International Journal of Contemporary Pediatrics
Patel A et al. Int J Contemp Pediatr. 2018 Jul;5(4):1334-1341
http://www.ijpediatrics.com
pISSN 2349-3283 | eISSN 2349-3291

DOI: http://dx.doi.org/10.18203/2349-3291.ijcp20182460

Original Research Article

Species wise incidence of malaria in pediatric age group of Raichur district, India

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Received: 26 May 2018
Accepted: 02 June 2018

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ABSTRACT

Background: The incidence of malaria is on the raise in Raichur district of Karnataka in the recent years and there is not much studies on malaria from this region. A hospital and community-based study was conducted to know the species wise incidence of malaria in pediatric age group of Raichur district and to know the efficacy of rapid diagnostic test for the diagnosis of malaria, against the gold standard 'Microscopic examination' of thick and thin smear.
Methods: Blood samples from 676 children with clinical suspicion of malaria were tested by PBS study and RDT. Differentiation of malaria parasite is based on antigenic differences between pLDH isoforms. Results from the RDT were compared to those obtained by PBS.
Results: A total of 302 (44.67%) samples were positive by PBS method of which 54 (8.0%) are *Plasmodium falciparum*, 248 (36.9%) are *Plasmodium vivax* and, while 218 (32.2%) were positive by RDT 37 (5.5%) Plasmodium falciparum, 181 (26.8%) *Plasmodium vivax*. In present study the overall incidence of *Plasmodium vivax* in Raichur district is 36.69% and *Plasmodium falciparum* incidence is 7.99% and none of the samples have tested positive for *Plasmodium malariae* and *Plasmodium ovale* species among the study group. The RDT showed sensitivities of 53.70% and 66.13% and specificities of 98.71% and 96.03%, respectively for the detection of *Plasmodium falciparum* and *Plasmodium vivax*.
Conclusions: Plasmodium vivax species remains the most common malarial parasite among the positive case by PBS method in Raichur district, but the incidence of plasmodium falciparum is on the rise which is a matter of concern. The RDT method has a low sensitivity and specificity for the diagnosis of malaria since the identification of the four-parasite species is not possible. The careful examination of a well-prepared and well-stained blood film currently remains the "gold standard" for malaria diagnosis.

Keywords: HRP-2, Plasmodium, PBS, RDT

INTRODUCTION

Malaria is the most common serious parasitic disease of human beings, killing one person every 12 seconds.[1] The disease now occurs in more than 90 countries worldwide. It is estimated that there are over 500 million clinical cases and 3 million malaria caused deaths per year in developing countries. The majority of these deaths being seen in the pediatric age group and pregnant women.[2-5] The magnitude of the problem is further enhanced by *Plasmodium falciparum* resistance to standard antimalarial drugs adding to increased morbidity and mortality. Important contributing factors of drug resistance are population movement, infrastructure deficiency, inadvertent use of antimalarial drugs.

It has been found that inadvertent use of antimalarial drugs is the single most important factor in the development of resistance following presumptive antimalarial treatment.[6]

Malaria is a significant and serious health problem in Karnataka state and particularly in Raichur district. In the recent years there has been a sharp rise in the incidence of malaria in this region due to rapid growth and development of various irrigation projects and migratory population, which poses major challenge for public health in both rural and urban areas.

There is a raise in the number of malaria cases with the onset of rainy season and so is the incidence of *Plasmodium falciparum* in the recent years, which is a matter of concern.

Among the four forms of malaria parasite *Plasmodium vivax* and *Plasmodium falciparum* are the two species of malaria parasite prevalent in Karnataka. And among the five Taluks of Raichur district Deodurga and Lingasugur Taluks contribute significantly to malaria incidence in Raichur district.

Hence, all-out effort should be made for more restrictive use of the drug and the prevention of irrational use of antimalarial drugs. Remarkable decrease in antimalarial drug use could be achieved by improving the diagnosis of malaria. i.e., early diagnosis and prompt treatment with national anti-malaria programme policy.

