *et al.*, 1996). Communities that are not normally exposed to high rates of malaria transmission are vulnerable to malaria outbreaks (Worell *et al.*, 2004). Malaria

epidemics can also be caused when people with low immunity enter as migrants to malaria prone or endemic areas (Kiszewski & Teklehaimanot, 2004). Transmission of malaria in a non-endemic area poses a challenge to local and public health authorities (Zoller *et al.*, 2009). The malaria outbreak in Nilagiri, a non endemic tribal block of Balasore district of Orissa during the month March-April 2010 was a challenge to State Health Department, Government of Orissa. Therefore, based on the request of the State Health Department for technical support, a detailed investigation was made by the Regional Medical Research Centre (RMRC), Bhubaneswar, Orissa. The investigating team comprising of medical and entomology experts, who visited the area and after conducting both epidemiological and entomological survey recommended necessary control measures to contain the malaria outbreak.

MATERIALS AND METHODS

Study area

Orissa is third highest malaria endemic state of India. It has four physiographical regions i.e, Northern plateau, Central tableland, Coastal belt, and Eastern Ghat. Balasore a coastal district broadly divided into three geographical regions, namely, the coastal belt, the inner alluvial plain and the North-Western hills. The Nilagiri block is situated in North-Western hills region of the district and located at 21° 28' North, 86° 46' 1" East. It is mostly hilly terrain and vegetated with tropical semi-ever green forests (Fig. 1). It has an average elevation of 23 m (75 ft). The climate of Balasore district is mostly hot and humid. The hot season starts from March till May and followed by rainy season from June to September. During this period, south-west monsoon causes maximum rain fall (32° temp & RF 1328mm). The Hills of Nilgiri has the highest peak of 543 meter above the sea level. The scheduled tribe people are mostly seen in this region of valuable forest resources and stone quarries. Nilagiri block has gravelly and

lateritic soil, which is less fertile. Thus the people of this region depend on wage earning, farming and forest product gathering for their livelihood. The malaria outbreak affected villages are located in the foot hill area and are inhabited by only one tribal group named "BHUMIZ". They avail the health services mainly from the grass root level providers like accredited social health activist (ASHA) and multi purpose health worker (MPHW) for any minor ailment. Besides, this tribal community receives health facilities from Berhampur Primary Health Centre (PHC) that is 25 KM, and Nilagiri Sub Divisional Hospital which is 13 Km away from the villages for any major health problem.

Study population and area

The tribal people of three affected villages under Sialimal sub-centre of Nilagiri block of Balasore district.

Clinical & Parasitological monitoring

A central camp was organized by the Regional Medical Research Centre (RMRC) team, and local health providers like ASHA

