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A cross-sectional survey of *Plasmodium falciparum* and *Plasmodium vivax* in India using rapid diagnostic test and microscopy across 12 sites of varying transmission, 2023–2024

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Abstract

Background In India, *Plasmodium falciparum* and *Plasmodium vivax* remain in circulation. Accurate detection of the parasite species remains crucial for prompt initiation of treatment and reducing onward transmission.

Methods A cross-sectional study across 12 sites of varying malaria endemicities was conducted from September 2023 to April 2024. Febrile participants were tested for malaria using rapid diagnostic tests (RDTs) and microscopy. Malaria positivity proportions along with 95% confidence intervals (95% CI) were presented separately by parasite species. The diagnostic performance of the RDT was compared against microscopy.

Results A total of 10,290 febrile participants were tested by both RDT and microscopy: 1,516 (14.7%, 95% CI 7.7–21.8%) malaria cases were identified by RDT and 1,436 (14.0%, 95% CI 6.9–21.1%) by microscopy. Of the 1,516 RDT positives, 1,105 (72.9%) had *P. falciparum* mono-infection, 290 (19.1%) had *P. vivax* mono-infection, and 121 (8.0%) had *P. falciparum* and *P. vivax* mixed infections. The sensitivity and specificity of RDT were 95.0% [95% CI 94–96%] and 99% [95% CI 98–99%], respectively, for detecting *P. falciparum* mono-infection, 83% [95% CI 78–87%] and 100% [95% CI 99–100%] for detecting P. *vivax* mono-infection, and 88% [95% CI 80–93%] and 100% for detecting a mixed infection of *P. falciparum* and *P. vivax*. Overall, 43 (0.4%) participants who were RDT negative were found to have malaria on subsequent microscopic examination.

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Conclusion Approximately 15% of the febrile participants tested were identified as malaria positive by RDT, of which nearly one-fifth were *P. vivax* mono-infections and 8% harboured *P. falciparum* and *P. vivax* mixed infections. Low sensitivity of the RDTs for identifying *P. vivax* underscores an urgent need for developing reliable diagnostics.

Keywords *Plasmodium falciparum*, *Plasmodium vivax*, India, Malaria elimination, Survey, Transmission

Background

Early and accurate disease diagnosis is one of the cornerstones of global malaria control and elimination. Microscopy of peripheral blood smears remains the 'gold standard' for malaria diagnosis [1, 2], but rapid diagnostic tests (RDTs) can be used where microscopy is not feasible. The operational suitability makes RDTs a feasible alternative for deployment in remote areas with limited access to healthcare and among hard-to-reach population [3, 4]. Therefore, RDTs remain a crucial component in the management and control of malaria globally. In 2022, approximately 345 million RDTs were distributed worldwide through the national malaria programmes, of which 12.1 million were distributed in the WHO South-East Asia region [5].

The WHO South-East Asia region has reported a consistent decline in malaria cases over the past 15 years, with confirmed cases reducing from 2.6 million in 2010 to 809,000 in 2022. Of the latter, India has accounted for approximately two-thirds, with both *Plasmodium falciparum* and *Plasmodium vivax* contributing to the disease burden [6]. A majority of the cases of *P. vivax* and *P. falciparum* in India have emerged from a few states: Odisha, Jharkhand, Chhattisgarh, West Bengal, Tripura, Mizoram, Maharashtra, and Uttar Pradesh [6]. Across these states, RDTs have remained the mainstay of malaria control efforts. However, several diagnostic-related challenges have emerged in some of these states in recent times, such as the failure to detect low-density parasitaemia and limited accuracy to detect mixed infections [7–9].

Some of the most widely used RDTs target histidine-rich proteins (PfHRP2) encoded by the *pfhrp2* gene for detection of *P. falciparum* malaria. Detection of *P. vivax* malaria utilizes *Plasmodium* lactate dehydrogenase (pLDH) enzymes, which is present in metabolically viable *P. vivax* parasites. Partial or a complete deletion of the *pfhrp2/3* genes results in minimal or no PfHRP2 production, and allows parasites to evade detection of *P. falciparum* malaria by RDTs leading to false-negative results [10]. Parasites harbouring partial or complete deletions of the *pfhrp2/3* genes have also been reported in several studies from India [11, 12]. The emergence of such "diagnostic-resistant" *P. falciparum* malaria has thus jeopardised malaria control efforts in India and the broader region. Therefore, timely and efficient monitoring of *pfhrp2/3* gene deletion status is crucial to inform the optimal diagnostic strategies. This is particularly important as the triad of resistance against antimalarials (in particular artemisinin) [13, 14], diagnostics, and insecticides threatens to seriously undermine the sustainability of the progress made over the past two decades. To evaluate the operational accuracy (diagnostic accuracy) of the current RDTs in field deployment and assess the current malaria status, a survey was undertaken across 12 different sites in India, representing varying levels of transmission endemicity, from September 2023 to April 2024.

This study provides an estimate of malaria positivity among screened febrile participants and evaluates the diagnostic performance of rapid diagnostic tests (RDTs) against microscopy. The findings will assist the national programme in identification of high-burden areas, aid policymakers in tracking progress towards malaria elimination goals, guide resource allocation, and ultimately contribute towards optimizing strategies and resources in the combat against malaria.

Methods

Study design

A cross-sectional study was conducted across 12 different sites in nine states of India of varying malaria endemicities between September 2023 and April 2024 (Fig. 1).

