

The footprints of relapsing malaria in southwest Delhi, India

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ABSTRACT

Background & objectives: Control of vivax malaria is challenging due to persistence of hypnozoites causing relapses and safety concerns with primaquine in G6PD deficient individuals. We present the epidemiology of malaria with emphasis on recurrence of vivax malaria over a period of four years in southwest Delhi among patients reporting to malaria clinic.

Methods: Microscopic examination of stained blood smears of fever patients attending malaria clinic was performed. Confirmed malaria cases were treated as per the national treatment guidelines. The epidemiological data of confirmed malaria cases including demographic characteristics, age, gender and past history of malaria were analysed. Patients were asked to report in case of occurrence of fever.

Results: From January 2011 to December 2014, 429 *Plasmodium vivax*, 24 *P. falciparum* and three mixed infection cases were reported to the Malaria Clinic at National Institute of Malaria Research, New Delhi. Malaria cases peaked in the months of August and September during all the four years. Recurrent episodes of vivax malaria were observed in 14.72% patients to whom primaquine was not dispensed, while the prevalence was 4.02% among those who received primaquine. The relapsing patterns observed were of both short as well as long latency *P. vivax* phenotypes. The entomological survey of area from where malaria patients reported, showed prevalence of *Anopheles stephensi*.

Interpretation & conclusion: The study showed presence of persistent *P. vivax* malaria with strains causing both frequent and long latency recurrences (probable relapses) in southwest Delhi. This highlights the need to evaluate primaquine regimens against both these strains and formulate strategies to improve compliance to 14-days primaquine treatment.

Key words Delhi; malaria; *Plasmodium vivax*; relapse

INTRODUCTION

Plasmodium vivax infection has the most widespread geographic distribution among all the human malaria parasites worldwide¹. Control of *P. vivax* malaria poses a major challenge to malaria elimination in Asia². India reports around one million malaria cases annually and *P. vivax* contributes to about half of them. Jharkhand, Madhya Pradesh, Gujarat and Uttar Pradesh states contribute to about half of the vivax cases of the country³. The recommended regimen for treating vivax malaria in India is chloroquine (25 mg/kg body weight) over three days and primaquine (0.25 mg/kg body weight per day for 14 days)⁴. The control of vivax malaria faces additional challenges due to persistence of hypnozoites responsible for relapses and safety issues of primaquine in glucose-6-phosphate dehydrogenase (G6PD) deficient individuals. The relapses caused by hypnozoites vary in frequency and pattern of occurrence in different geographical regions and occur weeks to months after the primary vivax infection⁵⁻⁶.

In India, both frequent and long latency relapses are observed⁷⁻⁹. Further, the relapses are polymorphic as three different patterns of relapses have been observed in northern India⁷. The relapse rates of up to 44.3% have been observed in different geographical regions of the country^{7, 10}, while a relapse rate of 23.3 to 44.3% has been observed in Delhi^{7, 11}.

We present the epidemiology of malaria in southwest Delhi with emphasis on malaria cases with recurrent malaria attacks.

MATERIAL & METHODS

The study is based on the analysis of data from Malaria Clinic located at the National Institute of Malaria Research, New Delhi from year 2011 to 2014. The clinic caters to the peri-urban areas of southwest Delhi covering a population of about one million. Malaria in Delhi is mostly due to *P. vivax* and transmission is mainly seasonal.

Thick and thin blood smears were made from the fe-

ver patients reporting to the clinic, stained with Jaswant Singh Bhattacharya (JSB) stain and examined under microscope. Their demographic characteristics, history of fever and past history of malaria were recorded. All the confirmed *P. vivax* malaria cases were treated with chloroquine 10 mg/kg body weight on Days 0 and 1; and 5 mg/kg body weight on Day 2. A qualitative test was performed to assess G6PD status in 373 patients. Primaquine was dispensed to all the G6PD non-deficient cases in the dose of 0.25 mg/kg body weight/day for 14 days. The patients were counselled regarding the importance of completing the full course of primaquine therapy. For convenience, age-wise schedule was followed. Cases of *P. falciparum* and mixed infections were treated with artemisinin-based combination therapy and primaquine in appropriate doses⁴. Patients were asked to report to malaria clinic in case of occurrence of fever.

Data regarding the patients' demographics, blood smear results, G6PD status and treatment given were entered in Microsoft Excel 2007. Chi-square test was used as test of significance. Patients with recurrent malaria were tracked by patient ID, report cards issued at the time of first attack, patient name and history of malaria in past.