Thick and thin peripheral smear examination is considered the 'gold standard' method for diagnosis of malaria, it requires up to 60 minutes time from specimen collection to result.[6]

It is labour intensive and requires considerable expertise for its interpretation, particularly at low levels of parasitemia.[6,7] The RDT method is said to have good sensitivity for the diagnosis of malaria. The major drawbacks are the cost of the equipment and a low specificity with respect to identification of all the four species.[8,9]

The objective of the present study was to know the species wise incidence of malaria in pediatric age group of Raichur district and to compare the RDT with microscopic examination of thick and thin peripheral smear for the diagnosis of malaria.

METHODS

In the present study of species wise incidence of malaria in pediatric age group of Raichur district the cases attending OPD / IPD with clinical features consistent with malaria during the study period formed the study sample.

The study was conducted in the Department of Pediatrics, Navodaya Medical College and the PHCs of the Raichur district after taking clearance from the Ethical committee.

Inclusion criteria

- Children in the age group of 1-18 years with clinical suspicion of Malaria.

Exclusion criteria

- Children who had received treatment for Malaria in the previous 4 weeks.

Details of the history and clinical examination of suspected malaria cases were recorded on a proforma. And an informed consent was taken from all the cases before drawing blood.

Sampling procedure

2 ml of venous blood was drawn with aseptic precautions and collected into a sterile EDTA test tube.

Peripheral blood smear study

Two slides were prepared from each sample, one slide with a thick block film and another with a thin blood film. The film was allowed to dry and labeled. After drying, only thin smear was fixed. Slide with thick smear was dehaemoglobinised using distilled water before staining.

Staining method

This is the standard method used by the laboratories under the National Malaria Eradication Programme in India. The duration of examination of the thick and thin smears were 5 minutes and 5 to 7 minutes respectively, which involved the visualization of 100 fields in each smear. The species and the stage of the parasite were reported after examining the thin blood smear. The parasite density determination by examination at the thick blood smear was not done routinely for all cases.

RDT

Plasmodium lactate dehydrogenase/HRP-2 immuno chromatographic assay was done by using the commercial one step malaria antigen rapid test (SD bioline malaria antigen pf/pan).

Interpretation

- Observe for the presence of any band and the corresponding letter.
- A band in relation to 'c' is a positive control band, while '2' corresponds to target plasmodium species (genus specific band) and '1' for Plasmodium falciparum species (species-specific band).

Following this observation, the result is interpreted as follows:

a. When only control band appears without test band, the test is considered negative and is also indicative that the test is working properly. The absence of this band indicates the test is invalid and necessitates the repeat of test with another strip.
b. When a single test band (genus specific band) appears with a control band the test is considered to be positive for *Plasmodium vivax*.
c. When 2 test bands (genus specific and species specific) appeared with a control band the test is considered to be positive for *Plasmodium falciparum*.
d. plasmodium species is indicated when both genus and species-specific bands appear, and the genus specific band is much darker and more intense than the species-specific band.

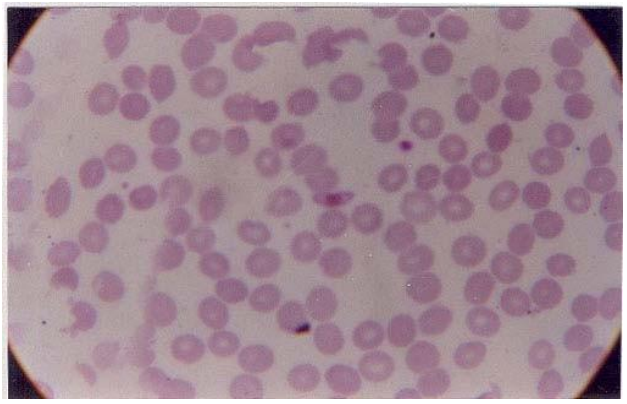

Figure 1: *Plasmodium falciparum* gametocyte in thin blood smear.

