J Vector Borne Dis 50, December 2013, pp. 248–257

Research Articles

Anopheline ecology and malaria transmission during the construction of an irrigation canal in an endemic district of Odisha, India

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ABSTRACT

Background & objectives: A new irrigation canal system is under construction in Dhenkanal district of Odisha, to increase the production of rice crop and thereby improve the living standard of farmers in the project area. Construction of canal may increase the transmission of malaria by creating vector breeding habitats. Knowledge about bionomics of vectors will support authorities for appropriate management of the disease in a changing ecological set up. The aim of this study was to assess the malaria transmission in the bank of the canal area under construction.

Methods: The entomological survey was carried out in three seasons, winter, summer and rainy during the period November 2008–October 2010 in the study area. Adult mosquitoes were collected by using suction tubes and flash lights. Mosquito species identification was done by using standard keys, separated according to abdominal conditions and were kept in an isopropanol for further molecular analysis of sibling species, presence of sporozoites and human blood meal. Larvae were collected by dippers and reared in the laboratory, and the emerged adults were identified to species. The epidemiology of malaria was evaluated from the data collected by the State Health Department. Insecticide succeptibility test was done by WHO method.

Results: The adult mosquito collection from the study area showed the prevalence of 14 species belonging to three genera, i.e. *Anopheles, Culex* and *Aedes*. The per man hour densities (PMHD) of *An. culicifacies* were 3.8, 1.4, 4.8; that of *An. annularis* were 2.1, 1, 2.1; and that of *An. fluviatilis* were 1.4, 0.3, 0.6 during winter, summer and rainy seasons respectively. Sibling species identified were: *An. culicifacies* A, B, C and D, *An. annularis* A and *An. fluviatilis* S. Sporozoite rates of *An. culicifacies* A and C were 1.1 and 0.5% respectively and that of *An. annularis* A was 2% (reported for the first time in the state). Both the vectors (*An. culicifacies* and *An. annularis*) showed resistance to DDT and malathion and were susceptible to deltamethrin, whereas *An. fluviatilis* was susceptible to all the three insecticides tested.

Interpretation & conclusion: Anopheles culicifacies, An. fluviatilis and *An. annularis* were prevalent in all the three seasons. The artificial ponds and seepage pools of canal are the major breeding sites for *An. culicifacies* and *An. annularis*. Thus, in the canal command area, control of malaria transmission requires use of insecticide-treated bednets and use of biolarvicides (seepage pools) and larvivorous fish (artificial ponds) wherever feasible.

Key words *Anopheles annularis; An. culicifacies; An. fluviatilis;* anophelines; irrigation; malaria

INTRODUCTION

Anopheline vectors have a pivotal role in increasing malaria incidence in an irrigation command area. The problem of vector-borne diseases associated with the construction of dams and irrigation canals is well known[1–3]. Worldwide, the role of irrigation in malaria transmission has received much attention and studies have yielded a complex picture[4]. In areas with stable malaria transmission where populations have developed immunity to malaria, the introduction of irrigation does not significantly worsen transmission[4]. Gratz[5] has pointed out that in Africa and Latin America, malaria and a number of arboviral infections, which can be readily transmitted by mosquito species breeding in rice fields, have been found to intensify with increased rice production. Both rice fields per se and the runoff water from rice fields support heavy vector breeding[5–6]. In the highlands of Tigray, northern Ethiopia, malaria incidence in young children was sevenfold higher in communities near small dams than in those who resided farther away[7]. The entomological survey done by Kibret *et al*[8] indicated that the most important prolific resided *Anopheles* larval habitats to be poorly constructed irrigation canals (with stagnant water), canal leakage pools and puddles in waterlogged fields, especially during the dry season. In Kenya, an increase in the

density of *An. gambiae* s.l., *An. funestus,* and *Culex quinquefasciatus* and a consequent increase in the prevalence of Bancroftian filariasis has been reported after introduction of irrigated agriculture[9]. A study in Ethiopia showed that children living in close proximity to the reservoir created by the newly constructed Gilgel-Gibe Dam are at a greater risk of *Plasmodium* infection than in children living farther away, possibly due to the creation of new vector habitats around the lakeshore[10].