RESULTS

During the study period, 9588 fever patients reported to the Malaria Clinic at NIMR (Fig. 1), out of which 426 patients were tested positive for malaria (slide positivity rate 4.44). The male to female ratio was 2.7 : 1. The proportion of children < 5 yr with malaria was 3.52% (Table 1). About 50% of the total malaria cases were reported from Raj Nagar II (38%) and Bagdola area (12%). The areas reporting malaria cases largely remained the same over the four years (Fig. 2). Vector breeding surveys of cemented, iron and plastic tanks, coolers, mud pots, etc. conducted in these areas showed presence of *Anopheles stephensi* immatures in low numbers (Table 2).

The slide positivity rate was consistent in all the years. There were 399 *P. vivax* and 24 *P. falciparum* malaria cases during the reported period. Three patients had mixed infections of *P. vivax* and *P. falciparum* (Table 1). Malaria cases were reported throughout the year, however, higher numbers of cases were observed from July to October with peak reporting during August and September (Fig. 3). The parasitaemia among *P. vivax* cases ranged from 40 to 49200 parasites/ml; while among *P. falciparum* patients, it was 800 to 345600.

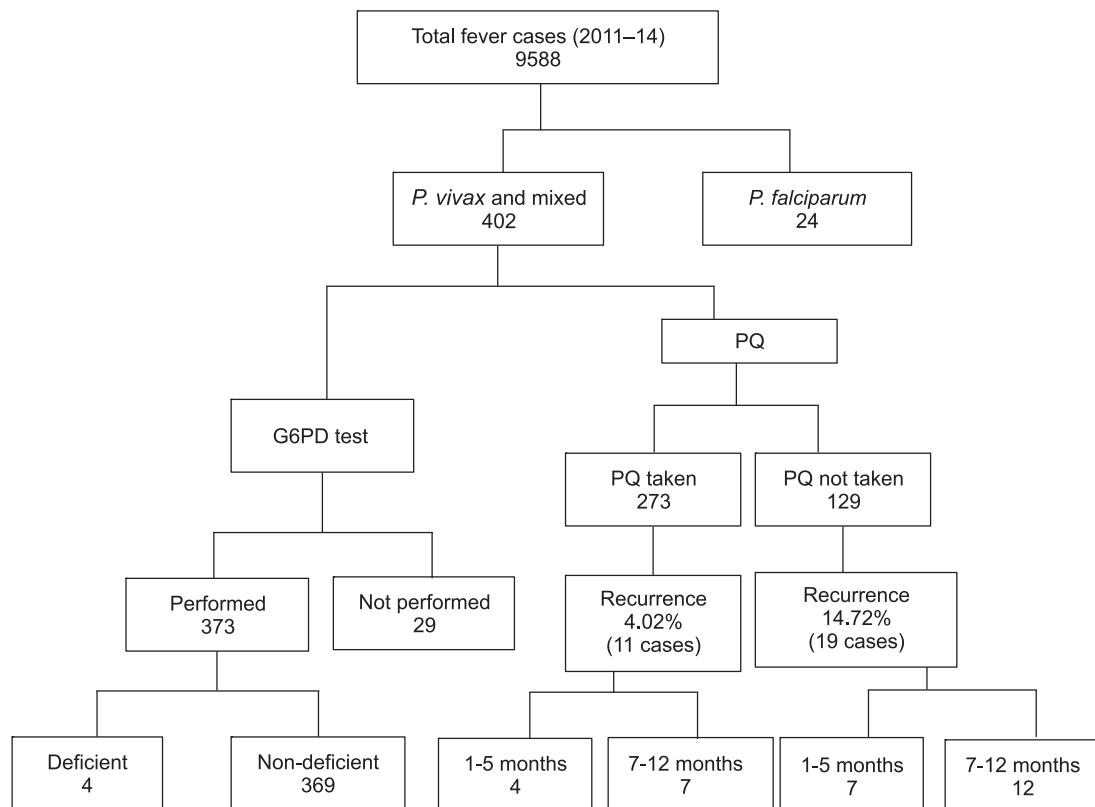


Fig. 1: Consort diagram showing distribution of recurrences of *P. vivax* infections.