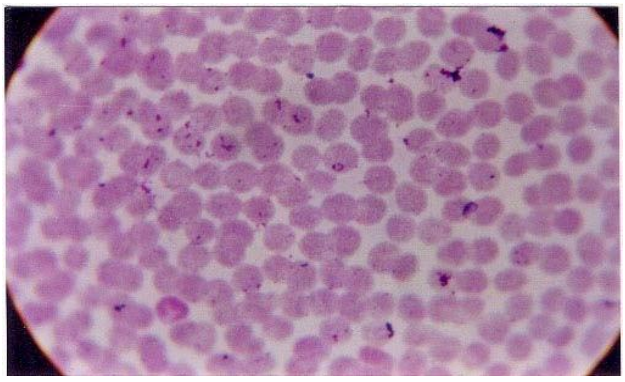

Figure 2: *Plasmodium falciparum* ring stage in thin blood smear.

Statistical analysis

Two methods were employed in the present study, which were Contingency coefficient analysis (CC) and Chi-square test.

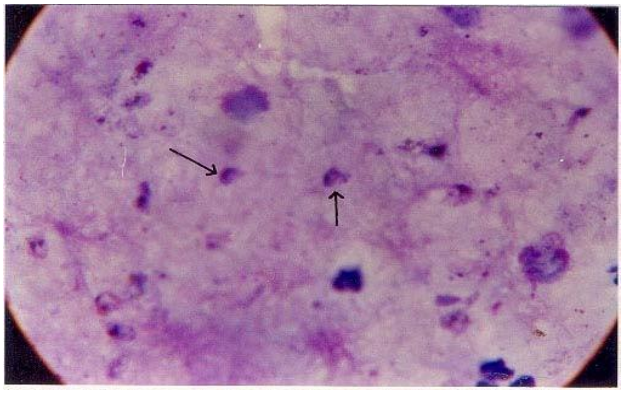

Figure 3: *Plasmodium vivax* trophozoites in thick blood smear.

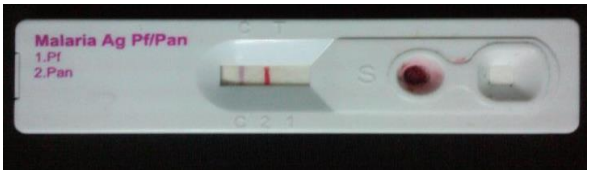

Figure 4: RDT being carried out.

RESULTS

During this study of 676 clinically suspected cases of malaria, 302 cases were confirmed positive for malaria by PBS study/ RDT. The following observations were made in relation with these confirmed malaria cases (302) (Table 1).

Table 1: Distribution of Malaria cases by age.

Age (years)	Total cases	+ve cases	Incidence
0-5	138	55	39.9
5-10	280	127	45.4
10-15	192	92	47.9
15-18	66	28	42.4
	676	302	44.7

Mean ±SD-8.2±4.11

Table 1 shows that the maximum positive malaria cases occurred between 5-10 years age group (127/302) and a total of 92 cases out of 302 were in the 10-15 years age group. Chi-square analysis revealed no significant difference in the frequencies of different age groups (χ^2 = 0.891, df=3, p=0.828).

Table 2: Distribution of malaria cases by gender.

Sex	Total cases	+ve cases	Incidence (%)
Female	256	115	44.9
Male	420	187	44.5
Total	676	302	44.7

$\chi 2$ = 0.067; P <.796 (Non - Significant)

Among the 302 confirmed malaria cases, there showed

almost an equal sex distribution. Further, Chi-square analysis revealed that there is no significant difference in the frequencies of male and female patients. (χ^2= 0.067; P < 0.796) (Table 2).

From the Table 3, it can be seen that the maximum Malaria cases encountered were from Deodurga (35.4%), followed by Lingasugur (28.8%), Raichur Taluk (26.5%), Manvi (7%) and Sindhanoor (2.3%) among the study group.

Table 3: Taluk wise distribution of malaria cases.

