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SEROEPIDEMIOLOGICAL STUDY OF PREVALENCE OF MALARIA IN VILLAGE SOLANA, UTTAR PRADESH, INDIA

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The roles of causative factors responsible for prevalence of malaria in the village of Solana, India, were studied. Mosquitoes and larvae density in and around the area were measured by process of random sampling and counting their numbers under microscopy. Malaria in population of the village was diagnosed by standard ELISA method and malaria antibody capturing level were measured against three *Plasmodium falciparum* antigens. The effect of insecticides for the control of malaria was also evaluated. Results of study showed that more than two third of village human populations (75%) were suffering from malaria, with 67.14% being children below 14 years of age. Similarly vectors identification study showed *Anopheles culicifacies and Anopheles stephensi* as the main source for infection transmission. Sporozoite positive rate estimated in *Anopheles culicifacies* was found to be 1.26%. Both vectors were resistant to DDT and Malathion insecticides. Antibody capturing by three different *Plasmodium falciparum* antigens study showed that glycophospholipid antigen (GPL) was able to capture and detect highest amount of malarial antibody followed by sonicated *Plasmodium falciparum (Pf)* antigen and ring infected erythrocyte surface antigen (RESA) i.e. 0.69±0.25, 0.60±0.22 and 0.59±0.23 respectively. Age specific antibody levels was found to gradually increase from lowest to highest age groups i.e. 0.29-1.18 for GPL, 0.26-0.94 for RESA and 0.25-0.97 for Pf. The study showed that infants and children are highly prone to malaria attacks than the adult population, which may be as a result of low level of Plasmodium antibody in their circulation.

Key words: Solana village, endemicity, malaria incidence, antibodies, ELISA, insecticides.

INTRODUCTION

Malaria endemicity is one of the major public health problems of India. Recent World Health Organization (WHO) report suggests that about 7 million human populations in India suffer from malaria annually (1). Incidence of *Plasmodium falciparum* is gradually rising according to National Malaria Eradication Program (NMEP) (2), and human death rate are increasing i.e. from 500-600 to more than 1000 in recent years. Epidemiological study of malaria also showed that *P. falciparum* infection is more

common in Uttar Pradesh, Bihar, Orissa and Northeastern states than other regions of India (3). In addition to this, malaria is a major cause of mortality and morbidity in developing countries with immense economic consequences on the afflicted population. These include considerable direct (medical consultations, hospitalizations, laboratory tests and medications) and indirect (work day lost) costs for households that are already socio-economically disadvantaged (4,5,6,7).

Several studies have shown malaria infection to be dependent upon life style and literacy in these areas. It has also been observed that human malaria parasites are becoming resistant to common anti-malaria drugs (8,9). For the first time in 1973, resistance to chloroquine in *P. falciparum* was detected in Assam with several hundred deaths occurring due to resistant malaria. Similarly *P. vivax* resistant cases were found in Bombay and South Bihar India (10). Recently, Chloroquine resistant *P. vivax* has been observed in different parts of the tropical region of the world (11,12,13). In spite of the increasing numbers of resistant strains, chloroquine is still recommended by the National Malaria Eradication Program of the Government of India as standard therapy for all types of malaria. Chemoprophylaxis with chloroquine, mosquito coils, insecticide sprays and bed nets are generally used for prevention. It has also been observed that mosquitoes are developing resistance to various insecticides, which is causing difficulties in the eradication of malaria (14). Poor socio economic conditions in certain areas are also causing immense set back in malaria eradication programs.

Transmission of malaria is predominantly common in rural India but also there is a growing concern about the urbanization of malaria (3). Unplanned growth of the cities without public services and hygiene has been found to be favourable conditions for anopheles breeding and appropriate environment for disease transmission (15). Risk factors associated with the transmission of urban malaria may be different from those identified in rural areas. However, most studies that have been carried out in rural areas throw very little light on the

failure of malaria eradication program and provide little information of the causative factors and no reasons for the continued spread of infection. It is important to understand the risk factors and reasons responsible for uncontrolled transmission of malaria infection in the rural areas so that remedial measures for prevention of disease can be suggested. Therefore, this epidemiological and experimental study was done in Solana village of Uttar Pradesh (UP), a village of India where eradication program for malaria started in 1941, in order to understand the causative factors responsible for the continued malaria transmission and to suggest methods for prevention of malaria in these areas.