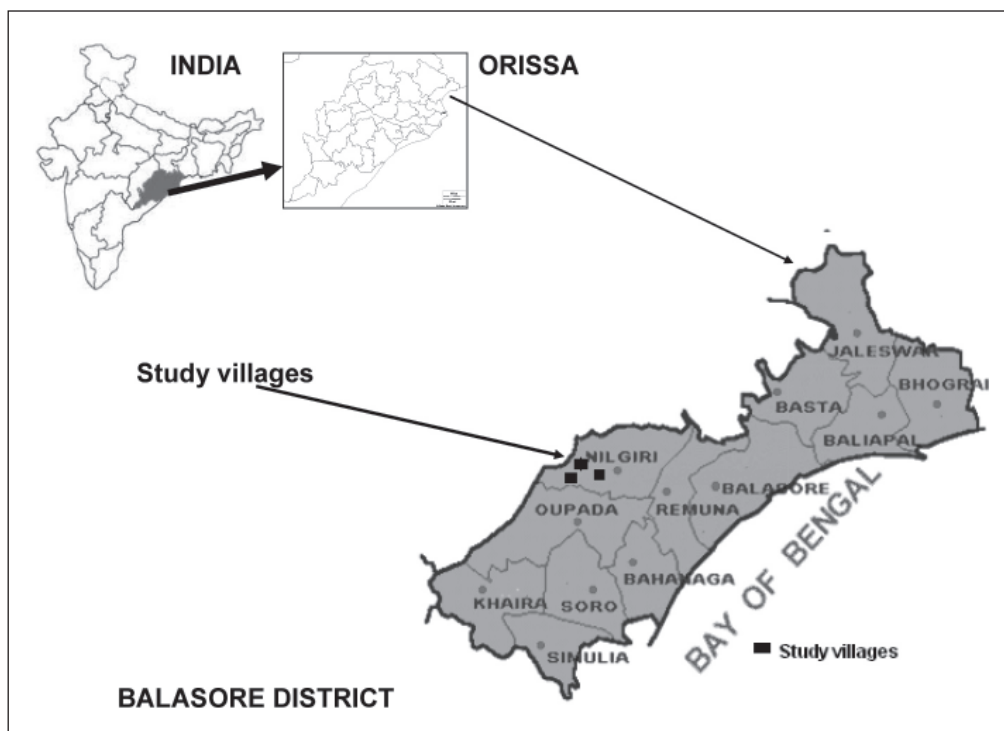


Figure 1. Malaria affected villages of Balasore district, Orissa

and MPHW (female), for early identification and treatment of the fever cases in the villages. The finger prick blood sample was collected from all the fever cases for detection of plasmodium parasite by rapid diagnostic test (RDT) and microscopy. Those found positive for *Plasmodium falciparum* (Pf) infection by RDT were treated with anti-malarial drug artemesinin combination therapy (ACT) as per the National Drug Policy immediately in the camp. The blood slide with thick and thin smears were brought to the RMRC laboratory and stained with JSB stain for microscopical examination.

Drug resistance test

The drug resistance of parasite to the standard anti-malarial drug chloroquine (CQ) was done by the molecular method of Ranjit *et al.* (2004). Drug resistant cases of *P. falciparum* were treated with ACT.

Entomological survey

Entomological survey was conducted in the affected villages following standard method (WHO, 1975). Indoor resting adult mosquitoes were collected in the morning from 6 am to 9 am from 30% of house holds using sucking tube. Each house was surveyed for 15 minutes. The mosquitoes were identified following the keys of Christophers (1933) and Per Man Hour Density (PMHD) of each species of mosquitoes were calculated (PMHD= Total no. of mosquitoes collected / No. of person x Time spent in hours). All the collected samples of *Anopheles culicifacies*, *Anopheles annularis* and *Anopheles subpictus* were preserved in individual vial containing isopropanol for molecular detection of sporozoites.

Larval survey

Larvae were collected from potential breeding sites including ponds, wells and slow- moving streams within half to one kilometers radius of the affected villages. The ponds were serially numbered. Anopheline mosquito larvae were searched using dippers of 10 centimeters diameter and 300 ml capacity (five dips at each site). Larval samples were brought to the laboratory

for rearing till the adult emergence and then the mosquitoes were identified up to species level. Larval densities were expressed per site as the number of larvae per five dips.

Sporozoite detection

Detection of sporozoite was done following nested PCR technique (Mahapatra *et al.*, 2006).

DNA template preparation

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

PCR amplification

Amplification of genus and species specific *Plasmodium* was done by using the primers: rPLU5, PLU6, rFAL1, rFAL2, rVIV1 & rVIV2. Each 20 µl reaction mixture for nest-1 amplifications contained 12 µl of template DNA, 250 nM of each primer (rPLU5 & rPLU6), 4 mM MgCL₂, PCR buffer (50 mM KCl, 10mMTris-HCl), 200 µM of each dNTPs and 0.4 units of Taq DNA Polymerase. The PCR conditions (nest-1) were as follows: step-1: 94°C for 4 min; 94°C for 30 sec, 55°C for one min; Extension at 72°C for one min; 35 cycles and final extension at 72°C for 4 min. About 8 µl of the nest-1-amplification products served as the DNA template for each of the 20 µl of second PCR (nest 2) amplification. The concentration of the nest 2 primers and other constituents were identical to nest 1 amplification, except that 0.3 unit 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. PCR amplified products (10 µl) were subjected to gel electrophoresis on a 2% agarose gel, stained with ethidium bromide, and visualized in an Alphamager gel documentation system.

Susceptibility Status

The susceptibility status of *An. culicifacies*, *An. annularis* and *An. subpictus* to various insecticides like DDT, Malathion and synthetic pyrethroid was determined using standard WHO method (WHO, 1975). Twenty five numbers of freshly fed mosquitoes were introduced to the holding tube of WHO susceptibility test kit. After one hour they were released into the exposure tube having insecticide impregnated papers. After one hour of exposure they were again transferred to the holding tube and the mortality was recorded after 24 hours.

RESULTS

The investigation revealed that three villages namely Kadamsul, Nuasahi, Bada Nuasahi under Sialimal sub-centre of the block Nilagiri were affected during this malaria outbreak. A total of 839 tribal populations reside in 157 households in these three tribal villages with average family size of 5 persons. The households are situated at a distance of more than 12 ft from each other and most of the cattle sheds are 5-10 ft away from the household. There are five ponds in and around and one stream running in the periphery of the villages that are used mostly for bathing, washing clothes and cleaning utensils by the villagers. The tube well facilities are available for drinking water purpose. The cattle population in the villages is 22.2% (186) of human population.

