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EPIDEMIOLOGIC INVESTIGATIONS OF A MALARIA OUTBREAK IN NORTHERN DELHI AREA

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ABSTRACT. Epidemiologic investigations revealed a 56.7 and 13.32% slide positivity rate in febrile and afebrile malaria cases, respectively. In both cases, *Plasmodium falciparum* was predominant. *Anopheles culicifacies* resistant to dichlorodiphenyltrichloroethane and benzene hexachloride (hexachlorocyclohexane) was found breeding profusely in pools and ponds created by excavation of earth around brick kiln in the region. Furthermore, children were not found to be producing significant levels of antibodies and a large percentage of patients harbored chloroquine-resistant parasites. Also, more than 1 *P. falciparum* strain was present in the population. We detected 2 strains, VI and VII, of which type VI was predominant.

KEY WORDS Malaria outbreak, *Anopheles culicifacies, Anopheles stephensi,* susceptibility to insecticides, serology, molecular typing of *Plasmodium falciparum* strains, chloroquine-resistant strains

INTRODUCTION

Malaria outbreaks are recorded periodically in metropolitan towns, either at construction sites where laborers from endemic areas are congregated, or in slum settlements without adequate health infrastructure. After release of a news item regarding deaths due to malaria in 1996, a comprehensive survey was carried out in Shahbad Daulatpur Dairy Colony, Rohini Zone, Circle 79, Municipal Corporation of Delhi (MCD), Delhi, National Capital Region, India. The investigations were directed to assess the magnitude of the problem by monitoring densities of immatures and adults of suspected vector species and their role in transmission of the disease, spleen and parasite rates in febrile and afebrile cases, susceptibility of parasites to drugs, seropositivity rate, and type of strains involved during the epidemic.

Study site

The area is a cluster of about 4,000 unauthorized huts with a population of about 15,000 distributed in different blocks on Bawana Road opposite Shiv Vihar. The slum dwellers, mainly laborers, artisans, craftsmen, and so on, have migrated from endemic states. The ratio of humans to cattle was 1:0.1. A low-lying area remained submerged with rainwater and was not accessible. Because of this, the investigation was conducted only in block A, which was accessible. This area consists of a population of 2,314. The predominant population was floating laborers living in temporary structures. No drug distribution center, fever treatment depot, and malaria clinic were present in the unauthorized human settlement, but consequent to the news item on the outbreak of the disease, malathion fogging and antilarval measures were carried out intensively. Temporary fever treatment centers were established by MCD and all positive cases were treated as per the National Anti Malaria Program (NAMP) drug schedule.

MATERIALS AND METHODS

Blood smears from febrile and afebrile cases were collected on glass slides and presumptive treatment (600 mg of chloroquine) was given at the time of blood smear collection. Slides were examined and positive cases were treated as per the NAMP drug schedule. Slides from persons having contact with the deceased also were made to determine the cause of death.

A spleen survey (in children aged 2–9 years) was also carried out and the average enlarged spleen value was calculated to classify the endemicity. An entomologic survey included mapping of breeding places, breeding association, adult density, vector incrimination, and susceptibility of vector species to insecticides according to the procedure described by Ansari et al. (1984). Epidemiologic data were analyzed according to procedures described earlier (Ansari et al. 1986).

Enzyme-linked immunosorbent assay: Enzyme-linked immunosorbent assay (ELISA) was performed with a synthetic nonapeptide (EENVEHDA-C) of ring-infected erythrocyte surface antigen (AR-1) and the total parasite antigens from the schizont stage of *Plasmodium falciparum* as described earlier by Roy et al. (1994). In brief, each well of a 96-well microtiter plate was coated with 50 ng of AR-1, derived from RESA/*Pf* 155 antigen and procured from Cambridge Research Biochemicals, London, United Kingdom; or 2 μg of *P. falciparum* (*Pf*) parasite sonicated antigen. The 1st antibody, filter paper elute, was used at a dilution of 1:40 and 1:1,000 for AR-I and *Pf* antigen, respectively. The 2nd antibody, rabbit anti-human immunoglobulin G (IgG) conjugated to horse radish

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peroxidase, was used at a 1:1,000 dilution and color was developed with the substrate *O*-phenylenediamine and H_2O_2. The optical density (OD) was measured at 490 μm in a Pasteur ELISA reader.

Drug sensitivity assay: The micro-in-vitro sensitivity assay of *P. falciparum* for the chloroquine test was carried out on the produced plates as per World Health Organization standard procedures (WHO 1990). The RPMI-1640 culture medium containing HEPES (30 mm) and 5% sodium bicarbonate (pH 7.2–7.4) was used. The plates were incubated for 24–28 h depending on parasite growth. Isolates showing less than 10% schizont maturation in controls were not included in the assay. Thick smears were prepared from cultures and stained with Giemsa. The number of schizonts was counted and the schizont maturation inhibition rate was calculated according to the procedure of Ansari et al. (1984).

