Malaria Parasite Bank

Malaria Parasite Bank established in 1992 is a supporting unit for research activities on different aspects of malaria. The main objective of establishing this facility is to strengthen researches at MRC and to establish a national resource of malaria parasites. The function of the parasite bank is to collect, cultivate, characterize and store these parasites for various studies. Research activities at MRC, other research organizations and universities on parasite biology, host-parasite interaction studies, immunochemistry, understanding drug resistance mechanisms, drug development and screening of various antimalarials got a big help from this facility. Malaria parasite bank is an unique setup of its kind in India.

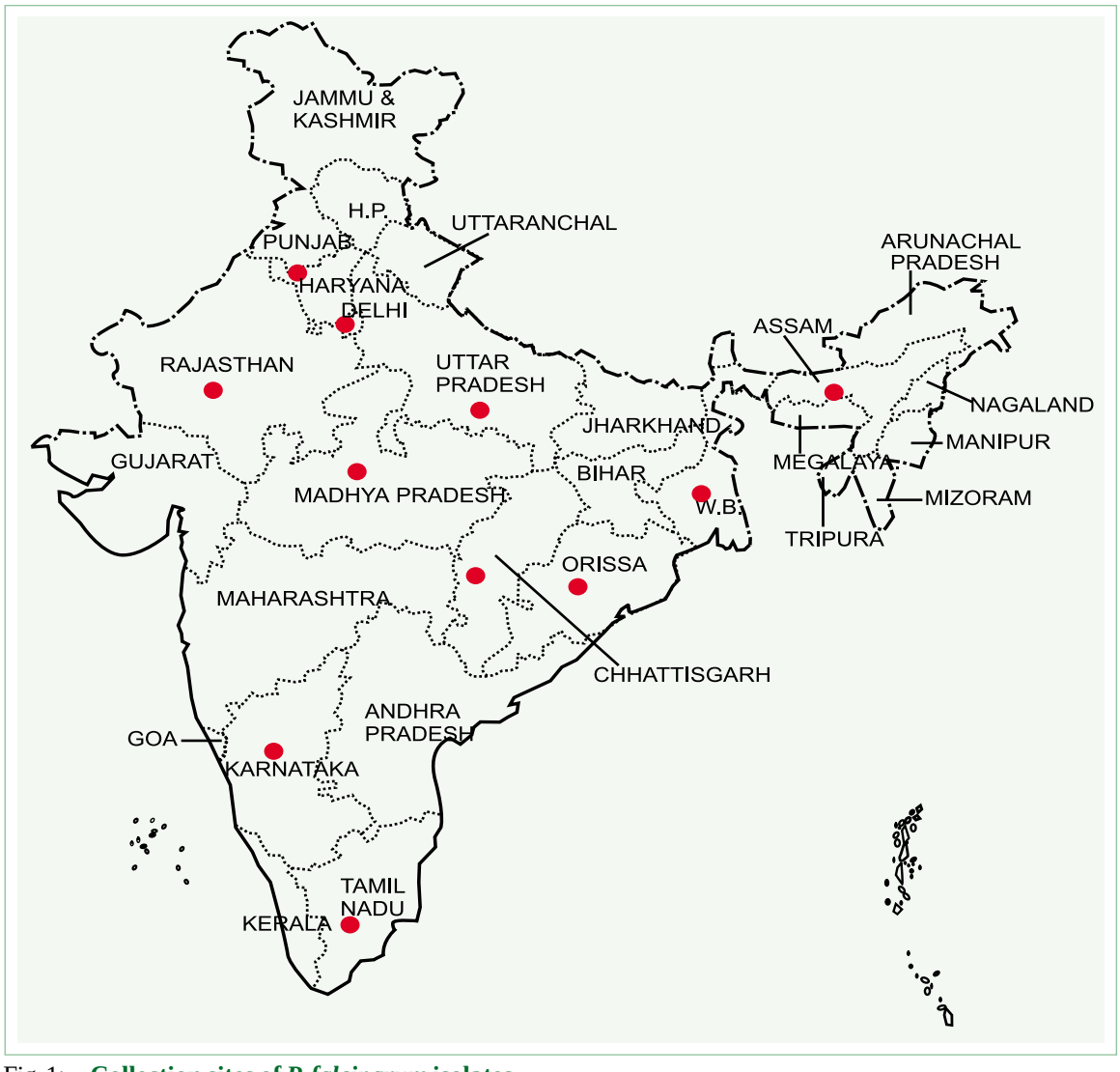

Fig. 1: **Collection sites of *P. falciparum* isolates**

Malaria Parasite Bank is a National Resource for both Human and Nonhuman Malaria Parasites

The parasite bank hosts human plasmodia—*P. falciparum, P. vivax, P. malariae* along with different species of nonhuman plasmodia. At present, the bank has 600 *P. falciparum* isolates collected from different geographical areas (Fig. 1), of which 188 isolates were adapted in culture. About 201 of these isolates were tested for their sensitivity to chloroquine of which 129 were found to be resistant to chloroquine. In addition, parasites characterized for enzyme electrophoretic variations, size variation at MSP-1, MSP-2 and GLURP antigen with family grouping analysis, with different binding properties to various molecules (ICAM-1, CD36), rosetting capacities, and which can use alternative invasion pathways, etc. are also available at the bank (Tables 1-3).

