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Estimation of vectorial capacity of *Anopheles minimus* Theobald & *An. fluviatilis* James (Diptera: Culicidae) in a malaria endemic area of Odisha State, India

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***Background & objectives*: *Anopheles minimus* and *An. fluviatilis* were incriminated as the major malaria vectors in Keonjhar district of Odisha State recently. This study was carried out to elucidate the potential role of these two vector species in transmission of malaria during different seasons, and vectorial capacity of these species was also estimated.**

***Methods*: Three hilly and forested villages of Keonjhar district were randomly selected. Vectorial capacity (C) was calculated using the Macdonald's formula as modified by Garret-Jones. The human landing density of the vector species was obtained from all night human landing collections (bait protected by bed-net). Man feeding habit was estimated by multiplying the human blood index with feeding frequency, which was obtained on daily basis from the duration of gonotrophic cycle. The probability of survival through the extrinsic incubation cycle was calculated from the probability of survival through one day and duration of sporogonic cycle.**

***Results*: The estimated vectorial capacity of *An. minimus* varied between 0.014 and 1.09 for *Plasmodium falciparum* (*Pf*) and between 0.1 and 1.46 for *P. vivax* (*Pv*). The C of *An. minimus* for both *Pf* and *Pv* was higher during rainy season than the other two seasons. The estimated C of *An. fluviatilis* varied between 0.04 and 1.28 for *Pf* and between 0.20 and 1.54 for *Pv*.**

***Interpretation & conclusions*: Based on the estimated values of vectorial capacity of the two vector species, the area could be stratified and such stratification would reflect the difference in the intensity of transmission between different strata and accordingly the appropriate control strategy could be adopted for each stratum.**

Key words *Anopheles minimus* - *An. fluviatilis* - India - malaria - Odisha - vectorial capacity

Malaria is a major public health problem in Keonjhar district of Odisha State (part of east-central India), which has been hyperendemic for falciparum malaria for many years[1]. *Anopheles minimus*, which was earlier believed to have disappeared from this area consequent to the introduction of DDT for indoor residual spraying under the National Malaria Eradication Programme (NMEP), was recorded in this district after a period of about 45 years[2]. Subsequently, *An. minimus* and *An. fluviatilis* were incriminated as the major malaria vectors in the district[3]. Human landing rates, host feeding behaviour and parity of both the vector species in the district have also been studied[4].

The areas of high malaria transmission in Keonjhar district include hilly and forested regions that are largely inhabited by tribal population. The problem of malaria persists in the district primarily due to ecological and geographical conditions that are favourable for the prevalence of *An. minimus* and *An. fluviatilis* resulting in the spread of malaria in the district. Understanding transmission dynamics is essential to plan for the control of malaria through implementing site specific and evidence based vector control measures. To elucidate the role of *An. minimus* and *An. fluviatilis* in the transmission of malaria during different seasons in a year, the vectorial capacity of the two species was estimated in Keonjhar district of Odisha State.

Material & Methods

Study area: The study was carried out between May 2006 and July 2007 in three randomly selected hilly and forested villages namely, Mamulipusi, Puradihi and Dhanakunia Sahi of Baunspal PHC in Keonjhar district, Odisha, India. The demographic profile of the study area has been given elsewhere[1]. During summer season, majority of the people sleep outdoors. The human to cattle ratio in the study villages was 1.0: 0.47. The climate of the study area is characterized by summer (March-June), rainy (July-October) and winter seasons (November-February). The minimum and maximum temperature during 2001-2006 ranged from 9.8° C in January to 25.6° C in May and from 24.5° C in December to 39.3° C in May, respectively[4]. The average relative humidity ranged from 30.6 per cent in March to 85.6 per cent in September[4]. The monthly rainfall varied from 0 to 456 mm, with an average of 1393 mm annually[4]. Malaria has been persisting since many years in the district with *Plasmodium falciparum* (*Pf*) being the predominant species (>95%)[1]. There are two major malaria vectors namely *An. minimus* (Species A) and *An. fluviatilis* (Species S)[1] involved in transmission of the disease in the study area[3]; among *An. fluviatilis*, species 'S' is the predominant one constituting 87 per cent and the remaining 13 per cent were species 'T'[1].