Polymerase chain reaction: The parasite DNA was isolated from infected blood with the method of Foley et al. (1992). The polymerase chain reaction (PCR) amplification was carried out for 2 marker genes called Knob-associated histidine-rich protein (KAHRP) and thrombospondin-related anonymous protein (TRAP) on this parasite DNA by using previously described primers and conditions (Contemn et al. 1995, Sharma et al. 1995, Bhutani et al. 1998). The PCR products were analyzed on 1.2% agarose gel.

Restriction digestion: The PCR product for the TRAP gene was digested with Taq I restriction enzyme. A 20-μl aliquot of PCR product was incubated with 5 units of enzyme in 50 μl of total volume. The reaction mixture was incubated at 65°C for 1 h and the product was separated on a 1.5% agarose gel.

RESULTS AND DISCUSSION

Table 1 depicts the numbers of febrile and afebrile cases during the period of the investigation. A total of 760 blood slides was collected from the febrile population. Of these, 89 were positive for *P. vivax,* 335 were positive for *P. falciparum,* and 7 were positive for both *P. vivax* and *P. falciparum* infections. The average slide positivity rate (SPR) and slide falciparum rate were 56.7 and 45.0, respectively. All age groups and sexes were affected with the disease; however, the SPR was highest (60%) in 1- to 14-year-old children. The infection in children >1 year old indicated recent action transmission, which was timely detected.

Results of afebrile cases revealed that malaria cases were prevalent in all age groups and both sexes. Percent positive cases were 10.6 and 16.5 with *P. vivax* and *P. falciparum* infections, respectively. Results of spleen survey (in children 2–9 years old) revealed that males and females were equally affected. Of 62 children examined, only 17

Table 1. Prevalence of malaria in Shahbad Daulatpur Colony, Municipal Corporation of Delhi, Delhi, India (September–December 1996).[1]

Age group (years)	Febrile									Afebrile[2]								
										Male			Female			Total		
	Male	Female	TBS	*Pv*	*Pf*	Mixed	Total positive	SPR	SfR	Male	Female	Total	*Pv*	*Pf*	*Pv*	*Pv*	*Pf*	*Pf*
0–1	12	10	22	2	2	1	5	22.7	13.6	3	2	5	0	0	0	0	1 (5.8)	1 (2.3)
2–4	35	31	66	18	26	1	45	68.1	40.9	33	29	62	1 (6.6)	2 (8.0)	2 (12.5)	3	1 (5.8)	3 (7.1)
5–8	56	72	128	21	52	1	74	57.8	41.4	53	39	92	3 (20.0)	4 (16.0)	1 (6.2)	4	3 (17.6)	7 (16.6)
9–14	69	35	104	15	52	1	68	65.3	50.9	66	34	100	6 (40.0)	5 (20.0)	7 (48.7)	13	2 (11.7)	7 (16.6)
15–25	117	61	178	16	73	2	91	51.1	42.1	58	58	116	2 (13.3)	7 (28.0)	1 (6.2)	3	2 (11.7)	9 (21.4)
26–50	133	107	240	16	125	1	142	59.1	52.5	72	90	162	3 (20.0)	6 (24.0)	5 (31.2)	8	8 (47.0)	14 (33.3)
>50	15	7	22	1	5	0	6	27.2	22.7	7	4	11	0	1 (4.0)	0	0	0	1 (2.3)
Total	437	323	760	89	335	7	431	56.7	45.0	292	254	548	15 (5.1)	25 (8.5)	16 (5.4)	31 (10.6)	17 (6.6)	42 (16.5)

[1] TBS, total blood slide; *Pv*, *Plasmodium vivax*; *Pf*, *Plasmodium falciparum*; SPR, slide positivity rate; SfR, slide falciparum rate.
[2] Percentage of afebrile cases is given in parentheses.

Table 2. Density of immature anophelines and vector species in block A, Shahbad Daulatpur Colony, Municipal Corporation of Delhi, Delhi, India.[1]

Month (1996)	Species	Average density/dip		Man-hour density	
		Pools	Ponds	*An. culicifacies*	*An. stephensi*
September	Anopheline	21.6 (80.0)	22.5 (100.0)	38.0	17.0
October	Anopheline	6.36 (65.0)	4.8 (100.0)	24.0	14.0
November	Anopheline	5.6 (61.0)	4.0 (100.0)	12.0	13.0
December	Anopheline	2.5 (41.6)	1.2 (100.0)	5.0	11.0

[1] Figures in parentheses indicate percent proportion. *An., Anopheles.*

(27.4%) were found with an enlarged spleen thereby, indicating low exposure to transmission.