Singh and Mishra[11] had documented high mosquitogenic and malariogenic conditions in Bargi Dam area of Madhya Pradesh, India. A study in Gujarat, India showed the importance of irrigation water release in maintaining high *An. culicifacies* adult density during the dry season[12]. The temporal advancement of both the anopheline fauna and the incidence of malaria synchronous with the availability of irrigation in the desert also have been explained by Tyagi[13]. The distribution of *Plasmodium falciparum* dominated malaria in the Thar Desert is more or less synchronous with the spread of Indira Gandhi Nahar Pariyojana (IGNP) related irrigated agriculture and *An. culicifacies* vector[14]. The surplus irrigation water that accumulates on irrigated land, large land areas along the course of the Indira Gandhi main canal perennially contain seepage water from the irrigation canals. The supply of canal water to the desert region over several decades has raised the water level and water-holding potential of the soil, resulting in the growth of hydrophytic weeds. Such extensive surface-water areas are the preferred breeding sites of many anophelines including *An. culicifacies,* the major vector of malaria in the Indian subcontinent[14]. From the review of malaria emergence in the Thar Desert in the wake of irrigated agriculture, it becomes more clear that *P. falciparum*-dominated malaria epidemics started to occur after the establishment of canalised irrigation, particularly under the IGNP[14].

The irrigation-mediated rise in the water level has resulted in the creation of perennial sources of still water in which malaria vectors breed[15]. Irrigation is also responsible for extending the transmission season of malaria and, as noticed in some parts of India, it has changed areas from epidemic to endemic malarious regions[16]. The Sardar Sarovar Project, an irrigation scheme along the Narmada River, intended to irrigate 1.8 million ha in drought-prone areas of Gujarat and Rajasthan, was invaded by *An. culicifacies* and *An. fluviatilis.* As a consequence, not only was the malaria season extended but also the area changed into an malaria endemic region, with nearly a 10–15-fold increase in disease incidence[15]. Although the numbers of *An. culicifacies* in the project area were similar to those seen in nearby villages, compared with their village counterparts, *An. culicifacies* in the project area was significantly more likely to be feeding on humans and significantly more likely to be efficient malaria vector. The poor housing conditions in the vicinity of the river and the relatively low cattle population at the project site may both have enhanced human-vector contact[17].

Construction of dam/irrigation canal, changes the ecology, environment and climatic conditions of the area pertaining to breeding and survival of vector mosquito species. Dhenkanal district of Odisha is highly endemic for malaria. Since, it is a drought prone area, to increase the cultivable area, a new irrigation canal system is under construction in this area. Though most of the literature reports on entomological and parasitological data are available after water release to the canal, but no report is available on the above parameters before the canal construction and release of water. So the main objective of the study is to find out the malaria transmission during the construction of the canal before release of the water.

MATERIAL & METHODS

Study area

Dhenkanal district is centrally located between the longitude of 85° 58′ to 86° 2′ E and latitude of 20° 29′ to 21° 11′ N with an average elevation of 80 m from the mean sea level. The economy of the district is predominantly agrarian. Over 70% of the population depends on agriculture. The present cultivable area in the district is 149,755 ha. The irrigated area is <24% of the total cultivable area which is much less than the state average of 35%. Therefore, to increase the cultivable area, a new irrigation canal system named Rengali Left Bank Canal is under construction in the Kamakhyanagar block of the district, where it passes through the foothill areas. Four villages located within 3 km buffer zone of the irrigation canal have been selected for the study.

The Rengali dam was constructed across the River Brahmani, in Angul district. The construction of dam was started during 1972 and was completed during the year 1985. A barrage was constructed on the Brahmani River at Samal located about 34 km downstream of the Rengali dam. Two canals, i.e. Left Bank canal (LBC) and Right Bank canal (RBC) are constructed from the Samal Barrage. The water releases made after the power generation through the Rengali dam powerhouse is picked up at the Samal Barrage to provide irrigation to 235,500 ha of land (121,200 ha from RBC and 114,300 ha from LBC). Construction of a new irrigation canal system, named Rengali Left Bank canal, of 141 km long has been planned during

1996, which would provide irrigation facilities 51,522 ha in the Dhenkanal district (District Statistical Handbook 2001). Till now, only 71 km of the canal has been constructed covering Parjang (0–30 km) and Kamakhya-nagar (31–71 km) Block. In Kamakhyanagar, the canal is under construction and water has not yet been released (Water Resource Department 2008).