Selection of study sites

The study sites were selected in two stages. In the first stage, seven sites were chosen: Dantewada (Chhattisgarh), West Singhbhum (Jharkhand), Lunglei & Lawngtlai (Mizoram), Balaghat (Madhya Pradesh), Kothagudem (Telangana), and South Tripura (Tripura). The selection was based on pragmatic considerations such as (i) ability to access the sites as some of the endemic regions are in remote forest areas with limited transportation network, (ii) capacity to collect and process study samples, and (iii) the capacity of the research team to diagnose and treat participants presenting with malaria. In the second stage, an additional five sites were selected based on outputs of a geostatistical modelling (manuscript in preparation) and these included: Bokaro (Jharkhand), North Goa (Goa), Uttara Kannada and Mangalore (Karnataka), and Lakhimpur (Assam).

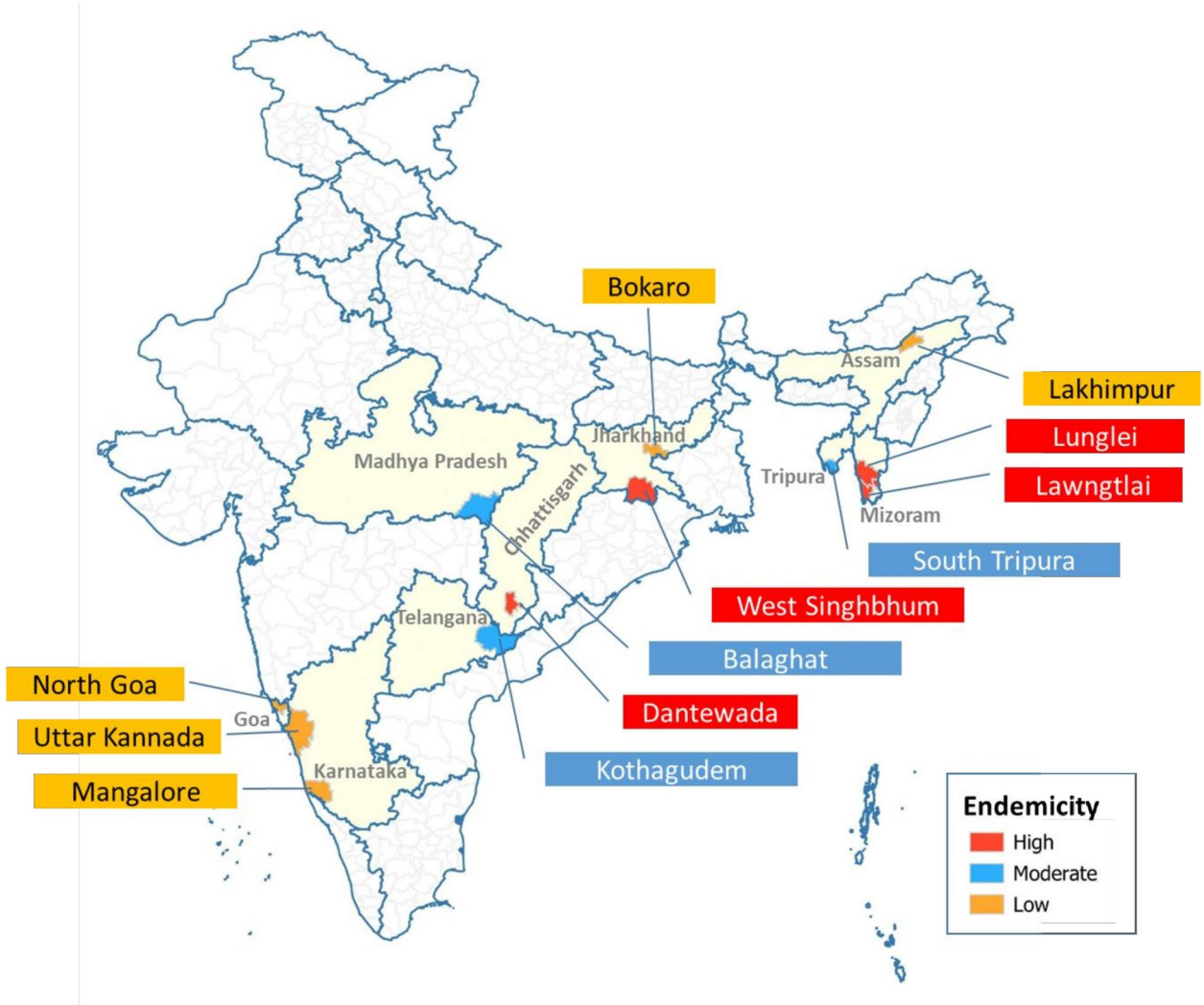

Fig. 1 Study sites and overall malaria endemicity

Description of study sites

Balaghat district in Madhya Pradesh has dense forests, hilly terrain, perennial streams, and experiences perennial malaria transmission. Villages in the district become inaccessible during the monsoon (July–September), and both *P. falciparum* and *P. vivax* are endemic. Dantewada in Chhattisgarh, along with six districts in the Bastar division has a year-round transmission, primarily of *P. falciparum*; the division has dense forest cover, and remote tribal settlements. In Jharkhand, West Singhbhum is a high-transmission zone, with intense seasonal peaks during the monsoon, whereas Bokaro has a low to moderate seasonal transmission. Kothagudem in Telangana has forested terrain and faces seasonal to perennial transmission. South Tripura has perennial transmission with peaks during monsoon, and has a large, susceptible tribal population. Lunglei and Lawngtlai in Mizoram also report perennial transmission, exacerbated by cross-border movement. Lakhimpur district in Assam experiences low seasonal transmission. In Karnataka, both Mangalore and Uttar Kannada report low transmission. Goa, although having achieved zero indigenous cases in 2023, faces the risk of imported transmission, particularly among migrant workers, necessitating continued surveillance. The status of malaria endemicity at the selected study sites is presented in Fig. 1.

