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Risk of malaria transmission in stone quarry sites of Villupuram district in Tamil Nadu, India

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Abstract
Movement of labourers from malaria endemic areas into non-malarious places for employment in quarry industries poses threat on transmission of malaria. Such situation was prevailing in Villupuram District of Tamil Nadu, where there were earlier reports of occurrence of malaria. Therefore, the risk of malaria transmission was assessed in the area with quarry industries. Malaria parasite (*Plasmodium* species) infection in human was diagnosed. Sixteen mosquito species were recorded from the larval samples and 13 species were recorded in adult collection. PCR assay showed that, out of 50 mosquito pools, 3 pools of *An. subpictus* were found positive for *P. vivax* infection (MIR: 1.09). Three *P. vivax* positives (asymptomatic) were detected among migrant labourers and one positive among the villagers. Two asymptomatic *P. falciparum* infections were found among the school children. Thus, the area with both vulnerability and receptivity was under risk of establishmant of a focus for indigenous transmission of malaria, necessitating surveillance and containment measures.

Keywords: Malaria transmission; *An. subpictus*; receptivity; vulnerability; vector infection; *Plasmodium falciparum*; *P. vivax*

1. Introduction
Malaria is a major public health problem in many States of India. Tamil Nadu State, situated in the southern most part of the Indian peninsula. The regional disparities in development influence interstate migration of malaria carriers for employment and trade. This internal migration within the country accounts for about 326 million population with 29% rural male migrants. Mainly, people from Uttar Pradesh, Bihar, Rajasthan, Madhya Pradesh, Andhra Pradesh, Chhattisgarh, Jharkhand, Odisha, Uttarakhand and Tamil Nadu are migrating. They are employed in subsectors such as, construction, domestic work, textile, brick-kilns, transportation, mines, stone quarries and agriculture [1, 2]. Due to lack of employment throughout the year in their area and expecting monitory gain, the rural labourers from Odisha, Chhattisgarh, Bihar and Uttar Pradesh prefer to migrate to Tamil Nadu to work in various sectors including mining and stone quarries.

Five stone quarries were functioning at Kunnam, Karasanur and Perrumbakkam villages in Villupuram district of Tamil Nadu. Both migrant and local labourers from the adjacent villages were working in those quarries. Since, the majority of the migrant workers is from the malaria endemic States and they enter into the Tamil Nadu State for work without undergoing any preliminary screening for malaria by the state health authority, there is an ample chance to introduce malaria, mainly through asymptomatic carriers [3] provided the area is receptive with a prevalence of potential/ recognized vectors. The prevalence of asymptomatic carriers is common for *Plasmodium falciparum* and very common for *P. vivax* infections [23]. Thus, chances of getting malaria from migrant labourers of *Pf* and *Pv* endemic areas to the healthy, non-immune local villagers/ workers residing near the quarries is expected to be high in the presence of vector species.

Malaria infection and infectivity, enhanced with suitable ecological and entomological factors, favour local and focal outbreaks of the disease [4-6]. There have been reports of outbreaks among different occupational groups such as construction, stone quarry and agriculture labourers. Critical analysis of malaria cases during a five year epidemiological study in stone quarry sites of Faridabad, Haryana confirmed that the occurrence of malaria was due to labourer migration with an infection rate of 38.7% [7].

Surveys conducted at different mines in Odisha state reported malaria cases and involvement of *Anopheles* in the transmission [8]. Drug resistance *P. falciparum* infections were detected among the pilgrims visiting the Rameswaram Island from southern parts of India [9]. Thus, the risk of malaria transmission in an area largely depends upon its vulnerability and receptivity. The movement of the higher percentage of malaria carriers to an area determines its vulnerability. Presence of potential vector species (having vector characteristics in optimum levels) and favourable climatic conditions make the area receptive. In such situation, movement of the higher percentage of parasite carries might be the source of introduction of infection that could be spread by the receptivity of the area. Thus, both vulnerability and receptivity of the area play an important role in the mechanism of the explosion of the disease [8, 10]. Drug resistance malaria parasites, improper chemotherapy, inadequate infrastructure, rapid movement of people, lack of easy accessibility of treatment facilities and environment changes in non-malarious zones are accredited greatly in malaria control programme worldwide [11]. Indian picture involves a complex system of transmission in rural, semi-urban and urban areas which increases the rate of annual parasite incidence (API) in the cases reported with *Plasmodium* species. Though, decreased trend of malaria cases has been reported in Tamil Nadu, some parts of the State have become vulnerable by attracting people from other malaria endemic States of the country as labourers.