Table 1. Characteristics of malaria patients presented at Malaria Clinic, NIMR, New Delhi

Category		No. of patients
Gender	Male	311
	Female	115
Age group (yr)	0 to <1	0 (0)
	1 to <5	15 (3.52)
	5 to <15	75 (17.60)
	≥15	336 (78.87)
Referral	Self	317
	Referred	109
Species	<i>P. vivax</i>	399
	<i>P. falciparum</i>	24
	Mixed	3
History of malaria in past one year	Present	21
	Absent	405

Figures in parentheses indicate percentages.

Out of 402 *P. vivax* malaria cases including mixed infections, primaquine was given to 273 cases of vivax and mixed infections. The reasons for not giving primaquine to remaining patients were pregnancy, G6PD deficiency, patients not willing to undergo G6PD test and patients not turning up for taking primaquine. G6PD deficiency was observed in 4 (1.1%) patients among the 373 tested. Of the four G6PD deficient patients, three were males while one was a female (Fig. 1).

Among the patients taking primaquine treatment ($n=273$), the recurrence rate of vivax malaria was 4.02%; while in patients not receiving primaquine, it was 14.72% (Fig. 1). This difference was statistically significant (Chi-square = 14.522, $p < 0.01$). During the year 2015, till the month of May, two patients had recurrence of vivax malaria of which, one reported with third recurrence after