Screening of the study participants and recruitment

Study participants were recruited from various community health centres (CHCs) within the district identified for surveillance. The selected CHCs had sufficient facilities and resources for case management. Those who

presented with signs and symptoms of malaria (including but not limited to headache, body aches, fever, chills, and weakness), aged older than 1 year, and providing informed consent/assent were screened for malaria status using an RDT and microscopy.

Diagnostics and sample collection

The bivalent SD Bioline Malaria Ag *P.f./P.v*. rapid diagnostic test (RDT), which detects *Plasmodium falciparum*-specific histidine-rich protein 2 (HRP2) and *Plasmodium vivax*-specific parasite lactate dehydrogenase (Pv-pLDH) antigens, was used alongside blood smear examination to screen febrile patients in field settings by trained staff. The slides prepared in the field were validated by two WHO certified level-1 microscopists at Indian Council of Medical Research-National Institute of Malaria Research (ICMR-NIMR), New Delhi. In case of discrepancy, a third independent microscopist served as a tie-breaker. For each sample, thick and thin smears were prepared and stained with 10% Giemsa stain. The smears were allowed to air-dry and were protected from dust, labelled with a unique ID. Basic information collected at the time of screening included the age and sex of the participant along with the date of testing and their geographic location. This study was a cross-sectional survey and there were no participant follow-up visits. Name, address, and other personal identifiers were not recorded in the study database. Fully anonymised data were archived indefinitely at the ICMR-NIMR Delhi, as per the institutional guidelines.

Quality assurance

All study personnel involved in the implementation first received on-site training on the principles of good clinical practice (GCP), informed consent/assent procedures, specimen collection, labelling, storage, and shipment before recruitment commenced. All the logistics were shipped to the study site in batches. Feedback on sample quality and suggestions for corrective action were sent to the site personnel.

Sample size approximation

The sample size at each participating site was determined by logistical limitations rather than statistical considerations. The primary limitation was the number of malaria cases, which is declining at many sites with ongoing malaria control efforts. This study aimed to detect approximately 203 *P. falciparum* positive patients per site. The precision of the estimated proportion of 5% (95% confidence interval, 95% CI) will be ± 3% with this sample size. Further assuming no results can be obtained from ~ 8–10% of the specimens, a target sample size of 220 *P. falciparum* positive cases was desired at each survey site.

Statistical analysis

Malaria positivity proportion was estimated separately for each study sites and presented with 95% CI computed using Wilson's method. An overall malaria positivity proportion was estimated by combining data across the 12 sites; 95% CI was estimated by adjusting for within-site correlation using Fleiss's method [15]. The results were presented separately by the diagnostic methodology used (RDT or microscopy), and separately by the parasite species (*P. falciparum*, *P. vivax*, mixed infection, or other species). The accuracy of the RDT was compared against the results from microscopy, and the following performance measures computed using *epiR* library in R software were reported: sensitivity, specificity, and negative and positive predictive values (NPVs and PPVs) [16]. Due to the occurrence of triple zeros across multiple sites, the performance measures were summarised by pooling data across the studies without undertaking meta-analysis and the results were presented separately for each of the parasite species. All analyses were conducted in R (R Studio) [17].

Informed consent

All prospective participants were informed about the study in both oral and written forms. The written consent documents embodied the elements of informed consent described in the current edition of the Declaration of Helsinki and adhered to the ICH guidelines. Prospective participants were informed that their decision regarding participation would have no impact on the care they received and those not consenting to participate received standard care per local protocol.

Ethics approval

Ethics approval for the study was granted by the Institutional Ethics Committee of NIMR (NIMR/IEC-M/2023/970/V2-OCT/14).

Results

During the study period (September 2023 to April 2024), 10,290 febrile participants who consented to inclusion in the study were tested by RDT and subsequently by microscopy across 12 different study locations (Fig. 1, Table 1). Of these, 4,237 (41.2%) participants were from four highly endemic sites (Lawngtlai, Dantewada, Lunglei, and West Singhbhum), 3,477 (33.8%) were from three moderately endemic sites (Kothagudem, South Tripura, and Balaghat), and 2,576 (25.0%) were from 5 sites with low malaria endemicity (North Goa, Lakhimpur, Mangalore, Uttara Kannada, and Bokaro). Overall,