Vectorial capacity (C): The vectorial capacity of *An. minimus* and *An. fluviatilis* was estimated using the formula of Macdonald as modified by Garrett-Jones[5]: Vectorial capacity (C) = $ma^2p^n/-\log_e p$; Where, ma = human landing density, a = human feeding habit, p = probability of daily survival, n = duration of sporogony, and $-\log_e p$ = expectation of life.

Human landing collections, where the bait (human volunteer) was protected by a bed-net, were carried out simultaneously indoors and outdoors at monthly intervals, at one site, in each of the three selected villages between 1800 and 0600 h for a period of 15 months from May 2006 to July 2007 (six months in summer, five months in rainy and four months in winter season). In total, 45 each of indoor and outdoor collections were made. Ethical clearance was obtained for engaging human volunteers as baits from the Institutional Ethical Committee of Vector Control Research Centre, Puducherry. Mosquitoes that landed on the net were collected using oral aspirators and flashlights. Human landing density was calculated from the number of female mosquitoes landed on the net or attempted to bite per bait per night. In the following morning, the mosquitoes were identified, grouped according to their gonotrophic conditions and dissected out to determine the parity status using ovariolar dilatation method[6].

Since the human landing density indoors and outdoors was low, the human landing density was obtained averaging indoor and outdoor results. Man feeding habit of the vector species was estimated by multiplying the human blood index (HBI) (proportion of blood meals taken on man) with feeding frequency, which was obtained on daily basis from the duration of one gonotrophic cycle (the interval in days between two consecutive blood meals). The gonotrophic cycle of *An. fluviatilis* was assumed to be two days for summer and three days for rainy and winter seasons based on the earlier study in Koraput district, Odisha[7]. The same duration was considered for *An. minimus* also. To determine the human blood index from blood meal analysis, diurnal resting catches were separately made (from 0600 to 0730 h) in the three study villages by randomly selecting six human dwellings and three cattle sheds in each village at monthly interval. Indoor resting *Anopheles* mosquitoes were collected for 10 min in each dwelling using a flash light and a mouth aspirator. The collections from each catching station were labelled separately, brought to the Vector Control Research Centre Camp Laboratory at Keonjhar, and identified to species. Blood meals of the fully fed *An. minimus* and *An. fluviatilis* females were analyzed using agar-gel diffusion method to find the source of feeding[8]. The proportion that actually fed on humans was taken as HBI.

The probability of survival through one day was computed by taking the g^{th} root of PP (where PP is the proportion of parous females in man-landing collections and 'g' is the duration of gonotrophic cycle). The proportion parous was determined by dissecting out ovaries of the female mosquitoes collected on human baits and looking for dilatations in pedicel part of the ovarioles that were separated from the ovaries[6]. The time (in days) taken by malaria parasite to complete its development in vector mosquitoes [sporogonic cycle (n)] was estimated as a function of temperature using the formula $n = T/t\text{-}t_{min}$ where, n is the duration of sporogony, T = total degree days required for completion of sporogony, (105 °C for *P. vivax* and 111 °C for *P. falciparum*), t is the actual mean diurnal temperature and t_{min} is the threshold temperature (14.5 °C for *P. vivax* and 16.0 °C for *P. falciparum*) required for development of malaria parasite[9]. Data on temperature during the study period were collected from the Research Station of the Odisha University of Agriculture and Technology located about 10 km from the study sites and used for estimating the duration of sporogonic cycle. Using the values of both probability of survival through one day (p) and the duration of sporogonic cycle (n), the probability of survival through extrinsic incubation cycle (p^n) was calculated.

