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PLASMODIUM VIVAX-DOMINATED MALARIA IN LOWER DIR, KHYBER PAKHTUNKHWA, PAKISTAN: A TWELVE-MONTH HOSPITAL LABORATORY STUDY OF SLIDE POSITIVITY, SEX DIFFERENCES AND THE INFLUENCE OF TESTING VOLUME IN 2020

Masood Shah[*1], Alia Amin[2], Muhammad Najeeb Ul Haq[3], Muqaddas Khalid[4], Iqra Hakim[5], Haider Ali[6], Sardar Alam[7], Anbareen[8], Asma[9]

[1] Department of Zoology, Shaheed Benazir Bhutto University, Dir Upper, Khyber Pakhtunkhwa, Pakistan 18050
[2] Department of Zoology, Abdul Wali Khan University Mardan, Khyber Pakhtunkhwa, Pakistan 23200
[3] Department of Zoology, University of the Punjab, Pakistan 54590
[4,5,6] Department of Zoology, Quaid-i-Azam University, Islamabad, Pakistan 45320
[7]Department of Zoology, Government Post Graduate College Timergara, Lower Dir, Khyber Pakhtunkhwa; University of Malakand 18000, Pakistan
[8]Department of Zoology, University of Peshawar, Pakistan 25120
[9] Department of Zoology, Islamia College Peshawar, Khyber Pakhtunkhwa, Pakistan 25120

[*1]masoodshahpk2018@gmail.com, [2]aliaamin901@gmail.com, [3]mnajeebulhaq@gmail.com, [4]muqaddas.22411017@zoo.qau.edu.pk, [5]iqrahakim.zoo.edu.pk@gmail.com, [6]ha7193095@gmail.com, [7]sardarstu0340@gmail.com, [8]ambreenyusafzai@gmail.com, [9]asmamehmood92@gmail.com

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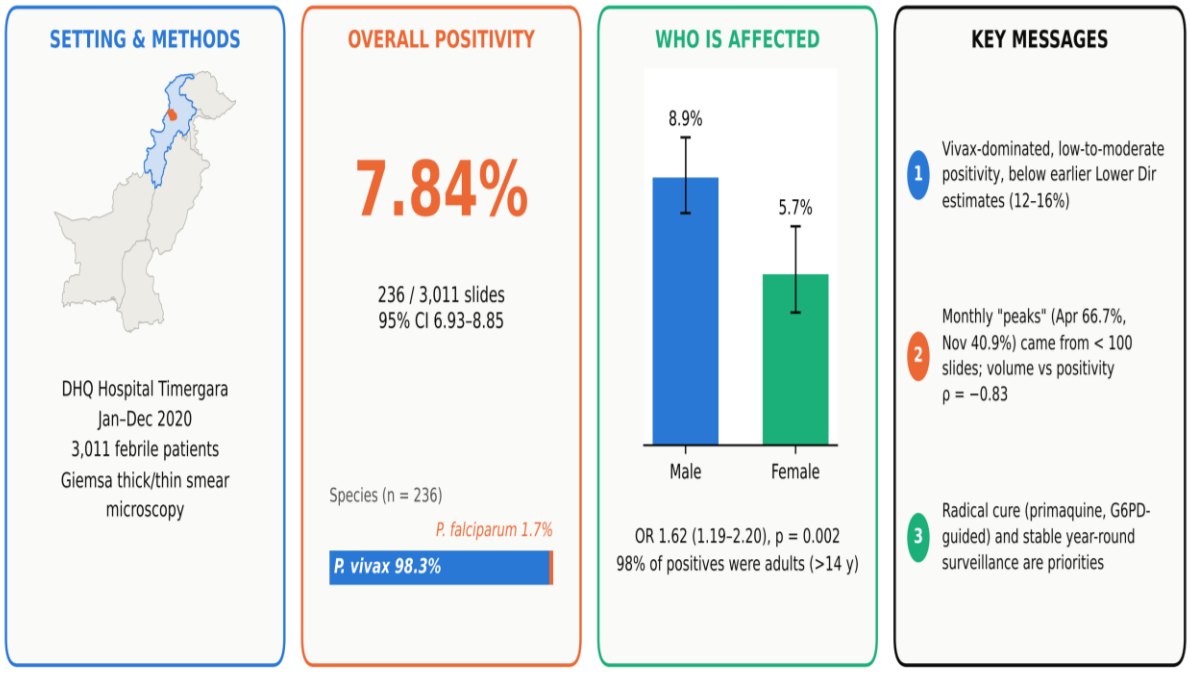
Keywords:
Malaria; Plasmodium vivax; Plasmodium falciparum; slide positivity rate; microscopy; seasonality; Lower Dir; Khyber Pakhtunkhwa; Pakistan; COVID-19.

Abstract

Malaria remains endemic in Khyber Pakhtunkhwa (KP), Pakistan, where Plasmodium vivax predominates. District-level data from the northern hill districts are sparse, and few studies have accounted for how changes in testing volume distort monthly positivity. We estimated the slide positivity rate of malaria, its species composition and its distribution by month, season, sex and age group among febrile patients in Lower Dir in 2020. We conducted a retrospective, hospital-based cross-sectional study of all patients referred with clinically suspected malaria to the laboratory of the District Headquarters (DHQ) Hospital, Timergara, from 1 January to 31 December 2020. Giemsa-stained thick and thin blood films were examined by oil-immersion microscopy. Positivity was calculated with Wilson 95% confidence intervals (CIs) and compared by sex and season using χ^2 tests and odds ratios (ORs). Of 3,011 patients (1,986 males, 1,025 females), 236 were smear-positive (7.84%; 95% CI 6.93–8.85). P. vivax accounted for 232 infections (98.3%; 95% CI 95.7–99.3) and P. falciparum for 4 (1.7%); no mixed, P. malariae or P. ovale infections were detected. Positivity was higher in males (8.86%) than in females (5.66%; OR 1.62, 95% CI 1.19–

2.20; p = 0.002). The highest monthly figures (April 66.7%, 4/6; November 40.9%, 9/22) were therefore statistically unstable. Adults (>14 years) made up 98.3% of positives with recorded age. Malaria in Lower Dir is overwhelmingly due to P. vivax, with moderate slide positivity and a significant excess in males. Monthly positivity from hospital laboratories must be read alongside testing volume. Control should prioritize radical cure of vivax, steady year-round testing, and routine recording of age and sex.