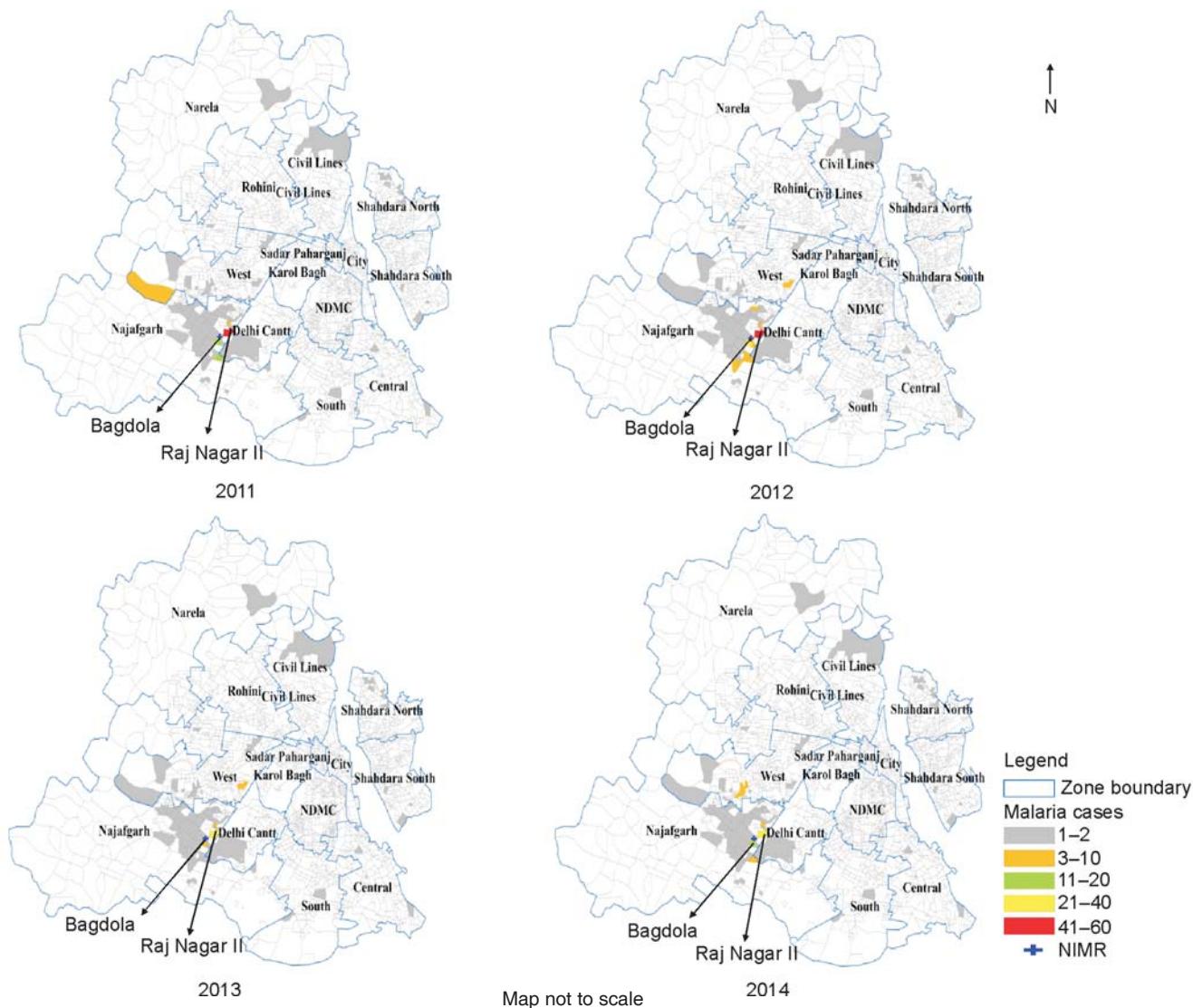


Fig. 2: Map of southwest Delhi showing distribution of malaria cases reported to NIMR clinic during 2011–2014.

Table 2. Entomological survey from draining area during 2014–15

Containers	2014			2015		
	Examined	Positive for mosquitoes	Positive for <i>An. stephensi</i>	Examined	Positive for mosquitoes	Positive for <i>An. stephensi</i>
Over head tanks	1141	6	1	550	9	2
Coolers	1226	21	1	495	10	1
Cemented tanks	386	3	2	40	4	1
Mud pots	137	6	—	61	1	—
Tyres	17	2	—	9	0	—
Iron tanks	16	6	1	8	2	1
Plastic tanks	322	13	1	140	6	—
Bird pots	8	2	—	17	1	—
Others	44	0	—	4	3	—
Total	3305	59	6	1449	36	5

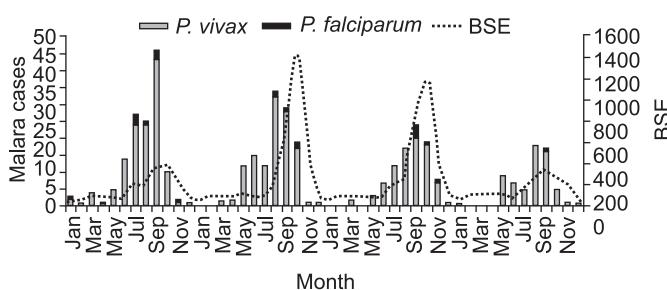
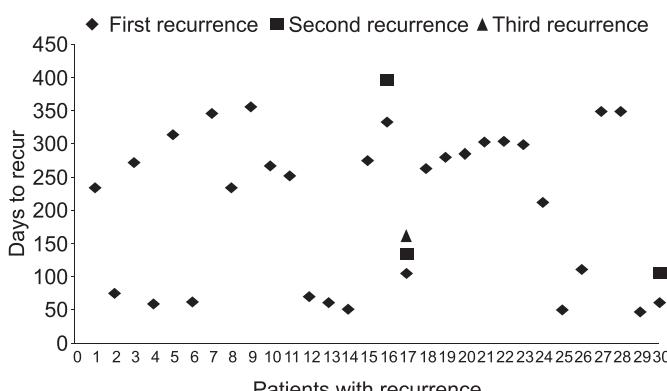


Fig. 3: Month-wise distribution of fever and malaria cases [BSE—Blood slides examined].

primary vivax infection while the another patient reported after eight months of primary infection. Both the patients had not taken primaquine. Among the 30 patients with recurrent attacks, 11 (36.6%) had first recurrence within five months while remaining 19 (63.3%) experienced recurrence between 7 to 12 months of primary attack. Two patients had two recurrent attacks (after two and four months of primary attack and 11and 13 months of primary attack, respectively); while another had three recurrent attacks (after 3, 4 and 5 months of primary infection) (Fig. 4). Of the 34 attacks of *P. vivax* recurrence, 16

Fig. 4: Graph showing days to recur and number of recurrences of *P. vivax* malaria episode.

(47.05%) occurred in non-transmission season (December to June).

Entomological surveys were conducted in the residential areas of malaria patients in the years 2014 and 2015. The breeding of *An. stephensi* was observed in low numbers mostly in overhead, cemented, and iron tanks (Table 2).

DISCUSSION

The results show that the transmission of vivax malaria in Delhi is highly seasonal with peak cases in August and September. This finding is consistent with previous reports from Delhi^{7, 12}. A time series analysis from Delhi has shown that the vector breeding and malaria transmission depend upon rainfall and humidity which are significant predictors for forecasting malaria cases¹².

