Int. J. Curr. Res. Biosci. Plant Biol. 2015, 2(7): 20-25

EXCELLENT PUBLISHERS

International Journal of Current Research in Biosciences and Plant Biology

ISSN: 2349-8080 Volume 2 Number 7 (July-2015) pp. 20-25

www.ijcrbp.com

Original Research Article

Molecular Detection and Epidemiological Studies of *Plasmodium vivax* and *Plasmodium falciparum* Isolates from Endemic Regions of Jharkhand, India

Abhash Sikdar[1]* and M. Raziuddin[2]

[1]Department of Zoology, Vinoba Bhave University, Jharkhand-825301, India
[2]Pro-Vice Chancellor, Ranchi University, Ranchi-834 001, Jharkhand, India

**Corresponding author.*

Abstract

Malaria is the most prevalent communicable disease and is hyper endemic throughout India. The complexity and magnitude of malaria in Central-Eastern part of India including Jharkhand, deserves a special attention as the central eastern states contribute 15-20% of the total malaria cases in the country (Draft on National Policy on Tribal by Govt. of India). Accurate information on the distribution and clinical prevalence of *P. vivax* and *P. falciparum* malaria in endemic areas is essential to develop integrated control strategies. To measure the impact of any intervention there is a need to detect malaria properly. Mostly, decisions still rely on microscopy diagnosis. But sensitive diagnosis tools enabling to deal with a large number of samples are needed. The molecular detection approach offers a much higher sensitivity and flexibility to be automated and upgraded. Here we present a systematic effort to diagnose the presence of *P. vivax* and *P. falciparum* at molecular level among the age stratified samples from endemic regions of Jharkhand, India.

Keywords

Age distribution
Geographical distribution
Malaria
Plasmodium species

Introduction

Malaria is a disease caused by *Plasmodium* parasites, and has a history as old as that of the human race. According to the 2008 report of the World Health Organization (WHO), half of the words population is at the risk of malaria; in 2006, 250 million cases were encountered with approximately 1 million deaths (WHO, World Malaria Report, 2008). There has been a resurgence of malaria, one of the most important public health problems in India despite on-going malaria control programmes. The province of Jharkhand in Eastern India is one such area where malaria is rampant. Moreover, the state of Jharkhand lies in the tropical zone with favourable geo-climatic and ecological conditions conducive for perennial malaria transmission.

In the natural and untreated intermediate hosts, *Plasmodium* infections are maintained for extended and often lifelong periods. Parasitemia generally reaches its highest peak after inoculation, and only rises thereafter during increasingly brief and interspersed episodes. As the host acquires tolerance to higher numbers of multiplying parasites, the severity and duration of the clinical episodes that coincide with the parasite peaks diminish.

Microscopic examination of blood has until recently been the sole rapid and practical method to detect and

identify *Plasmodium* parasites unequivocally. The main limitation of this method is the difficulty in detecting very low levels of parasites. In human infections, the probability of detecting the parasite diminishes rapidly as the parasitemia falls below 0.0005% or 25 parasites/microliter of blood. Medically this relative insensitivity is of little consequence because clinical episodes are only rarely associated with such low parasitemias. However, the fact that circulating parasites are undetectable throughout the major part of the infection is of some consequence to the epidemiological and biological perception of malaria (Brown et al., 1992; Snounou et al., 1993; Black et al., 1994; Roper et al., 1996).

Amplification of DNA by polymerase chain reaction (PCR) has provided the opportunity to devise highly sensitive methods of parasite detection (Jaureguiberry et al., 1990; Sethabutr et al., 1992; Barker et al., 1992; Snounou et al., 1993; Tirasophon and Panwyim, 1993; Tirasophon et al., 1994), and the specificity inherent to this method allows the unequivocal identification of the parasite species. The efficiency of the assay is markedly improved when a nested PCR strategy is adopted (Snounou et al., 1993; Arai et al., 1994; Khoo et al., 1996). In this strategy two rounds of amplification are carried out, with the product of the first reaction serving as a template for a second reaction where the oligonucleotide primers used hybridize to sequences contained with that product. In this manner, a single parasite genome can be detected routinely and reproducibly, and the sensitively then depends solely on the quantity and nature of the initial DNA template.

Materials and methods

Ethical statement

The present study was approved by the Institutional Ethics Committee of Vinoba Bhave University, Hazaribag (No.VBU/R/502/2011) and all participants gave informed consent.