Table 1 Malaria diagnosis using rapid diagnostic test and microscopy

Study site	Endemicity	Sample period	Method	Tested	Negative	*Pf* monoinfection	*Pv* monoinfection	*Pm* monoinfection	*Pf* + *Pv*	*Pf* + *Pm*
Bokaro (Jharkhand)	Low	11/2023–12/2023	RDT	241	241 (100%)	0 (0.0%)	0 (0.0%)	–	0 (0.0%)	–
			Microscopy	215	215 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Lakhimpur (Assam)	Low	10/2023–02/2024	RDT	644	641 (99.5%)	1 (0.2%)	2 (0.3%)	–	0 (0.0%)	–
			Microscopy	644	641 (99.5%)	1 (0.2%)	2 (0.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Mangalore (Karnataka)	Low	11/2023–04/2024	RDT	642	620 (96.6%)	4 (0.6%)	18 (2.8%)	–	0 (0.0%)	–
			Microscopy	642	620 (96.6%)	4 (0.6%)	18 (2.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
North Goa (Goa)	Low	01/2024–03/2024	RDT	647	636 (98.3%)	11 (1.7%)	0 (0.0%)	–	0 (0.0%)	–
			Microscopy	647	636 (98.3%)	11 (1.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Uttar Kannada (Karnataka)	Low	01/2024–02/2024	RDT	402	401 (99.8%)	1 (0.2%)	0 (0.0%)	–	0 (0.0%)	–
			Microscopy	402	401 (99.8%)	1 (0.2%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
South Tripura (Tripura)	Moderate	11/2023–03/2024	RDT	906	869 (95.9%)	29 (3.2%)	7 (0.8%)	–	1 (0.1%)	–
			Microscopy	904	876 (96.9%)	22 (2.4%)	6 (0.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Balaghat (Madhya Pradesh)	Moderate	09/2023–10/2023	RDT	890	618 (69.4%)	227 (25.5%)	17 (1.9%)	–	28 (3.1%)	–
			Microscopy	888	632 (71.2%)	222 (25%)	10 (1.1%)	0 (0.0%)	24 (2.7%)	0 (0.0%)
Kothagudem (Telangana)	Moderate	10/2023–11/2023	RDT	1,681	1,549 (92.1%)	121 (7.2%)	6 (0.4%)	–	5 (0.3%)	–
			Microscopy	1,681	1,571 (93.5%)	104 (6.2%)	5 (0.3%)	0 (0.0%)	1 (0.1%)	0 (0.0%)
West Singhbhum (Jharkhand)	High	11/2023–02/2024	RDT	614	310 (50.5%)	259 (42.2%)	30 (4.9%)	–	15 (2.4%)	–
			Microscopy	614	317 (51.6%)	248 (40.4%)	34 (5.5%)	0 (0.0%)	15 (2.4%)	0 (0.0%)
Dantewada (Chhattisgarh)	High	09/2023–10/2023	RDT	1,059	834 (78.8%)	182 (17.2%)	23 (2.2%)	–	20 (1.9%)	–
			Microscopy	1,058	865 (81.8%)	163 (15.4%)	16 (1.5%)	0 (0.0%)	14 (1.3%)	0 (0.0%)
Lawngtlai (Mizoram)	High	10/2023–03/2024	RDT	1,671	1,286 (77%)	191 (11.4%)	146 (8.7%)	–	48 (2.9%)	–
			Microscopy	1,668	1,271 (76.2%)	176 (10.6%)	174 (10.4%)	2 (0.1%)	45 (2.7%)	0 (0.0%)
Lunglei (Mizoram)	High	11/2023–03/2024	RDT	893	769 (86.1%)	79 (8.8%)	41 (4.6%)	–	4 (0.4%)	–
			Microscopy	891	773 (86.8%)	73 (8.2%)	39 (4.4%)	0 (0.0%)	5 (0.6%)	1 (0.1%)
Overall	**Overall**	**09/2023–04/2024**	**RDT**	**10,290**	**8,774 (85.3%)**	**1,105 (10.7%)**	**290 (2.8%)**	–	**121 (1.2%)**	–
			Microscopy	**10,254**	**8,818 (86.0%)**	**1,025 (10.0%)**	**304 (3.0%)**	**2 (0.0%)**	**104 (1.0%)**	**1 (0.0%)**

Percentage in parenthesis represents row percentages. All percentage rounded to 1 decimal place. *RDT* rapid diagnostic test. The bold test represents overall data pooled from all the study sites. *Pf* = *P. falciparum*. *Pv* = *P. Vivax*. *Pm* = *P.malariae*

5,349 (52%) participants were female and 4,941 (48%) male. A total of 1,022 (9.9%) participants were < 5 years old, 2,774 (27.0%) were between 5 and < 15 years old, and the remaining 6,494 (63.1%) were aged 15 years or older (See supplemental file S1).

Overall malaria positivity using RDT and microscopy

Of the 10,290 participants, 1,516 (14.7%, 95% confidence interval (CI) 7.7–21.8%) tested positive for malaria (any species; either mono-infection or a mixed infection) by RDT (Fig. 2). Paired microscopic slide results were not available in 36 (0.3%) participants; reasons included damage during transportation, staining issues, and poor-quality smear. Of the 36 with missing microscopic results, 34 was RDT negative (any malaria) and 2 were RDT positive for *P. falciparum*. Among 10,254 participants in whom microscopy slides were available, 1,436 (14.0%, 95% CI: 6.9%–21.1%) tested positive for malaria (any species).

Malaria positivity estimates across the study sites are presented in Table 1 and Fig. 2. In the high endemicity sites, the proportion of participants who tested positive for malaria by RDT and microscopy were 21.2% (225/1,059) and 18.2% (193/1,058) respectively in Dantewada, 23.0% (385/1,671) and 23.8% (397/1,668) in Lawngtlai, 13.9% (124/893) and 13.2% (118/891) in Lunglei, and 49.5% (304/614) and 48.4% (297/614) in West Singhbhum. The corresponding estimates in moderately endemic sites were 30.6% (272/890) and 28.8% (256/888) in Balaghat, 7.9% (131/1,681) and 6.5% (110/1,681) in Kothagudem, and 4.1% (37/906) and 3.1% (28/904) in South Tripura. The corresponding estimates in areas of low endemicities were: 0% (0/241) and 0% (0/215) in Bokaro, 0.5% (3/644) and 0.5% (3/644) in Lakhimpur, 3.4% (22/642) and 3.4% (22/642) in Mangalore, 1.7% (11/647) and 1.7% (11/647) in North Goa, and 0.2% (1/402) and 0.2% (1/402) in Uttara Kannada. Further details are presented in supplemental file S1.