Taluk	Frequency	Percent
Deodurga	107	35.4
Lingsur	87	28.
Manvi	21	7
Raichur	80	26.5
Sindhanoor	7	2.3
Total	302	100

Maximum number of malaria cases i.e *Plasmodium vivax* and *Plasmodium falciparum* by PBS are from Deodurga taluk (107) followed by Lingsur taluk (87), Raichur taluk (80), Manvi (21) and Sindhanoor (7) among study group (Table 4).

Table 4: Taluk wise distribution of results of PBS test employed in diagnosis of malaria with respect to species.

Taluka	*Plasmodium falciparum*	*Plasmodium vivax*	Total
Deodurga	18	89	107
Lingsur	15	72	87
Manvi	4	17	21
Raichur	16	64	80
Sindhanoor	1	6	7
Total	54	248	302

Maximum number of Malaria cases i.e *Plasmodium vivax* and Plasmodium falciparum by RDT test are from Deodurga taluk followed by Lingsur taluk, Raichur taluk, Manvi and Sindhanoor among study group.

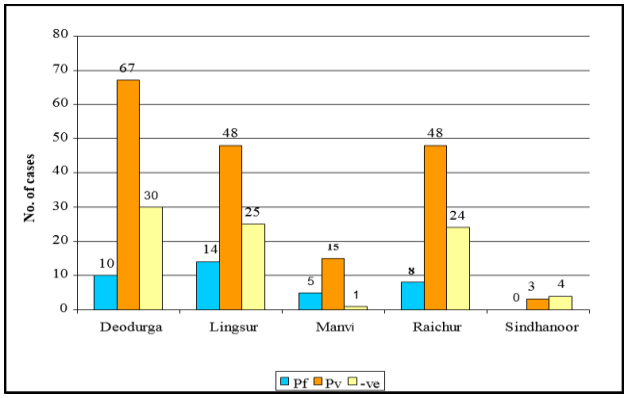

Figure 5: Taluk wise distribution of results of RDT employed in diagnosis of malaria with respect to species.

From the Figure 5, it can be seen that incidence of malaria is more in Sindhanoor Taluk (50%) followed by Deodurga (46.9%), Lingasugur (46.8%), Raichur (41.5%) and Manvi (38.2%).

Table 5: Taluk wise incidence of malaria among the study group.

Taluka	Total cases	+ve cases	Incidence (%)
Deodurga	228	107	46.9
Lingasugur	186	87	46.8
Manvi	55	21	38.2
Raichur	193	80	41.5
Sindhanoor	14	7	50.0
Total	676	302	44.7

From the Table 5 it is observed that Incidence rate of *Plasmodium falciparum* is more in Raichur (8.29) followed by Lingasur, Deodurga, Manvi, Sindhanoor and Incidence rate of *Plasmodium vivax* is more in Sindhanoor (42.86) followed by Deodurga, Lingasur, Raichur, Manvi among study group (Table 6).

Table 6: Species wise incidence of malaria in the study group among the 5 Talukas of Raichur district.

Taluka	Total cases	*Plasmodium falciparum*	Incidence (%)	Total cases	*Plasmodium vivax*	Incidence (%)	Total
Deodurga	228	18	7.89	228	89	39.04	107
Lingasur	186	15	8.06	186	72	38.71	87
Manvi	55	4	7.27	55	17	30.91	21
Raichur	193	16	8.29	193	64	33.16	80
Sindhanoor	14	1	7.14	14	6	42.86	7
Total	676	54	7.99	676	248	36.69	302

Table 7 show the symptomatology in malaria cases. Fever was the most common symptom followed by chills and rigor. Chi-square analysis revealed no significant difference in the presence and absence of symptoms like sweating and convulsions, where lesser number of patients manifested with these symptoms.

Table 7: Symptoms of children with malaria.

Symptoms	Frequency	%	X^2	p-value
Fever	301	99.7	0.157	0.692
Chills and rigor	158	52.3	2.244	0.134
Vomiting	62	20.5	0.474	0.491
Sweating	61	20.2	0.299	0.585
Headache	96	31.8	3.661	0.056
Alt sensorium	27	8.9	1.678	0.195
Convulsions	23	7.6	0.038	0.845

Table 8: Signs manifested in children with malaria.