Graphical Abstract



1. INTRODUCTION

Malaria is a febrile illness caused by protozoan parasites of the genus Plasmodium and transmitted by the bite of infected female Anopheles mosquitoes. The World Health Organization (WHO) estimated 229 million cases and 409,000 deaths worldwide in 2019. Children under five accounted for about two-thirds of the deaths, and the WHO African Region carried roughly 94% of the burden [1]. Its most recent global report describes a continuing burden and a stalling of progress in several regions [2]. The parasite was first seen in human blood by Laveran in 1880, and Ross showed mosquito transmission in 1897–1898. That history laid the basis for modern diagnosis and vector control [3].

Five Plasmodium species routinely infect humans, but P. falciparum and P. vivax cause most of the disease. P. vivax is the most widely distributed species. It forms dormant liver stages (hypnozoites) that cause relapses weeks to months after the first infection, so blood-stage treatment alone does not clear it [4,5]. Vivax malaria was long called "benign tertian" malaria. It is now known to cause severe anemia, respiratory distress and death, and its burden is underestimated wherever it is common [6,7]. Global modelling places most of the clinical vivax burden in South and South-East Asia, Pakistan included [8]. Malaria also costs endemic economies through lost productivity and spending on care [9].

Pakistan is a moderately endemic country in the WHO Eastern Mediterranean Region. P. vivax accounts for about 70–80% of confirmed cases, and the burden is concentrated in Baluchistan, Sindh, KP and the former Federally Administered Tribal Areas (FATA) [10–12]. A meta-analysis of studies published from 2006 to 2021 estimated a pooled malaria prevalence of 23.3% among those tested in Pakistan, with P. vivax making up 79.1% of infections [13]. Within KP, household surveys and facility studies report high vivax predominance and wide variation between districts [14,15]. Districts bordering Afghanistan carry a

large share of the national burden, a pattern linked to population movement and weak health infrastructure [10,16]. After the 2022 floods, reported cases rose more than fourfold, from about 400,000 in 2021 to over 1.6 million in the districts the Global Fund supports. More than two million cases a year were still being reported in 2024 [17,18].

Lower Dir is a hilly district in north-western KP, bordering Bajaur and Afghanistan. Three earlier studies from the district reported microscopy positivity of 12.2% in 2013, 16.1% in 2018, and about 12% in a combined Lower Dir and Swat sample, with P. vivax dominant in all three [19–21]. None covered 2020. That year the COVID-19 pandemic changed the reported incidence of several endemic infections in Pakistan [22]. None of these studies assessed whether changes in testing volume affected their monthly or seasonal estimates. That question matters because hospital-based slide positivity depends on who is tested as well as on transmission.

This study aimed to (i) estimate the overall slide positivity rate (SPR) of malaria among febrile patients tested at the main public hospital of Lower Dir during 2020; (ii) describe the species composition of infections; (iii) describe variation in positivity by month and season and examine its relation to testing volume; and (iv) compare positivity between sexes and describe the age distribution of cases.

2. Materials and methods

2.1 Study area

Lower Dir (approximately 34°36′–35°02′ N, 71°29′–72°12′ E) lies in the Malakand Division of KP (Fig. 1). It borders Upper Dir to the north, Swat to the east, Malakand to the south and Bajaur to the west. The Panjkora River and its tributaries drain the district, and its narrow alluvial valleys are cultivated with irrigation, surrounded by mountains. In the 2017 census the district had 1,436,082 residents, 96% of them rural and 47% aged 0–14 years [23]. The climate is sub-tropical to temperate. Summers are warm (June–July are the hottest months), winters are cold with sub-zero minima from December to February, and rain falls in both winter and the summer monsoon. Timergara, the district headquarters, hosts the DHQ Hospital, the main public referral facility for the district and parts of neighboring districts.

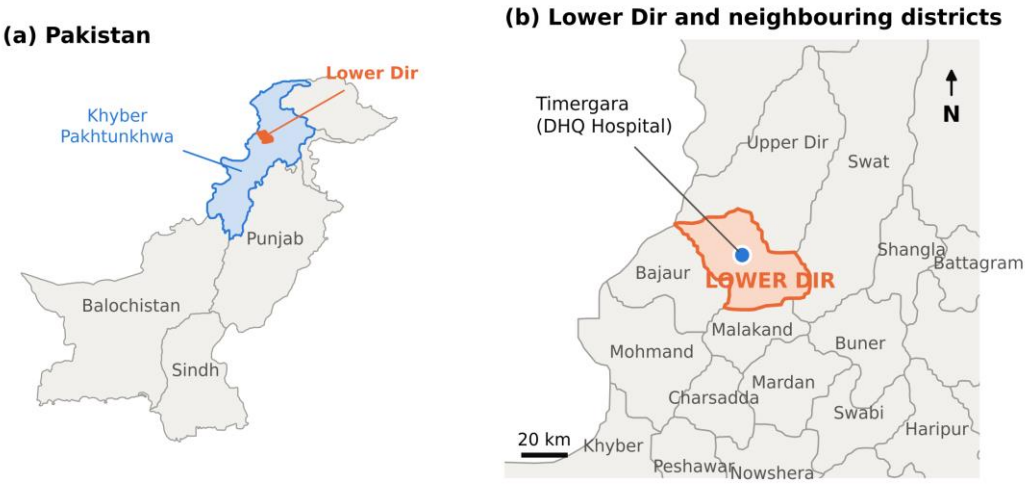


Fig. 1. Location of the study area. (a) Pakistan, with Khyber Pakhtunkhwa province (blue) and Lower Dir district (orange). (b) Lower Dir and neighboring districts, showing the study site at the District Headquarters Hospital, Timergara. Administrative boundaries from geoBoundaries [24].

2.2 Study design and setting

This was a retrospective, hospital-based cross-sectional study of laboratory records. It is reported according to the STROBE guidelines for observational studies [25]. All patients referred to the pathology laboratory of DHQ Hospital Timergara for malaria microscopy from 1 January to 31 December 2020 were eligible. Physicians in the outpatient, emergency and inpatient departments referred patients who had fever, chills, rigors, sweating or a history suggestive of malaria.

2.3 Participants and data collection

Each referred patient had one blood examination. For each patient, laboratory staff recorded sex, age, locality, the microscopy result (positive or negative) and, if positive, the species on a standard data sheet. Positive patients were classed as children (≤14 years) or adults (>14 years). Age was recorded only as these two groups, and the number of children tested was not recorded. Monthly totals were compiled from the data sheets.