Selection and description of study area

The province of Jharkhand in eastern India is one such area where malaria is rampant. Jharkhand had a yearly average slide positivity rate (SPR) for symptomatic individuals of 6 .8% over the last three years with *P.vivax* accounting for 44% of the cases (Malaria Control Program Annual Report Ranchi, Jharkhand, Directorate of Health Services; 2007). Study has been conducted in Jharkhand state emphasizing tribal dominant area as shown in Fig. 1, including Palamu- semi-urban mix population of tribal, rural and urban, accounting highest malaria prevalence and malaria associated morbidity as per the Malaria Control program Annual Report, Ranchi, Jharkhand, Directorate of Health Services; 2007 in Jharkhand, Latehar- rural and ethnic tribal population, geographically adjacent to Palamu, accounting severe cerebral malaria and death associated with malaria.

Fig. 1: Map showing the study area.

Bokaro, Ranchi district, were to represent an urban district with low transmission of malaria and the SPR in Ranchi district was 7.2% in 2005 (Ministry of Health and Family Welfare. National Vector Born Disease control Program (Government of India, 2008); Hazaribag, a semi urban district, lies in the tropical zone with an annual rainfall of 1234.5 mm with favourable geo-climatic and ecological conditions conducive for perennial malarial transmission. Hazaribag had a yearly SPR for symptomatic individuals of 7.3% over the last three years with *P. vivax* accounting for 84% of the cases(State Malaria Control Program Annual Report Ranchi, Jharkhand, Directorate of Health Services; 2007).Thus, the selected sites were meant to provide a reasonable representation of typical conditions that would be found in Jharkhand.

Blood sample collection

Blood specimens were collected from all age groups during different transmission periods of the year from positive cases of *P. vivax and P. falciparum* malaria, which had undergone clinical investigation and confirmed on the basis of clinical symptoms and parasite blood film examination. 2-3 spots(each containing about 100 microliters of blood) was collected on Whatman-3mm filter paper from 126 malaria symptomatic patients attending hospitals located in five different areas of Jharkhand. The spots were dried and brought to the laboratory.

Extraction of DNA

Routine protocols were followed to isolate malaria parasite DNA from the dried blood spots using QIA amp mini DNA kit (Qiagen, Germany).Only one blood spots for each sample was used to isolate DNA at a time. The DNA was eluted with Tris-EDTA buffer (pH 8.0).

Plasmodium species identification

The isolated DNA was subjected to diagnostic PCR (primary and nested). Using primary PCR, the *Plasmodium's* DNA was amplified using primers-Pla1 (5' TTA AAA TTG TTG CAG TTA AAA CG3') (Forward) and Pla2(5' CCT GTT GTT GCC TTA AAC TTC 3') (reverse) with the following cycling parameters: a 5 min initial denaturation step at 95^{o}C was followed by 35 cycles with a 1min denaturation step at 94^{o}C,a 1min annealing step at 55^{o}C,a 1 min extension step at 72^{o}C, and a final 7 min extension step at 72^{o}C. This primary PCR product was diluted 10 times and used as a DNA template for nested PCR to amplify *P. falciparum's* DNA using primers (P.fal-1 5'TTA AAC TTG TTT GGG AAA ACC AAA TAT3')(forward) and (P.fal-2 5'ACA CAA TGA ACT CAA TCA TGA CTA CCC GTC3') (reverse) and for *Plasmodium vivax*'s DNA, the primers used are (P.viv.1- 5'CGC TTC TAG CTT AAT CCA CAT AAC TGA TAC3') (Forward) and (P.viv.2- 5'ACT TCC AAG CCG AAG CAA AGA AAG TCC TTA3')(reverse) with the following cycling parameters: a 5 min initial denaturation step at 95^{o}C was followed by 35 cycles with 30-s denaturation step at 94^{o}C,a 30-s annealing step at 58^{o}C, a 1 min extension step at 75^{o}C, and a final 10 min extension step at 75^{o}C (Singh et al., 1999). The identification was further confirmed by carrying out agarose gel electrophoresis. A diagnostic band of 205bps and 102 bps in the gel showed the presence of *P. falciparum and P. vivax* respectively (Snounou et al., 1993).

Results and discussion

The use of PCR-based methods to detect malaria parasites in blood samples increase the sensitivity of detection compared to microscopy. Even the most skilful morphological analysis of stained parasites on blood film is not a very reliable basis for determining the identity of a malaria parasite species. Difficulties are compounded when infections contain more than one parasite species or when an unusual species is present. Qualitative PCR protocols that are robust, sensitive and species specific have been available since 1990s (Singh et al., 1999; Andrews et al., 2005) and there are now several quantitative PCR methods that allow estimation of parasitemia levels as well as positivity (Mangold et al., 2005; Rougemont et al., 2004).