Entomological survey

Entomological survey was conducted in three seasons, i.e. winter (November–February), summer (March–June) and rainy (July–October) during November 2008–October 2010.

Adult survey

Entomological survey was conducted in 10% households (60/550) of the study villages following standard method[18]. Indoor and outdoor resting adult mosquitoes were collected from human dwellings and cattlesheds in the morning and evening (0600 to 0900 hrs) using suction tubes and flash lights. Each household was surveyed for 15 min. The collected mosquitoes were identified following the standard keys[19]. Per man hour density (PMHD) of each species of mosquitoes was calculated as follows:

$$\text{PMHD} = \frac{\text{Total no. of mosquitoes collected} \times \text{Time spent in hours}}{\text{No. of persons}}$$

Abdominal conditions

Collected females were categorised according to abdominal status, unfed (UF), fullyfed (FF), semigravid (SG) and gravid (G). All the collected samples of *An. culicifacies, An. annularis* and *An. fluviatilis* were dissected into two parts—the head-thorax and the abdomen, and kept in an isopropyl alcohol for molecular processing such as identification of sibling species, detection of human blood and sporozoites.

Identification of sibling species

Total DNA from individual mosquito was extracted following a modified standard method[20]. The vector species *An. culicifacies* s.l., *An. annularis* s.l. and *An. fluviatilis* s.l. were processed for the detection of sibling species.

Anopheles culicifacies s.l.: Anopheles culicifacies specimens were first assayed by the Allele-specific polymerase chain reaction (ASPCR) assay that distinguishes the five species into two categories: A/D and B/C/E[21]. The specimens belonging to A/D group were subjected to the AD-PCR assay[22] to differentiate species A (359 bp) from species D (166 + 359 bp). The samples belonging to B/C/E group were subjected to the BCE-multiplex PCR assay to differentiate species B (248 bp), C (95 + 248 bp) and E (178 + 248 bp) from each other using targeted primers for mitochondrial *COII* gene[22].

Anopheles fluviatilis s.l.: An ASPCR assay was done to identify the sibling species of *An. fluviatilis* complex following the standard protocol[23], to observe the common amplicon of 375 bp for all the three species and S, T specific bands of 295 bp and 128 bp respectively and for U no specific band (only 375 bp common amplicon).

Anopheles annularis s.l.: Similarly, the sibling species of *An. annularis* were differentiated by the following standard protocol[24]. The D3-PCR assay was done using the specific primers. The PCR product was digested with *Alw261* and *KpnI* enzymes in a 25 µl reaction mixture. After digestion with *Alw261* enzyme two diagnostic fragments of 210 and 200 bp were observed for the presence of species A. Similarly, for species B, two diagnostic fragments of 344 and 66 bp were observed when digested with *KpnI* enzyme.

Blood meal identification

Human blood meal identification was done by PCR method[25]. Samples showing 519 bp were taken as positive for human blood (the presence of human DNA). The numbers of identified human blood meals were recorded to calculate the anthropophilic index (AI), i.e. the percentage of vector species positive for human blood.

Sporozoite detection

Detection of sporozoite was done following nested PCR techniques[26]. Samples containing *P. falciparum* sporozoites gave fragments of 205 bp (the presence of *P. falciparum* DNA). *Plasmodium falciparum* sporozoites were used to calculate the sporozoite rate (SR).

Insecticide susceptibility status

The susceptibility status of *An. culicifacies* and *An. annularis* to various insecticides like DDT, malathion and deltamethrin has been determined using standard WHO method[18].

Larval survey

Larval surveys were carried out in each village in all the three seasons using standard dippers (250 ml capacity) (BioQuip Products, USA). Larvae were collected from the main canal, artificial ponds, pools, wells, paddy fields and cemented tanks. Artificial ponds (area approx. 100

m^2) are the diggings done on both sides of the canal in the process of canal bank construction. About 5–20 dips were taken from the breeding places according to the area of the breeding habitats. Larval samples were brought to the laboratory and kept till the adult emergence and the mosquitoes were identified up to species level. Larval densities were calculated per site as:

$$\text{Larval density} = \frac{\text{Total number of larvae}}{\text{Number of dips}}$$

Information collected on meteorological data

Data on temperature, rainfall and relative humidity of the study area were collected from the Meteorological Department, Govt. of India.