In Kunnam Primary Health Centre (PHC) of the Villupuram district in Tamil Nadu, there are granite quarries adjoining to villages. Labourers from other malaria endemic States migrate to this area seasonally for quarry work. There were different types of breeding sources of the known vector species available perennially. On earlier occasions, this district in general and the villages of Kunnam PHC lying adjacent to the quarry sites, in particular, have recorded malaria cases. Therefore, vulnerability and receptivity of the quarry sites and the adjacent villages were studied to assess the risk of local transmission of malaria in the area.

2. Materials and Methods

2.1 Study area & demography

The study was conducted during March to July 2014 in Kunnam (N 12° 08'& E 079° 67'), Karasanur (N 12°07'& E 079°67') and Perrumbakkam (N 11°78'& E 079° 35') villages of Kunnam Primary Health Centre (PHC) in Vanur administrative block of Villupuram district, Tamil Nadu State. The district consists of 11 blocks, 51 PHCs and one Government Hospital that has been recognized as the Medical College Hospital. The area contains metamorphic and sedimentary rocks such as fire clay, silica sand, black granite, multi coloured granite and blue metal etc. that created interest in establishing quarry industries and recruiting labourers (migrants) from malaria endemic States. Five quarries namely, Satheesh mine and industry, Enterprising enterprises in Kunnam, Katti-maa and Ashwani enterprises in Karasanur and B.G quarry in Perumbakkam were in operation during the study period. The selected three villages, with a total of 1,308 households and a population of 6, 352 were relatively closer with variation just about 2-3 km, forming a triangle (Fig. 1).

