An investigation into the recent malaria outbreak in district Gurgaon, Haryana, India

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We present results of entomological and parasitological surveys carried out on a recent outbreak of malaria in district Gurgaon, Haryana. *An. culicifacies sensu lato* the major vector of malaria was found in high densities and was incriminated. The species was resistant to DDT and dieldrin and was susceptible to malathion and deltamethrin in laboratory tests and these results were in agreement with the observations on their field-efficacy. High incidence of *P. falciparum*, break down of surveillance, favourable mosquitogenic conditions were some factors responsible for this outbreak. Suggestions for measures to avoid such outbreaks in future are discussed.

In October 1996, high incidence of malaria associated with fever-related deaths was reported from villages of community health centres (CHCs), Nuh and Ferozepur Jhirka of Mewat region of district Gurgaon, Haryana (Source: Directorate of National Malaria Eradication Programme, Delhi). Twenty-three confirmed deaths occurred in CHC Nuh from September to November. Tables 1 and 2 give month-wise parasitological data of CHCs Nuh and Ferozepur Jhirka. High prevalence of *P. falciparum* was encountered during the transmission months in September and October. Parasitological indices observed in Nuh (population: 2.8 lakhs) for 1996 were: annual parasite index (API) 24.3; slide positivity rate (SPR) 6.03; slide falciparum rate (SfR) 3.26 and percentage of *P. falciparum* (%Pf) 54.1 and indices for the same year for CHC, Ferozepur Jhirka (population: 1.78 lakhs) were: API 37.00; SPR 8.0; SfR 4.89 and %Pf 61.1 (Source: District Malaria Officer, Gurgaon).

In November 1996, when these CHCs were surveyed by us, the Health Department of the Haryana Government had already stepped up malaria control operations. In addition to three rounds of HCH indoor residual spray @ 200 mg/m^2 during May to September, special rounds of insecticide-spray were organized as follows: in Nuh, HCH 50% (October 1–31); malathion 25% @ 2 g/m^2 (November 1–17); deltamethrin 2.5% @ 20 mg/m^2 (November 17–December 12) and in Ferozepur Jhirka, HCH 50% (October 1–16); malathion 25% (October 17–December 5) (source: District Malaria Officer, Gurgaon, Haryana). Both human dwellings and cattle sheds were sprayed with the insecticide. The affected villages were also fogged with technical malathion. Radical treatment was given to all fever cases (FRT) (Source: Chief Medical Officers, Nuh and Ferozepur Jhirka).

This paper presents results of spot entomological and parasitological surveys carried out in villages of CHCs, Nuh and Ferozepur Jhirka of Gurgaon district, Haryana in November, 1996.

Table 1. Parasitological data of CHC Nuh during 1996

Month	No. of blood films collected	No. of positives			SPR	SfR	%Pf
		Total	Pv	Pf			
Jan.	2628	97	29	68	3.7	2.58	70.00
Feb.	2551	8	0	8	0.31	0.31	100.00
Mar.	2933	22	10	12	0.75	0.41	54.50
Apr.	2214	25	19	6	1.12	0.27	24.00
May	2860	66	61	5	2.30	0.17	7.57
Jun.	3044	111	108	3	3.60	0.10	2.70
Jul.	3031	234	228	6	7.70	0.19	2.56
Aug.	5478	213	210	3	3.90	0.05	1.40
Sep.	21,497	1071	722	349	4.98	1.62	32.58
Oct.	38,430	2831	1180	1651	7.36	4.29	58.31
Nov.	20,017	1674	460	1214	8.36	6.06	72.52
Dec.	7737	433	87	346	5.60	4.47	79.90
Total	112,420	6785	3114	3671	6.03	3.26	54.10

Source: DMO, Gurgaon, Haryana.
Pv, *Plasmodium vivax*; Pf, *P. falciparum*; SPR, Slide positivity rate = (No. of positives/total blood films examined); SfR, Slide falciparum rate = (No. of Pf positives/total blood films examined); %Pf = (No. of Pf cases/No. of positives).