Thick film microscopy can allow the examination of ~0.1 to 1 µl of blood and thus the detection of more than ~10 parasites µl^{-1}. Most applications of PCR typically involves amplification of DNA template from the equivalent of 1 to 10 µl blood and are thus either slightly more or up to 100 times more sensitive than microscopy. DNA template can be prepared from large volumes of blood to give even higher sensitivity, with detection of ~20 parasites µl^{-1} being achieved, which is useful in clinical vaccine trials in which the time to first detectable blood stage parasitemia is the end point. Sensitive PCR methods are better adapted to automation of the process and objective readings of results by machines. This

potential makes them a valuable option for large-scale epidemiological studies.

In the present study, we have successfully isolated genomic DNA of malaria parasites for all the 252 blood samples from malaria symptomatic patients. Out of these 252 samples, only 211(83.7%) were found to be infected with malaria parasites following PCR diagnostic assay (Table 1). As expected, majority of infections were due to *P. vivax* and only few cases were due to *P. falciparum* and mixed (Pv and Pf) infection. Taking all these five districts into consideration [Fig.1-5], Palamu (73.9%), Bokaro (73.7%) and Latehar (71.2%) showed considerably high Pv infections. As far as maximum Pf infection (19.4%) and mixed infection (12.9%) is concerned, Ranchi showed the higher prevalence comparatively. Apart from the geographical differences, the age differences have also found to affect the percentages of *P. vivax* and *P. falciparum* infections among males and females (Table 2).

Table 1. Distribution and prevalence of *Plasmodium Vivax* (Pv) and *Plasmodium falciparum* (Pf) infections among the clinical samples collected from the five centres/hospitals across the endemic regions of Jharkhand.

Locality	No. of samples	Total infection	No. of Pv infection	No. of Pf infection	No. of Pv and Pf infection
Hazaribag	54	40(74%)	27(50%)	8(14.8%)	5(9.3%)
Ranchi	62	54(87%)	34(54.8%)	12(19.4%)	8(12.9%)
Bokaro	38	30(78.9%)	28(73.7%)	2(5.3%)	0(0%)
Latehar	52	48(92.3%)	37(71.2%)	7(13.5%)	4(7.7%)
Palamu	46	39(84.8%)	34(73.9%)	4(8.7%)	1(2.2%)

Fig. 1: Relative distribution of Pv, Pf and mixed infections in Hazaribag.

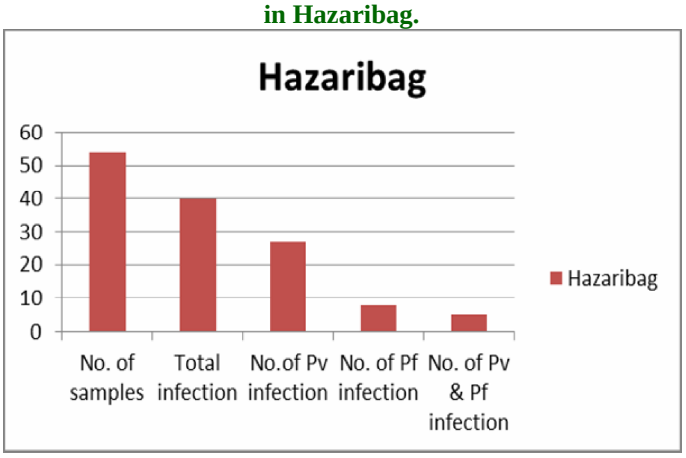


Fig. 2: Relative distribution of Pv, Pf and mixed infections in Ranchi.

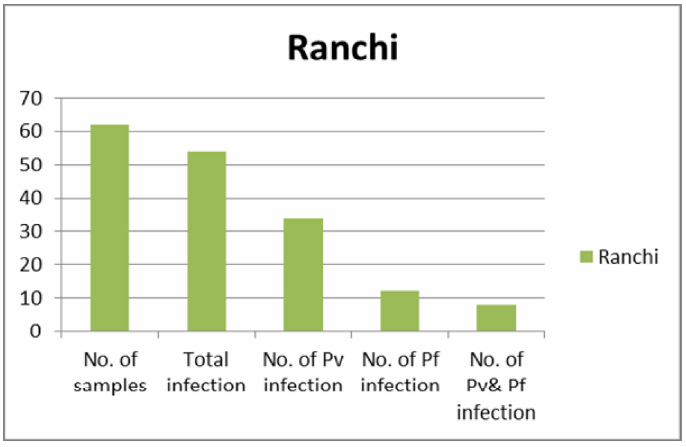


Fig. 3. Relative distribution of Pv, Pf and mixed infections in Bokaro.