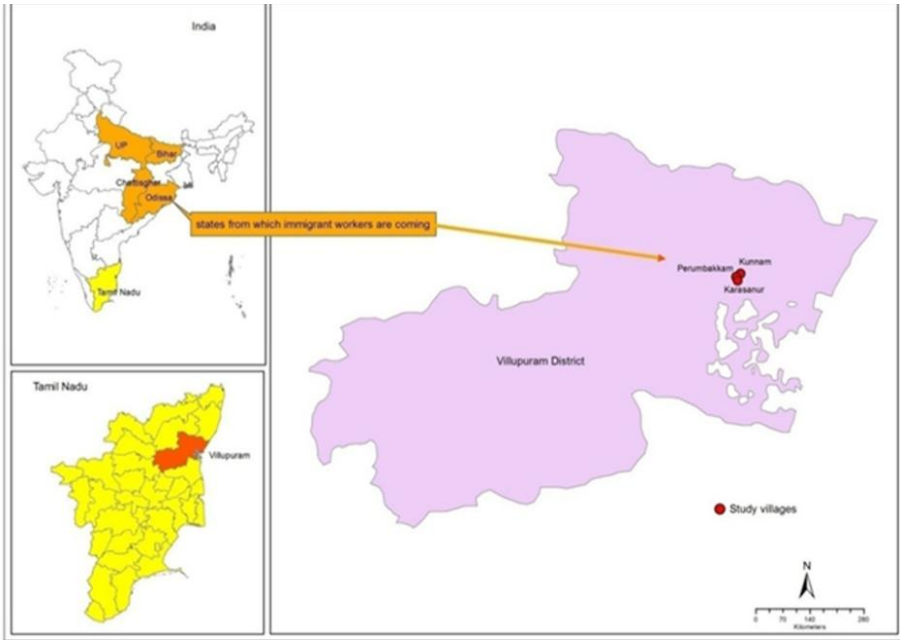

Fig 1: Map showing study area in Villupuram district of Tamil Nadu state

2.2 Climate, vegetation and vectors

The climate in the district is mostly humid and hot. The district received an average minimum rainfall of 715 mm and a maximum of 1115 mm through southwest and northeast monsoons for the last five years from 2008 to 2012. Paddy, groundnut and sugarcane were the major crops in this district. Seasonal paddy cultivation during pre- and post-monsoon supports the breeding of *Anopheles* mosquitoes. Seven *Anopheles* species, including three of the six recognized primary vectors of malaria viz., *An. culicifacies*, *An. stephensi* and *An. fluviatilis*, one secondary vector species *An. subpictus* and three non-vector species viz., *An. barbirostris*, *An. pallidus* and *An. vagus* have been reported in Tamil Nadu [12]. However, no information is available about the prevalence of malaria vector species, their breeding habitats and potential role in transmission in the current study area.

2.3 Ethical clearance
Ethical clearance for conducting blood survey among migrant labourers and villagers in the three study villages was obtained from the VCRC Human Ethics Committee.

2.4 Entomological surveys
All breeding habitats in the three study villages and the quarry sites were enumerated. Assessment of mosquito breeding was made in all the potential breeding habitats and the positive ones with the presence of mosquito immature were selected for sampling at weekly interval. Accordingly, breeding habitats such as used wells, abandoned wells, agriculture wells, ponds, cement tanks and quarry pits were surveyed to obtain information on mosquito species composition, preferential breeding habitat(s) and immature density of the known malaria vectors.
Immature samples along with water from the respective breeding habitat were collected in 300/500 ml plastic containers and brought to the VCRC laboratory, Puducherry and reared. After emergence, the adult mosquitoes were kept habitat-wise and identified to species under a dissection microscope (10X objective) using the standard taxonomic keys and protocols [13].

2.5 Indoor resting, light trap & dusk biting collections
Indoor resting adult mosquitoes were collected from six human dwellings and six cattle sheds randomly selected in each study village using a mouth aspirator and a flash light, spending 10 minutes in each shelter. The number of indoor collections (in human dwelling and cattle sheds) varied from 3 to 8 in different study villages during the study period. Mosquitoes collected were labelled habitat-wise and brought to the laboratory for identification to species and to record species composition and density (number per man hour). The modified version of the CDC lights trap was also used to collect mosquitoes from indoors, setting one trap for three hours during dusk in one randomly selected cattle shed in each study village. In Kunnam village, two light trap collections were carried out. The density was expressed as number per trap-hour. Dusk biting collection included cattle biting and man landing to describe host preference pattern of malaria vectors. Cattle biting collection was done in randomly selected cattle shed in each study village spending one hour on a cattle using a mouth aspirator and a flash light. Similarly, man landing collection was done in a randomly selected human dwelling in each study village using self-bait to collect the landing mosquitoes for one hour. During the study period, two dusk biting collections each on man and cattle were conducted in each study village, except Karasanur where only one collection was done. Biting/landing density was calculated as number per cattle per hour or number per man per hour. Mosquitoes collected were brought to the laboratory in test tubes wrapped with wet clothes for further processing.

2.6 Laboratory processing of mosquitoes
2.6.1 Identification & dissection of female anophelines
Adult mosquitoes obtained from field were kept in a deep freezer (-40°C) for 5- 10 minutes to immobilize them for identification to species and preparation of their blood meal on filter paper for identifying the source of feeding. Male and female *Anopheles* mosquitoes were separated and the females were categorized according to their gonotrophic conditions and dissected out for determination of parity status. Dissection of mosquitoes was performed under a dissection microscope with 10X objective resolutions, on a drop of physiological saline (0.90% w/v of NaCl) and the proportion of porous mosquitoes was recorded. Presence of at least one dilatation in pedicle part of the ovariole of the dissected mosquito confirmed its parous status, mosquitoes without any dilatation were the nulliparous ones.

