Am. J. Trop. Med. Hyg., 59(1), 1998, pp. 175–179

STUDIES ON THE *PLASMODIUM VIVAX* RELAPSE PATTERN IN DELHI, INDIA

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Abstract. A five-year epidemiologic study of patients attending a malaria clinic in Delhi was conducted to find the relapse rate of infections with *Plasmodium vivax,* its seasonal correlation between the primary infection and subsequent relapses, the duration of the incubation period, and the patterns of relapse. By our definition, the relapse rate ranged from 23% to 44% depending on the duration of follow-up. The relapse pattern observed in the study clearly suggests the existence of both tropical and temperate zone types of *P. vivax* in the population characterized by distinct incubation periods and the possible existence of *P. vivax* subpopulations characterized by primary long incubation periods. The implication of different incubating forms of *P. vivax* on the epidemiology and control of malaria is also discussed.

Plasmodium vivax malaria constitutes about 60–65% of total malaria cases in India with, pronounced morbidity particularly in the economically weaker sections of the society.[1] The clinicoepidemiologic picture of *P. vivax* is not well understood due to the phenomenon of latency/relapse. Due to the persistence of the hepatic or hypnozoite form of the parasite, relapses occur in *P. vivax* infections and it is difficult to predict their timing.[2] *Plasmodium vivax* exhibits two primary types of incubation/relapse patterns that apparently depend on their tropical or temperate zone origin.[3–5] The classic example of the tropical type is the Chesson strain (New Guinea-South Pacific), characterized by an early primary attack, followed by a short latent period before appearance of frequent relapses during the next year or more, whereas the St. Elizabeth (United States) strain of the temperate type exhibits an early primary attack, followed by a long latent period of 6–14 months, and thereafter succeeded by a series of relapses at short intervals.

The present study is an attempt to understand the composition of *P. vivax* populations exhibiting different types of incubations in relation to the phenomenon of latency and relapses to elucidate their transmission dynamics for planning vector control strategies and chemotherapeutic measures in *P. vivax* foci.

MATERIALS AND METHODS

Study site. The malaria clinic of the Malaria Research Centre, at 2-Nanak Enclave, Delhi is located in northeastern Delhi. The clinic attracts patients mostly from 8–9 periurban villages that are 4–5 km from the clinic and have an area of approximately 25 km^2. The Yamuna River is located approximately 3–4 km from these villages. The inhabitants belong mainly to low socioeconomic strata and are employed in small-scale industries as laborers.

The climate of Delhi is divided into three distinct seasons: summer (April–June), monsoon (July–October), and winter (November–March). The average temperature, rainfall, and relative humidity during the three seasons are as follows: summer: 19.5–41.9°C, 0.1–71.4 mm, and 17–58%; monsoon: 18.5–39.6°C, 1–494.6 mm, and 34–83%; winter: 6.5–29.9°C, 0.3–123.5 mm, and 30–86%.

Malaria in Delhi is transmitted by *Anopheles culicifacies* and *An. stephensi.*[6–8] *Anopheles stephensi* breeding occurs mainly in the central parts of the city in a variety of sites such as pools, borrow pits (dried out pits), overhead tanks, temporary cement tanks, and ditches at construction sites. *Anopheles culicifacies* breeds in the periphery of the city, mainly in pools, ponds, borrowpits and river bed pools.

In Delhi, there is sporadic transmission of malaria from the end of April until the end of May that is interrupted during June. With the onset of the monsoon season in early July, active transmission resumes by the middle or end of the month, reaching a peak in September and ultimately terminating with the advent of winter in December. This has been repeatedly corroborated by vector incrimination studies.[7–10]

Diagnosis, treatment, and record of cases. The study was reviewed and approved by the Scientific Advisory Committee of the Malaria Research Centre. All patients were given an explanation of the drug schedule and verbal consent was obtained before the drug was administered.