2.4 Blood collection and microscopy

Blood was collected under aseptic conditions by venipuncture from older children and adults, or by heel prick from infants under six months. Specimens were processed without delay by a trained laboratory technologist. For each patient, a thick and a thin film were prepared on clean, grease-free slides. Thin films were spread with a slide edge held at about 45°, air-dried and fixed in absolute methanol for one minute. Thick films were made from a drop of blood spread in a small circle and air-dried horizontally for 4–6 minutes without fixation. Both films were stained with Giemsa, for 4–6 minutes with a strong working solution or 8–10 minutes with a dilute one, then rinsed gently in tap water and air-dried [26,27]. Slides were examined under oil immersion (×100 objective). The thick film was used to detect parasites and the thin film to identify species from parasite and red-cell morphology [27,28]. Slides were reviewed by an experienced microbiologist and kept for re-examination.

2.5 Outcomes and definitions

The primary outcome was the slide positivity rate (SPR): smear-positive patients divided by all patients examined, as a percentage. Because the denominator is febrile patients referred for testing, the SPR measures positivity among suspected cases, not community prevalence. Species share was the proportion of positive patients infected with each species. Seasons followed the meteorological convention: winter (December–February), spring (March–May), summer (June–August) and autumn (September–November). Months with fewer than 100 slides examined were labelled low-volume a priori, because a single positive in such a month moves the SPR by more than one percentage point.

2.6 Statistical analysis

Proportions are given with Wilson score 95% confidence intervals, which stay accurate when counts are small or proportions are near 0% or 100% [29,30]. Positivity was compared between sexes using Pearson's χ^2 test and an unadjusted odds ratio (OR) with a Woolf 95% CI. Differences across seasons and months were tested with χ^2 tests of homogeneity. Because several monthly cells had expected counts below five, monthly comparisons are descriptive. The relationship between monthly testing volume and SPR was assessed with Spearman's rank correlation. Positives with missing sex or age were excluded from those analyses only, and the numbers excluded are reported. Two-sided $p < 0.05$ was taken as significant. Analyses used Python 3 (SciPy and statsmodels).

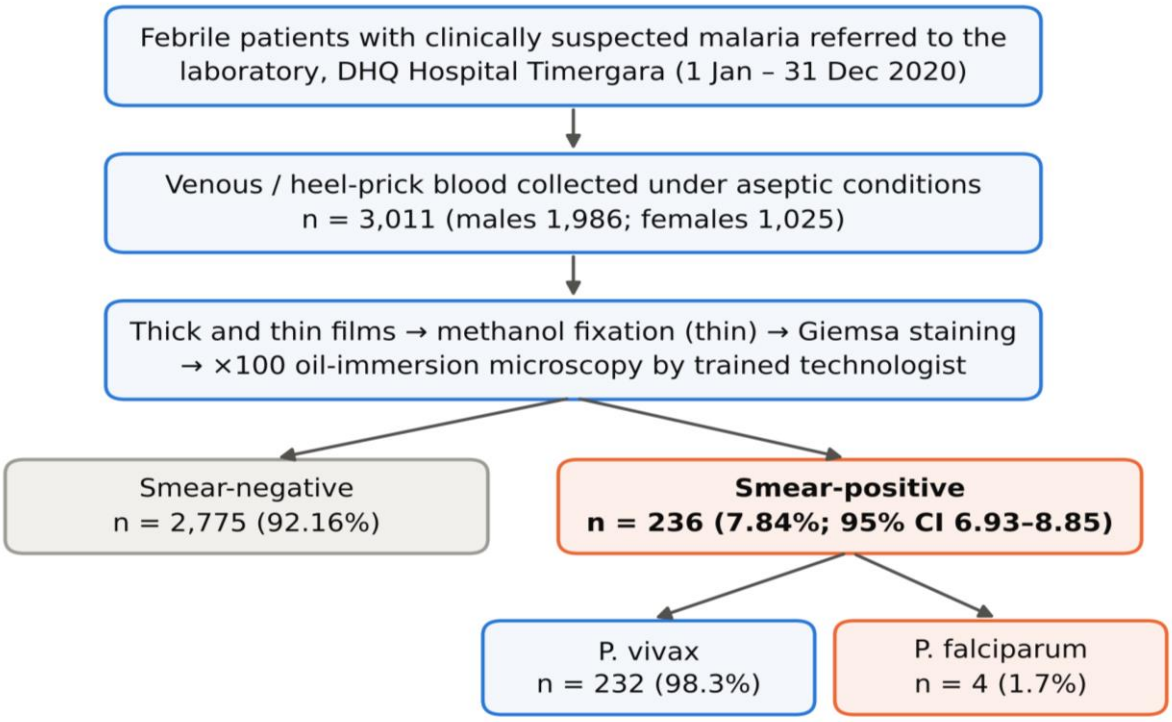


Fig. 2. Study flow and laboratory workflow, from referral of patients with suspected malaria to species identification. Percentages for species are of positive patients.

3. Results and Discussion

3.1 Overall positivity

During 2020, 3,011 febrile patients were examined: 1,986 males (66.0%) and 1,025 females (34.0%). Of these, 236 were smear-positive, an overall SPR of 7.84% (95% CI 6.93–8.85) (Fig. 2; Table 1). The SPR for P. vivax was 7.71% (232/3,011; 95% CI 6.81–8.71) and for P. falciparum 0.13% (4/3,011; 95% CI 0.05–0.34).

Table 1. Malaria slide positivity among febrile patients tested at DHQ Hospital Timergara, Lower Dir, January–December 2020, overall and by sex.

Group	Tested, n (%)	Positive, n	SPR, % (95% CI)	OR (95% CI)	p value
Overall	3,011 (100)	236	7.84 (6.93–8.85)	—	—
P. vivax	—	232	7.71 (6.81–8.71)	—	—
P. falciparum	—	4	0.13 (0.05–0.34)	—	—
Male	1,986 (66.0)	176	8.86 (7.69–10.19)	1.62 (1.19–2.20)	0.002
Female	1,025 (34.0)	58	5.66 (4.40–7.25)	1.00 (reference)	
Sex not recorded*	—	2	—	—	—

3.2 Monthly and seasonal variation

Monthly testing volume varied about 84-fold, from 6 slides in April to 504 in February (Table 2; Fig. 3a). In eight months, 297–504 slides were examined. In four months, fewer than 100 were examined: April (6), May (40), November (22) and December (60). The most positive cases were recorded in June (46), October (34), February (30) and September (28).