2.7 Blood meal analysis
The midgut of fully fed *Anopheles* mosquitoes obtained from indoor resting and light trap collections was drawn and placed on a circle marked on Whatman No.1 filter paper and squashed to make a round smear, and labelled. Each filter paper with mosquito blood meal smears was kept separately in a self-sealing cover and stored at +4°C until analysis was done for blood meal source using agar gel diffusion method [14]. The proportion that fed on human was used to calculate human blood index (HBI) for each village. Laboratory processing was restricted only to anophelines as the primary aim of this study was to analyse the risk of malaria transmission in quarry sites and the adjoining villages.
HBI= number fed on human/ total number tested

2.8 Sample blood survey
Written informed consent for finger pricking and conducting rapid diagnostic test (RDT), blood sample collection on slides for microscopy and on filter paper for PCR assay was obtained from participants selected for sample blood survey in the study villages on the basis of proportionate population sampling (PPS). Blood smears on slides and filter paper blood samples were brought to the VCRC laboratory for further processing.

2.9 Detection of malaria parasite
2.9.1 Microscopy
The blood smears were stained with Giemsa satin and examined under microscope with 5x100 magnifications for presence of malaria parasites. The slide was considered positive if at least one asexual/sexual form of parasite was detected during examination. All migrant labourers were screened for malaria infection especially for detecting asymptomatic parasite carriers.

2.9.2 PCR assay: Malaria parasite infection in anopheline mosquitoes and filter paper blood samples
Adult anopheline mosquitoes collected from the study villages were categorised species-wise and pooled. DNA was extracted from each dried mosquito pool containing minimum 2 to maximum 8 mosquitoes per pool (pool size subjected to the availability of vector species). Each pool was homogenized in 1.5 ml microtube for 3 minutes with 20µl of ATL buffer using cordless motor with reusable sterile pellet pestle. After completion of homogenization, the pestle was washed carefully with 160 µl of ATL buffer carefully. The microtube was closed tightly and incubated in a dry bath at 85 °C for 10 minutes. After incubation, the content was centrifuged @ 8000 rpm for 1 minute. The supernatant was collected in a fresh 1.5 ml microtube and DNA was extracted following QIAamp DNA mini kit (Qiagen) protocol.
For extraction of DNA from filter paper blood sample, three blood spots were punched using a single paper punching

machine, cleaned with spirit and heated on spirit lamp for each sample. Extraction of DNA was done as per QIAamp DNA mini kit protocol. Following the method described by the Snounou *et al*., 1993 [15], nested PCR assay was conducted to detect malaria parasite species, *P. falciparum* and *P. vivax*. One each of negative control (PCR mix without DNA) and positive control (PCR mix with DNA known to work in amplification) were included in every PCR assay. *Plasmodium falciparum* and *P. vivax* DNA extracted from human blood samples were used as positive control.

PCR assay was carried out in 0.2 ml microtubes in BIO-RAD (T100) thermal cycler. After electrophoresis of PCR products, the gel was photographed using UVP GelDoc-It Imaging System, UVP, LCC, Upland, CA 91786. The PCR results were checked by comparing with *P. falciparum* (205 bp) and *P. vivax* (120 bp) positive controls and100 bp DNA marker (Sigma, USA) loaded alongside the samples. The parasite infection in *An. subpictus, An. culicifacies, An. annularis, An. varuna* and *An. vagus* was calculated using the CDC software Pooled Inf Rate, Version 4.0 for pools of mosquitoes [16]

2.10 Sociological survey

The number of migrant workers in each quarry site, their movement pattern and structure of their shelters, human and cattle population and structure and types of houses in the study villages were recorded. Pre-tested structured questionnaires were used for the survey. In addition, key informant interviews were conducted to describe the knowledge and practices of migrant labourers regarding malaria, malaria vectors and their control.