Table 2. Parasitological data of CHC Ferozepur Jhirka during 1996

Month	No. of blood films collected	No. of positives			SPR	SfR	%Pf
		Total	Pv	Pf			
Jan.	1501	37	10	27	2.46	1.79	72.90
Feb.	1594	11	3	8	0.69	0.50	72.70
Mar.	1448	6	4	2	0.41	0.14	33.73
Apr.	1288	12	11	1	0.93	0.07	8.33
May	1638	38	37	1	2.32	0.06	2.63
Jun.	846	42	40	2	4.96	0.23	4.76
Jul.	4765	317	309	8	6.65	0.16	2.52
Aug.	2633	113	113	–	4.29	–	–
Sep.	22,005	1015	642	373	4.61	1.69	36.74
Oct.	18,780	3045	1181	1864	16.21	9.92	61.21
Nov.	20,482	1479	173	1306	7.22	6.37	88.30
Dec.	5275	471	38	433	8.92	8.20	91.93
Total	82,255	6586	2561	4025	8.00	4.89	61.11

Source: DMO, Gurgaon, Haryana.

Materials and methods

Entomological and parasitological surveys were carried out in four villages of CHCs Nuh and six villages of CHCs Ferozepur Jhirka (see Table 3). The selected villages also included those villages that are most affected during this current outbreak of malaria. Anopheline mosquitoes resting indoors in human dwellings and cattle sheds were collected with the help of a flashlight and an aspirator[1] and were brought to the MRC Laboratory in Delhi in cloth cages covered with a wet towel. Blood films were made from fever cases and were given presumptive dose of chloroquine according to the National Malaria Eradication Programme Drug Policy (10 mg base/kg body wt).

In the laboratory, mosquitoes were identified to species based on morphological characters with the help of an identification key[2]. The total number of each species of mosquitoes collected from human dwellings and cattle sheds were pooled and their abundance was expressed in man hour densities (MHD)[3]. *An. culicifacies sensu lato* were processed for various studies as follows: (i) Midgut and salivary glands were examined by dissections for oocysts and sporozoites respectively. (ii) Mosquitoes were exposed to DDT-, dieldrin- and deltamethrin-impregnated papers procured from WHO and to malathion papers, prepared at MRC[4]. Mosquitoes were exposed to DDT 4% for 1 h, dieldrin 0.4% for 1 h, malathion 5% for 1 h and deltamethrin 0.025% for 1 h. In another test, mosquitoes were exposed to graded times[5] from 15 min to 75 min and the time-mortality response data was subjected to probit-regression analysis[6] to calculate LT_{50}, time to kill 50%, LT_{90} to kill 90% and LT_{99} to kill 99% of the exposed population. (iii) From malathion-susceptibility tests dead and alive mosquitoes were individually processed for DNA isolation for the identification of sibling species by polymerase chain reaction (PCR) assay[7]. These data were used to calculate the susceptibility/resistance levels to malathion in sibling species. Blood films were stained with JSB stain[8] and examined under microscope to identify malaria-positive slides and parasite species.

Results

Table 3 gives results of entomological studies on the prevalence of anophelines, man hour densities (MHD) and information on the insecticides, dates and rounds sprayed in villages of CHCs, Nuh and Ferozepur Jhirka. The most prevalent species in villages of CHCs Ferozepur Jhirka were *An. culicifacies* followed by *An. annularis*, while in Nuh *An. annularis* was followed by *An. culicifacies*. The highest MHD of *An. culicifacies* was 78.0 in village Tekri (Ferozepur Jhirka), followed by 47.5 in village Salamba (Nuh). In village Salamba, deltamethrin was sprayed as indoor residual spray in the third week of November and during our survey in the fourth week, MHD of *An. culicifacies* was 1.5, which was about 33 times less than the density observed in earlier surveys. Similar observations were made in villages of Ferozepur Jhirka where malathion was sprayed as indoor residual spray. The sibling species composition of *An. culicifacies* was 61% species A and 39% B (ref. 7) ($n = 57$). Of a total of 87 *An. culicifacies s.l.* mosquitoes dissected for malaria parasites, one mosquito was found positive for sporozoites in salivary glands (sporozoite rate of 1.15%) and none was found positive for oocysts. Laboratory susceptibility tests against different insecticides on field-collected *An. culicifacies* indicated moderate to high degree of resistance to DDT, and almost complete resistance to dieldrin (Table 4). The same population was 78–88% susceptible to malathion while to deltamethrin it was fully susceptible. In time-mortality response studies with malathion, the populations have shown slight tolerance as observed