Causative parasite species

Of the 1,516 RDT positives, 1,105 (72.9%) were *P. falciparum* mono-infection, 290 (19.1%) were *P. vivax* mono-infection, and the remaining 121 (8.0%) had *P. falciparum* and *P. vivax* mixed infections. Of the 1,436 who tested positive using microscopy, 1,025 (71.4%) had *P. falciparum* mono-infection, 304 (21.2%) had *P. vivax* mono-infection, 2 (0.1%) were identified as *P. malariae* mono-infection, 104 (7.2%) presented with a mixed *P. falciparum* and *P. vivax,* and 1 (0.1%) patient had a mixed *P. falciparum* and *P. malariae*. Species specific breakdown of the malaria status is presented in Table 1.

Performance of RDT for detecting *P. falciparum* mono-infection

The diagnostic accuracy of the RDTs in detecting malaria by causative parasite species is presented in Tables 2, 3, and 4. Among 10,254 participants for whom both

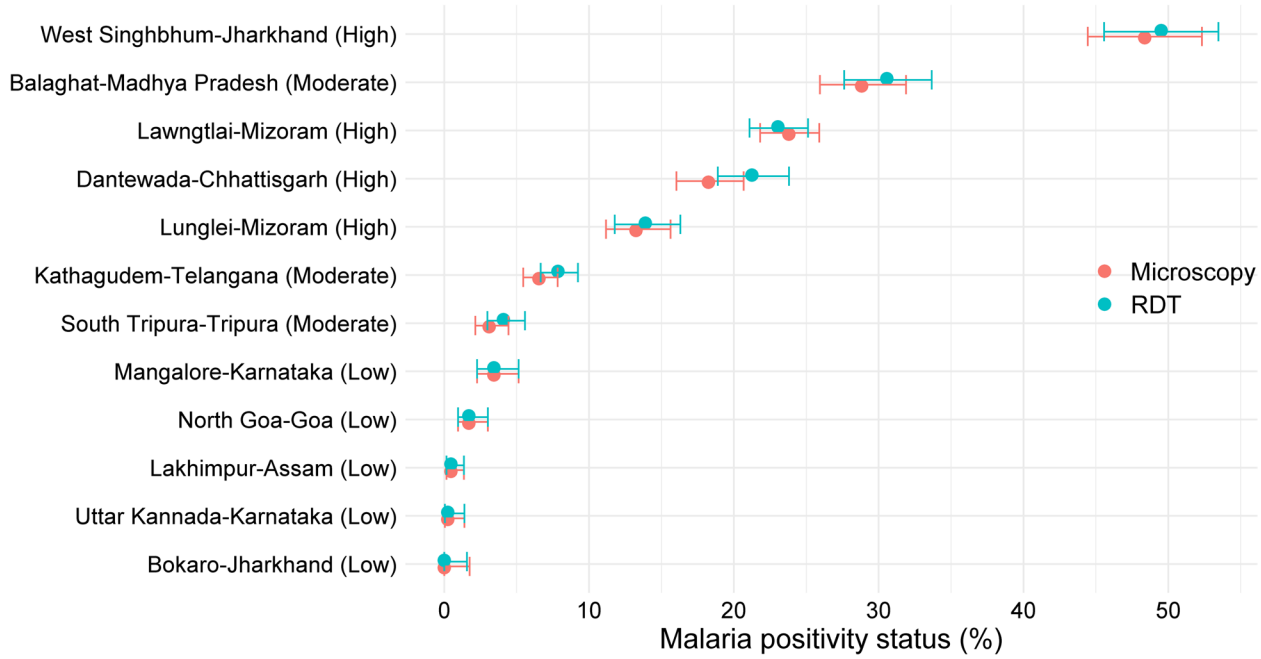

Fig. 2 Malaria prevalence using microscopy and RDT for each of the study sites. 95% CI estimated using Wilson's method. Current endemicity status for each site shown in parenthesis

Table 2 Accuracy of rapid diagnostic test for detecting malaria infection, by parasite species

Parasite species	Microscopy (positive/tested)	RDT (positive/tested)	Sensitivity [95% CI]	Specificity [95% CI]	PPV [95% CI]	NPV [95% CI]
Pf mono-infection	1,025/10,254	1,103/10,254	0.95 [0.94–0.96]	0.99 [0.98–0.99]	0.88 [0.86–0.90]	0.99 [0.99–1.00]
Pv mono-infection	304/10,254	290/10,254	0.83 [0.78–0.87]	1.00 [0.99–1.00]	0.87 [0.82–0.90]	0.99 [0.99–1.00]
Pf + *Pv* mixed infection	104/10,254	121/10,254	0.88 [0.80–0.93]	1.00	0.75 [0.67–0.83]	1.00
Any malaria	1,436/10,254	1,514/10,254	0.96 [0.95–0.97]	0.98 [0.98–0.99]	0.91 [0.89–0.92]	0.99 [0.99–0.99]