MATERIALS AND METHODS

Study area: The study was conducted in Solana village of District Meerut, Uttar Pradesh situated near the river Ganga (Fig. 1). A canal has been built from river Ganga for irrigation of fields and the village is situated 500 meter from the west side of the riverbank. This canal irrigates the village fields and supplies water for drinking. Also, four water pond surround the village area and the village surroundings are fully covered with trees, water plants, grasses and household garbage. One pond has been cleaned and used for fish culturing. The village is not connected to the city road system and mode of transportation is bullock cart, horse driven cart, bicycles and tractors. There is no hospital or public health center in the village. There are about 500 families living in the village with a population of 2000 to 3000. Ninety percent of the villagers are farmers and about 10% are petty shopkeepers, labourers and civil servants. Several jaggery-manufacturing units are located in the west side of the village. The main cultivable crops of the area are, sugar cane, paddy, mustard, animal feeding plants and beans. Three temples and one mosque are located in the village. Two primary schools and one middle school are present in the village and one high school is located 5 kilometer from the village. Most of the village children attend primary and middle education in the village only. Almost all families maintain one or more domestic animals with most families keeping their animals inside the house

95% of the population did not use mosquito net. Previous general clinical survey had shown most villagers to be suffering from fever, headache, cough, eye infection and polio.

Mosquito collection: Mosquitoes in and around cow sheds and houses were collected with the help of cattle bate CB and human bate HB for 20 minutes by WHO sucking tube between 4 and 7am. Identification of the mosquito types (genera and species) was done according to WHO guidelines (16, 17, 18, 19, 20, 21).

Mosquito larva collection: Mosquito larvae were collected from 10 locations in each pond and creek.

Blood fed Mosquitoes collection: Blood fed mosquitoes resting on the wall under the bed and corner of the house and also outdoors were collected. All mosquitoes' blood were collected on a 3 mm Whatman filter paper and dried in room temperature and stored at -20^0C till use.

Human blood collection: Finger prick, thick and thin blood smear on glass slides, and blood soaked filter paper samples were collected from subjects with symptoms of malaria. Thick smear was used for the detection of malaria parasites, thin smear for identification of malaria parasite species, and filter paper sample for ELISA immunoassay. Thick and thin blood films were stained with 10% Giemsa stain for 10 minutes and slides were washed with buffer solution and dried in room temperature. Blood films were examined under high power oil immersion lens of Olympus microscope. Parasites were counted against 300 white blood cells. ELISA immunoassay was performed on the filter paper blood using GPL (glycolipid) antigen, Pf sonicated antigen and RESA synthetic peptide antigen. A 1:100 dilution of serum was made with phosphate buffered saline (PBS) and standard ELISA procedure performed as described by Roy *et al* (23). Final agglutination results were read using LP 300 Pasteur ELISA reader.

Spleen examination: All febrile subjects were clinically examined by qualified physicians for splenic enlargement according to Hackett's Index (22).

Mosquito blood meal examination: Blood meals were collected from recovered mosquitoes for de-

tection of animal and human blood. ELISA dots method was used to determine human and animal inoculation rate according to Roy and Sharma (24).

Mosquito sporozoite detection rate: Mosquitoes were dissected in 5% normal saline for sporozoite detection in salivary gland under dissecting microscope after species identification.

Insecticide susceptibility test: The WHO method (25) was used. Test kits provided by WHO/TDR were used and batches of anopheline mosquitoes were exposed to standard insecticide impregnated papers with 4% DDT, 0.1% Icon, 0.025% Deltamethrin and 5% Malathion. The standard exposure time of one hour was used.

Statistical analysis of data: Data obtained were statistically analyzed using student 't' test and p values were calculated for level of significance with cut off value as control mean + 2 SD.

RESULTS

The results of epidemiological study of malaria in Solana village are presented in Table 1. It was found that 44% of the village population were suffering from different types of malaria infection and 79% of those infected were found to be positive for *P. falciparum* malaria, 18.7% for *P. vivax* and 2.3% were from mixed infections. Children of age group below 14 years showed high prevalence of malaria i.e. infant parasite rate was found to be more than 65%. Gamatocyte prevalence rate in the blood smear of the subjects were found to be 2.06%. Total parasite density index (PDI) was also high i.e. 5.3%. Enlargement of spleen in children below age 2-9 years was 51.4%.