The secondary data on health facilities and malaria prevalence were collected from Chief District Medical Office of Balasore district. The malaria epidemiological data of the sub centre for the past three years indicates that the area is of low transmission with average Annual Blood Examination Rate (ABER) of 10.1%, Slide Positivity Rate (SPR) 2.0% and Annual Parasite Incidence (API) of 0.8 (Table 1).

Epidemiological Survey

A total of 172 cases affected with fever which includes 79 (45.9%) male and 93 (54.0%) female during this outbreak. The median age of these cases was 22 years. The microscopy

examination revealed parasitaemia positivity in 123 (71.5%) cases. Of which only 6 cases were positive for *Plasmodium vivax* (*Pv*), two cases found having mixed infection and 115 (93.5%) found having *Pf* infection. The age and sex distribution of the fever cases with malaria parasitaemia positivity is shown in Table 2. All age groups were affected in this out break. The age specific attack rate of *P. falciparum* was found to be high with 23.5% among the group of people aged 15-30 years. These cases presented with the clinical signs and symptoms like headache, chill, rigor and fever. Other associated symptoms like vomiting and diarrhoea was also marked in very few cases. A total of 11 blood samples were tested for the CQ sensitivity using drug resistance test by molecular technique. The parasite count of these cases ranged from than 1000 to 30,000. The result showed 7 (63.6%) samples having the *Pfcr1* K76T mutation which was an important determinant of CQ resistance.

Socio-economic status and knowledge attitude behavior and practice (KABP)

The information on socio economic status of the community indicates, out of total house hold studied, 101(64.3%) depends on wage earning, 52 (33.1%) on farming and 4 (2.4%) on forest gathering. There is no electrification in the village. All the houses are kuchha house with thatched roof having a big veranda. The community is with a habit of outside sleeping after the whole day's tiresome activities. The practice of mosquito-net use amongst the community showed 75 (47.8%) house holds use mosquito net. Of

Table 1. Malaria epidemiological data of Sialimal sub-Centre for last three years

Year	Population	ABER	SPR	API
2007	3025	4.5	0.7	0.03
2008	3178	12.6	4.4	1.2
2009	3345	13.3	0.9	1.2
Average	3182	10.1	2.0	0.81

ABER- Annual Blood Examination Rate

SPR- Slide Positivity Rate

API- Annual Parasite Incidence

Table 2. Age sex distribution of total fever cases and parasitaemia positives

Age	Male	Female	Total (%)	Parasite Male	Positives Female	Total Positive (%)
≤ 5 years	8	7	15 (8.7%)	6	5	11 (8.9%)
> 5 to 15 years	27	23	50 (29.1%)	14	13	27 (22.0%)
> 15 – 30 years	43	31	74 (43.0%)	22	31	53 (43.1%)
> 30 years	15	18	33 (19.2%)	15	17	32 (26.0%)
Total	93	79	172	58	65	123

Figures in the brackets show the percentage of the total

which 35 (46.6%) have only one net, 27 (36.0%) have two, 8 (10.6%) have three and 5 (6.6%) have four mosquito-net in the families. Government supplied mosquito-net is available in only 5 (6.6%) families. These net have been received by the children, who stay in residential school run by Tribal Welfare Department, Government of Orissa. The rest 93.4% house holds owned self procured mosquito-net. The health seeking behaviour indicated that the affected cases received treatment from local health providers like ASHA 59 (34.3%), Sub divisional hospital (SDH) 40 (23.3%), medical camp 52 (30.0%), Quack or private practitioner 9 (5.2%) and traditional healers 2 (1.2%). The KABP of the communities shows that, more than 60% people know the disease malaria. But the cause is not known by the people. They perceive that the malaria caused by tick bite as they often get tick bite while working in the forest and in the cattle shed at house hold level. None of the community members are aware about the actual cause of malaria and its transmission.

Entomological observation

In total, 414 mosquitoes belonging to three genera viz. *Anopheles*, *Culex* and *Armigeres* were collected. Anophelines were most dominant genus comprising 76.1% (315) of the total collection and the species were *An. culicifacies*, *An. annularis*, *Anopheles subpictus*, *Anopheles vagus* and *Anopheles barbirostris*. *Anopheles subpictus* was the most dominant species followed by *An. vagus*, *An. culicifacies* and *An. annularis* (Table 3). The PMHD of all species were given

in table 3. The PMHD of the primary vector *An. culicifacies* was 4.2 which was higher than the critical density (3.3).

