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## Status of Plasmodium Falciparum and Vivax in Jharkhand: A Five Year (2004-08) Retrospective Study at Rajendra Institute of Medical Sciences, Ranchi

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### ABSTRACT

Malaria is well known for its fatalities worldwide. In India, it is still endemic in many areas where two species of Plasmodium namely *Plasmodium vivax* and *Plasmodium falciparum* are reported. *P. vivax* is widespread, creating lots of morbidities across the country. *P. falciparum*, on the other hand, though comparatively narrow in its infectious volume, is a serious cause of mortalities in India. A five year survey was conducted from 2004 to 2008 in a high malaria-hit district, Ranchi. Thick and thin blood smears were made at the Department of Clinical Pathology, Rajendra Institute of Medical Sciences (RIMS), where the microscopic examinations were carried out. The overall reported and examined cases at RIMS included 36643 suspected malaria cases, out of which, 21833(59.5%) were found positive. Out of these positive cases, 6842(31.3%) were confirmed as *P. falciparum* patients and 14991(68.6%) as *P. vivax* cases respectively. Number of negative cases was 14811 (40.4%). In this study, it was observed that after the year 2005, incidence of malaria suddenly dropped by 50% and remained almost static on the same level in the following years with only some seasonal variations. However, it was observed that *P. falciparum* steadily became more dangerous. It is therefore highly necessary to take immediate and effective measures to minimize the complications of *P. falciparum* along with *P. vivax* to prevent death toll in these areas.

**Keywords:** Malaria, *Plasmodium falciparum*, *Plasmodium vivax*

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## INTRODUCTION

Jharkhand is surrounded by Bihar, West Bengal, Odissa & Chhattisgarh and is highly endemic for malaria. RIMS, Ranchi has registered a big number of malaria cases during the past few years due to which this study was planned and conducted from 2004 to 2008. Human race has been struggling against these parasites for centuries and *Plasmodium* is still one of the most threatening, causing malaria worldwide (Schlitzer, 2007). Globally, 243 million malaria cases are reported every year. More awakening figures tell us that approximately 8, 63,000 deaths were caused by malaria in 2008 (WHO, 2009a). In Eastern Mediterranean Region (EMR), there were 18.4 million confirmed malaria cases (WHO, 2012a). In India, two species of *Plasmodium* are rife: *P. vivax* (74%), the most prevalent and *P. falciparum* (26%), the most dangerous (WHO, 2009). Main factors involved in its transmission are the improper diagnosis and control measures taken against vectors species. The results of the retrospective analysis not only provide a more accurate, baseline estimation of the burden of malaria in an urban area of India but also clearly indicate the need for a much more efficient health-information system, for recording and managing malaria in such a setting.<sup>1</sup> With the prevailing situation of malaria in perspective, this study was designed to assess the prevalence of *P. falciparum* in the study subjects.

### Literature Review

A Cross-sectional survey was carried out in health care facilities at four locations: two urban areas (Libreville and Port-Gentil), one semi-urban area (Melen) and one rural area (Oyem), between 2005 and 2011. Febrile paediatric patients, aged less than 11 years old were screened for malaria using microscopy. Between 2005 and 2008, malaria prevalence dropped significantly from 31.2% to 18.3%, followed by an increase in 2011 in Libreville (24.1%), Port-Gentil (6.5%) and Oyem (44.2%) ( $p < 0.01$ ). The malaria burden in the country highlights the importance of maintaining various malaria control strategies and redefining their implementation.” [2] “The primary focus of malaria research and control has been on *Plasmodium falciparum*, the most severe of the four *Plasmodium* species causing human disease. However, in presence of both *P. falciparum* and *Plasmodium vivax* occurs in several countries, including India. From this, we determined that increased use of bednets contributed the most to case reduction. During the enhanced control period, the best model predicts that *P. vivax* is out-competing *P. falciparum* [3]. However, the reproduction numbers are extremely close to the invasion boundaries. This procedure, applied to the enhanced control period, revealed that the best model predicts that *P. vivax* outcompeting *P. falciparum* is the most likely outcome, whereas the remaining candidate models predict the opposite. Moreover, the predictions of the top model are counter to what one expects based on the case data alone. Although the proportion of cases due to *falciparum* has been increasing, the best fitting model reveals that this observation is insufficient to draw conclusions about the long term competitive outcome of the two species.” We estimate that the global burden of malaria due to *Plasmodium vivax* is approximately 70-80 million cases annually [4]. Because malaria transmission rates are low in most regions where *P. vivax* is prevalent, the human populations affected achieve little immunity to this parasite; as a result, in these regions, *P. vivax* infections