Thick and thin blood smears of patients attending the malaria clinic were prepared by fingerprick, stained with Jaswant Singh and Bhattacharya stain,[11] and microscopically examined under an oil-immersion lens. All *P. vivax*-positive cases were treated only with 900 mg of chloroquine base (600 mg on day 0 and 300 mg on day 1; adult dose). The dose for children was adjusted accordingly. Primaquine was not given for the radical cure of *P. vivax* infection. The patients registered in the clinic were advised not to take any drugs, including antimalarials, from other sources if they subsequently got a fever; only after ensuring that these directions were followed were their subsequent visits recorded.

To determine the pattern of *P. vivax* relapse, each patient was identified individually by name, address, and subsequent treatment; other epidemiologic information, e.g., movement and social conditions, was also recorded. On reporting to the clinic, blood smears were collected from patients and examined microscopically for the presence of malaria parasites and each case was entered into the existing database of the clinic.

Cases recorded between July and December 1988 were followed up to five years while those recorded between January 1989 and December 1992 were followed for 1–4 years, i.e., until December 1993. Data entry and analysis was carried out using a computer program developed in a dBASE package.

The following criteria were used in classifying the patients into primary cases and nonrelapse and relapse categories in

TABLE 1

Plasmodium vivax (*Pv*)/*P. falciparum* (*Pf*) cases recorded at the Malaria Research Center clinic, Delhi

Month	1988*		1989		1990		1991		1992		1993		Total	
	Pv	*Pf*	*Pv*	*Pf*	*Pv*	*Pf*	*Pv*	*Pf*	*Pv*	*Pf*	*Pv*	*Pf*	*Pv*	*Pf*
Jan	–	–	6	3	10	3	3	1	8	2	13	4	40	13
Feb	–	–	6	3	14	3	5	1	9	0	22	2	56	9
Mar	–	–	18	1	15	1	9	0	11	1	33	3	86	6
Apr	–	–	40	0	15	0	37	0	13	0	52	0	157	0
May	–	–	76	0	47	0	69	0	45	0	87	0	324	0
Jun	–	–	121	1	60	0	70	0	47	0	138	1	436	2
Jul	3	0	102	0	84	0	120	0	75	0	160	0	544	0
Aug	29	5	117	0	101	4	104	2	153	9	347	2	851	22
Sep	83	3	133	2	125	5	139	3	234	12	600	9	1,314	34
Oct	140	9	60	4	114	8	140	19	187	31	441	16	1,082	87
Nov	69	15	22	3	53	10	50	8	65	29	243	11	502	76
Dec	22	5	11	3	11	3	18	4	22	9	52	7	136	31
Total	346	37	712	20	649	37	764	38	869	93	2,188	55	5,528	280

* Clinic started in July.

the present study. Patients reporting to the clinic for the first time (having no history of malaria) with acute illness and showing symptoms such as high fever, severe headache, loss of appetite, occasional vomiting, and microscopic evidence of *P. vivax* infection were considered primary cases. Some patients in this group who had no clinical symptoms of malaria or parasitologic evidence of *P. vivax* infection following their primary infection during the entire study period were considered nonrelapse cases. Those patients who reported back to the clinic within 1.5 months to one year with renewed clinical symptoms (mild) along with a periodic alternate day fever (not observed in the primary cases) and found to be microscopically positive for *P. vivax* infection were considered relapse cases.

The time interval between the primary attack (date of first attack with a confirmed *P. vivax*-positive smear) and first relapse (date of second attack) was calculated as lag months: 30.4 days was considered to be one month, 0.5 months to < 1.50 months (one lag month), > 1.51 to < 2.50 months (two lag months), and so on.

The variations in the relapse pattern with reference to mean lag month were measured by calculating the coefficient of variation (CV). CV = (SD/X) × 100, where X is the mean of the group and SD is the standard deviation.

RESULTS

Table 1 shows month and year data on *P. vivax* cases in the present study. The incidence of *P. falciparum* cases is also given in Table 1 to provide information on seasonality.