Gonotrophic conditions

The proportion of unfed (UF), full fed (FF), semi gravid (SG) and gravid (G) condition of vector species are recorded (Table 3). It was seen that 87.5%, 100% and 12.7% of *An. culicifacies*, *An. annularis* and *An. subpictus* were full fed. Unfed populations were 12.5%, and 9.1% for *An. culicifacies* and *An. subpictus*. No unfed *An. annularis* was found during this study period. All the four stages of the gonotrophic cycles were found in *An. subpictus* with a predominance of half gravid population. The high percentage of the FF population of the vector exhibit were endophagic.

Larval survey

The anopheline breeding was seen in five ponds (1-5), one stream and one well. The detail of *An. culicifacies*, *An. annularis*, *An. subpictus*, *An. vagus* breeding in the above habitat are presented in Table 4. Anopheline larval density per dip revealed highest density (21) in the pond 5, followed by 19 in the pond 4. All the four species of anophelines, *An. culicifacies*, *An. annularis*, *An. subpictus* and *An. vagus* were found in pond 5 (Table 4).

Plasmodium falciparum (Pf) sporozoites were detected in two *An. culicifacies* (Sporozoite Rate (SR) is 3.45%, n=58). One sample each from *An. annularis* (SR is 2.5%, n=40) and *An. subpictus* (SR is 1.05%, n=95) were found positive for Pf.

Table 3. Mosquito species prevalence in the affected village

Species Name	Abdominal condition				Total	PMHD
	UF (%)	FF (%)	HG (%)	G (%)		
<i>An. culicifacies</i>	8 (12.5)	56 (87.5)	0	0	64	4.2*
<i>An. annularis</i>	0	47 (100)	0	0	47	2.8
<i>An. subpictus</i>	10 (9.1)	14 (12.7)	82 (74.5)	4 (3.6)	110	10.8
<i>An. vagus</i>	0	18 (19.1)	73 (77.7)	3 (3.2)	94	7.0
<i>Cx. quinquefasciatus</i>	0	10 (20.8)	38 (79.2)	0	48	3.0
<i>Cx. vishnui</i>	10 (27.8)	22 (61.1)	4 (11.1)	0	36	2.0
<i>Armigeris</i>	15 (100)	0	0	0	15	1.0
Total	43 (10.4)	167 (40.3)	197 (47.6)	7 (1.7)	414	

* Critical density >3.3 PMHD, PMHD: Per Man Hour Density

Table 4. Larval survey in the study areas during the malaria outbreak

Type of breeding place	Nos. of larvae collected	Larval density/dip	Species of larvae
Pond 1	183	18	A.c, A.s, A.a
Pond 2	146	12	A.v, A.a, A.s
Pond 3	169	14	A.c, A.v, A.a
Pond 4	210	19	A.a, A.s, C.v, C.q
Pond 5	252	21	A.c, A.a, A.s, A.v
Stream	98	18	A.c, A.a
Well	51	12	A.c, A.s

A.c – *An. culicifacies*

A.a – *An. annularis*

A.s – *An. subpictus*

A.v – *An. vagus*

C.q – *Culex quinquefasciatus*

C.v – *Culex vishnui* group

None of the samples were positive for *P.* The high sporozoite rate in the above three vectors confirms their roles in the indigenous transmission during the outbreak.

The vectors susceptibility tests showed *An. culicifacies*, *An. annularis* and *An. subpictus* were resistant to DDT but susceptible to synthetic pyrethroid.

DISCUSSION

Malaria is the major public health problem of Orissa which contributes 23% of total malaria cases, 40% of *P. falciparum* cases and 50% of malaria deaths in the country. The coastal belts of the state show low malaria

incidence (<2 API) compared to the other regions of the state (>2 API). *Anopheles culicifacies*, *An. fluviatilis* and *An. annularis* are the three major vectors of the state (Tripathy *et al.*, 2010). *Anopheles subpictus* was found to be the local vector of Orissa (Kumari *et al.*, 2009). All the vector control programmes are run in the state by National Vector Borne disease Control Programme (NVBDCP) in the area with API more than two.