affect people of all ages. Although the effects of repeated attacks of *P. vivax* through childhood and adult life are only rarely directly lethal, they can have major deleterious effects on personal well-being, growth, and development, and on the economic performance at the individual, family, community, and national levels. Features of the transmission biology of *P. vivax* give this species greater resilience than the less robust *Plasmodium falciparum* in the face of conditions adverse to the transmission of the parasites" [5]. There are various other factors begetting the increasing incidence of *P. falciparum* in Jharkhand ; one of them is the people dwelling here from surrounding states for doing business or visiting relatives . Surrounding states are endemic for *P. falciparum* (WHO, 2001). Carme B, Demar M.et al. 2013, January "Between 2000 and 2010, 12,254 bouts of malaria were confirmed at the Parasitology Laboratory of CHG: *P. vivax*: 56.2%, *P. falciparum*: 39.5%, co-infection with both species: 3.4%, *P. malariae*: 0.9%. HP was observed in 262 cases, at a frequency of 4.9% for *P. falciparum* and only 0.041% for *P. vivax*, with no recorded cases for *P. malariae*". [6].

This study describes patterns of *falciparum* and *vivax* malaria in a private comprehensive-care, multi-specialty hospital in New Delhi from July 2006 to July 2008. Malarial morbidity by *Plasmodium* species (*Plasmodium falciparum* or *Plasmodium vivax* were confirmed using microscopy and antigen tests [7]. The influence of seasonal factors and selected patient demographics on morbidity was evaluated. The national data reported a smaller proportion of malaria cases caused by *P. falciparum* in the national capital region than was observed in a private facility within the region. *Plasmodium vivax* also caused a large proportion of the cases presenting clinically at the private hospital during the summer and monsoon seasons. The proportion of *P. falciparum malaria* cases tends to be greatest during the post-monsoon season while the proportion of *P. vivax malaria* cases tends to be greatest in the monsoon season. "The changing epidemiology of malaria since 1975 was studied in a tribal forested belt of central India, Chhattisgarh state, which is the second most highly malarious state in India [8]. Chhattisgarh, which accounts for 2% of the total population of the country, contributed more than 16% of the total malaria cases, 23% of *Plasmodium falciparum* and 7% of deaths due to malaria in the country. Retrospective analysis further revealed that, in 1975--76, *P. vivax* was the predominant species (58%); however, since 1979, *P. falciparum* showed a steady upward trend (50%), and in 2002. *P. vivax* reduced to 28%" 5

#### **India to see decrease in malaria cases: WHO [9]**

In the south-east Asia Region, Bhutan, Korea, Nepal, Sri Lanka and Thailand have registered decreases of 75 per cent or more in the incidence of microscopically confirmed malaria incidence rates between 2000 and 2011."India, the country with the highest number of cases in the region, is projected to achieve decreases of 50-75 per cent in malaria case incidence by 2015," it said. India, Nepal and Thailand could also potentially move from the "control" to the "pre-elimination" phase by continuing their progress, assuring that "all malaria cases are laboratory confirmed and including the private sector in the health reporting system."India has been taking measures like providing insecticide-treated mosquito nets (ITNs). The number of patients tested by microscopic examination increased to a peak of 171 million in 2011, with India accounting for over 108 million blood slide examinations. This report indicates



that international funding for malaria appears to have reached a plateau well below the level required to reach the health-related Millennium Development Goals and other internationally-agreed global malaria targets.

## MATERIALS AND METHODS

Blood samples were collected from the patients attending OPD, emergency and indoors who had malaria specific symptoms such as fever, persistent headache, vomiting, jaundice & others [10]. Finger pricked blood was used for slide preparation. Thick and thin blood smears were prepared and stained by Giemsa stain (WHO, 1991). Microscopic examinations of thick and thin films were carried out to identify *P. falciparum* & *P. vivax*.

**Thick films:** The thick film is first de-hemoglobinised in water and then stained with Giemsa.

**Rapid Giemsa:** Prepared a 10% Giemsa in buffered water at pH 7.1. Immersed the slide in the stain for 5 minutes. Rinsed gently for 1 or 2 seconds in a jar of tap water. Drained, dried and examined.

**Thin films:** (Thin film examination is the gold standard in diagnosis of malarial infection.)

**Leishman's stain:** Added 7-8 drops of the stain and left for 1-2 minutes. Then added 12-15 drops of buffered distilled water, mixed thoroughly, left for 4 - 8 minutes. Then washed off with clean water, drained, dried and examined.

**Jaswant Singh Battacharya (JSB) Stain for thick and thin films:** This is the standard method used by the laboratories under the National Malaria Eradication Programme in India.

**Staining technique:** After dehemoglobinisation, the thick smear is dipped in JSB II stain two to three times, washed by dipping in buffer water two to three times. Then kept dipped in JSB I stain for 40-60 seconds, washed with buffer water- drained, dried and examined.

### Established a parasite count by following methods

Either of the two methods were used, which were found to be convenient at the time of examinations to establish the parasite count.