Vector/larvae identification survey: Four Anopheles species and one Culex species were found i.e. *An. culicifacies, An. stephensi, An. annularis, An. sapitus* and *Culex quenquefasiastus. An. culicifacies* and *An. sapitus* were found in maximum numbers during 4 to 7 am indoor resting collections (Table 2). Three *An. culicifacies* mosquitoes were found positive to sporozoite detection study. All the four types of Anopheles and the Culex mosquito larvae were found in three of the four village ponds, pools and creeks (Table 3). The fourth pond, which has been

cleansed and used for culturing fishes, did not contain mosquito larvae.

Insecticide susceptibility test: *An. annularis* and *An. sapitus* were found to be 100% sensitive to 4% DDT, 0.1% Icon, 0.025% Deltamethrin and 5% Malathion but *An. culicifacies* and *An. stephensi* showed 30-40% resistance to 4% DDT and 40-50% resistance to 5% Malathion.

Mosquitoes inoculation rate (Man biting rate): 86 blood fed mosquitoes were collected indoors and outdoors. Animal/human blood identification test found 24 mosquitoes containing human blood meal in their body. Human inoculation rate was 27.91%.

Immunological sensitivity study: A total of 389 blood samples were collected out of which 205 were from fever cases and the remaining from patients having no history of malaria/fever. ELISA results of the three antigens showed very high OD value in subjects above 15 years, moderate among subjects 10-14 years age group and a very low value in 0-11 months age group. The ELISA values of antigens titer are presented in Table 4.

Table 1: Microscopic examination of age specific parasite, gametocyte and spleen positive

Clinical findings of subjects					Microscopic examination for malaria				
Age group	No	Spleen +ve	Parasite +ve	Age specific +ve	Parasite Species			% +ve	Pf G +ve
					Pf	Pv	Mixed (Pf+Pv)		
0-11 mon	6	-	4	66.67	3	1		2.33	
12-23 mon	4	1	2	50.00	1	1		1.16	
2-4 yrs	26	19	10	38.46	8	2		5.81	1
5-9yrs	114	60	59	51.75	42	15	2	34.30	3
10-14yrs	84	42	40	47.62	34	5	1	23.25	1
15 and above	155	38	57	36.77	48	8	1	33.14	3
	389	160 41.13%	172 44.21%	44.21	136 79.06%	32 18.60%	4 2.33%	100%	8 2.06
2-9 yrs	140	79 56.43%	69 49.29%	49.29%	50 72.46%	17 24.64%	2 2.9%	100%	4 2.86%

Table 2: Details of mosquitoes/larvae and sporozite positive rate.

Details of mosquito/larvae			
Mosquito/Larves	No. of larvae	No. of mosquitoes	Sorozoite +ve rete (%)
An culicifacies	184	238	3(1.26)
An stephensi	97	169	0
An sapitus	283	305	0
An annularis	166	147	-
Culex quenquefasciatus	209	156	
	939	1015	3(0.3492)

Table 3: Mosquitoes larval collection and identification of breeding

S. No	Name of areas	Types of mosquitoes species emerge from Larvae
1	Pond (1)	*An annularis, An sapitus and Culex quenquefasciatus*
2	Pond (2)	*An stephensi, An annularis, An sapitus and Culex quenquefasciatus*
3	Pond (3)	*An annularis, An sapitus and Culex quenquefasciatus*
4	Pond (4) (Fish culture pond)	Larvae absent
5	Creek	*An culicifacies, An stephensi, An annularis.*

Table 4: Age specific ELISA OD value of fever cases vs *P. falciparum* antigens.

Particular of subjects (Age Group)	Fever cases	Positive cases	ELISA OD value of antigen		
			GPL	RESA	Pf
0-11 months	4	3	0.29	0.26	0.25
12-23 months	2	2	0.37	0.38	0.35
2-4 yrs	15	10	0.59	0.50	0.48
5- 9yrs	71	58	0.78	0.65	0.62
10-14 yrs	50	40	0.92	0.87	0.86
15 and above	63	59	1.18	0.94	0.97
Total	205	172	0.69±0.25	0.6±0.22	0.5[illegible]±023