Information collected on epidemiological data

The epidemiological data pertaining to annual blood examination rate (ABER), slide positivity rate (SPR), slide falciparum rate (SFR) and annual parasite incidence (API) were collected from the Government Health Department. Estimates for 2006–10 for the area are based on the data pooled for all 12 months in an year. These estimates are primarily based on active case detection (where a malaria worker goes to the community and takes blood smears from suspected malaria cases) and also from passive case detection (where blood smears were made from suspected malaria cases among patients visiting a health centre or a hospital). Accredited social health workers (ASHA) collect the blood slides every month from the fever cases. Health workers from the subcentre collected slides from ASHA and sent them to the primary health centres where the blood slides were examined by the experienced technicians for the presence of *P. falciparum* and *P. viva*x. Some fever cases also came to the health centre directly. Both the data were recorded to calculate the SPR, SFR, API, ABER, etc.

$$\text{SPR} = \frac{\text{Total slides found positive}}{\text{Total blood slides examined}} \times 100$$

$$\text{SFR} = \frac{\text{Total } \textit{P. falciparum} \text{ slides}}{\text{Total blood slides examined}} \times 100$$

$$\text{API} = \frac{\text{Total slides found positive}}{\text{Total population}} \times 1000$$

$$\text{ABER} = \frac{\text{Total blood slides examined}}{\text{Total population}} \times 100$$

Data analysis: One way ANOVA was applied to analyze the significance of spatio-temporal variations of adult density of the vectors in different seasons. Correlation analysis was done to analyze the significance of environmental parameters (temperature, rainfall and humidity) on adult density.

RESULTS

Entomological survey

The adult mosquito collection from the study area showed the prevalence of 14 species belonging to three genera, i.e. *Anopheles, Culex* and *Aedes*. A total of 1147 anopheline mosquitoes comprising three efficient malaria vectors— *An. culicifacies* s.l., winter (85), summer (33) and rainy (114); *An. annularis* A— winter (48), summer (22), rainy (50); *An. fluviatilis* S— winter (25), summer (8) and rainy (12) along with *An. subpictus* s.l.— winter (166), summer (106) and rainy (230); and *An. vagus*— winter (89), summer (56) and rainy (96) were collected. In addition, very few *An. hyrcanus* (1)*, An. karwari* (2)*, An. splendidus* (2) and *An. tessellatus* (2) were also collected along with *Culex quinquefasciatus* (128)*, Cx. vishnui* group (65), *Cx. whitmorei* (10), *Cx. gelidus* (12) and *Aedes albopictus* (8). *Anopheles subpictus* s.l. was found to be predominant (43.8%) in all the three seasons (winter, rainy and summer) followed by *An. vagus* (21%), *An. culicifacies* s.l. (20.2%), *An. annularis* A (10.5%), *An. fluviatilis* (3.9%), and other anopheline species (0.6%). The PMHDs of *An. culicifacies*, *An. annularis* A*, An. subpictus* s.l. and *An. vagus* in rainy season were 4.8, 2.1, 9.7 and 4.3 respectively (Table 1). The one way ANOVA test results revealed that the density variations of *An. culicifacies* s.l. ($p < 0.05$), *An. annularis* A ($p < 0.05$) and *An. subpictus* s.l. ($p < 0.001$) between the seasons were significantly high during rainy season (Table 1).

Indoor collected female anopheline mosquitoes were graded according to abdominal conditions. The proportion of unfed (UF), fullyfed (FF), semigravid (SG) and gravid (G) condition of vector species has been presented in Table 2. All the four stages of the abdominal conditions were found in all the four species. It was seen that 43.5 and 55% of *An. culicifacies* s.l. and *An. annularis* s.l. respectively were fullyfed. Unfed populations were 22 and 11.7% respectively. The ratio of blood seeking stages to resting stages for *An. culicifacies* s.l. was 1.9 and *An. annularis* s.l. was 2.0.