3. Results

The numbers of the potential breeding habitats of mosquitoes, including the known malaria vector species (primary/secondary) namely *An. culicifacies, An. varuna, An. annularis* and *An. subpictus* were relatively higher in Karasanur (Table. 1) followed by Kunnam among the three study villages.

Table 1: Habitats surveyed and found positive for mosquito breeding

Habitat	Kunnam		Karasanur		Perumbakkam		Total surveyed	Total +ve
	No. surveyed	No. +ve	No. surveyed	No. +ve	No. surveyed	No. +ve		
Domestic wells	15	2	19	5	3	1	37	8
Agri wells	4	2	8	1	3	1	15	4
Abandoned wells	4	2	9	2	3	2	16	6
Ponds	3	1	3	1	1	0	7	2
Quarry pits	7	0	5	1	3	1	13	2
Cement tanks	9	2	11	2	7	0	27	4
Shallow water body	0		2	0	0	0	2	0
Total	42	9	57	12	20	5	117	26

The immature density (number per dip) of mosquito species recorded in different habitats was also greater in these two villages (3.0 and 2.9) than in Perumbakkam (0.7). The immature density of mosquitoes of different genera varied between habitats. In higher number of freshwater habitats, anopheline breeding was recorded, whereas, more number of contaminated (polluted organic) habitats were found with *Culex* or *Aedes* breeding. Out of the 16 mosquito species recorded from larval samples, seven were anophelines. In Karasanur village, next to *An. varuna*, *An. vagus*, *An. subpictus* and *An. culicifacies* were the predominant species. In Kunnam village, *An. subpictus* was predominant followed by *An. annularis*; *An. culicifacies* was not recorded from immature samples. The known malaria vector species were collected relatively in lower numbers in Perumbakkam village (Table 2).

Table 2: Species composition of mosquitoes from immature collections in the study villages

S. No	Name of the species	No. emerged			Total	Species composition (%)
		Kunnam	Karasanur	Perumbakkam		
1	*An .culicifacies*	0	58	0	58	3.8
2	*An. annularis*	71	38	13	122	8.0
3	*An. varuna*	9	85	2	96	6.3
4	*An. subpictus*	80	74	17	171	11.3
5	*An. jamesii*	0	0	6	6	0.4
6	*An. vagus*	8	85	12	105	6.9
7	*An. barbirostris*	18	0	9	27	1.8
8	*Cx. quinquefasciatus*	24	8	293	325	21.4
9	*Cx. tritaeniorhynchus*	13	6	0	19	1.3
10	*Cx. vishnui*	1	2	0	3	0.2
11	*Lp. minuttismus*	346	25	33	404	26.6
12	*Ae. aegypti*	4	0	52	56	3.7
13	*Ae. albopictus*	0	0	15	15	1.0
14	*Ae. vittatus*	107	0	0	107	7.1
15	*Ad. catastica*	3	1	3	7	0 .5
16	*Ar. subalbatus*	3	0	0	3	0.2
Total		687	382	455	1517	

Overall, *An. subpictus* was the predominant species followed by *An. annularis, An. vagus, An. varuna* and *An. culicifacies.*
The adult mosquitoes, obtained from different types of collections in the three study villages, belonged to 13 species and among them six were anophelines. The *Anopheles* species composition recorded from the larval and the adult samples were the same except that *An. jamsii* was not obtained from adult collections. The proportion of *An. subpictus* collected from Perumbakkam village was the maximum (24.7 %) followed by Kunnam (13.5%). *An. annularis* and *An. culicifacies* were collected in greater proportion in Kunnam than the other two villages (Table 3).