from the calculated lethal times (LT_{50}–37.05 min, LT_{90}–50.88 min and LT_{99}–65.9 min) and time to kill all susceptibles was beyond the WHO diagnostic time of 60 min. In time-response studies with deltamethrin, *An. culicifacies* showed complete mortality within 5 min of exposure. In the present study using PCR, the dead and alive *An. culicifacies* mosquitoes ($n = 32$) were identified to sibling species A and B with species-specific primers[7]. These data were used to calculate malathion-resistance in sibling species A and B, which was respectively 13.3% and 17.6%. High resistance to dieldrin (75%) observed in *An. culicifacies s.l.* (Table 4) suggests that species A and B were resistant to HCH. Observations on the ineffectiveness of HCH-indoor spray were confirmed by the presence of high densities of *An. culicifacies*, *An. annularis* and other anophelines in sprayed villages. Effectiveness of malathion- and deltamethrin-indoor sprays was dramatic and spraying brought down the densities of *An. culicifacies* to negligible numbers and there was almost complete disappearance of other species. These observations were in agreement with the results obtained from insecticide susceptibility tests in laboratory (Table 4). Results of the parasitological surveys (Table 5) indicated a high prevalence of *P. falciparum* malaria in the area. Of the 191 slides examined, 118 were positive, of which 13% (15) were *P. vivax* and 87% (103) cases were of *P. falciparum*. The SPR was in the range of 43–70%, SfR – 35–71% and %Pf was 54–100%. Agewise distribution of positive cases among the patients of both the sexes was: no case in 0–1 yr age group, 22% (26) cases in 1–4 yrs; 23.7% (28) in 4–8 yrs, 12.7% (15) in 8–14 yrs and 41.5% (49) in 14 yrs and above age group.

Discussion

In our earlier studies in Haryana and the neighbouring states, species A and B were found sympatric[9] and only species A was incriminated as a vector[10] and later by

Table 3. Results of the entomological monitoring in villages of CHCs, Nuh and Ferozepur Jhirka of district Gurgaon, Haryana

Village	Anopheline species	No. collected	MHD	Insecticide sprayed (spray round)
CHC Nuh				
Salamba (2 surveys)	*An. culicifacies*	392	47.50*	HCH (III) 16–9–96
	An. annularis	925	123.33	
	An. subpictus	42	7.00	
	An. stephensi	2	0.33	
Salamba	*An. culicifacies*	3	1.50	Deltamethrin (I) 21–11–96
Ghasera	*An. culicifacies*	5	1.11	HCH (III) 16–9–96 Malathion (I), NA
Akeda	*An. culicifacies*	0	–	Deltamethrin (I) 19–11–96
	An. annularis	10	6.66	
Dhillana	*An. culicifacies*	15	30.00	NA
CHC Ferozepur Jhirka				
Ferozepur Namak	*An. culicifacies*	45	15.00	NA
Newli	*An. culicifacies*	1	0.66	Malathion (I), NA
Tekri (2 surveys)	*An. culicifacies*	390	78.00**	HCH (III), NA
	An. subpictus	51	6.37	
	An. annularis	3	0.75	
	An. aconitus	14	4.66	
Pratapbas	*An. culicifacies*	206	29.42	NA
	An. subpictus	35	11.60	
	An. annularis	10	3.30	
Gohana	*An. culicifacies*	82	20.50	NA
	An. annularis	147	36.75	
Khedli kurd	*An. culicifacies*	3	2.00	Malathion (I), NA

MHD: Man hour density = $n \times 60/t \times p$ (where n = no. of mosquitoes; t = time in min; p = no. of persons).
*: MHDs of the two surveys were 47.3 and 47.7.
**: MHDs of the two surveys were 82.5 and 60.0.
NA: Information not available at the time of spray.