The molecular identification of *An. culicifacies* showed the presence of four sibling species, i.e. A, B, C and D. Prevalence of species B was more (51.7%) followed by species C (27.6%), A (11.6%) and D (9.1%). The siblings species of the *An. culicifacies* showed different patterns of seasonal abundance. The prevalence

Table 1. Per man hour density of indoor-resting anophelines and their percentage in the study area

Anopheline species	Average PMHD ± SD			Between seasons	
	Winter	Summer	Rainy	F-value	Significance
An. culicifacies	3.8 ± 0.31 (20.6)	1.4 ± 0.08 (14.7)	4.8 ± 0.13 (22.4)	103.4	p <0.05
An. annularis	2.1 ± 0.13 (11.6)	1 ± 0.10 (9.8)	2.1 ± 0.02 (9.8)	77.7	p <0.05
An. subpictus	7.2 ± 0.23 (40.2)	5 ± 0.12 (47.1)	9.7 ± 0.02 (45.2)	480.5	p <0.001
An. vagus	5.3 ± 0.93 (21.5)	2.3 ± 0.3 (24.9)	4.3 ± 0.29 (18.9)	11.4	p <0.05
An. fluviatilis	1.4 ± 0.05 (6)	0.3 ± 0.05 (3.5)	0.6 ± 0.05 (2.3)		

Figures in parentheses indicate total percent collected; SD—Standard deviation; Others (*An. hyrcanus, An. karwari, An. splendidus* and *An. tessellatus*).

Table 2. Abdominal conditions of indoor collected female anophelines from the study area

Species	UF	FF	SG	G	UF+FF	SG+G	UF+FF/SG+G
An. culicifacies	51 (22)	101 (43.5)	41 (17.7)	39 (16.8)	152 (65.5)	80 (34.5)	1.9
An. annularis	14 (11.7)	66 (55)	18 (15)	22 (18.3)	80 (66.7)	40 (33.3)	2
An. subpictus	109 (21.3)	197 (38.5)	132 (25.8)	74 (14.5)	306 (59.8)	206 (40.2)	1.5
An. vagus	94 (39)	81 (33.6)	42 (17.4)	24 (10)	175 (72.6)	66 (27.4)	2.7
An. fluviatilis	10 (22.2)	15 (33.3)	12 (26.6)	8 (17.7)	25 (55.5)	20 (44.4)	1.2

Figures in parentheses indicate percentages; UF— Unfed, FF—Fullyfed, SG—Semi gravid, G—Gravid.

Table 3. Distribution of sibling species (% of total), anthropophilic index and sporozoite rate of *Anopheles* mosquitoes

Sibling species	No. of abundance per season				Anthropophilic index (%)	Sporozoite rate
	Winter	Summer	Rainy	Total		
An. culicifacies						
A	10 (11.8)	5 (15.2)	12 (10.5)	27	5.5	1 (8.33) Rainy
B	51 (60)	15 (45.5)	54 (47.4)	120	1.7	0
C	16 (18.8)	10 (30.3)	38 (33.3)	64	2.8	3 (4.68) Summer
D	8 (9.4)	3 (9.1)	10 (8.8)	21	1.1	1 (4.76) Winter
An. annularis A	48 (40)	22 (18.33)	50 (41.66)	120	14	1 (2.08) Winter, 1 (4.54) Summer*
An. fluviatilis S	25 (55.5)	8 (17.77)	12 (26.66)	45	65	1 (4) Winter

*Represents *Plasmodium vivax* sporozoite in the mosquito; Figures in parentheses indicate percentages.

of species A was higher in summer (15.2%), species B (60%) and species D (9.4%) in winter and species C (33.3%) in rainy seasons. The AI values for all the sibling species of the complex (A, B, C and D) were found to be 5.5, 1.7, 2.8 and 1.1%, respectively. Season-wise sporozoite rate was found to be 8.33 in rainy, 4.68 in summer and 4.76% in winter seasons in *An. culicifacies* A, C and D respectively (Table 3). *Anopheles annularis* sibling species showed the presence of only species A in the study area. AI of this species was found to be 14% and sporozoite rate was found to be 4.54% in winter. Among *An. fluviatilis* S, T, U and V only species S was present in the study area. AI of this species was found to be 65% and sporozoite rate was 4% in winter.

Insecticide susceptibility status

Results of insecticide susceptibility tests are presented in Table 4. *Anopheles culicifacies* B showed highest mortality (50%) to DDT, 40% to malathion and 100% to deltamethrin. Species A was found to be less susceptible among A, C and D to DDT (23%) and malathion (25%) whereas C was found to be more susceptible to malathion compared to DDT. However, all the species showed 100% mortality to deltamethrin. Similarly, *An. annularis* A showed 30% susceptibility to DDT and 45% to malathion. *Anopheles fluviatilis* S showed 100% mortality to deltamethrin, malathion and DDT.