TABLE 2

Yearly distribution of *Plasmodium vivax* relapse patients

Year	*P. vivax* patients	Nonrelapse patients	Relapse patients	Relapse rate (%)	Follow-up (years)
1988	316	176	140	44.3	5
1989	487	340	147	30.2	4
1990	497	365	132	26.6	3
1991	524	375	149	28.4	2
1992	669	513	156	23.3	1
Total	2,493	1,769 (70.96%)	724	29.04	

Plasmodium vivax infections were predominant and were recorded in all months of the year, with a similar seasonal pattern during the five-year study period. They showed a gradual increasing trend from April onwards, reaching a peak in September soon after the rainy season, and then decreasing sharply to very low levels in December. *Plasmodium falciparum* started appearing in August, and showed a peak in October–November and decreased sharply with the onset of winter.

Table 2 shows yearly analysis of *P. vivax* patients, nonrelapse versus relapse patients, and the relapse rate (%) with different follow-up durations. In 1988, the total number of *P. vivax* patients was 316. Of these, 176 did not have any further relapses, whereas 140 patients had relapses in five-year follow-up study, giving a relapse rate of 44.3%. Similarly, for the years 1989–1992, the relapse rates calculated were 30.2%, 26.6%, 28.4% and 23.3%, respectively, and 29.04% for the five-year period.

Table 3 shows the frequency distribution of 724 patients with relapses identified in the present study. Of these, 442 patients (61.05%), which comprised the largest group, had only one relapse, 25.55% had two, 7.18% had three, and the remaining 6.22% had 4–13 relapses during the study period. The monthly relapse rate from January to December ranged between 17.64% and 36.92% for the five-year period.

Table 4 shows lag month analysis for patients with relapses (only the first relapse) within 1–12 months. Of a total of 724 relapse patients, the largest group of 582 patients (80.39%) had relapses within 12 months, 73 patients (10.08%) had them in the following year (13–24 months), 31 patients (4.28%) in the third year (25–36 months) of follow-up, and remaining 38 patients had relapses beyond 36 months (beyond 13 months is not presented in Table 4). Although the intervals between the primary attack and first relapse ranged widely, the most common intervals were 1–2 and 8–9 lag months.

Figure 1 shows a summary of patterns of relapse in *P. vivax*. The pattern was derived from the patient's lag month estimated from the time interval between the primary attack and the first relapse detected within 12 calendar months in 582 of 724 patients from the all relapsing categories (relapse 1–13, Table 4). Three distinct patterns emerged from groups

TABLE 3
Frequency distribution of *Plasmodium vivax* patients with relapses (five years pooled data)

No. of relapses	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total	% relapses
1	2	3	7	11	37	32	38	66	97	100	41	8	442	61.05
2	1	0	2	8	9	7	16	34	45	39	17	7	185	25.55
3				1	3	4	4	3	12	15	9	1	52	7.18
4				0	1	1	5	2	6	3	4	1	23	3.18
5				1	2	2	1	2	5	1	1	1	16	2.21
6								1		2		1	4	0.55
11										1			1	0.14
13								1					1	0.14
Total	13	17	28	68	158	195	247	388	605	525	195	54	2,493	
Relapses	3	3	9	21	52	46	64	109	165	161	72	19	724	
% relapses	23.07	17.64	32.14	30.88	32.91	23.58	25.91	28.09	27.27	30.66	36.92	35.18	29.04	

I (CV = 92.2%), II (CV = 75.14%), and III (40.67%) representing 105 (18.0%), 132 (22.7%) and 345 (59.3%) patients with three (2.73 ± 0.24), five (5.18 ± 0.33), and seven (7.33 ± 0.16) lag months (mean ± SE), respectively. The CVs of groups I, II, and III were 92.2%, 75.14% and 40.67%, respectively, indicating that the variability with reference to the duration of lag months was maximum in group I, minimum in group III, and intermediate in group II.