Monthly SPR ranged from 2.92% in July (10/343) to 66.67% in April (4/6) (Fig. 3b). The three highest monthly SPRs all came from low-volume months: April (66.67%; 95% CI 30.0–90.3), November (40.91%; 23.3–61.3) and May (32.50%; 20.1–48.0). Their confidence intervals were very wide. In the

eight months with at least 297 slides, SPR ranged from 2.92% (July) to 12.17% (June), and positivity still differed between months ($\chi^2 = 46.2$, df = 7, $p < 0.001$). Among these high-volume months, positivity was highest in June (12.17%), October (11.45%) and September (9.36%), and lowest in July (2.92%) and January (3.31%).

Table 2. Monthly malaria testing, slide positivity, species and sex distribution of positive patients, DHQ Hospital Timergara, 2020.

Month	Slides examined	Positive	SPR, %	95% CI	P. vivax	P. falciparum	Male	Female	Sex NR
January	423	14	3.31	2.0–5.5	13	1	11	3	0
February	504	30	5.95	4.2–8.4	30	0	20	10	0
March	302	20	6.62	4.3–10.0	20	0	17	3	0
April†	6	4	66.67	30.0–90.3	4	0	1	3	0
May†	40	13	32.50	20.1–48.0	12	1	7	6	0
June	378	46	12.17	9.2–15.9	45	1	31	15	0
July	343	10	2.92	1.6–5.3	9	1	8	2	0
August	337	20	5.93	3.9–9.0	20	0	17	3	0
September	299	28	9.36	6.6–13.2	28	0	23	5	0
October	297	34	11.45	8.3–15.6	34	0	28	6	0
November†	22	9	40.91	23.3–61.3	9	0	9	0	0
December†	60	8	13.33	6.9–24.2	8	0	4	2	2
Total	**3,011**	**236**	**7.84**	**6.93–8.85**	**232**	**4**	**176**	**58**	**2**

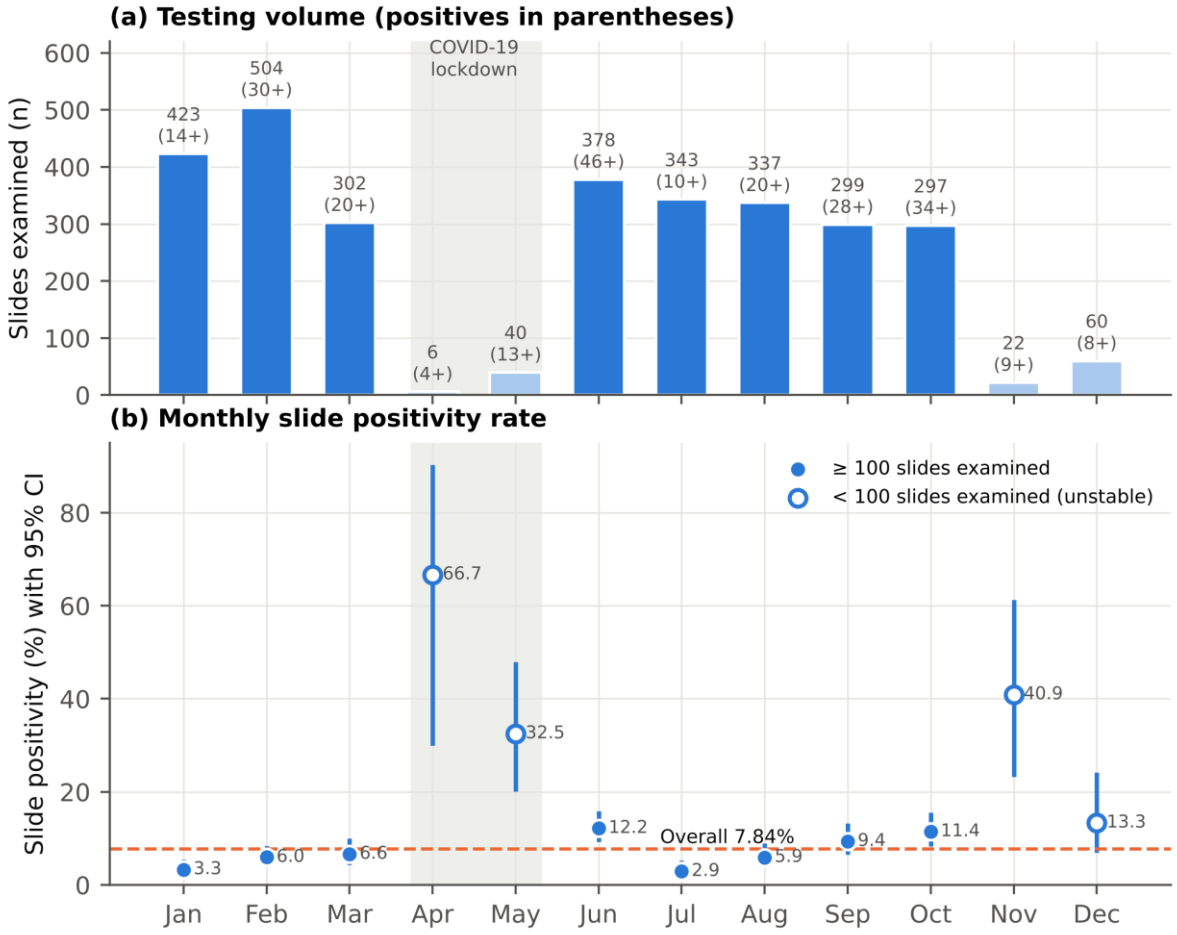


Fig. 3. Monthly malaria testing and slide positivity at DHQ Hospital Timergara, January–December 2020.

(a) Number of slides examined per month; positives are shown in parentheses. Light bars mark months with fewer than 100 slides. (b) Monthly slide positivity rate with Wilson 95% confidence intervals. Open circles mark low-volume months, whose estimates are unstable. The dashed line marks the annual SPR (7.84%). The shaded band marks the approximate period of the national COVID-19 lockdown (late March to early May 2020).

By season, positivity was lowest in winter (5.27%; 52/987) and highest in autumn (11.49%; 71/618) and spring (10.63%; 37/348). Summer was intermediate (7.18%; 76/1,058) (Table 3; Fig. 4). The difference across seasons was significant ($\chi^2 = 24.8$, df = 3, $p < 0.001$). The spring estimate rests mainly on March (302 slides), because only 46 slides were examined in April and May combined.