The breeding habitats found were artificial ponds, paddy fields, canals, pools, wells and cemented tanks.

Out of 164 larval habitats surveyed, 86 (52.4%) were found positive for anopheline larvae, i.e. *An. culicifacies* s.l. and *An. annularis* s.l., *An. subpictus* s.l. and *An. vagus* (Tables 5 and 6). Contribution of different breeding habitats for anopheline larvae was, artificial ponds 48 (65.8%) followed by canals 17 (19.8%), paddy fields 12 (14%), wells 4 (4.7%), seepage pools of canal 3 (3.5%) and cemented tanks 2 (2.3%). A total of 688 dips were taken and 327 (47.5%) were found positive. The average number of larvae per dip was 2.6. Artificial ponds were found to be the most predominant breeding spots and those alone contributed 48 (55.8%) positive larval habitats. Out of the anophelines collected from this breeding habitat, the major vector *An. culicifacies* contributed 60%. Abundance of the major vector *An. culicifacies* was found more in artificial ponds 126 (60%) followed by seepage pools of

Table 4. Susceptibility status of the *An. culicifacies, An. annularis* and *An. fluviatilis* in the study area

Insecticide (%)	*An. culicifacies* s.l.				*An. annularis* A			*An. fluviatilis* S		
	Sibling species	No. exposed	No. dead	Mortality (%)	No. exposed	No. dead	Mortality (%)	No. exposed	No. dead	Mortality (%)
DDT (4)	A	13	3	23	30	9	30	10	10	100
	B	12	6	50						
	C	15	5	33						
	D	10	4	40						
Malathion (5)	A	8	2	25	20	9	45	7	7	100
	B	15	6	40						
	C	10	5	50						
	D	7	2	28.5						
Deltamethrin (0.05)	A	5	5	100	20	20	100	8	8	100
	B	12	12	100						
	C	10	10	100						
	D	3	3	100						

No. dead (post 24 h).

Table 5. Distribution of anopheline larvae in different breeding habitats in the study area

Larval habitats	No. of habitats examined	No. of habitats positive	No. of dips taken	No. of dips positive	Average no. of larvae per (+)ve dip
Artificial ponds	72	48 (55.8)	360	125 (38.2)	3.1
Paddy fields	25	12 (14)	125	112 (34.3)	1.9
Canals	30	17 (19.8)	120	68 (20.8)	1.8
Seepage pools of canal	12	3 (3.5)	20	6 (1.8)	4.2
Wells	16	4 (4.7)	36	8 (2.4)	2.3
Cemented tanks	9	2 (2.3)	27	8 (2.4)	2.3
Total	164	86 (52.4)	688	327 (47.5)	

Figures in parentheses indicate percent positive.

Table 6. Anopheline species breeding in different breeding sites in the study area

Breeding sites	Number of anopheline larvae				Total
	An. culicifacies	*An. annularis*	*An. subpictus*	*An. vagus*	
Artificial ponds	126 (60)	22 (10.5)	44 (21)	18 (8.6)	210
Paddy fields	115 (29.8)	67 (17.4)	164 (42.5)	40 (10.4)	386
Canals	58 (46.8)	30 (24.2)	24 (19.4)	12 (9.7)	124
Seepage pools of canal	12 (48)	8 (32)	5 (20)	2 (7.4)	27
Wells	2 (10)	4 (22.2)	9 (50)	5 (27.8)	20
Cemented tanks	3 (14.28)	5 (27.8)	10 (55.6)	3 (16.7)	21
Total	311 (39.8)	136 (17.4)	256 (32.8)	78 (10)	781

Figures in parentheses indicate percentages.

canal 12 (48%), canals 58 (46.8%) and paddy fields 115 (29.8%). *Anopheles annularis* was found more in seepage pools of canal 8 (32%) followed by cemented tanks 5 (27.8%), and canals 30 (24.2%). In most of the breeding habitats, *An. subpictus* and *An. vagus* were found in association with *An. culicifacies* and *An. annularis.*

Epidemiological data

To know the five years prevalence of malaria in the study area (2006–10) epidemiological data were analyzed. During 2006–10, the malaria prevalence (SPR) was 11.6% (9.3–15.6%). Decreasing trend of SFR was recorded from 15.15% in 2009 to 13.5% in 2010 (Fig. 1). There was a significant correlation (R=0.84; p <0.05) between seasonal vector density (*An. culicifacies, An. annularis* and *An. fluviatilis*) and malaria prevalence (slide positivity rate). With the increase in vector density malaria prevalence also increased.