Four deaths (3 females and 1 male) were recorded from September 8 to 18, 1996. Of these, 3 deaths occurred in children 1–12 years old and a 4th death was of a 35-year-old person. Eighteen contact slides were collected from family members of the deceased. Of these, 1 was positive for *P. vivax* and 7 were positive for *P. falciparum.* Because the *P. falciparum* proportion was 87.5%, the most likely cause of deaths could have been falciparum malaria.

Table 2 depicts the density of immature mosquitoes during the study period. Pools and ponds were the main sources of breeding *Anopheles culicifacies* Giles and *Anopheles stephensi* Liston. The percent positive rate varied from 80 to 41.6% in pools. The percent positive rate in ponds was 100% during the study period. The average density per dip in pools varied from 21.6 to 2.5 as compared to 22.5 to 1.2 per dip in ponds. This was also reflected when adult densities of *An. culicifacies* and *An. stephensi* were compared.

The highest density of *An. culicifacies* (38.0) and *An. stephensi* (17.0) was recorded in September, followed by gradual decline in subsequent months. Efforts also were made to incriminate the vector species. Dissection of 98 *An. culicifacies* and 115 *An. stephensi* did not reveal oocysts and sporozoites in guts and salivary glands. This could be due to knockdown of most of the parous females under the intensified malathion fogging operation carried out by MCD after detection of the epidemic. However, *An. culicifacies* is the established vector of the area. *Anopheles stephensi* was identified as var. *mysoriensis* and is known to be a poor vector in the rural area.

To determine the susceptibility of vector species, 15 females in each replicate were exposed to a discriminatory dose (4% dichlorodiphenyltrichloroethane [DDT]) for 1 h. Ten replicates were made with each species. Results of susceptibility tests revealed that both *An. culicifacies* and *An. stephensi* were resistant to DDT. The corrected mortality was 60 and 65 for *An. culicifacies* and *An. stephensi,* respectively.

To determine the malaria exposure in the community, we performed ELISA on filter paper blood samples for anti-AR-1 and anti-*Pf* IgG antibody levels. Results are shown in Table 3. We found that the majority of this population had a low seropositivity rate and low IgG antibody levels. The average OD value for anti-AR-1 IgG was 0.26 and for anti-*Pf* IgG was 0.25. These values are below the cut-off points, which were 0.35 and 0.45 for AR-1 and *Pf* antigens respectively (Roy et al. 1995). In the ELISA test, pooled seropositive samples from Shankargarh (Roy et al. 1995), a high endemic

Table 3. Correlation of AR-1 and *Plasmodium falciparum* (*Pf*) enzyme-linked immunosorbent assay (ELISA) optical density (OD) in Shahbad Daulatpur Colony, Municipal Corporation of Delhi, Delhi, India.

Age group (years)	No. examined			ELISA OD			
				Male		Female	
	Male	Female	Total	AR-1	*Pf*	AR-1	*Pf*
0–1	1	—	1	0.25	0.19	—	—
2–4	9	9	18	0.27 ± 0.05	0.26 ± 0.05	0.25 ± 0.07	0.21 ± 0.08
5–8	19	15	34	0.25 ± 0.07	0.26 ± 0.07	0.26 ± 0.08	0.26 ± 0.05
9–14	33	20	53	0.26 ± 0.08	0.25 ± 0.07	0.23 ± 0.06	0.28 ± 0.08
15–25	25	24	49	0.28 ± 0.06	0.27 ± 0.10	0.28 ± 0.05	0.29 ± 0.09
26–50	31	46	77	0.28 ± 0.08	0.28 ± 0.07	0.28 ± 0.09	0.25 ± 0.07
>50	2	3	5	0.28 ± 0.05	0.24 ± 0.03	0.25 ± 0.05	0.18 ± 0.04
Total	120	117	237	0.26	0.25	0.28	0.24

Table 4. Polymerase chain reaction typing and drug sensitivity of *Plasmodium falciparum* strains from Shahbad Daulatpur Colony, Municipal Corporation of Delhi, Delhi, India.[1]

Sample or case	Genotype KAHRP	Genotype TRAP[2]	Strain type	Chloroquine MIC in 10^6 M	Chloroquine sensitivity status (in vitro)
D1	H	A	VI	4.2	R
B34	H	A	VI	3.5	R
BSN5	H	A	VI	1.0	S
BSN8	H	B	VII	0.44	S
D/9	H	B	VII	3.7	R
B15	H	A	VI	0.47	S
C23	H	A	VI	0.49	S
C15	H	A	VI	4.0	R
16	H	A	VI	1.6	R
29	H	A	VI	0.5	S
33	—	—	—	0.46	S
42	—	—	—	1.8	R

[1] KAHRP, Knob-associated histidine-rich protein; TRAP, thrombospondin-related anonymous protein; MIC, minimum inhibitory concentration; H, high molecular weight allele of KAHRP; R, resistant; S, susceptible.