Table 3. Seasonal slide positivity rate of malaria, DHQ Hospital Timergara, 2020.

Season (months)	Slides examined	Positive	SPR, % (95% CI)	P. vivax	P. falciparum
Winter (Dec, Jan, Feb)	987	52	5.27 (4.04–6.84)	51	1
Spring (Mar, Apr, May)	348	37	10.63 (7.81–14.31)	36	1
Summer (Jun, Jul, Aug)	1,058	76	7.18 (5.78–8.90)	74	2
Autumn (Sep, Oct, Nov)	618	71	11.49 (9.21–14.24)	71	0
Total	**3,011**	**236**	**7.84 (6.93–8.85)**	**232**	**4**

χ^2 test of homogeneity across seasons: $\chi^2 = 24.8$, df = 3, $p < 0.001$. Winter combines December 2020 with January–February 2020 (calendar-year data). Spring includes April and May, in which only 46 slides were examined.

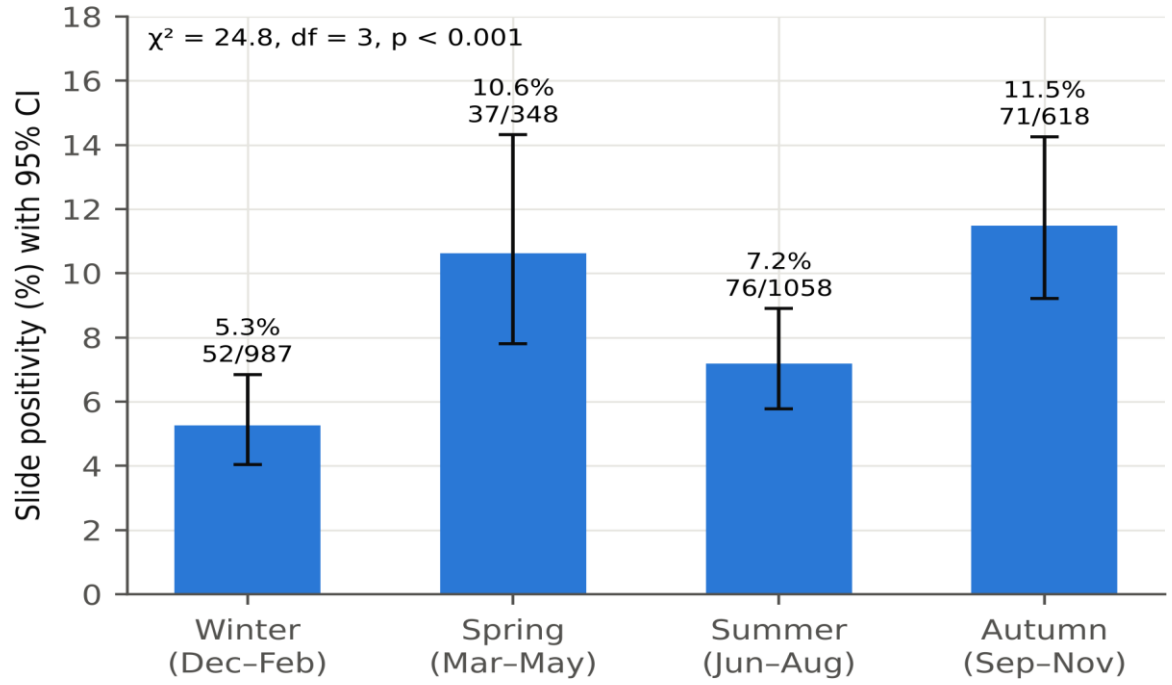


Fig. 4. Seasonal slide positivity rate with Wilson 95% confidence intervals. Winter, December–February; spring, March–May; summer, June–August; autumn, September–November.

3.3 Testing volume and positivity

Monthly SPR was strongly and inversely correlated with the number of slides examined (Spearman $\rho = -0.83$, $p = 0.001$; Fig. 5). Taken together, the four low-volume months had an SPR of 26.6% (34/128), more than three times the 7.0% (202/2,883) of the eight high-volume months. When testing fell, the patients still being tested were therefore much more likely to be positive.

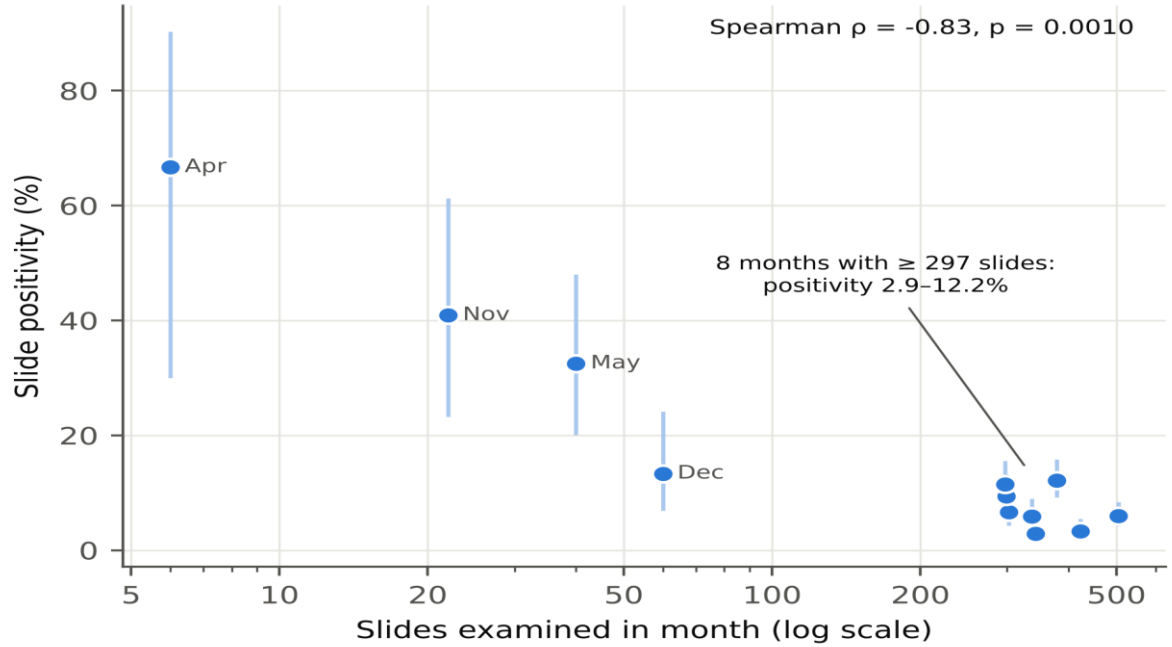


Fig. 5. Relationship between monthly testing volume (log scale) and slide positivity rate. Vertical lines show Wilson 95% confidence intervals. The four low-volume months are labelled; the eight high-volume months cluster at 2.9–12.2%.