Malaria incidence: Incidence of malaria in the study villages was recorded through fortnightly door to door active case detection method. From all fever cases as well as persons suffering from fever within last 15 days from the date of earlier survey, blood smears were collected and the patients were treated presumptively with chloroquine. The microscopically proved malaria positive persons were administered with radical treatment (chloroquine and primaquine) as per the guidelines of National Drug Policy on Malaria[10].

Statistical analysis: Pearson correlation analysis was carried out to see the relationship between the values of C and malaria cases during different seasons and the significance of correlation co-efficient was tested using t test.

Results

The monthly data of the number of *An. minimus* and *An. fluviatilis* collected, dissected, number of fever cases and malaria positive cases of the three study villages were combined and presented in Table I. Tables II and III summarize the average man biting density (ma), the probability of survival through one day (p), the sporogonic period (n) and the estimated vectorial capacity (C) of the two vector species together with temperature and number of malaria cases for the study period during the three seasons. The average proportion parous $\pm$ SD of *An. minimus* was relatively higher during rainy months (0.69±0.11) than winter (0.55±0.17) and summer months (0.55±0.13). Taking into account the proportion parous and duration of gonotrophic cycle, the average probability of survival of *An. minimus* through one day was found to be the highest during rainy months (0.88±0.04) followed by winter (0.81$\pm$0.09) and summer months (0.74±0.09). The average proportion parous of *An. fluviatilis* during rainy, winter and summer months were 0.63±0.27, 0.52±0.35 and 0.7±0.24, respectively. The corresponding probability of daily survival of this species was 0.85±0.12, 0.66±0.44 and 0.83±0.15 during the months of the three seasons. Of the 161 blood meal samples of *An. minimus* tested, 97.5 per cent (HBI of 0.98) reacted with human antiserum and the remainder with bovine antiserum. A total of 104 samples of *An. fluviatilis* were tested, of which 92.0 per cent (HBI of 0.92) were positive for human blood.

Using the mean diurnal temperature recorded during the study period, the estimated 'n' value ranged from 9.2 to 34.7 for *Pf* and 7.76 to 22.34 for *Pv* in different seasons. Sporogony took the minimum time for completion during summer followed by rainy and winter months (Tables II and III).

There was a wide variation in the vectorial capacity of *An. minimus* and *An. fluviatilis* between the months. The estimated C of *An. minimus* for both *Pf* and *Pv* was higher during rainy season than the other two seasons (Table II). Although, the man biting density of *An. minimus* did not show any marked difference between rainy and winter months, the vectorial capacity was relatively higher during rainy season and this could be due to a shorter sporogonic cycle coupled with a higher survival rate. Like in winter season, the probability of survival through one day was higher during rainy season as favoured by a low temperature prevailed in the later part of this season. The sporogonic cycle, which is primarily a function of temperature, was shorter during rainy season than that estimated for winter season. As a result, the higher daily survival rate together with a shorter sporogonic cycle favoured the vector species to have a higher vectorial capacity during rainy season.

The estimated vectorial capacity of *An. fluviatilis* varied between 0.04 to 1.28 for *Pf* and between 0.2 to 1.54 for *Pv* (Table III). This vector species exhibited a different seasonal pattern in its vectorial capacity,

Table I. Number of parous females of the two vector species, and number of fever and malaria cases in the study villages