Signs	Frequency	%	X^2	p-value
Pallor	137	45.4	0.513	0.474
Icterus	26	8.6	0.887	0.346
Edema	3	1.0	1.913	0.167
Hepatomegaly	23	7.6	0.566	0.452
Splenomegaly	204	68.5	0.389	0.533

Table 8 shows, pallor being present in 137 out of 302 confirmed cases of malaria (45.4). Splenomegaly was present in 204 (68.5%) malaria cases, Hepatomegaly was present in 23 cases (7.6%) of malaria. Chi square analysis reveal no significant difference in frequencies of signs among the study group ($p > 0.05$).

Of the 676 clinically suspected cases of malaria, 302 (44.7%) cases were positive for malaria and 374 (55.3%) cases negative by PBS study. The RDT showed a positivity percentage of 32.2%, with 218 cases being positive and 458 cases negative for malaria. By taking in to consideration of positivity of the standard PBS method employed the number of cases positive for malaria were 302.

Table 9: Results of the 2 tests employed in the diagnosis of malaria among the study sample.

Test	Positive (%)	Negative (%)	X^2	p-value
PBS	302 (44.7)	374 (55.3)	14.914	P<0.0001
RDT	218 (32.2)	458 (67.8)	169.0	P<0.0001

Table 10: Results of the 2 tests employed in the diagnosis of malaria with respect to the species.

Test	*P. falciparum*	*P. vivax*	X^2	p-value
PBS	54	248	160.47	P<0.0001
RDT	37	181	113.40	P<0.0001

A significantly more number of Plasmodium vivax infections were observed in both the tests compared to *Plasmodium falciparum*. Chi-square analysis revealed a highly significant difference between the frequencies of these different tests. Of the 302 smear confirmed cases, 54 (17.9%) cases were positive for Plasmodium *falciparum* and 248 (82.1%) cases for *Plasmodium vivax*. By taking into consideration of positivity by PBS as the standard method employed, the number of malaria cases positive for *Plasmodium falciparum* and *Plasmodium vivax* were 54 and 248 respectively. Of the 218 cases tested positive for malaria by RDT, 37 (17%) cases were positive for *Plasmodium falciparum* and 181 (83%) cases for *Plasmodium vivax* (Table 10).

RDT had a sensitivities of 53.70% and 66.13% and specificities of 98.71% & 96.03% for the detection of *Plasmodium falciparum* and *Plasmodium vivax*, respectively, when compared with the PBS study. RDT had a PPV's of 78.38% & 90.61% and NPV's of 96.09% & 83.03% for the detection of *Plasmodium falciparum* and *Plasmodium vivax*, respectively, when compared with the PBS study (Table 11).

Table 11: Comparison of RDT with peripheral blood smear examination for malaria parasite detection.

Species	Plasmodium blood film result			
	RDT	**+ve**	**-ve**	**total**
Plasmodium vivax	+ve	164	17	181
	-ve	84	411	495
	total	248	428	676
	X^2 =306.25, df =1, p<0.001			
Plasmodium falciparum	+ve	29	8	37
	-ve	25	614	639
	total	54	622	676
	X^2 =263.86, df =1, p<0.001			

DISCUSSION

In the present study of 'species wise incidence of malaria in pediatric age group of Raichur district, 676 children clinically suspected of malaria were chosen for the study. Of this, 302 children were confirmed positive for malaria by taking into consideration of positivity by standard PBS method employed. About 41% of the confirmed malaria cases were distributed in the 5-10 years age group, with a mean age 8 years. There was almost an equal sex distribution among the confirmed malaria cases.