Table 3: Species composition of mosquitoes from adult collections in the study villages

S. No.	Name of the species	Number collected			Total	Species composition (%)
		Kunnam	Karasanur	Perumbakkam		
1	*An .culicifacies*	9	2	0	11	1.7
2	*An. annularis*	23	7	0	30	4.6
3	*An. varuna*	3	0	2	2	0.8
4	*An. subpictus*	88	45	161	306	45.2
5	*An. vagus*	4	5	4	11	2.0
6	*An. barbirostris*	2	2	0	4	0.6
7	*Cx. quinquefasciatus*	15	91	51	157	24.1
8	*Cx. tritaeniorhynchus*	2	16	24	42	6.5
9	*Cx. vishnui*	4	4	4	12	1.8
10	*Cx. gellidus*	1	0	0	1	0.2
11	*Ae. aegypti*	11	57	8	76	11.7
12	*Ae. albopictus*	0	0	1	1	0.2
13	*Ad. catastica*	4	1	0	5	0.8
Total		166	230	255	651	

Dusk biting collection on cattle yielded six anopheline species and among them *An. subpictus* and *An. culicifacies* were collected relatively in higher number. From indoor resting collections also, *An. subpictus* was the predominant species; *An. culicifacies* and *An. annularis* were also collected indoors, but their density was too low. Dusk biting collection on man did not yield any anopheline mosquitoes. This could be due to smaller number of collections (only six collections were done). Light traps collected only five anophelines which was lower than that obtained with the other two collection methods. Overall, adult collections using the three methods yielded six anopheline species including the known malaria vectors, *An. culicifacies, An. varuna, An. annularis and An. subpictus; An. subpictus* was the predominant species (Table 4).

Table 4: Density and parity status of adult anophelines obtained from different types of collections in the three study villages

Species	No. collected	No. of parous	Type of collection	No of collections	Time spent in hrs	Total collected	Adult density
Indoor resting collection in human dwelling							
An. annularis	2	0	Hand catch	14	14	43	3.1 per man-hr
An. vagus	1	0					
An. subpictus	40	9					
Indoor resting collection in cattle shed							
An. culicifacies	3	1	Hand catch + light trap	18	26	176	6.8 per man-hr
An. annularis	9	2					
An. subpictus	151	29					
An. vagus	10	2					
An. barbirostris	3	0					
Dusk biting collection on cattle							
An. culicifacies	8	3	Biting collection	5	5	138	27.6 per cattle per hr
An. varuna	5	0					
An. annularis	19	4					
An. subpictus	103	22					
An. vagus	2	0					
An. barbirostris	1	0					

Most of the field collected anophelines fed on cattle, whereas out of 35 *An. subpictus* tested for the source of their blood meal, four were found positive for human blood with a HBI of 0.11. None of the blood meals of *An. annularis* (*n*=16), *An. culicifacies* (*n*=5), *An. vagus* (*n*=1) and *An. varuna* (*n*=2) was found positive for human blood. The number tested for these species was, however, too small to make a solid conclusion on feeding behaviour. Out of 36 pools of *An. subpictus* tested, three pools were positive for *Plasmodium vivax* infection with a minimum infection rate of (MIR) 1.09. None of the pools of *An. culicifacies* (1 pool), *An. annularis* (5 pools), *An. varuna* (1 pool) and *An. vagus* (3 pools) was found positive for human *Plasmodial* infection.
From the blood surveys conducted in the three study villages, 496 slides with thick and thin blood films, and 310 blood samples on filter papers were collected for detection of

malaria parasites using microscopy and PCR assay respectively. Among them, one villager (Kunnam) working in a quarry was found positive for *P. vivax* by microscopy, RDT and PCR assay. Two *P. falciparum* positive cases were detected in Karasanur by PCR assay. In addition, a total of 58 migrant workers were screened for malaria infection through the blood survey conducted at five stone quarry sites functioning adjacent to the three study villages. While microscopy and RDTs did not detect any malaria positives, PCR assay detected three asymptomatic *P. vivax* positives among the migrant workers (Table 5).