RDT rapid diagnostic test, *NPV* negative predictive value, *PPV* positive predictive value; 95% confidence interval (95% CI) are based on exact method

Table 3 Accuracy of the rapid diagnostic test for detecting *Pf* mono-infection, by malaria endemicity

Endemicity	Microscopy (positive/tested)	RDT (positive/tested)	Sensitivity [95% CI]	Specificity [95% CI]	PPV [95% CI]	NPV [95% CI]
High (n = 4,231)	660/4,231	710/4,231	0.95 [0.93–0.97]	0.98 [0.97–0.98]	0.88 [0.86–0.91]	0.99 [0.99–0.99]
Moderate (n = 3,473)	348/3,473	376/3,473	0.95 [0.92–0.97]	0.99 [0.98–0.99]	0.88 [0.84–0.91]	0.99 [0.99–1.00]
Low (n = 2,550)	17/2,550	17/2,550	1.00 [0.80–1.00]	1.00	1.00[0.80–1.00]	1.00

RDT rapid diagnostic test, *NPV* negative predictive value, *PPV* positive predictive value; 95% confidence interval (95% CI) are based on exact method

Table 4 Accuracy of the RDT for detecting *Pv* mono-infection, by malaria endemicity

Endemicity	Microscopy (positive/tested)	RDT (positive/tested)	Sensitivity [95% CI]	Specificity[95% CI]	PPV [95% CI]	NPV [95% CI]
High (n = 4,231)	263/4231	240/4231	0.81 [0.76–0.86]	0.99 [0.99–1.00]	0.89 [0.85–0.93]	0.99 [0.98–0.99]
Moderate (n = 3,473)	21/3473	30/3473	0.81 [0.58–0.95]	1.00 [0.99–1.00]	0.57 [0.37–0.75]	1.00
Low (n = 2,550)	20/2550	20/2550	1.00 [0.83–1.00]	1.00	1.00 [0.83–1.00]	1.00

RDT rapid diagnostic test, *NPV* negative predictive value, *PPV* positive predictive value; 95% confidence interval (95% CI) are based on exact method

the RDT and microscopy results were available, 8,987 (87.3%) tested negative by both methods i.e., microscopy and RDT, and 1,087 (10.6%) tested positive by both methods. Further 43 participants (0.4%) were RDT negative but were subsequently found to be microscopy positive, while the remaining 137 (1.3%) were RDT-positive but subsequently tested negative by microscopy. Overall, this resulted in sensitivity and specificity estimates for the RDT (compared to microscopy) of 95.0% [95% CI 93.0–97.0%] and 98.0% [95% CI 97.0–98.0%] respectively in high endemicity areas, 95.0% [95% CI 92.0–97.0%] and 99.0% [95% CI 98.0–99.0%] in the areas of moderate endemicities, and 100% sensitivity and specificity were observed in the areas of low endemicities (Table 3). Pooled across the study sites, the overall sensitivity and specificity estimates of the RDT was 95.0% [95% CI 94.0–96.0%] and 99.0% [95% CI 98.0–99.0%] respectively (Table 2). See Tables 2 and 3 for the predictive values and further accuracy measures.

Performance of RDT for detecting *P. vivax* mono-infection

The sensitivity and specificity of the RDT were respectively 81.0% [95% CI 76.0–86.0%] and 99.0% [95% CI 99.0–100%] in the areas of high endemicity, 81.0% [95% CI 58.0–95.0%] and 100% [95% CI 99.0–100%] in the areas of moderate endemicities, and 100% sensitivity and specificity were observed in the areas of low endemicities (Table 4). Pooled across the 12 study sites, the overall sensitivity and specificity of the RDT for detecting a *P. vivax* mono-infection were 83.0% [95% CI 78.0–87.0%] and 100% [95% CI 99.0–100%] (Table 2). See Tables 2 and 4 for the predictive values and further accuracy measures.

Performance of RDTs for detecting mixed *P. falciparum* and *P. vivax* infection

The overall sensitivity and specificity of the RDT for detecting a mixed *P. vivax* and *P. falciparum* infection were 88% [95% CI: 80%–93%] and 100% respectively (see Table 2). See Table 2 for the predictive values.

Relationship between age, sex and malaria status

In a multivariable logistic regression containing age, sex, and transmission endemicity, the following variables were associated with increased odds of test positivity by RDTs: every 5 yearly increase in age was associated with 12% lower odds of testing positive (adjusted odds ratio (AOR): 0.88, 95% CI 0.86–0.90), being male (AOR: 1.24, 95% CI 1.10–1.39) (compared to females), and residing in the areas of high malaria endemicity (AOR: 17.33, 95% CI 12.55–24.68) or moderate endemicity (AOR: 7.81, 95% CI 5.62–11.21) (compared to areas of low transmission) (Fig. 3 and see supplemental Table 1 and supplemental Table 2).

Discussion

Overall, the proportion of participants who were RDT positive was approximately 15% in this study. This estimate is similar to previous studies conducted over multiple sites in India that had reported approximately 18% positivity [18]. Malaria positivity proportion was found to be higher among males who had 1.24-fold increased odds of testing positive compared to females; this finding are in line with a previous review that indicated a generally higher disease burden (DALYs and deaths) among males [19] and also concur with the findings from a longitudinal survey among adult population [20].