There is local malaria transmission in Delhi as both *An. culicifacies* and *An. stephensi* have been reported to be present in the state and higher densities of the vectors were found during the post-monsoon months¹³. However, entomological surveys conducted by us have shown presence of *An. stephensi* only (Table 2). The possible reasons for increased vector breeding in urban areas could be unplanned urbanization, demographic and societal changes, developmental projects etc. Higher prevalence of malaria was observed in males compared to females. Such observations have been reported earlier and possible reason may be more exposure to mosquito bite due to outdoor activities¹⁴, and possible role of sex hormones for increasing susceptibility in adult males, though ambiguous¹⁵.

The control of *P. vivax* is challenging due to several biological adaptations of this parasite particularly the relapses responsible for increasing the disease burden. The malaria transmission season in Delhi is reported to be

from July to September by time series analysis¹² and from July to November by Adak *et al*⁷ based on vector incrimination studies. We observed 47.05% of the recurrences in non-transmission season from December to June, which can be considered as true relapses.

Lower rate (4.02%) of relapse was observed with the use of primaquine compared to those not receiving primaquine (14.72%). The recommended antirelapse therapy for vivax in India is primaquine 0.25 mg/kg per day for 14 days. Though studies have reported this primaquine regimen to be significantly efficacious compared to no primaquine arm in preventing relapse^{16–17}, a study from eastern India has questioned the efficacy of primaquine as primaquine arm showed a recurrence rate of 16.5% against 26.7% recurrence rate in no primaquine arm¹⁸, and a study from western India has also reported probable resistance to primaquine¹⁹. This could be due to the lack of compliance or reduced efficacy of primaquine. Treatment with effective dose of hypnozoitocidal drug is required to control the *P. vivax* burden and eventually for control of malaria. Thus, it would be good to introduce observed therapy of primaquine or formulated strategies to improve compliance.

The relapsing phenotypes reported from India include frequently relapsing as well as long latency phenotypes. The frequently relapsing Chesson strain causes relapse from 1 to 5 months while long latency temperate strain causes relapse from 7 to 13 months after primary infection²⁰. Both the patterns of recurrence were observed in our study. 36.7% recurrences occurred within five months while 63.3% of the recurrences occurred after seven months of primary attack. This finding could be important in planning the trials for antirelapse medicines to evaluate their efficacy against both the parasite strains. In areas with prevalence of long latency vivax strains there is need to follow up patients for longer duration than the routine six-month follow up to capture the relapses caused by long latency strains and assess efficacy against them. A recent clinical trial of antirelapse medicine with six-month follow up showed recurrence rate of 10% in no primaquine arm; while there was no recurrence in primaquine arm²¹.

It is reported that temperate strains causing long latent relapses are susceptible to primaquine while frequent relapses caused by Chesson strain of *P. vivax* are known to be resistant to 8-aminoquinolines (primaquine). Hence, a higher dose of primaquine(0.5 mg/kg/day for 14 days) is required for preventing frequent relapses^{20, 22}. Since, the present study showed such frequent relapses having a proportion of almost 36.7%, there is need to evaluate such regimens in India.

Currently, there is no reliable method for genotyping to differentiate between relapse and new infection. This is supported by the observation of Imwong *et al*²³, that most of the relapses are caused by hypnozoites which are genetically heterologous to the parasite which caused the primary infection. However, the first relapse of malaria as observed in infants is very commonly caused by homologous hypnozoites²⁴. The reason for heterologous relapse may be that the relapses are caused by hypnozoites which are derived from genetically different sporozoites inoculated by infected mosquito previously^{16, 23–25}. Even a recent study from India, compared two different methods of genotyping for differentiating relapse from reinfection and discordance in results was observed¹⁶.

Chloroquine continues to be efficacious in *P. vivax* malaria in India²⁶. Hence, the recurrence we observed might be due to either re-infection or relapse rather than drug failure, as all the recurrences occurred after Day 28.

Limitation

The patients could not be followed up actively in this study. Instead, they were asked to report in case they develop fever. Hence, the actual recurrence rates could be higher than reported.

CONCLUSION

The study showed presence of *P. vivax* malaria with both short and long latency recurrences (relapses) in Delhi. This highlights the need to evaluate primaquine regimens against both these strains and formulate strategies to improve compliance to 14-days primaquine.

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