Balasore a coastal district of Orissa is considered to have low malariogenic potential, based on the epidemiological data (SPR of Balasore district-1.4, API of PHC-3.7 and API of studied subcenter data- 1.2, NVBDCP, 2009). As per the government

record, the month wise sub-centre data showed no malaria cases during the month of February to June in the year 2009. But in the year 2010, a sudden increase in malaria incidence up to 34.4% was seen in March. However no malaria cases were detected in the preceding months of January and February. The outbreak investigation following the request from State Health Department revealed the SPR of 24.4% among the fever cases with a high incidence of *P. falciparum* malaria having Slide Falciparum Rate (SFR) of 81% which is considerably higher than the usual rate suggesting recent malaria outbreak in the area.

Three vectors involved in the out break were *An. culicifacies*, *An. annularis* and *An. subpictus* with sporozoite rates of 3.45%, 2.5% and 1.05% respectively. *Anopheles culicifacies*, *An. annularis* and *An. subpictus* played the major role in the transmission during outbreak. It was marked that, in spite of high temperature of 42°C the ponds in the affected area were having water up to three feet depth which was unusual and in contrast to the ponds of neighbouring villages (not affected) which were dried during the same period. Sudden rise in the larval population might be due to high temperature resulting in the high concentration of water, which favoured the development of microorganism (acts as a larval food) in the pond. Soon before the outbreak there was heavy rainfall for three days. That might have caused increased humidity in the area which was suitable for vector to survive long and parasite to grow inside the vector. As the proportion of human: cattle were very low, man vector contact increased and transmission of malaria was high, resulting in the outbreak. Several reports are available on malaria outbreak in non endemic area. Zoller *et al.* (2009) opined that climate change affects improving conditions for competent Anopheline vectors in non-endemic areas, resulting in outbreaks due to *P. falciparum* infection. The study from India, district Korea of Chhattisgarh, during 2009 reported malaria out break in the month of March with SPR 22.2% and showed the average PMHD of *An. culicifacies* (5.5) followed by *An. subpictus* (2.0) and *An. fluviatilis* (1.5) (Mishra & Chand, 2007). The

desert of Rajasthan has also experienced unprecedented rain during 1994 (45.9cm) as compared to the preceding years (8.4 cm) leading to an outbreak in small foci with stable malaria and the PMHD of *An. culicifacies*, *An. stephensi* were 3.4 and 3.7 respectively and were found to be the major vectors during that period. High value of SPR 60.1%, SFR 56.9% were observed, which might be due to stable malaria (Shukla *et al.*, 1995). The presence of high sporozoite rate in *An. culicifacies* and *An. annularis* and increased incidence of Pf cases in our study area proofs the active malaria transmission indicating malaria outbreak. Despite of active involvement of the grassroot level health providers in identifying and treating the fever cases with standard anti-malarial drug chloroquine (CQ), the outbreak could not be controlled. Since mosquito control was highly essential at that point of time, the vectors susceptibility test were done using WHO kit and *An. culicifacies* *An. annularis* and *An. subpictus* were found to be resistant to DDT but susceptible to synthetic pyrethroid. This important information was communicated to the State Health Department and necessary control measures taken by the department for spraying and medicating the bed nets with synthetic pyrethroid (Cyfluthrin). The drug resistance of parasite to the standard anti-malarial drug chloroquine (CQ) was done by molecular method and detected 63.6% of resistance. Basing on this finding the anti-malarial drug treatment was changed from CQ to artemesinin combination therapy (ACT) by both State Government and RMRC to treat the confirmed malaria cases. The outbreak could be controlled by taking immediate action by the state health department as per the recommendation given by the investigating team. After the intervention, not a single vector species were found.

The reason for the outbreak in most of the studies reported were (i) breakdown of surveillance, (ii) consistently low API, (iii) villages not covered under indoor residual spray and (iv) favourable weather conditions for vectors to grow rapidly. But from our investigation the possible reasons for this outbreak were i) abnormal and sudden

climatic change resulting in sudden increase of temperature to 42°C and unusual rain favouring the breeding of the vector, ii) population migration from the non endemic area to endemic area, iii) area not covered under IRS programme, iv) vector resistant to DDT and v) Chloroquine resistance in *P. falciparum* parasite.

With the experience from this study the following recommendations were made to avert malaria outbreak in future. i) Long-term and systematic monitoring of environmental risk factors, vector prevalence and disease surveillance at sub-centre level, ii) intensified Early Detection and Prompt Treatment (EDPT) of malaria cases, iii) early prediction of outbreak using the surveillance data, iv) implementation of situation specific malaria control strategies and v) promotion of the use of personal protection measures with long-lasting insecticide-treated bed nets. The above recommendations will help the local and public health authorities to face any challenges of malaria outbreak occurring in the non endemic area.

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