Of those who tested positive for malaria, nearly three-quarters had *P. falciparum* mono-infection and approximately one-fifth tested positive for *P. vivax* mono-infection and 8% harboured mixed infections. However, there was a marked heterogeneity in the dominant causative species across the study sites. For example, *P. vivax* accounted for 80% (18/22) of total malaria cases in Mangalore-Karnataka and more than one-third across the two Mizoram sites. For the remaining sites, malaria was largely caused by *P. falciparum*. In particular, Lawngtlai, Mizoram—which is an area of high malaria endemicity, had substantially higher proportion of *P. vivax* infection compared to the rest of the sites. This is consistent with the known uneven distribution of *P. vivax* in India with a regional variation in burden and previously reported fluctuations in the dominance of *P. falciparum* and *P. vivax* as the leading causative species for malaria in India. *Plasmodium falciparum* accounted for 63% of all reported malaria in 2017, and this dropped to 46% in 2019, followed by a resurgence to 64% in 2020, and has since consistently remained above 50% [21]. Overall, identification of substantial proportion of *P.* vivax infections in this survey may indicate an increasing importance of *P. vivax* malaria in the country that historically had a large burden of *P. falciparum* malaria (See Fig. 1 in [22]). These shifts and fluctuations highlight ongoing challenges in malaria control and the need to implement

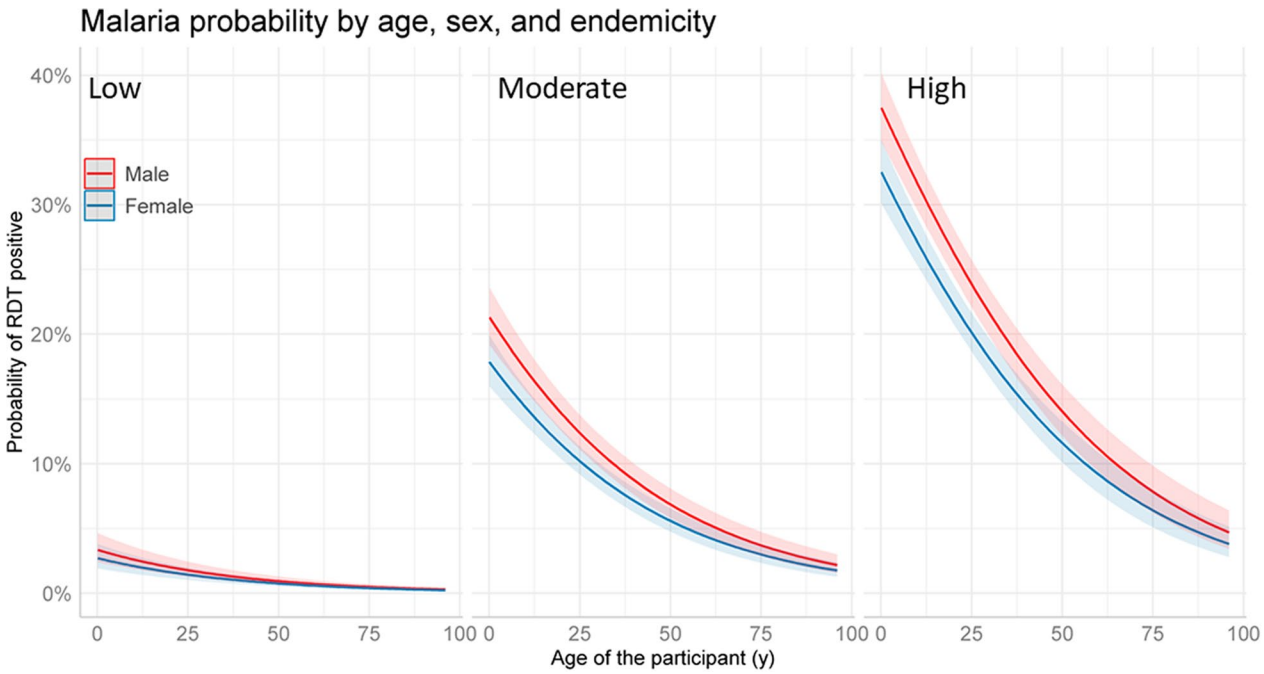

Fig. 3 Probability of RDT confirmed malaria status by age, sex, and transmission setting. Predictions obtained from a multivariable logistic regression that contained age, transmission setting and sex. The model had age as a linear effect which was the most parsimonious model. For comparison of different model fits, see supplemental file 1

site-specific malaria control strategies tailored to the local needs.

The observed malaria positivity proportion in this study while largely corroborated with the underlying disease endemicity, some results were notably distinct. First, West Singhbhum, a site with known high transmission, had nearly half of the patients testing positive which suggests a substantial malaria burden in this location beyond what was previously observed. The high positivity proportion observed could be due to the timing of the survey which coincided with the peak of the malaria season (November to February), right after the monsoon leading to a surge in the cases. Second, Balaghat, currently considered a moderately endemic site, had a malaria positivity of approximately 30% among screened participants. This contrasts with previous findings at the site, which reported monthly slide positivity rates of < 5% between 2008 and 2020 [23]. If true, this finding likely suggests the need for re-consideration of endemicity status of the Balaghat. A potential explanation for this discordance could be the inclusion of higher proportion of the children < 10 years enrolled at this site relative to other study sites (See supplemental Figs. 1–4). However, it remains unclear whether the 30% positivity in this site reflects a true increase in transmission over the years or remains simply an artefact of the sampling strategy; this warrant further epidemiological investigation.