3.4 Species composition

Of the 236 positive patients, 232 had P. vivax (98.31%; 95% CI 95.72–99.34) and 4 had P. falciparum (1.69%; 95% CI 0.66–4.28) (Fig. 6). No mixed infections, P. malariae or P. ovale were detected. P. vivax was found in every month. The four P. falciparum infections occurred singly in January, May, June and July (Table 2; Fig. 6a).

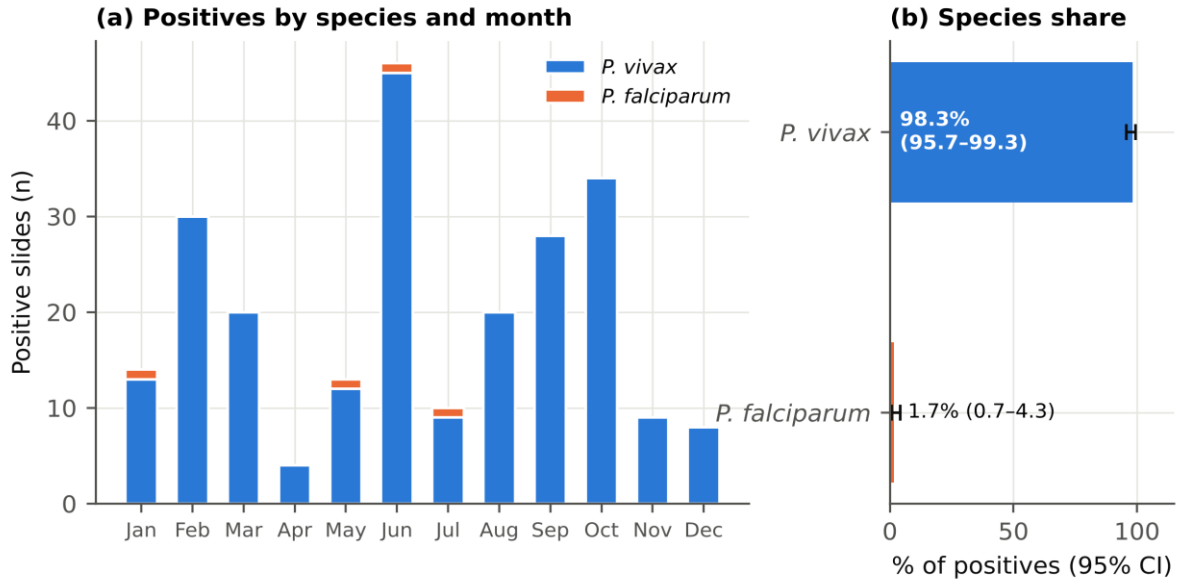


Fig. 6. Species composition of malaria infections. (a) Monthly positive slides by species. (b) Proportion of all positives due to each species, with Wilson 95% confidence intervals.

3.5 Sex

Sex was recorded for 234 of the 236 positives. The two positives without recorded sex were both in December. Positivity was 8.86% in males (176/1,986; 95% CI 7.69–10.19) and 5.66% in females (58/1,025; 95% CI 4.40–7.25) (Table 1; Fig. 7a). This difference was significant (χ^2 = 9.68, df = 1, p = 0.002). Males had 1.62 times the odds of a positive smear (95% CI 1.19–2.20). Males made up 66.0% of those tested and 75.2% of positives with recorded sex. Males outnumbered females among positives in every month except April (1 vs 3) (Fig. 7b).

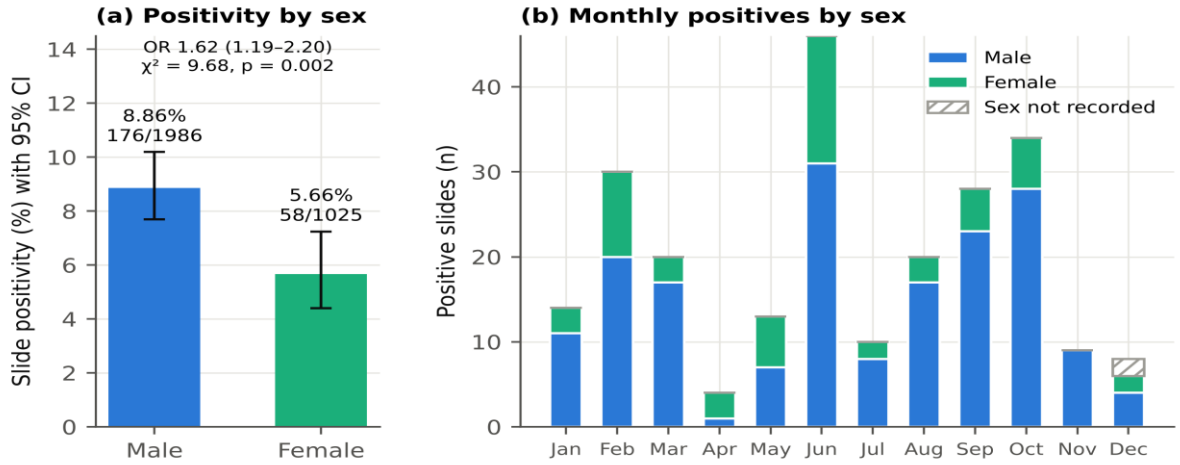


Fig. 7. Malaria by sex. (a) Slide positivity rate by sex with Wilson 95% confidence intervals; odds ratio and χ^2 test compare males with females. (b) Monthly positives by sex. The hatched segment shows the two December positives without recorded sex.

3.6 Age group

Age group was recorded for 234 positives. Of these, 230 (98.3%) were adults (>14 years) and 4 (1.7%) were children (≤14 years), one each in January, September, October and December (Table 4). The number of children tested was not recorded, so positivity by age could not be calculated. Children aged 0–14 years make up about 47% of the district population [23], so children are strongly under-represented among recorded cases.