Table 5: Details of sample blood survey conducted in the three study villages

Name of the Village	Population		Blood slides collected		Filter paper blood samples collected		Result			
							Microscopy +ve		PCR +ve	
	Villagers	Migrants	Villagers	Migrants	Villagers	Migrants	Villagers	Migrants	Villagers	Migrants
Kunnam	1727	22	19	22	40	22	1*(*Pv*)	0	1*(*Pv*)	3 (*Pv*)
Karasanur	2582	20	152	20	150	20	0	0	2 (*Pf*)	0
Perumbakkam	2043	16	154	16	120	16	0	0	0	0
Total	6352	58	496	58	310	58	1(*Pv*)	0	3 (*Pv*-1, *Pf*-2)	3 (*Pv*)

Although, agriculture was the major occupation in the study villages, some villagers also involved in stone quarry work along with migrant labourers. More than half of the houses were made up of concrete and bricks (Pucca) but not ventilated well. Man to cattle ratios was very low in Kunnam (1:0.03) & Perumbakkam (1:0.03) than Karasanur (1:0.43). Mosquito coil was stated to be the primary personal protection measure used by the villagers against mosquito bites, the however, migrant workers reported to use cloths to cover their whole body as a method of personal protection (Table 6).

Table 6: Major occupation, type of houses and man to cattle ratio

Name of the village	No. of key Informants	Major occupation			Types of houses			Man to cattle ratio		
		Agri culture	Business	Labour	Thatched	Tiled	RCC*	Human population	Cattle population	Man to cattle ratio
Kunnam	13	11	1	1	2	2	9	1727	52	1: 0.03
Karasanur	20	15	1	3	4	5	11	2582	112	1: 0.43
Perumbakkam	10	10	0	0	4	2	4	2043	72	1: 0.03
	43	36	2	4	10	9	24	6352	236	1: 0.04

The villagers knew little about the breeding habitats of mosquitoes. The proportion of people having knowledge on malaria and experiencing mosquito bites did not differ significantly between the villagers and the migrant labourers (x^2=0.248 & 0.04, p=0.618 & 0.522 respectively). Cent percent of migrant labourers were from malaria endemic states namely Odisha, Chhattisgarh, Bihar and Uttar Pradesh and the frequency of their movement to their native places was more because their duration of stay at quarry sites was reported to be lesser (Table 7 & 8).

Table 7: Knowledge of villagers on mosquito breeding habitats and their practice of using personal protection measures.

Name of the village	No. of key Informants	Major occupation			Types of houses			Man to cattle ratio		
		Agriculture	Business	Labour	Thatched	Tiled	RCC*	Human population	Cattle population	Man to cattle ratio
Kunnam	13	11	1	1	2	2	9	1727	52	1: 0.03
Karasanur	20	15	1	3	4	5	11	2582	112	1: 0.43
Perumbakkam	10	10	0	0	4	2	4	2043	72	1: 0.03
	43	36	2	4	10	9	24	6352	236	1: 0.04

Table 8: Duration and staying details of immigrant works at quarry sites

Name of the village	Native State of migrants	Districts	No. of migrants	Duration of stay at quarry sites				
				<1 month	1-3 months	4-6 months	6-9 months	>9 months
Kunnam	Odisha	Ganjam & Berhampur	23	1	10	3	6	3
Karasanur	Chhattisgarh	Jashpur	12	6	10	4	1	1
	Bihar	Bettiah	8					
	Uttar Pradesh	Gorakhpur	2					
Perumbakkam	Odisha	Ganjam	13	1	8	4	0	0
Total			58	8	28	11	7	4

4. Discussion

The study was conducted in villages (of Villupuram district of Tamil Nadu), adjoining to which there were stone (granite) quarries that attract migrant labourers every year from other states including malaria endemic ones. There were earlier reports of occurrence of malaria cases in the study villages. In and around the villages, there were potential habitats supporting breeding of known primary and secondary vectors of malaria viz., *An. culicifacies, An. varuna, An. annularis* and *An. subpictus*. Breeding of anophelines was also found in the quarry pits. Collection of adult mosquitoes in the villages also showed the presence of the known malaria vectors.