The maximum malaria cases encountered were from Deodurga (35.4%) followed by Lingasugur (28.8%), Raichur Taluk (26.5%), Manvi (7%), Sindhanoor (2.3%). In this study Taluk wise incidence of malaria among the study group showed the incidence of 46.9% in Deodurga, 46.8% in Lingasugur, 38.2% in Manvi, 41.5% in Raichur Taluk and 50% in Sindhanoor. Among the study group Incidence of *Plasmodium vivax* is more than the *Plasmodium falciparum* species in all the 5 Talukas of

Raichur district. In Deodurga Taluk incidence of *Plasmodium vivax* is 39.4% and *Plasmodium falciparum* is 7.89%. In Lingasugur incidence of *Plasmodium vivax* is 38.71% and *Plasmodium falciparum* is 8.06%, In Manvi incidence of *Plasmodium vivax* is 30.91% and *Plasmodium falciparum* is 7.27, In Raichur Taluk incidence of *Plasmodium vivax* is 33.16% and *Plasmodium falciparum* is 8.29%. In Sindhanoor incidence of *Plasmodium vivax* is 42.86% and *Plasmodium falciparum* is 7.14%. In this study overall incidence of *Plasmodium vivax* in Raichur district is 36.69% and *Plasmodium falciparum* incidence is 7.99% and none of the samples have tested positive for *Plasmodium malariae* and *Plasmodium ovale* species among the study group.

Fever was the most common symptom (99.7%) followed by, chills and rigor (52.2%), headache (31.8%), vomiting (20.5), sweating (20.2%), altered sensorium (8.9%), convulsions (7.6%).

On examination, 45.4% of the malaria cases had pallor, 68.5% splenomegaly and 7.6% had hepatomegaly. There were few cases with features suggestive of complicated malaria. This could be explained by the fact that the study population was involved from a high endemic area and that the majority of these malaria cases were in the 5 - 10 years age group. In area with the constant high-level transmission (stable transmission area), severe malaria occurs predominantly between 6 months to 3 years of age; mild symptoms are seen in older children and adults are asymptomatic.[10,11] *Plasmodium vivax* is the most common malarial parasite accounting for 82.1% and *Plasmodium falciparum* accounted for 17.9% of the confirmed cases.

In the Present study 44.9% of the clinically suspected cases were positive for malaria by peripheral blood smear, which is comparable with the study of Kodisinghe et al (1997) 46.9%. and other studies like Rickman et al, (1989), Shiff et al,26 (1993), Tarimo et al (1999), Chayani et al (2004) showed 55.5%, 50.8%, 52%, 52.5% respectively.[12-16] The number of *Plasmodium falciparum* and *Plasmodium vivax* positive cases were 54 and 248 respectively as per PBS study.

The present study had a positivity percentage of 44.7% for PBS study and 32.2% for RDT test. This is comparable with studies done by Carol J. Palmer et al17 1998 and Jamshaid Iqbal et al (2002) which also had a difference in percentage of positivity between PBS study and RDT.[18] The present study had a high percentage of positivity since the study population was from a high endemic are for malaria.

In the present study, the RDT showed sensitivities of 53.70% and 66.13% and specificities of 98.71% and 96.03% respectively, when compared with the traditional peripheral blood smear for the detection of *Plasmodium falciparum* and *Plasmodium vivax* malaria. These results are comparable with Jelini et al (1999) and Iqbal et al.[19,20]

RDTs for the diagnosis of Plasmodium falciparum malaria generally achieve a sensitivity of >90% at densities above 100 parasites per µL blood and the sensitivity decreases markedly below that level of parasite density. Many studies have achieved >95% sensitivity at parasitemia of ~500 parasites/µL, but this high parasitemia is seen in only a minority of patients. For the diagnosis of. *Plasmodium vivax* malaria, the PfHRP2 test has a lower sensitivity compared to that for *Plasmodium falciparum* malaria; however, the pLDH test has an equal or better sensitivity for *Plasmodium vivax* malaria compared to *Plasmodium falciparum* malaria. For the diagnosis of *Plasmodium malariae* and *Plasmodium ovale* infections, the sensitivity is lower than that of *Plasmodium falciparum* malaria at all levels of parasitemia on both the PfHRP2 and the pLDH tests. The specificity appears to be better with the pLDH test than the PfHRP2 test for both *Plasmodium falciparum* and non-falciparum malaria.