The lag month analysis revealed that the majority of the primary cases registered during January to June relapsed within 1.5 ± 0.45 (mean ± SE) to 3.67 ± 1.51 lag months, while the cases recorded between September and December relapsed after 6.35 ± 1.17 to 7.74 ± 0.94 lag months. In cases recorded during July and August, an intermediate value was obtained, ranging from 4.38 ± 1.89 to 5.61 ± 1.62 lag months. These observations clearly suggest the existence of a polymorphic *P. vivax* population in this study area, characterized by three types of incubation periods following primary attack.

The *P. vivax* primary attack recorded in individuals who did not have any previous malaria history during nontransmission months, particularly from December to June, may be due to the infection acquired during the previous transmission period (July–November) and became clinically and parasitologically positive after a prolonged period, suggesting the existence of a *P. vivax* subpopulation characterized by a primary long incubation period.

DISCUSSION

In the present investigation, clinicoepidemiologic data on *P. vivax* over a five-year period have been analyzed to determine the relapse rate, life span of infection, correlation between the month of primary attack and subsequent relapses, duration of incubation period, relapsing pattern, and its implications on control of *P. vivax* foci.

On interpretation of the results, one may disagree with the differentiation of primary attack versus relapse or reinfection, particularly during the peak transmission season. However, in the absence of any clinical or parasitologic marker, the following observations are considered as very relevant.

In the present study, malaria cases detected between December and June (the supposed nontransmission season) could be grouped in three categories: 1) infections acquired in the previous transmission season, i.e., between July and November but remained undetected and thus untreated; 2) infections acquired during the previous transmission season that were detected, treated, and subsequently reappeared (relapse); or 3) infections acquired during the previous transmission season that became clinically and parasitologically positive after a prolonged period (delayed primary attack). It may be pointed out that the community studied was sensitized and made health conscious through health education; as a result, even patients with mild symptoms with or without fever reported to our clinic for blood examination. It was observed that in relapse cases clinical symptoms were noticeably milder compared with those observed in the primary attack and the delayed primary attack, in which they were found to be acute. In addition, the periodicity of the fever in relapsed patients was typically tertian from the very onset of the infection.

The study area in Delhi is under the influence of two malaria vectors: *An. culicifacies* and *An. stephensi*. *Anopheles culicifacies* is a monsoon-associated species and is predominantly involved in transmission from July to November, whereas *An. stephensi* is an intradomestic species that is found nearly throughout the year but it attains epidemiologic significance only during the monsoon and post-monsoon periods when climatic factors are favorable. This ensures its longevity and completion of sporogony, which is probably affected during the summer and winter months. Sporadic transmission by *An. culicifacies* may occasionally occur during April–May depending on the amount of winter rain occurring in the previous year. This phenomenon, however, is localized and restricted to microhabitats of riverine belts only.

The likely malaria transmission season and transmission potential of these two vectors was aptly supported by several vector incrimination records of Delhi and nearby areas in which both *An. culicifacies* and *An. stephensi* were found to be sporozoite-positive from July to November,[6–10] whereas between December and June, *An. culicifacies* was incriminated only twice.[7] Furthermore, longitudinal vector incrimination studies carried out in the study area where sporozoite-positive vectors were found only from July to November, and no mosquitoes were found to be positive from December to June (Adak T, unpublished data).