Month & yr	*An. minimus*						*An. fluviatilis*						Number of fever cases	Number positives for malaria
	Indoor			Outdoor			Indoor			Outdoor				
	1	2	3	1	2	3	1	2	3	1	2	3		
2006														
May	1	1	0	6	6	3	2	2	1	5	5	3	8	2
June	2	2	1	4	4	2	2	2	2	5	5	4	11	5
July	3	3	1	3	3	3	4	4	4	0	0	0	23	11
August	9	9	8	4	4	3	10	10	5	3	3	0	25	15
September	11	11	7	7	7	4	11	11	8	9	9	5	24	12
October	13	12	7	24	24	16	9	9	4	13	13	7	19	10
November	13	13	8	11	11	8	10	10	7	8	8	5	15	7
December	7	6	4	2	1	1	13	13	8	8	7	5	16	10
2007														
January	2	2	1	0	0	0	1	1	0	0	0	0	13	4
February	4	3	1	3	3	1	6	6	4	2	2	2	12	5
March	8	6	3	3	3	1	2	2	0	1	1	1	14	5
April	1	1	0	5	5	4	2	2	1	3	3	2	19	9
May	2	2	2	2	2	1	4	4	4	5	5	5	16	5
June	0	0	0	2	2	1	4	4	4	8	8	6	17	8
July	3	3	0	1	1	0	0	0	0	0	0	0	20	8
Total	79	74	43	77	76	48	80	80	52	70	69	45	252	116

1, number collected; 2, number dissected; 3, number parous

Table II. Estimated vectorial capacity (C) of *An. minimus* in the study villages

Season	Mean temperature (°C)	ma	a	p	n (days)		C		New malaria cases	
					Pf	*Pv*	*Pf*	*Pv*	*Pf*	*Pv*
Rainy	25.9	2.6	0.33	0.86	11.2	9.19	1.09	1.46	51	5
Summer	28.0	1.0	0.49	0.73	9.2	7.76	0.08	0.13	32	2
Winter	19.2	1.8	0.33	0.85	34.7	22.34	0.014	0.10	20	6

ma, man landing rate; a, man feeding habit; p, probability of daily survival; n, duration of sporogony; *Pf, Plasmodium falciparum; Pv, Plasmodium vivax*

Table III. Estimated vectorial capacity (C) of *An. fluviatilis* in the study villages

Season	Mean temperature (°C)	ma	a	p	n (days)		C		New malaria cases	
					Pf	*Pv*	*Pf*	*Pv*	*Pf*	*Pv*
Rainy	25.9	1.96	0.31	0.82	11.2	9.19	0.35	0.53	51	5
Summer	28.0	1.2	0.46	0.88	9.2	7.76	1.28	1.54	32	2
Winter	19.2	2	0.31	0.87	34.7	22.34	0.04	0.20	20	6

ma, man landing rate; a, man feeding habit; p, probability of daily survival; n, duration of sporogony; *Pf, Plasmodium falciparum; Pv, Plasmodium vivax*

having relatively higher values in summer months, a period when there was a higher survival rate and life expectancy but a lower value of 'n'. A higher survival rate was recorded in summer and this was probably a reflection of dissection of a relatively lesser number of mosquitoes in this season.

During the study period, a total of 118 (*Pf*-103, *Pv*-13 and *Pf* +*Pv*-2) new malaria cases were detected in the study villages [slide positivity rate (SPR) = 46.8%]. *P. falciparum* was the predominant malaria parasite constituting 87.3 per cent of the total cases. Number of malaria cases peaked in August. Of the total malaria cases, 49.6 per cent were recorded in rainy months followed by 28.6 per cent in summer and 21.8 per cent in winter months. The estimated vectorial capacities of *An. minimus* during the corresponding seasons were 1.09, 0.08 and 0.014 for *Pf*, and 1.46, 0.13 and 0.10 for *Pv*, indicating that the maximum number of *Pf* cases were recorded in rainy months when the vectorial capacity of *An. minimus* was the highest. This was followed by summer and winter months. There was a significant positive association ($P<0.05$), between the estimated C for *An. minimus* and number of malaria cases in the study villages in different seasons. Such relationship was not seen for *An. fluviatilis* as its vectorial capacity was higher during summer season followed by rainy and winter. When the vectorial capacities of the two vector species were combined season-wise, with an increase in vectorial capacity the number of malaria cases, particularly of *P. falciparum*, also showed an increasing trend (C: 1.44, 1.36 and 0.054 for *Pf* and 1.99, 1.67 and 0.3 for *Pv*; cases due to *Pf*: 51, 32 and 20 and *Pv*: 5, 2 and 6 during rainy, summer and winter season, respectively).