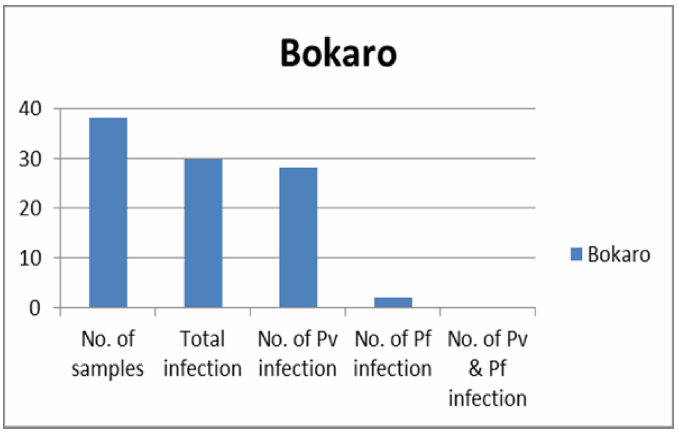


Fig. 4: Relative distribution of Pv, Pf and mixed infections in Latehar.

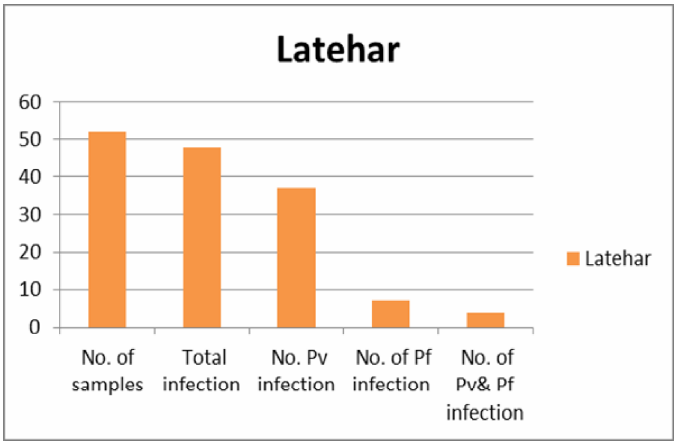
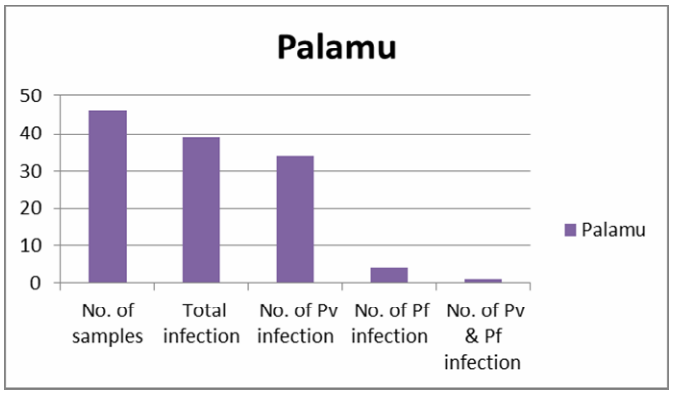


Table 2. Patient details and differential malaria species infection in males and females of different age groups in five regions of Jharkhand.

Sampling site (sample size)	Gender(sample size)	Age range(min-max)	No. of infected patients		
			Pv	Pf	Pv+Pf
Hazaribag (40)	Male (28)	(7 months-55 years)	18	7	3
	Female (12)	(2 months-77 years)	9	1	2
Ranchi (54)	Male (33)	(3 years-50 years)	23	7	3
	Female (21)	(5 months-60 years)	11	5	5
Bokaro (30)	Male (19)	(8 months- 65 years)	19	0	0
	Female (11)	(6 months-76 years)	9	2	0
Latehar (48)	Male (21)	(1 year-80 years)	20	1	0
	Female (27)	(5 years- 57 years)	17	6	4
Palamu (39)	Male (21)	(8 months-66 years)	19	1	1
	Female (18)	(1.8 years- 71 years)	15	3	0

Fig. 5. Relative distribution of Pv, Pf and mixed infections in Palamu.



Conclusion

Geographical and age differences influence the distribution of *P. falciparum* and *P. vivax* in endemic regions of Jharkhand. This work is a first step on the way to develop high-throughput parasite detection approaches for large scale field studies (Sethabutr et al., 1992). These findings may offer some evidence based guidelines in targeting malaria control efforts in this state.

Acknowledgement

This work was supported by Dr. Noor Alam (Head), University Department of Zoology, Vinoba Bhave University, Hazaribag and Dr. T.K Saha, Department of Biotechnology, Annada College, Hazaribag, Jharkhand.