#### Method 1: parasites per microlitre of blood

The number of parasites per microlitre of blood in a **thick film** was counted in relation to a standard number of leukocytes (8000) by using two tally counters, one to count parasites and the other to count leukocytes.

**Step 1**

(a) If, after 200 leukocytes were counted, and 10 or more parasites had been identified and counted, results were counted in terms of the number of parasites per 200 leukocytes.

(b) If, after 200 leukocytes were counted, and 9 or fewer parasites had been counted, we continued to count until we reached 500 leukocytes on our tally counter; then counted the number of parasites per 500 leukocytes.

**Step 2**

In each case, the number of parasites relative to the leukocyte count converted to parasites per microlitre of blood by the simple mathematical formula:

$$\frac{\text{Number of parasites} \times 8000}{\text{Number of leucocytes}} = \text{Number of parasites per microlitre}$$

Meaning that if 200 leukocytes were counted, the number of parasites was multiplied by 40 and if 500 leukocytes were counted the number of parasites was multiplied by 16.

**Method 2: the plus system**

A simpler method of counting parasites in thick blood films was used in the plus system. (Though this system was less satisfactory, this was used only when it was not possible to carry out the more acceptable count of parasites per microlitre of blood.)

Note: In mass screening in remote village areas this method is very useful & very easy to follow.)

The system entailed by using a code of between one and four plus signs, as follows:

- + = 1-10 parasites per 100 thick film fields
- ++ = 11-100 parasites per 100 thick film fields
- +++ = 1-10 parasites per single thick film field
- ++++ = more than 10 parasites per single thick film field

**RESULTS**

The present study was conducted during 2004 to 2008. Finger pricked whole blood specimens were taken from 36643 malaria suspected cases (ageing 1-60 years) in Rajendra Institute of Medical Sciences (RIMS), Ranchi.

The distribution of *P. falciparum* & *vivax* in the population is shown in Table-1.

Table -1: Tests done for MP from the year 2004 to 2008

Year	Total cases for screening	No. of Malaria positive cases	Percentage of Malaria positive cases	No. of P. Vivax cases	Percentage of P. Vivax cases	No. of P. Falciparum cases	Percentage of P. Falciparum cases	No. of Negative cases	Percentage of Negative cases
2004	9666	7849	81.2	4979	63.5	2870	36.5	1817	18.8
2005	11083	8265	74.5	6205	75.0	2060	25.0	2818	25.5
2006	6859	1862	27.1	1314	70.5	548	29.5	4997	72.9
2007	4539	2274	50.1	1592	70.0	682	30.0	2265	49.9
2008	4496	1582	35.1	901	56.9	681	43.1	2914	64.9
Total	36643	21833	59.5	14991	68.6	6842	31.4	14811	40.5

In the first year of the study, a total of 9666 suspected malaria cases were examined and of which 7849 were positive. Out of positive cases *P. falciparum* cases were 2870(36.5%) and *P. vivax* cases were 4979(63.5%). While in the 2nd year of study 11083 suspected cases were reported, of which 8265 were testified as positive malaria. Number of *P. falciparum* and *P. vivax* were 2060(25%) and 6205 (75%) respectively. In the 3<sup>rd</sup> year suspected patients reported for the examination were only 6859 out of which 1862 patients were found to be malarial positive. In the year 2006, though total cases for screening dropped to nearly 50% and total no. of positive malaria cases were 1862 but incidence of *P. Vivax* & *falciparum* remained up to 70.5 % & 29.5 % respectively. In the 4<sup>th</sup> year, total suspected cases for screening were 4539 and positive malarial cases were 2274. Out of which incidence of *P. vivax* was 1592 (70.0 %) and *P. falciparum* was 682 (30%). In the fifth year, there was a sharp rise in the spectrum of *P. falciparum* - 43.0%.