Discussion

The concept of vectorial capacity, a mathematical expression, is considered to be a useful indicator to measure the effectiveness of vector control measures and also the receptivity of both endemic and non-endemic areas[11]. In endemic areas, it is a major determinant in evaluating different methods of vector control, whereas in non-endemic areas, which are vulnerable and receptive, it can be used to monitor transmission potential[12]. Among the variables used for the estimation of vectorial capacity, the two important and sensitive entomological parameters *viz*., feeding frequency and longevity that determine the efficiency of a vector in disease transmission, are incorporated. Therefore, it stands as a valuable index in malaria epidemiology.

Although *An. minimus* has been incriminated as a major malaria vector in several parts of India, including the current study site, estimation of its vectorial capacity has not been done. However, vectorial capacity, which is not an absolute number but varies both temporally and spatially[13], has been worked out for some other Indian malaria vectors such as *An. fluviatilis* in Jeypore hill tracts, Odisha[7], *An. stephensi* in Salem town, Tamil Nadu[14], *An. culicifacies* in Rameswaram Island[15] and *An. dirus* in Assam[16].

The difference in the estimated vectorial capacity of a particular mosquito species for different *Plasmodium* parasites is a reflection of difference in the estimated values of sporogonic period. As a result, higher values have been obtained for *P. vivax*, which has a shorter sporogonic period than *P. falciparum*. However, *P. falciparum* has been the predominant species in the study area forming >95 per cent of the total malaria cases[3], which will be contrary to the higher vectorial capacity estimated for *P. vivax* during the current study. A comparatively longer duration of gametocytaemia in untreated persons and lesser stimulation of immune response have been reported to favour the preponderance of *Pf* in areas where there is prolonged transmission[7]. The vectors may also have different susceptibilities to different *Plasmodium* species[7]. In Koraput district of Odisha State, the vectorial capacity of *An. fluviatilis* varied between 0.049 and 2.379 for *P. falciparum* and 0.110 and 4.362 for *P. vivax*[7]. The higher values of this vector species reported in the Koraput compared to the present study was due to a relatively higher man biting density and a higher survival rate recorded there throughout the year[7].

The sporogonic period calculated for both the human malaria parasites was justifiable for rainy and summer seasons. However, the duration for *P. falciparum* in winter season was 34.7 and for *P. vivax* 22.3, which appeared to be overestimated. This overestimation would be due to the minimum temperature recorded (10°C) in December.

When vectorial capacity of *An. fluviatilis* was compared with *An. minimus*, the values varied between the seasons. While C of *An. minimus* was higher in rainy months, *An. fluviatilis* had a higher C in summer months compared to other seasons. This difference was due to the higher human landing density and survival rate of *An. minimus* in rainy months and the higher survival rate of *An. fluviatilis* in summer months. The season-wise analysis showed that vectorial capacity of both the vector species was relatively lower in winter season. There was an increase in number of malaria cases, particularly *P. falciparum,* with the increase in the vectorial capacity of *An. minimus* in different seasons. In other words, *An. minimus,* with *An. fluviatilis* as a supplementary vector, could be the principal vector of transmission of malaria in rainy season, whereas in summer season, *An. fluviatilis,* supplemented by *An. minimus* could play a primary role in transmission. Ideally, when two vectors involve in malaria transmission, the vectorial capacity of both the vector species need to be combined and the total C value to be related with the number of malaria cases.

In the study area, *An. minimus* and *An. fluviatilis* were incriminated with human *Plasmodium* infection with an overall sporozoite rate of 3.3 and 3.4 per cent, respectively[3]. The area was highly receptive for malaria. Based on the estimated value of vectorial capacity of the vector species, the area could be stratified and such stratification would reflect the difference in the intensity of transmission between different strata and accordingly the appropriate control strategy could be adopted for each stratum[17].

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