DISCUSSION

Application of DDT was started in Solana area of District Meerut by malaria control team in 1941. The establishment of NMCP in 1953 and NMEP in 1958 showed drastic decline in malaria and density of mosquitoes in these areas. Also, *P. vivax* and *P. falciparum* endemicity in these regions were also reduced considerably due to extensive DDT spray. In 1960s, NMEP epidemiological survey reported for the first time that *Anopheles culicifacies* was resistant to DDT. Analysis of malaria situation revealed that malaria was never eradicated but had only declined to low levels and returned with increased vigor (2). Furthermore, reversions from consolidation and maintenance phases coincided with the DDT shortages. In the next decade (1970s), these problems further multiplied and as a result malaria resurgence was widespread. But in the 1980s and 90s, malaria problem became serious and several large scale out breaks of malaria were reported. Exophilic vector behaviour became more pronounced and indoor residual spraying (IRS) had poor impact on vectors. NMEP also identified hard-core areas of about 51 million populations where indoor residual spraying (IRS) had failed (3).

In our study, only two malarial parasite species i.e. *P. falciparum* and *P. vivax* were present in village Solana and *P. falciparum* was the predominant parasite specie in the area. Several epidemiological studies also showed the presence of *P. falciparum*, *P. vivax* and *P. malariae* in Terai region of UP (26,27). Mosquito larvae of *An. culicifacies*, *An. stephensi*, *An. annularis*, *An. sapitus* and *Culex quenquefastiatus* larvae were found in three main ponds and in all creeks but no larvae of mosquitoes were seen in cleaned fishpond.

Infant parasite rate was found to be very high i.e. 66.67% in the area and common in both male and female population of village. Dev and Sharma (28) found in three years study, an infant parasite rate between 25.81 – 41.59% and the highest in 1991 at Sonapur, Assam. A similar result was shown by different authors in endemic area of Myanmar with 75% in Oktwin village of Myanmar (29). The gametocyte positive rate

in this study was found to be 2.06%, which is much higher than 0.85% in the Myanmar study (30).

Four types of mosquitoes were collected from cowsheds and houses of village Solana. In the mosquito collection study maximum number of *An. culicifacies* and *An. stephensi* were recovered. *An. culicifacies* is main vector for transmission of malaria in rural and semi rural areas of India and accounts for about 60% malaria cases annually (31). The sporozoite positive rate was found to be 1.26% in *An. culicifacies* by salivary gland dissection study. This is lower than studies of other workers, which showed salivary gland sporozoite positive rate of 4.04% (4/99) in *An. dirus* in Oktwin village, Myanmar (30).

Serological study of village population for presence of malaria and antibody titer showed a very high ELISA OD values with GPL and Pf antigens but RESA-R1 antigen showed low OD titer. RESA-R1 antigen has been used for the measurement of endemicity level of malaria and anti-RESA-R1 antibody levels correlate well with level of endemicity (32, 33). This study showed village Solana as a mesoendemic area according to RESA-R1 ELISA OD results, but spleen diagnostic test showed more than 50% enlargement of spleen in children between 2-9 years of age pointing toward high endemicity (22). There is indication from the study that children above the age of 4 do not have repeated infection due to high level of antibody present in their body contributing to protection against *P. falciparum* malaria. Also, recent GPL antigen studies of Remasamy and Reece (34) and Schofield *et al* (35) showed that GPL and GPI antibodies help in protection against *P. falciparum* malaria.

CONCLUSIONS

This study has identified several factors responsible for the high endemicity of malaria in the village Solana UP, India. The village and surrounding area is near a major riverine swampy area with large amount of shrubs and bushes, which form a favourable breeding site for mosquitoes. Also, there is no proper sanitation system and no proper disposal of household garbage, animal and human excreta. The primary health care (PHC) cen-

ter of the village is ill equipped with no qualified doctor or trained medical technician. Chloroquine is given as a treatment for most of the fever cases at PHC without any laboratory investigation, so that drug resistant strain of parasite might have developed in the village population. In addition vector mosquitoes have been found to be resistant to various insecticides further complicating the situation. It is suggested that proper cleaning of surrounding area, good sewage disposal and hygiene, which are main principles for healthy and good living should be strictly followed. Cattle shed should be located outside human residence. Regular use of Deltamethrin impregnated bed nets and /wall residual spay (with Icon) may reduce the incidence of parasite transmission. Lastly, malaria biomedical engineering procedures of NMEP and WHO should be implemented for better management and prevention of malaria.

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