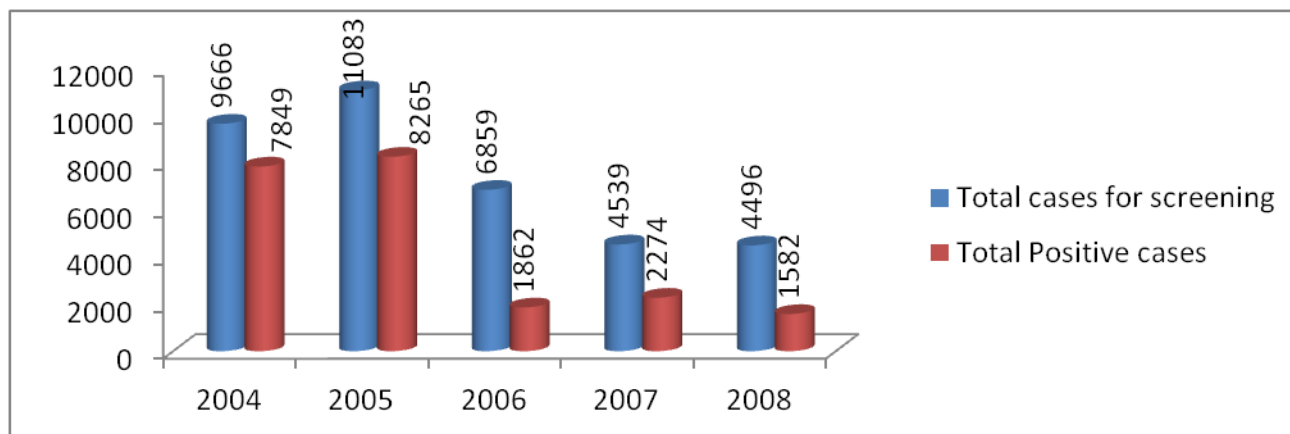


Figure1: Year wise distribution total cases for screening and total positive cases

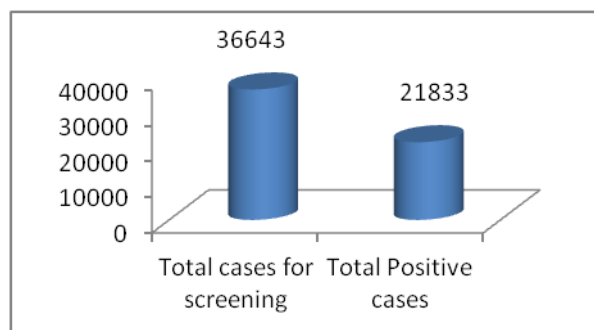


Figure2: Total positive cases

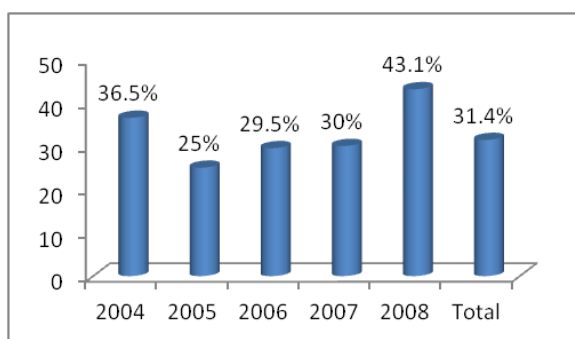


Figure3: Percentage of P. falciparum in year 2004-2008

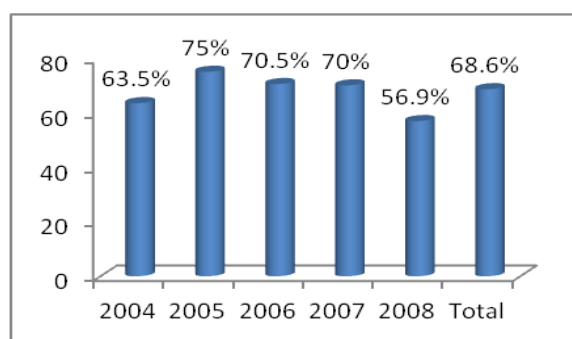


Figure4: Percentage of P. Vivax in year 2004-2008

### DISCUSSION

The overall reported cases at RIMS included 36643 suspected malaria cases, of which, 21833(59.5%) were positive. Out of positive cases, P. falciparum cases were 6842 (31.4 %) and P. Vivax cases were 14991(68.6 %). Numbers of negative cases were 14811 (40.4%) and similar finding was observed in retrospective epidemiological study, carried out in Ahmedabad city, having a population of about 3 million [2]. When the surveillance data and health records of the major public and private health facilities in the city were analyzed, for the period between 1991 and 1998, P. vivax was found to account for 69% of all malaria cases and P. falciparum for the other 31%” [8].

Incidence of P. falciparum slightly increased to 29.5% in 3<sup>rd</sup> year of the study, significantly higher than 2<sup>nd</sup> year indicator. This rapid rise might have been caused by the flood disaster in surrounding state of Bihar. Similar situation was reported from Sind during 2006 and from Baluchistan during 2009, where a sharp spike of malarial incidence was evidenced after floods (WHO, 2010b). All round the world, the wake of flooding epidemics are common as it has been reported from Costa Rica (1991), India (1999) and Dominican Republic (2004) (Singh et al. 1997; WHO 2010c).

### CONCLUSION

This study concludes that malarial infection is still very much common in Jharkhand. P vivax and P falciparum both are prevalent and responsible for huge losses in terms of morbidity



& mortality. Appropriate measures for proper reporting, surveillance, awareness campaigns / health education and control measures need be taken and strengthened at all levels so as to curtail the huge death toll as well as the economic losses out of this disease.

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