Table 4. Response of *An. culicifacies* to different insecticides

Village	1 h exposure to impregnated paper	Nos exposed (no. of replicates)	Nos dead	Percentage mortality*
CHC Nuh				
Salamba	DDT 4%	30 (2)	23	73.0
	Malathion 5%	133 (8)	117	87.9
	Deltamethrin 0.025%	30 (2)	30	100.0
CHC Ferozepur Jhirka				
Pratapbas	Dieldrin 0.4%**	44 (2)	11	25.0
	Malathion 5%	50 (3)	39	78.0
Tekri	DDT 4%	61 (4)	28	45.9
	Dieldrin 0.4%	91 (4)	22	24.1
	Malathion 5%	92 (4)	73	79.3
	Deltamethrin 0.025%	147 (9)	147	100.0

Mortalities after 24 h of holding have been corrected by Abbott's formula where necessary.
**Used to test susceptibility of mosquitoes to HCH.

Table 5. Results of parasitological surveys

Village	No. of blood films collected	No. of positives			SPR	SfR	%Pf
		Total	Pv	Pf			
CHC Nuh							
Ghasera	20	13	6	7	65.0	35.0	53.8
Salamba	13	6	1	5	46.1	38.5	83.5
Malab	11	7	2	5	63.6	45.4	71.4
CHC Ferozepur Jhirka							
Ferozepur Namak	28	12	0	12	42.8	42.8	100.0
Tekri	14	8	0	8	57.1	57.1	100.0
Newli	45	31	1	30	68.9	66.6	96.7
Gohana	10	7	0	7	70.0	70.0	100.0
Pratapbas	13	9	0	9	69.2	69.2	100.0
Khedli Kurd	37	25	5	20	67.5	54.0	80.0
Total	191	118	15	103	61.8	53.9	87.2

immunoradiometric assay both *P. vivax* and *P. falciparum* sporozoite antigens were detected in species A[11]. However, in this study, the sporozoite-positive *An. culicifacies* specimen was not identified to sibling species, 61% of species A found in this area suggests the possibility that there was indigenous transmission of malaria due to species A. Of the other four prevalent anopheline species, the role of *An. stephensi* in rural areas is limited and this species attains significance in the transmission of malaria in urban areas. *An. annularis* is considered to be a secondary vector and only in some localized areas in eastern India, *An. aconitus* is considered as a secondary vector in coastal plains of Orissa and *An. subpictus* is a non-vector[12]. High slide positivity rate in the population and 22% of the cases among children in < 4 yrs age group also supports that there was indigenous transmission of malaria. The gametocyte rate among the Pf cases was 50%, i.e. a high percentage of carriers. High gametocyte rates among the patients indicated that the Pf cases remained untreated or were not given prompt and proper treatment or may also be due to lower susceptibility of the earlier (asexual) stages of parasite to chloroquine. Field-studies on the *in vivo* response of *P. falciparum* to chloroquine in Haryana State[13] indicated complete susceptibility to this drug during 1978–1984, while during 1985–1989 it was 71% susceptible and during 1990–1994 it was 66% susceptible. In a recent study by MRC on the *in vitro* response of *P. falsiparum* isolates to chloroquine in this area during the current outbreak of malaria, 66% of the isolates tested were found susceptible (MRC-Annual Report, 1996). Though the decreased susceptibility to chloroquine was observed in this area, high gametocyte rate among the patients may not be due to this single factor alone and other factors mentioned above might also have contributed. In a study carried out in 1981–82 in Kharkoda PHC, Haryana[14], it was observed that *P. vivax* cases start increasing from March and show a peak in

May and August before declining. While *P. falciparum* cases were very slow from March to May and slowly started to increase peaking in October and cases were found up to January before declining by March. Persistence of *P. falciparum* malaria throughout the year in these CHCs (Tables 1 and 2) indicated a breakdown in surveillance. Added to this, heavy rains and floods due to breaches in two earthen dams created favourable mosquitogenic conditions and thus increased the mosquitogenic conditions during post-monsoon months. Spraying of three rounds of HCH (50%) and a special round of the same insecticide did not control malaria as *An. culicifacies* was resistant to it (Table 4). Instead of a special round of HCH, malathion or deltamethrin would have effectively controlled *An. culicifacies* and interrupted transmission. This finding is supported by susceptibility tests and field observations.