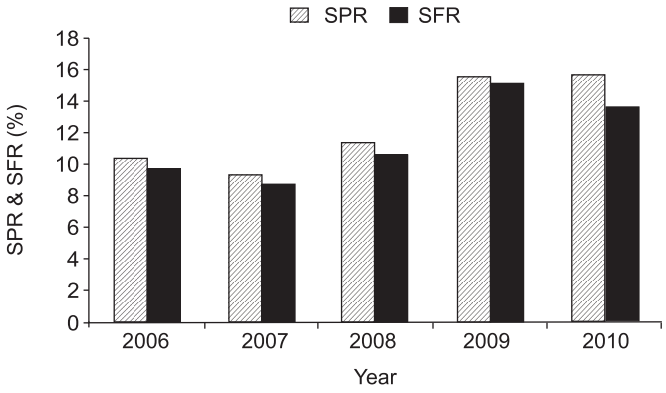

Fig. 1: SPR and SFR of the study area. *Source:* State Health Department Odisha.

Correlation between vectors (*An. culicifacies* s.l. and *An. annularis* A) density with maximum temperature revealed that with the increase in maximum temperature the mosquito density decreased significantly (R=0.88; p <0.05). There was no significant impact of minimum temperature and relative humidity on the density pattern in the study area.

DISCUSSION

The epidemiology of malaria is the product of complex interaction between host, vector and parasite, factors that are specific to each location in which malaria occurs[27]. Malaria is a "local and focal problem" which needs to be studied as a system of interaction of man, mosquito and environment[28]. It is generally believed that irrigation development offers ideal habitats for the proliferation of anophelines, including vectors of malaria. Irrigation projects may also result in the development and maintenance of mosquito-borne diseases[3]. The history of malaria control in India is in fact history of control of *An. culicifacies*[29]. The study area shows high malaria prevalence during the construction of irrigation canal.

Our study revealed higher larval and adult vector abundance and a higher prevalence of malaria in the study area. The study showed the presence of nine anopheline species, with the predominance of *An. subpictus* followed by *An. vagus, An. culicifacies, An. annularis,* and *An. fluviatilis* in all the three seasons. However, the previous study in the district had shown the presence of nine anophelines including the local vectors *An. aconitus* and *An. varuna*[30]. But in the present study, these two important local vectors were not found. This may be due to the deforestation in the area. Previous reports also showed *An. subpictus* to be the most prevalent species in several districts of Odisha[30–31]. The association of *An. culicifacies* with *An. annularis* in both the canal irrigated and non-irrigated areas and also with *An. subpictus* in Gujarat, India was established[32]. *Anopheles culicifacies* and *An. annularis* are the two important malaria vectors of Odisha[31, 33–34]. Seasonal analysis showed the presence of two main vectors *An. culicifacies* and *An. annularis* in all the seasons, thus responsible for perennial transmission.

The variations showed fullyfed were significantly high among the stages of different abdominal conditions (UF, FF, SG and G). It signifies the endophagic nature of these two malaria vectors of this region. Accurate identification of anopheline mosquitoes is necessary for planning effective vector control strategies and for better understanding of their potential role in malaria transmission. Bionomics and behaviour of mosquitoes are changing with respect to the ecological conditions[35]. All the three important vectors in the study area *An. culicifacies, An. annularis* and *An. fluviatilis* are species complexes. There are five sibling species of *An. culicifacies,* i.e. A, B, C, D and E and A, C, D, E are vectors whereas B is considered as a poor vector if at all. A, B, C and D were prevalent in our study area and their prevalence was 11.6, 51.7, 27.6, and 9.1 respectively. Previous study conducted in the Dhenkanal district showed the prevalence of *An. culicifacies* A, B, C, D as 4.45, 64.67, 28.34 and 2.54% respectively[30]. This showed that densities of species A and D had increased three fold in the present study, this might be due to availability of the large breeding areas developed during the construction of the canal. *Anopheles culicifacies* (Giles) (species B of Green and Miles) was incriminated as a vector of malaria in Sri Lanka during the early part of this century[36]. The studies conducted