False positivity

False positive tests can occur with RDTs for many reasons. Potential causes for PfHRP2 positivity, other than gametocytemia, include persistent viable asexual-stage parasitemia belo the detection limit of microscopy (possibly due to drug resistance), persistence of antigens due to sequestration and incomplete treatment, delayed clearance of circulating antigen (free or in antigen-antibody complexes) and cross reaction with non-falciparum malaria or rheumatoid factor. Proportion of persistent positivity has been linked to the sensitivity of the test, type of test, degree of parasitemia and possibly the type of capture antibody.

False negativity

On the other hand, false negative tests have been observed even in severe malaria with parasitemias >40,000 parasites/µl. This has been attributed to possible genetic heterogeneity of PfHRP2 expression, deletion of HRP-2 gene, presence of blocking antibodies for PfHRP2 antigen or immune-complex formation, prozone phenomenon at high antigenemia or to unknown causes.

Cross reactions between Plasmodia species and problems in identifying non-falciparum species

Cross reaction of PfHRP2 with non-falciparum malaria could give false positive results for *Plasmodium falciparum* and mixed infections containing asexual stages of *Plasmodium falciparum* could be interpreted as negative in about one third of the patients. Another major difficulty still encountered by the use of RDTs is the correct identification of Plasmodium species, particularly in areas where non-falciparum malaria is prevalent.

Table 12: Positive predictive value and negative predictive value of RDT for *Plasmodium falciparum* malaria.

Study series	PPV	NPV
Palmer et al	88%	99%
Jelini et al	97.9%	96.7%
Iqbal et al	94%	99%
Iqbal et al	89-97%	96-98%
Chayani et al	92%	98.5%
Present study	53.70%	96.03%

Table 13: Positive predictive value and negative predictive value of RDT for *Plasmodium vivax* malaria.

Study series	PPV	NPV
Palmer et al	100%	96%
Jelini K et al	100%	97.8%
Iqbal et al	91%	93%
Iqbal et al	88-95%	90-93%
Chayani N et al	100%	97.8%
Present study	66.13%	96.03%

In the present study the RDT showed a PPV's of 78.38% and 90.61% and NPV's of 83.03% and 96.09%, when compared to PBS study for detection of *Plasmodium falciparum* and *Plasmodium vivax* malaria respectively. These results are comparable with studies done b Iqbal et al and Iqbal et al.[18,20] Cases of malaria detected by blood film was missed by RDT.

This may be explained by the fact that increased awareness of malaria among the general public has led to a rampant miss use of antimalarial drugs in inadequate doses empirically for any fever. Since RDT detects pLDH and HRP-2 which are produced only by living parasites, the blood samples judged negative by RDT might have had dead parasites and not yet cleared from the host.[21] This can also be explained by insufficient enzyme production, which occurs during early malarial infection or the patient blood samples contained parasites at concentration below the RDT test detection level.[16,21]

CONCLUSION

In Raichur district the maximum Malaria cases encountered were from Deodurga (35.4%), followed by Lingasugur (28.8%), Raichur Taluk (26.5%), Manvi (7%) and Sindhanoor (2.3%). Plasmodium vivax species still remains the most common malarial parasite contributing 82.1% and Plasmodium falciparum contributing 17.9% among the positive cases by PBS method in Raichur district but the incidence of plasmodium falciparum is on the rise which is a matter of concern. In spite of advances in detection and management of malaria and its complications are still a major public health problem especially in children.

The RDT method has a low sensitivity and specificity for the diagnosis of malaria, and the identification of the four-parasite species is also not possible. Thus, we conclude that the direct microscopic visualization of the parasite on the thick and/or thin blood smears has been the accepted method for the diagnosis of malaria in most settings, from the clinical laboratory to the field surveys. The careful examination of a well-prepared and well-stained blood film currently remains the "gold standard" for malaria diagnosis.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Patel A, Srinivasa K, Manjunath GA. Species wise incidence of malaria in pediatric age group of Raichur district, India. Int J Contemp Pediatr 2018;5:1334-41.