During the 1981–82 epidemic of malaria in district Sonepat, Haryana, HCH was replaced by malathion and this strategy successfully controlled *An. culicifacies* species A and B[15]. Later, repeated spraying of malathion, though there was no transmission, resulted in *An. culicifacies* becoming resistant although species A and B have different levels of resistance[16]. Though the efficacy of malathion as a residual spray in decreasing the vector-densities in this region cannot be doubted at present, its continuous spray would hasten the development of resistance as has happened in Sonepat. To overcome this problem, as a strategy for the management of insecticide-resistance, alteration of insecticides with different groups, e.g. organophosphate and synthetic pyrethroids, would be advantageous in vector control programmes. In this context we quote A. W. A. Brown[17]: 'Long-term sequential selection, where the change of insecticide is arbitrarily made several generations after its introduction, has an advantage over the present practice of waiting until resistance develops before making a switch: making the change before that happens has the effect of denying the target population the opportunity of developing fitness alleles to counteract the reduction of fitness which characterizes the incipient stage of resistance development.'

To avoid malaria outbreaks in future, entomological surveillance is essential. Data should be used to implement situation-specific malaria vector control strategies in conjunction with parasitological surveillance.

1. *Manual on Practical Entomology in Malaria*, WHO, Part II, 1975, pp. 2–6.
2. Wattal, B. L. and Kalra, N. L., *Bull. Nat. Soc. Ind. Mal. Mosq. Dis.*, 1961, **9**, 85–138.
3. Subbarao, S. K., Vasantha, K., Raghavendra, K., Sharma, V. P. and Sharma, G. K., *J. Am. Mosq. Control Assoc.*, 1988, **4**, 29–33.
4. Hemingway, J., *Trans. R. Soc. Trop. Med. Hyg.*, 1983, **77**, 477–480.
5. *Instructions for determining the susceptibility or resistance of adult mosquitoes to organochlorine, organophosphate and carbamate insecticides: Establishment of base-line*, WHO, VBC/81.805, 1981, pp. 1–7.
6. Finney, D. J., *Probit Analysis*, Cambridge University Press, Cambridge, England, 1971, 3rd edn, pp. 333.
7. Cornel, A. J., Subbarao, S. K., Chandra, D., Raghavendra, K., Nanda, N., Sharma, V. P. and Collins, F. H., *J. Med. Entomol.*, 1997, (communicated).
8. Singh, Jaswant., *Bull. Nat. Soc. Ind. Mal. Mosq. Dis.*, 1959, **7**, 79–94.
9. Subbarao, S. K., Vasantha, K. and Sharma, V. P., *Biosystematics of Haematophagus Insects* (ed. Service, M. W.), Oxford University Press, London, 1988, pp. 25–37.
10. Subbarao, S. K., Adak, T. and Sharma, V. P., *J. Commun. Dis.*, 1980, **12**, 102–104.
11. Subbarao, S. K., Adak, T., Vasantha, K., Joshi, H., Raghavendra, K., Cochrane, A. H., Nussenzweig, R. S. and Sharma, V. P., *Trans. R. Soc. Trop. Med. Hyg.*, 1988, **82**, 394–397.
12. Nagpal, B. N. and Sharma, V. P., *Indian Anophelines*, Oxford and IBH, New Delhi, 1995, pp. 416.
13. Mishra, S. P., in *Proceedings of National Academy of Sciences of India* (eds Sharma, V. P. and Kamboj, V. P.), 1996, **66**(B), spl issue, pp. 123–138.
14. Sharma, V. P., Choudhury, D. S., Ansari, M. A., Malhotra, M. S., Menon, P. K. B., Razdan, R. K. and Batra, C. P., *Indian J. Malariol.*, 1983, **20**, 21–34.
15. Subbarao, Sarala K., Sharma, V. P., Vasantha, K. and Adak, T., *Indian J. Malariol.*, 1984, **21**, 109–114.
16. Raghavendra, K., Subbarao, Sarala K., Vasantha, K., Pillai, M. K. K. and Sharma, V. P., *J. Med. Entomol.*, 1992, **29**, 183–187.
17. Brown, A. W. A., *J. Am. Mosq. Control Assoc.*, 1986, **2**, 123–140.

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