after irrigation development of Mahawali project area of Sri Lanka only species B and E of *An. culicifacies* complex have been detected with species E being incriminated as the major vector of *P. falciparum* and *P. vivax* malaria[37]. Among *An. annularis* A and B sibling species, *An. annularis* A was detected from the study area. The sporozoite rate was found to be 2%. Species A had been incriminated as a vector in Odisha and Asom but not in Rajasthan and Haryana[26, 38]. In Sri Lanka, due to development of Mahawali irrigation project *An. annularis* and *An. subpictus* emerged as major vectors of malaria due to change in the ecosystem from dry zone forest to irrigated cultivated land[39]. *Anopheles annularis* B has not been found to have its role in malaria transmission in the country. Although both the species have sympatric distribution in Uttar Pradesh, none of them are vectors in the region[38].

The ability of an *Anopheles* species to feed on human blood is generally defined as anthropophily and represents the success of a species and its capacity to transmit malaria[40]. The AI is one of the important parameters to give the actual frequency of human-mosquito contact and thus measures the transmission probability. The blood-feeding behaviour of anopheline mosquitoes have been studied for the estimation of vectorial capacity and other epidemiological purposes[41]. In this study, the AI of *An. culicifacies* sibling species varied from 1.1–5.5%, species A showed highest AI (5.5%). Previous study had shown low AI of species C (0.8%) but it had increased to 2.8% in the present study. The AI of *An. annularis* A was 14%. The sporozoite rate was found to be 1.1 and 0.5% in *An. culicifacies* A and C respectively. The sporozoite rate as low as 0.25% in *An. culicifacies* was observed in Bargi Dam area, India[5]. The sporozoite rate of *An. annularis* A was 2% and this rate was observed to be 3.4% in *An. annularis* s.l. in Keonjhar district of Odisha[26].

The larval survey indicated that the artificial ponds and seepage pools of canal were the major breeding sites for *An. culicifacies* and *An. annularis*. This may likely to facilitate breeding of anopheline species, thus increasing the adult density in the nearby canal villages. Inside the digging canal, water is generally accumulated during rainy season and that prolongs up to winter season. This accumulated water may facilitate breeding of *An. culicificies*. *Anopheles culicifacies* s.l., the major vector of malaria in the Indian subcontinent is generally regarded to be intolerant to salinity, preferring to breed in newly-dug freshwater pits, domestic wells and pits used for plantation of coconuts and casurina in India[37]. Rapid breeding of the relatively inefficient malaria vector *An. annularis* in newly built irrigation canals in Sri Lanka was suggested to have triggered malaria epidemics[39].

Proper water management and canal maintenance for source reduction through environment management could help to reduce mosquito-breeding sites and thus, malaria transmission[8]. Health issues seem to have been neglected in many development projects. Poor engineering design is difficult to correct after construction, and hence early planning is critical[14]. Previous study by the authors found that with the construction of irrigation canal, simultaneous with specific malaria control programme with monitoring and surveillance system helped to control the malaria transmission in the command area[42]. All the developmental activities must look at the associated health issues and ensure that appropriate and durable safeguards are in place.

In conclusion, a clear understanding of vector density pattern, their seasonal prevalence and resting behaviour helps to plan appropriate and timely vector control measures. In our study area, *An. culicifacies*, *An. annularis* and *An. fluviatilis* were the main vectors prevalent during all the seasons. The artificial ponds and seepage pools of canal are the major breeding sites for *An. culicifacies* and *An. annularis*. Thus, in the canal command area, control of malaria transmission requires use of insecticide-treated bednets, use of biolarvicides in seepage pools and introduction of larvivorous fishs in artificial ponds.

ACKNOWLEDGEMENTS

The authors are thankful to the Indian Council of Medical Research (ICMR), New Delhi for financial support and to the Director, Regional Medical Research Centre, Bhubaneswar for constant encouragement and valuable suggestions during the study as well as providing necessary facilities. The authors are also thankful to Dr Rajnikant Srivastava for his valuable suggestions. The authors are thankful to the team members of Medical Entomology Division for their support and activities during the field survey. Authors are also thankful to Department of Water Resources, Government of Odisha for providing valuable information on the Rengali Irrigation Canal System Network.

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Received: 8 November 2012 *Accepted in revised form:* 30 July 2013