Table 1. Biological Material available at the Parasite Bank

Human Plasmodia	• Nonadapted cryopreserved isolates of *P. falciparum, P. vivax* and *P. malariae* • Sera/plasma from infected patients

P. falciparum	• Adapted/characterized isolates
	• Different stages of the parasite from culture
	  • Merozoites (from culture supernatant)
	  • Ring (by synchronization)
	  • Gametocytes (by Hypoxanthine treatment)
	  • Free parasites for antigen preparation (by Saponin lysis and ultrasonication)

P. vivax	• Sporozoites harvested from artificially fed mosquitoes
Nonhuman Plasmodia	• Different species of avian, simian and rodent plasmodia • Rodent plasmodia infected rats/mice • Sera/plasma from respective vertebrate hosts
Cell Lines	• Hepatoma cell line: Hep G2 A16 used in the *in vitro* cultivation of pre-erythrocytic stage malaria parasites • Myeloma cell line: SP2 • Hybridomas: 2A 10 (anti-*P. falciparum* sporozoite antibody secreting cells) 2 F2 1 A7 (anti-*P. vivax* sporozoite antibody secreting cells)

Table 2. Details of *P. falciparum* isolates collected and adapted *in vitro*

Place of collection	No. of isolates collected	Adapted/Cryopreserved
Delhi	175	70
Ghaziabad (Uttar Pradesh)	27	22
Shankargarh (Uttar Pradesh)	39	27
Baharaich (Uttar Pradesh)	21	–
Gautam Budh Nagar (Uttar Pradesh)	39	–
Shahjahanpur (Uttar Pradesh)	6	6
Mandla (Madhya Pradesh)	23	15
Jagdalpur (Chhattisgarh)	14	6
Sonapur (Assam)	25	2
Rourkela (Orissa)	33	9
Rameswaram (Tamil Nadu)	1	1
Jaisalmer (Rajasthan)	39	27

Contd...

Table 2. *Contd...*

Place of collection	No. of isolates collected	Adapted/Cryopreserved
Bharatpur (Rajasthan)	35	1
Alwar (Rajasthan)	25	–
Nuh (Haryana)	25	2
Kolkata (West Bengal)	19	–
Visakhapatnam (Andhra Pradesh)	12	–
Bissam Cuttack (Orissa)	22	–
Total	**580**	**188**

Table 3. Details of characterized *P. falciparum* parasites

Species/Strains of parasite	No. of isolates
Adapted isolates susceptible to chloroquine	54
Adapted isolates resistant to chloroquine	52
NF-54: an infective gametocyte producing strain of *P. falciparum*	1
3D 7A : a clone of NF-54	1
A-4 : a clone with binding property to CD36	1
Dd2: a clone which can invade trypsin treated erythrocytes	1
Field isolates which can invade trypsin treated erythrocytes	3
Field isolates which can invade neuraminidase treated but not trypsin treated erythrocytes	3
Field isolates which can invade normal erythrocytes but not neuraminidase or trypsin treated erythrocytes	3
Field isolates which can invade both neuraminidase treated and trypsin treated erythrocytes	5
Field isolates which can form rosettes	3
Field isolates which can bind to CSA	1
Field isolates which can bind to CD36	9
Field isolates which can bind to ICAM-1	2
Isolates with isoenzyme profile of GPI, GDH, ADA and LDH markers	22
Isolates with MSP-1, MSP-2 and GLURP markers	40

In addition to human malaria parasites, rodent parasites (*P. berghei*, *P. yoelii*, *P. vinckei petteri* and *P. chabaudi*) and avian parasite, *P. gallinaceum* are being maintained at the bank (Table 4). To keep the infectivity of these parasites, the parasites are cyclically transmitted through suitable vector species in the laboratory.

Cultivation of *P. vivax*

In addition, parasite bank has facilities for *in vitro* cultivation of *P. vivax* parasite.

Cultivation of Pre-erythrocytic Stages of P. vivax

Procedures were standardized to develop pre-erythrocytic schizonts *in vitro* in hepatic cells (Fig. 2).