Overall, there were 43 (0.4%) participants in whom RDT was negative but were identified as malaria positive subsequently by microscopic examination. Of these 43, some reported *hrp2* gene deletion. Such finding warrants further caution to be exercised and emphasizes the need for continuously monitoring the diagnostic performance of RDTs in India.

In this study, a higher sensitivity of RDTs was observed in the areas of low transmission for both *P. falciparum* and *P. vivax*, and the sensitivity progressively declined with increasing endemicity status. The performance of the RDTs was also generally better for *P. falciparum* than for *P. vivax*. In addition, the precision of the estimated performance measures was also much higher for *P. falciparum* than for *P. vivax.* The poor sensitivity of RDTs in identifying *P.* vivax and mixed infections is a particular note of concern, as the underlying drug for treatment of *P. falciparum* and *P. vivax* malaria remains different. In particular, the sensitivity of the RDT for *P. vivax* observed in this study is much lower than the 95.5% reported earlier [24]. It is known that SD Bioline RDT have a sensitivity below 90% for *P. vivax* at parasite density < 100/µL. In this study, explicit quantification of the parasitaemia levels was not undertaken and thus, low parasite density among the participants could not be ruled out as a potential explanation for the lower sensitivity observed. Taken together, these results suggest that a better and more accurate diagnostic test is warranted for *P. vivax* in the context of India. However, the specificity of RDT was high regardless of the parasite species along with the negative predictive values close to 100% suggesting a high accuracy in ruling out the disease.

There are some key limitations of this study. This study aimed to detect approximately 220 *P. falciparum* cases per site and this was not achieved across majority of the sites, especially in the areas of low and moderate transmission. The selection of the study sites was also not random and was determined based on operational feasibility in the first stage and guided by mathematical modelling in the second stage. In addition, limited participant level covariates were collected and therefore evaluation of predictors of test positivity was limited to age, sex, and transmission setting. Influence of predictors such as history of malaria in the recent past, travel history and other key factors such as housing conditions and bed-net usage could not be assessed. Information regarding the number of participants who were invited for screening and reasons for exclusion were also not explicitly recorded. Further investigation of the reasons for exclusion (such as declining consent or non-eligible age range) could have provided further insights. Data on *hrp2* gene deletion is not explicitly reported here and this will be presented as a subsequent report (manuscript in preparation). Finally, while *hrp2* deletion explained some of the discordant results between RDT and microscopy, few additional factors that could have led to the discordance were not investigated. In particular, *hrp2* antigen is known to persist in patients for several weeks even after the resolution of infection which could have explained some of the discordance between RDT and microscopy [20]. However, as stated earlier, additional information regarding participant's recent malaria history wasn't collected and this remains a limitation. Additional information regarding parasite density could have been potentially useful as previous research indicates a decreasing accuracy of RDT at low parasite density (See Fig. 4 in [25]), and this can be considered as part of future research, particularly for *P. vivax*.

Conclusions

India has made substantial gains in controlling malaria over the past two decades, attributed to roll-outs of vector and parasite control interventions. However, there are still some major challenges as identified in this study. For example, the finding of a high slide positivity proportion in Balaghat, a site currently considered as moderately endemic, suggests the need for further investigation and future studies at this site. Approximately one-fifth of those who tested positive for malaria had microscopically

confirmed *P. vivax* infections and 7.2% of the microscopically confirmed malaria cases harboured *P. falciparum* and *P. vivax* mixed infections. Low sensitivity of the RDTs for identifying *P. vivax* cases, and a further 43 (0.4%) RDT negatives who were subsequently confirmed as microscopy positive suggests need for more robust diagnostics for detection of *P. vivax.*

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12936-025-05556-7.

Supplementary material 1: Figure 1: Age-distribution by study sites. Figure 2: Age-distribution by study sites, with age presented as 10y bins. Figure 3: *Plasmodium falciparum* malaria slide positivity proportion by age-bins and site using microscopy. Y-axis depicts age-range, and X-axis presents the percentage of the slides identified as positive using microscopy. Figure 4: Age distribution by rapid diagnostic testresults, across areas of high, low and moderate endemicities. Table 1: Comparison of different functional forms of the covariates included in logistic regression model for malaria positivity by a rapid diagnostic test. Table 2: Coefficients from a logistic regression model for malaria positivity by a rapid diagnostic test.

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Author contributions
Study Conception: PKB, SSP, PJG; Data curation: PKB, SN, SSP; Formal analysis: PD, SSP, SN, PKB; Funding acquisition: PKB, MD, PJG; Investigation: PD, SSP, SN, PKT, WAM, DPS, AM, VHC, SBM, SPS, PKS, KS, RR, RKB, RC, MN, AD, RSK, AG, MR, JAF, NN, MD, AA, PJG, PKB; Methodology: PD, SSP, SN, PKB; Project administration: SSP, PKB; Resources: PKB, PJG; Software: PD, SSP; Supervision: PKB, PJG; Validation: PD, SSP, SN, PKB; Visualization: PD, SSP, SN, PKB; Writing—original draft: PD, SSP, SN, PKB; Writing—review and editing: All authors were involved in reading and critical revision of the initial draft and approved the final manuscript.

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Data availability
The data supporting the findings of this study are available from the ICMR-NIMR, New Delhi. The data can be obtained upon reasonable request submitted to the ICMR-NIMR, New Delhi through the corresponding author: saprapbs@yahoo.co.in.

Declarations

Competing interests
The authors declare no competing interests.

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