[2] A and B are the RFLP patterns of the TRAP allele.

area, were included for comparison with the test sample, whose ELISA OD value for anti-AR1 IgG was 0.83, whereas for anti-*Pf* IgG the OD was 0.84. The correlation between anti-AR-1 and anti-*Pf* IgG was significant. These findings also correlate with low spleen rates in the population. We suggest that residents in the outbreak area had experienced a different strain of parasites, whereas the floating population harbored the new resistant type VI strain as per PCR results.

Results of the in vitro chloroquine susceptibility test in *P. falciparum* patients revealed the presence of both RII and RIII levels of resistance. Of 12 samples tested, 6 (50%) showed chloroquine resistance. Prevalence of a high rate of chloroquine resistance may be 1 of the factors for the outbreak of the disease and deaths (Sharma et al. 1996).

Ten samples included in the above-mentioned drug sensitive study were also used for strain typing by PCR. Two variable marker genes (KAHRP and TRAP) were employed in this PCR typing. This was carried out based on the size of the PCR product for the KAHRP gene and restriction fragment length polymorphism (RFLP) pattern for the TRAP gene combined, as described by Bhutani et al. (1998). Based on this typing, 2 strain types (type VI and VII) were present among the analyzed samples (Table 4). Among these samples, strain type VI was more predominant because it was found to be present in 8–10 clinical isolates (Contemn et al. 1995). Both of these strain types were also found in the Rajasthan epidemic, where chloroquine resistance was highly prevalent (Sharma et al. 1996, Bhutani et al. 1998). In fact, the predominant strain (type VI) in Delhi was also the predominant strain in Rajasthan (Bhutani et al. 1998). However, the other strain type VII was found only in Rajasthan and Delhi and not in Haryana, Assam, Uttar Pradesh, and Orissa (Bhutani et al. 1998). In contrast, strain type VI was found in Rajasthan, Delhi, Uttar Pradesh, and Orissa. The results shown in Table 4 indicate that no correlation occurred between these strain types and chloroquine resistance. These observations were similar to those reported earlier (Sharma et al. 1996, Bhutani et al. 1998).

Malaria in rural Delhi is associated with unplanned developmental activities, human settlements, and inadequate maintenance of water supply storage and drainage systems resulting in the breeding of vector species. Lack of intersectorial coordination and inadequate legislative management of solid waste, particularly in unauthorized, notified and nonnotified slums, are also important factors in maintaining malaria endemicity in the capital city of the country (Kalra 1987). The unauthorized area under investigation is a low-lying area surrounded by several abandoned brick kilns. However, malaria resurgence in rural Delhi is primary caused by seasonal temporary rainwater bodies, which support the breeding of *An. culicifacies*. Heavy precipitation in August 1996 resulted in filling of numerous depressions of brick kilns and adjoining areas. This provided breeding places for *An. culicifacies*. Adult densities started building up in absence of any control measures before the epidemic. Initial densities (September 1996) of anopheline larvae in pools and ponds were 21.6 and 22.5 per dip, which subsequently were reduced by intensive antilarval and fogging operations. Similarly, the density of adult *An. culicifacies* was high (38.0) (September 1996) but was reduced considerably in subsequent months because intensified intervention measures against targeted species resulting in knockdown of parous females. This was the reason that vector incrimination could not be established in spite of dissecting females of both *An. stephensi* and *An. culicifacies*. Further observations on egg ridges of *An. stephensi* revealed that it was var. *mysoriensis*, which is considered to be a poor vector. In view of this, *An. culicifacies* may have played the initial role in active transmission during August and September. However, timely detection of the epidemic and application of intervention measures reduced the intensity of the disease and partially interrupted disease transmission in successive months. Chloroquine resistance (50%) could be responsible for the sudden spurt of the disease. The mortality in children may be due to inadequate treatment or due to complications. Results of the serologic survey revealed a low antibody titer in the floating population, thereby suggesting the presence of a less immune population because of their lack of exposure to malaria in the past. At the time of the outbreak, the majority of the population could have had falciparum malaria for the 1st time.

Probable cause of outbreak

The conclusion is made that the probable causes of outbreaks were settlement of migrant laborers in excavated land with inadequate health infrastructure, harboring of chloroquine-resistant strains of *P. falciparum,* and predominant presence of the type VI strain, as revealed by PCR. The study emphasized the need to create infrastructure facilities to monitor drug resistance, to monitor species specificity, to improve surveillance, and to create a viable health infrastructure to avoid deaths of children in the floating slum population.

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