Although the possibility of reinfection, particularly during the main transmission season could not be ruled out, various longitudinal vector incrimination data provide enough cir-

TABLE 4

Lag month analysis of *Plasmodium vivax* first relapse (1–12 months follow-up, five years pooled data)

Reporting month	Total relapses	Relapses first year	Time lag (in months) of first relapse												Total	Mean	SE	SD	CV(%)*
			1	2	3	4	5	6	7	8	9	10	11	12					
Jan	3	3	1	1		1									3	2.33	1	1.53	65.47
Feb	3	3	0	2					1						3	3.67	1.51	2.89	78.73
Mar	9	6	3	3											6	1.5	0.45	0.55	36.51
Apr	21	17	5	2	5	2	1	1	1						17	2.94	1.06	1.82	61.85
May	52	43	17	16	1	4	3	1		1					43	2.26	1.08	1.62	71.8
Jun	46	33	10	12	4	2							1	4	33	3.42	1.99	3.69	107.8
Jul	64	45	9	16	4	3	1			1	2	2	3	4	45	4.38	1.89	3.94	90.1
Aug	109	87	15	17	7	3	3	1	4	8	10	8	9	2	87	5.61	1.62	3.83	68.31
Sep	165	130	14	13	5	3	2	5	7	23	23	19	8	8	130	7.08	1.31	3.48	49.12
Oct	161	137	5	5	3	4	5	8	21	25	29	18	8	6	137	7.74	0.94	2.6	33.62
Nov	72	61	2	1	1	7	4	5	8	14	9	4	4	2	61	7.25	0.95	2.56	35.33
Dec	19	17		2	2	1	2	1	3	2	1	1	2		17	6.35	1.17	2.96	46.54
Total	724	582	81	90	32	30	21	22	45	74	74	52	35	26	582	6.02	1.47	3.6	59.85
Percentage			11.18	12.43	4.42	4.14	2.9	3.04	6.22	10.22	10.22	7.18	4.83	3.59	80.39				
Cumulative percentage			0	23.61	28.03	32.17	35.07	38.11	44.33	54.55	64.77	71.95	76.78	80.39					

*CV = coefficient of variation.

cumstantial evidence to conclude that real transmission from December to June is probably occasional and at a very low level, if it occurs. Therefore, the majority of cases detected during the supposed nontransmission period with a definite history of malaria were considered to be relapses rather than reinfections, whereas those detected with a history of malaria were considered delayed primary attacks. However, some uncertainty still exists regarding the possibility of reinfection during the supposed nontransmission season, which probably will persist until some diagnostic tool is developed that can distinguish the primary attack and subsequent relapse from reinfection. In spite of this limitation, the data obtained are still being used to understand the relapse characteristics of *P. vivax* infections in this study area, and this is the basis on which the data were analyzed.

Based on the foregoing epidemiologic features, three distinct relapse patterns were observed in the present study, and it can be concluded that the *P.* vivax population in northern India is polymorphic. Group I is the tropical or Chesson strain type of relapsing *P. vivax* with a short period of latency between the primary attack and the first relapse, which is similar to other Southeast Asian strains such as those from Thailand and Vietnam. Group III is the temperate or St. Elizabeth–type strain that has a long period of latency between the primary attack and the first relapse. Group II is intermediate between these two types.

Similar studies on the characterization of relapsing patterns of *P. vivax* strains by Contacos and others[12] and by Mason[13] in Central America revealed the the presence of only one type of relapse pattern conforming to the temperate zone type, which is characterized by an early primary attack followed by a long latency before appearance of frequent relapse activity. In the present study, *P. vivax* relapse patterns distinctly differ from the pattern reported by Bray and Garnham[14] in the northern Indian strain, in which only the temperate zone pattern was observed, whereas we noticed two distinct types, i.e., the temperate type and the tropical type, with possible existence of a third type. However, it may be pointed out by Bray and Garnham[14] did not report the origin of the strain, the season/duration of the study, and size of the sample on which the strain was characterized.