Table 4. Age group of malaria-positive patients by month, DHQ Hospital Timergara, 2020.

Month	Children (≤14 y)	Adults (>14 y)	Age not recorded	Total positive
January	1	13	0	14
February	0	30	0	30
March	0	20	0	20
April	0	4	0	4
May	0	13	0	13
June	0	46	0	46
July	0	10	0	10
August	0	20	0	20
September	1	27	0	28
October	1	33	0	34
November	0	9	0	9
December	1	5	2	8
Total (%)*	**4 (1.7)**	**230 (98.3)**	**2**	**236**

*Percentages are of the 234 positives with recorded age group. The number of children and adults tested (negatives) was not recorded, so age-specific positivity cannot be calculated.

4. Discussion

4.1 Principal findings

In this twelve-month hospital laboratory study from Lower Dir, 7.84% of febrile patients tested for malaria in 2020 had smear-confirmed infection. Nearly all infections were P. vivax (98.3%). Positivity was significantly higher in males, and almost all recorded cases were adults. Our most important methodological finding is that monthly positivity in 2020 depended strongly on testing volume. The months that appear to be peaks in a simple monthly tabulation were the months in which the fewest slides were examined.

4.2 Comparison with earlier studies in Lower Dir and the region

Our SPR of 7.84% is lower than earlier microscopy estimates from Lower Dir: 12.2% in 2013 [19], 16.1% in 2018 [20] and about 12% in a combined Lower Dir and Swat sample [21] (Table 5; Fig. 8). It is also lower than recent estimates from neighboring Swat (10.1%) [31] and Bajaur (13.1%) [32], and from household RDT surveys in southern KP (13.8%) [14]. It is similar to the 6.86% reported from the general population of Buner [33]. All of these figures are far below the pooled national estimate of 23.3% [13], which is dominated by hospital studies from high-transmission districts of Sindh and Baluchistan.

The lower figure in our study should not be read as proof of declining transmission. Study designs differ. Earlier Lower Dir studies sampled several clinics and tehsils [19,20], whereas ours drew on a single referral laboratory whose patients include many non-malarial febrile illnesses. An SPR is also sensitive to the threshold at which clinicians request a smear: liberal testing of all fevers lowers positivity even when transmission is unchanged. Finally, 2020 was a disrupted year (Section 4.4). At most, our result is consistent with moderate, vivax-dominated transmission that is no higher than in earlier years. Whether transmission is falling can only be judged with consistent multi-year data from the same facilities.

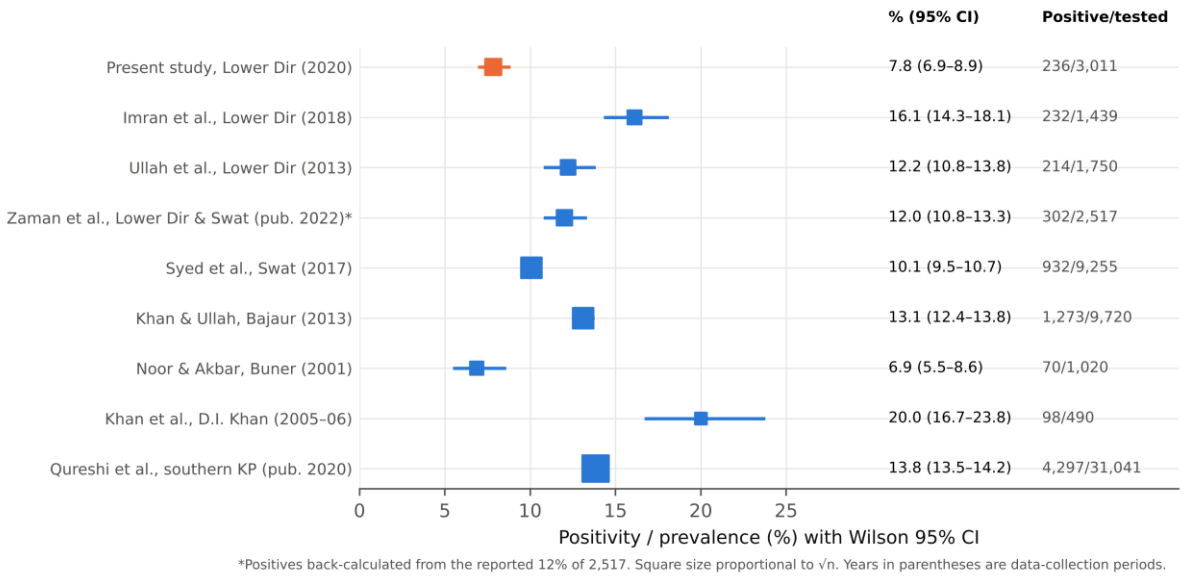


Fig. 8. Slide positivity or prevalence of malaria reported by the present and previous studies in Khyber Pakhtunkhwa, with Wilson 95% confidence intervals calculated from the published numerators and denominators. Designs, settings and diagnostic methods differ (see Table 5), so the estimates are shown for context and were not pooled.

Table 5. Malaria positivity and species composition reported from Lower Dir and other districts of Khyber Pakhtunkhwa, compared with the present study.

Study	Area	Data period	Setting / method	Tested (n)	Positivity, %	P. vivax share of positives
Present study	Lower Dir	2020	DHQ Hospital; microscopy	3,011	7.84	98.3%
Ullah et al. [19]	Lower Dir (7 tehsils)	2013	Hospitals/labs; microscopy	1,750	12.2	90.2%
Imran et al. [20]	Lower Dir	Apr–Nov 2018	Clinics, THQ Samarbagh; microscopy	1,439	16.1	Not reported
Zaman et al. [21]	Lower Dir & Swat	Not stated (publ. 2022)	Facility; microscopy	2,517	≈12	99.1%
Syed et al. [31]	Swat	Jan–Jul 2017	Facility; microscopy + RDT	9,255	10.1	100%
Khan & Ullah [32]	Bajaur	2013	Facility; microscopy	9,720	13.1	80.8%
Noor & Akbar [33]	Buner	Mar–Aug 2001	Community; microscopy	1,020	6.86	84.3%
Khan et al. [34]	D.I. Khan	2005–2006	Hospital adults; microscopy	490	20.0	P. falciparum > P. vivax (≈1.5:1)
Qureshi et al. [14]	Bannu, D.I. Khan, Lakki Marwat	Not stated (publ. 2020)	Household survey; RDT	31,041	13.8	92.4%

Values are as reported by each study; positivity for Zaman et al. is the reported overall rate. Studies differ in design, population and diagnostic method, so figures are not directly comparable and were not pooled. RDT, rapid diagnostic test; THQ, Tehsil Headquarters hospital.