Table 4. Nonhuman malaria parasites available at the Parasite Bank

Parasite species	Source	Susceptibility to antimalarials
Simian malaria		
P. cynomolgi bastianelli	NICD, Delhi	Not done
P. knowlesi	–do–	–do–
P. fragile	CDRI, Lucknow	–do–
Avian malaria		
P. gallinaceum	NICD, Delhi	Not done
P. relictum	Wild, Delhi	–do–
Rodent malaria		
P. berghei NK-65	PGI, Chandigarh	Not done
P. berghei NK-65*+	CDRI, Lucknow	CQ sensitive
*P. berghei**	–do–	CQ resistant
P. berghei	–do–	Quinine resistant
P. chabaudi	INSERM, Paris	Not done
P. vinckei petteri 279 BY	–do–	–do–
P. yoelii yoelii 265 BY**	–do–	–do–
*P. yoelii nigeriensis***+	LSHTM, London	–do–
P. yoelii nigeriensis	CDRI, Lucknow	Multi resistant
P. yoelii	ICGEB, New Delhi	Not done

*Oocyst positive in *An. stephensi;* **Oocyst and sporozoite positive in *An. stephensi;* +Infective gametocyte producing strain.

For the first time in India, *P. vivax* pre-erythrocytic schizonts were developed in hepatoma cell line. MRC has well established insectary facilities for the production of sporozoites in the laboratory by artificial feeding of mosquitoes. These sporozoites from artificially fed mosquitoes were used for inoculating the hepatocytes/hepatoma cell line for the development of pre-erythrocytic stage parasites.

Cultivation of Erythrocytic Stages of P. vivax

After the successful *in vitro* continuous cultivation and adaptation of *P. falciparum* in 1976, several attempts have been made to cultivate and adapt *P. vivax in vitro*, but this parasite could not be maintained in continuous culture as is being done for *P. falciparum* (Fig. 3). At MRC, attempts were made to cultivate erythrocytic stages of *P. vivax in vitro* in

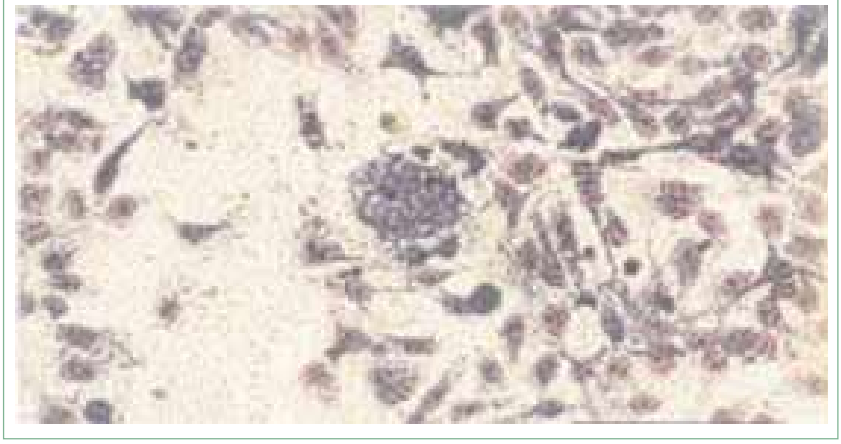

Fig. 2: **Geimsa stained pre-erythrocytic schizonts of *P. vivax***

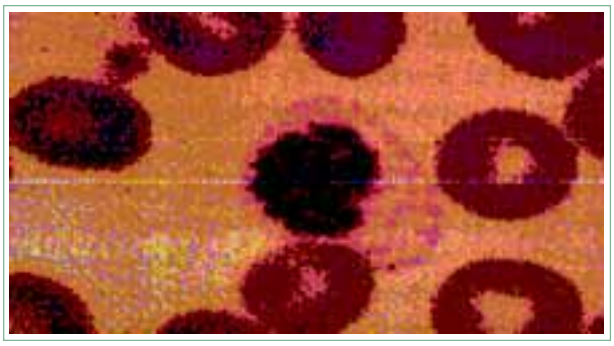

Fig. 3: **Erythrocytic stages of *P. vivax***

different combinations of media and culture conditions. A low level of parasitaemia could be maintained up to 52 days and healthy growth of the parasites was observed for 2–3 cycles (7–8 days). Even though adaptation of the parasite *in vitro* is extremely difficult, short-term *in vitro* cultivation of erythrocytic stages of *P. vivax* has been accomplished (Usha Devi *et al*., 2000). The *in vitro* culture system could be used for studies on parasite metabolism, testing of antimalarials, vaccine development, etc. The method for *in vitro* chloroquine sensitivity testing of *P. vivax* has also been standardized. ■