Blood meal analysis indicated human feeding behaviour of anophelines in this area as out of 35 blood meals of *An. subpictus* tested, four were found positive for human blood. All these entomological findings suggested that the villages were receptive with potential vectors. The receptivity of the area was further confirmed with detection of human malaria parasite infection in the vector mosquitoes. Out of 50 pools of *An. subpictus* tested in PCR assay, three were positive for *P. vivax* infection with a minimum infection rate of 1.09.There could be a seasonal increase in the receptivity of the area as the breeding surface area of the habitats such as irrigation canals, borrow pits and rice fields was found increased during post-rainy season with enhanced breeding of *An. culicifacies*, the recognized primary vector of malaria in rural areas. As a result, the adult density of *An. culicifacies* would increase and reach the threshold necessary for transmission of malaria [17].

The distance from the potential breeding sources of anophelines to the villages and also to the quarry sites (migrant settlements) was within the average anopheline flight range of 500 m^R. This close association between breeding habitats and blood feeding human sources favours malaria transmission. Further, the close proximity of human dwellings to cattle sheds and paucity of or the very low cattle population (with a man cattle ratio of 1:0.04) in the study villages might induce the vector species to switch from zoophagy to anthropophagy thereby enhancing man vector contact and transmission potential [18].

Risk of malaria transmission is a complex amalgamation of four basic components, namely host (human), causative agent (*Plasmodium* species), environment (tropical) and vector (primary or secondary). Vector involvement in transmission of malaria depends on its innate characteristic and bionomics and source of malaria parasite [19]. In this context, being receptive it was important to assess the vulnerability of the area. The stone quarries attract migrant labourers who were found staying nearer to the quarry sites. The blood survey conducted among the villagers and the migrant workers showed malaria positives. Among the 496 villagers screened, two were found positive for *P. falciparum* infection (11 year old) and one with *P. vivax* infection, with an overall parasite rate of 0.6%, and out of 58 migrant workers tested, three were found infected with *P. vivax* in PCR assay. It was obvious from the results that the area was also vulnerable; receiving migrant workers with malaria infection and facilitating local transmission.

Even though the migrant workers in the current study had *P. vivax* infection, which is considered to be benign, recent studies in India reported *P. vivax* cases with severe symptoms resembled to *P. falciparum* infection [20, 21]. The three *P. vivax* positive migrant workers did not show any symptom of malaria (asymptomatic) at the time of screening. Therefore, even if the surveillance system is in operation, they will be missed from being detected. Further, when mosquitoes were allowed to feed on *P. vivax* infected blood drawn from asymptomatic persons, the mosquito infection rate for *P. vivax* was 1.2% after 7 days, although the parasitaemia was below the threshold of microscopic detection [22]. Thus, the migrant positive persons could be a highly potential source of disseminating the infection to local people through the vector population.

Regarding diagnosis, of the total six malaria positives, only one was detected by all the three methods employed viz., microscopy, rapid diagnostic test (RDT) and PCR and the remaining were detected only by PCR assay. Moreover, compared to microscopy, ELISA and RDT, PCR assay detected higher proportion of malaria positives with low parasitaemia [22]. Therefore, any surveillance system using diagnostic methods other than PCR would lead to survival of parasites in the human host.

5. Conclusion

The current study was conducted in villages, which were found to be receptive for malaria transmission. The adjacent quarry sites attracted migrant workers from other states, including malaria endemic ones. The migrant workers used to stay near the quarry sites which were closer to the villages. The migrants were detected to be positive for malaria parasite infection, and as a result the area became vulnerable too. Further, the malaria positive persons were asymptomatic at the time of screening. Since, the villages were both receptive and vulnerable there exist risk of local transmission of malaria which would result in creation of new foci. In view of these facts, proper tracking of immigrant workers, screening them for malaria infection at the time of entry, and treating them with appropriate antimalarial drugs have become essential for the public health department of the state government in order to prevent the establishment of new foci of malaria transmission. Considering the sensitivity of the diagnostic methods and the presence of asymptomatic parasite carriers, PCR assay should be the choice for diagnosis in this type of special situation.

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