References

Andrews, L., Andersen, D., Webster, S., Dunachie, R.M., Walther, P., Bejon, A., Hunt-Cooke, Bergson, F., Sanderson, A., Hill, V.S., Gilbert, S.C., 2005. Quantitative real time polymerase chain reaction for malaria diagnosis and its use in malaria vaccine clinical trials. Am. J. Trop. Med. Hyg. 73, 191-198.

Arai, M., Mizukoshi, C., Kubochi, F., Kakutani, T., Wataya, Y., 1994. Detection of *Plasmodium falciparum* in human blood by a nested polymerase chain reaction. Am. J. Trop. Med. Hyg. 51, 617-626.

Barker, R.H.J., Banchongaksorn, T., Courval, J.M., Suwonkerd, W., Rimwungtragoon, K., Wirth, D.F., 1992. A simple method to detect *Plasmodium falciparum* directly from blood samples using the polymerase chain reaction. Am. J. Trop. Med. Hyg. 46, 416-426.

Black, J., Hommel, M., Snounou, G., Pinder, M., 1994. Mixed infections with *Plasmodium falciparum* and *P. malariae* and fever in malaria. Lancet 343, 1095.

Brown, A.E., Kain, K.C., Pipithkul, J., Webster, H.K., 1992. Demonstration by the polymerase chain reaction of mixed *Plasmodium falciparum* and *P. vivax* infections undetected by conventional microscopy. Trans. R. Soc. Trop. Med. Hyg. 86, 609-612.

Jaureguiberry, G., Hatin, I., d' Aurio, L., Galibert, G., 1990. PCR detection of *Plasmodium falciparum* by oligonucleotide probes. Mol. Cell. Prob. 4, 409-414.

Khoo, A., Furata, T., Abdullah, N.R., Bah, N.A., Kojima, S., Wah, M.J., 1996. Nested polymerase chain reaction for detection of *Plasmodium falciparum* infection in Malaysia. Trans. Royal Soc. Trop. Med. Hyg. 90, 40-41.

Mangold, K.A., Manson, E.S., Koay, L., Stephens, M., Regner, R.B., Thomson, J., Peterson, L.R., Kaul, K.L., 2005 Real time PCR for detection and identification of *Plasmodium species*. J. Clin. Microbiol. 43, 2435-2440.

Roper, C., Elhassan, I.M., Hviid, L., Giha, H., Richardson, W.A., Babiker, H.A., 1996. Detection of

very low level *Plasmodium falciparum* infections using the nested polymerase chain reaction and a reassessment of the epidemiology of unstable malaria in Sudan. Am. J. Trop. Med. Hyg. 54, 325-331.

Rougemont, M., Saanen V.M., Sahli, R., Hinrikson, H.P., Bille, J., Jaton, K., 2004 Detection of four *Plasmodium species* in blood from humans by 18 S rRNA gene subunit based and species specific real time PCR assays. J. Clin. Micobiol. 42, 5636-5643.

Sethabutr, O., Brown, A.E., Panyim, S., Kain, K.C., Webster, H.K., Echeverria, P., 1992. Detection of *Plasmodium falciparum* by polymerase chain reaction in a field study. J. Inf. Dis.166, 145-148.

Singh, B., Bobogare, A.J., Cox-Singh., Snounou, G., Abdullah, M.S., Rahman, H.A., 1999. A genus and species specific nested polymerase chain reaction malaria detection assay for epidemiological studies. Am. J. Trop. Med. Hyg. 60, 687-692.

Snounou, G., Pinheiro, L., Gonclaves, A., Fonseca, L., Dias, F., Brown, K.N., 1993. The importance of sensitive detection of malaria parasite in the human and insect hosts in epidemiological studies, as shown by the analysis of field samples from guinea Bissau. Trans. R. Soc. Trop. Med. Hyg. 87, 649-653.

Snounou, G., Viriyakosol, S., Zhu, X.P., Jarra, W., Pinheiro, L., Do Rosario, V.E., 1993) High sensitivity of detection of human parasites by the use of nested polymerase chain reaction. Mol. Biochem. Parasitol. 61, 315-320.

Tirasophon, W., Panwyim S., 1993. PCR for low level detection of malaria parasites in blood. Meth. Mol. Biol. 21, 205-211.

Tirasophon, W., Rajkulchai, P., Ponglikitmongkol, M., Wilairat, P., Boonsaeng, V., Panyim, S., 1994. A highly sensitive, rapid and simple polymerase chain reactionbased method to detect human malaria (*Plasmodium falciparum* and *Plasmodium vivax*) in blood samples. Am. J. Trop. Med. Hyg. 51, 308-313.