It was quite evident from the duration of follow-up study (1–5 years) that the duration of a *P. vivax* infection is at least three years, although in some patients infection has appeared to last more than three years, in which the probability of reinfection is greater. The average relapse rate was 29.04% in the five-year pooled data and ranged from 23.3% with a one-year follow-up to 44.3% with a five-year follow-up. Based on pooled data, the most valuable information obtained was that 70.96% of the *P. vivax*-infected patients never had a relapse after their primary infections. The data of such a large population with more than 70% nonrelapse, if some of the assumed relapse cases were actually primary infections, is very relevant in reference to their radical treatment. Therefore, the present drug policy of the National Malaria Eradication Program for the administration of primaquine (15 mg of base, once a day for five consecutive days) for radical cures of *P. vivax* infection warrants reconsideration.

The mechanism of long survival of *P. vivax* during nontransmission months through primary long incubation and relapses actually ensures transmission in the next season

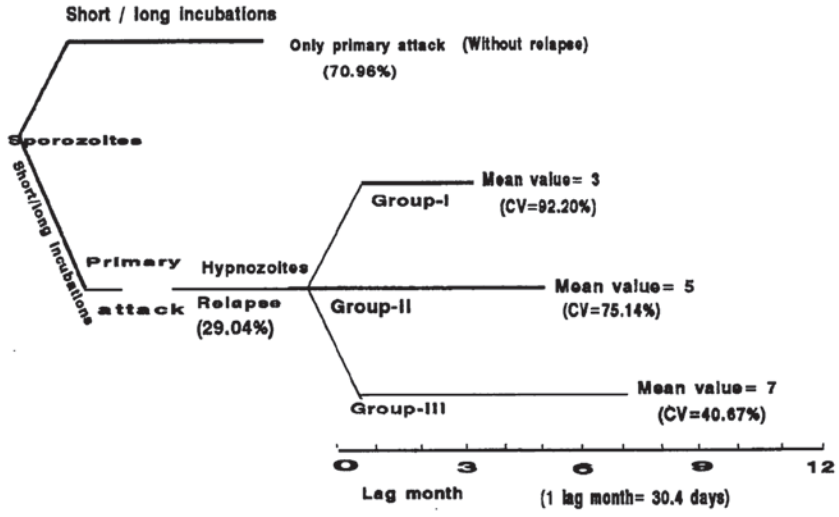

FIGURE 1. Patterns of incubation interval in *Plasmodium vivax*. Group I = primary attack between January and June (18.0%, n = 105); Group II = primary attack between July and August (22.7%, n = 132); Group III = primary attack between September and December (59.3%, n = 345). CV = coefficient of variation.

through the existence of a reservoir of *P. vivax*. It is assumed that the primary long and late relapse mechanism of *P. vivax* malaria might have evolved to survive into the next transmission season and be dormant to avoid the host-immune response. The data on *P. vivax* from Delhi is consistent with the hypothesis of overwintering of the parasite.[13]

In view of this information, it is suggested that the frequency distribution/ratios of different parasite forms responsible for different relapse patterns should be determined in different *P. vivax* ecosystems with reference to space and time, which are probably not constant and likely to be time dependent. In addition, the degree to which these parasite subpopulations interact with each other will no doubt have an impact on the maintenance of genetic diversity and regulation of the parasite population as a whole. However, in the absence of parasitologic and clinical markers, it may be difficult to characterize these forms. Perhaps amplification of specific DNA sequences by the polymerase chain reaction using specific oligonucleotide probes from different parasite isolates of relapsing and nonrelapsing patients could be used to analyze the genetic diversity of the *P. vivax* population and correlate this with epidemiologic findings. Therefore, there is a strong need for integrated laboratory and field studies as well as the use of mathematical models to interpret the complex transmission dynamics of *P. vivax* so that appropriate malaria control strategies, including chemotherapeutic measures, can be devised.

Acknowledgments: We express our sincere gratitude to Dr. N. N. Singh and Dr. D. S. Choudhury for critical and constructive suggestions. The diligent efforts of Kesab Pramanik, V. K. Soni, Pritam Singh, and Sarabjeet Kaur in compilation of data, and Ballabh Kumar, R. K. Srivastav, and Dr. Unni Kumar for data entry and computer analysis are also acknowledged.

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