4.3 Species composition and its implications

The near-total dominance of P. vivax (98.3%) matches recent data from the same district (90% in 2013; 99.1% in Lower Dir and Swat) [19,21] and from Swat, where all positives were vivax [31]. It also fits the national pattern of a north–south gradient: vivax predominates in the cooler northern districts of KP, while the share of P. falciparum rises towards Sindh and Baluchistan, including districts bordering Iran and eastern Baluchistan [10–12,35,36]. Hospital studies from southern Pakistan report much higher falciparum shares. These include a falciparum majority among children in Larkana [37], more falciparum than vivax among adults in Dera Ismail Khan [34], and mixed infections of about 2% in Multan and Quetta [38,39]. No mixed, P. malariae or P. ovale infections were found here, as in other northern KP series [32,40,41].

The predominance of vivax has three practical consequences. First, P. vivax sustains transmission through relapses from hypnozoites. These relapses can occur months after infection and outside the main vector season, which may explain why cases were recorded in every month, including the coldest [4,5]. Second, a lasting cure requires treatment of the liver stage. WHO recommends chloroquine or an artemisinin-based combination for the blood stage plus primaquine (or tafenoquine where available) for radical cure, after testing for glucose-6-phosphate dehydrogenase (G6PD) deficiency [42]. In a previous Lower Dir and Swat study, 95.7% of treated patients received chloroquine and primaquine use was not reported [21]. Radical cure may therefore be under-used locally, which would leave relapses untreated. Third, vivax is not benign: severe anemia and other complications are well documented and should not be dismissed in planning [6,7]. Microscopy can miss low-density or mixed infections and can misidentify species [28]. Molecular studies in Pakistan have found some microscopy-positive samples to be PCR-negative and have found mixed infections that microscopy missed [11]. The true falciparum share may therefore be slightly higher than 1.7%. Even so, it is very unlikely to change the conclusion that vivax dominates.

4.4 Seasonality and the influence of testing volume in 2020

A naive monthly tabulation of our data would show a malaria "peak" in April (66.7%) and November (40.9%). Such a reading would conflict with the known transmission biology of the region. In Pakistan, Anopheles density and malaria cases normally peak after the monsoon, from late summer into autumn [10]. Peshawar data show vivax arriving in spring and persisting through summer, followed by an autumn falciparum peak [43]. Earlier Lower Dir studies placed the peak in July–August [19,20]. Mosquito and parasite development are temperature-dependent, with transmission efficiency predicted to be highest at about 25 °C [44]. Climatic studies from the Himalayan foothills also link malaria incidence to temperature, humidity and rainfall [45].

Our data explain the discrepancy. The four months with the highest SPRs were the four months with fewest slides examined (6, 22, 40 and 60). Across all twelve months, positivity fell steeply as testing rose ($\rho = -0.83$). The fall in testing in April–May 2020 coincided with the first nationwide COVID-19 lockdown, when outpatient services were curtailed and many febrile patients stayed away from hospitals or were assessed for COVID-19 instead. A second fall in testing came in November–December 2020. Under these conditions, the patients still tested were probably sicker or more clearly malarial, which inflates positivity. National surveillance analyses show that COVID-19 altered the reported incidence of several endemic infections in Pakistan [22]. Studies in Africa document how the pandemic disrupted malaria diagnosis and services [46,47]. We could not measure the reasons for the falls in testing directly, so attributing them to COVID-19 is a plausible explanation rather than a demonstrated one.

When analysis is limited to the eight high-volume months, the seasonal pattern is more consistent with the literature. Positivity rose from a winter low (January 3.3%, February 6.0%) to a June high (12.2%) and stayed elevated in September–October (9.4–11.5%). The very low SPR in July (2.9%) is unexpected, given that June and August were higher. It may reflect a large volume of non-malarial fevers being tested in July, but we cannot confirm this. Seasonal comparisons in this study should therefore be read as

descriptive. Surveillance programmes that report monthly SPR should always report the denominator alongside it.

4.5 Sex and age

The male excess (OR 1.62) is consistent with most Pakistani studies, including those from Lower Dir, Bajaur and Swat [20,31,32]. The usual explanations are that men in this agricultural district spend more time outdoors and in irrigated fields in the evening and at night, sleep outdoors in hot months, and travel for work. In clinical series from hypo endemic India, the excess appears after puberty, which suggests that behavior and possibly hormonal factors both contribute [48]. The direction of this difference depends on context. In Uganda, diagnosed malaria was more common in women, largely because women used health facilities more [49]. Health-seeking behavior shapes both who is tested and who tests positive. In our study, two-thirds of those tested were male, which may itself reflect restricted access for women in a conservative rural setting. Male excess was present for both the numerator and the denominator, and the OR addresses only the second.

Children made up only 1.7% of positives, although under-15s are about 47% of the population [23]. In KP and FATA, other studies have found children to be the group at highest risk [15,19,32]. Our finding most likely reflects how cases reached this laboratory, through separate pediatric pathways, private clinics, or incomplete recording of age. It is unlikely to show true protection of children. Because neither the number of children tested nor exact ages were recorded, we cannot estimate age-specific positivity. We do not interpret this result as showing lower risk in children.

4.6 Implications for malaria control

Malaria in Lower Dir is a vivax problem. The priorities are: (i) G6PD-guided radical cure with primaquine for confirmed vivax cases, in line with WHO guidance [42]; (ii) species-specific diagnosis at every testing point, since falciparum cases are rare and need different treatment; (iii) steady, year-round febrile testing, with monthly denominators reported alongside positivity, so that disruptions such as those in 2020 or after the 2022 floods [17,18] do not distort trend interpretation; (iv) routine recording of exact age, sex, locality and travel history, which would allow age-specific rates and mapping of hotspots; and (v) vector control (long-lasting insecticidal nets and indoor residual spraying), with behavior-change messaging for men working outdoors and for households that sleep outdoors in summer.

4.7 Strengths and limitations

This study's strengths are a full calendar year of consecutive data from the district's main public laboratory, a reasonably large sample, microscopy by trained staff, and reporting of denominators and confidence intervals for every estimate. We also explicitly assessed how testing volume affected monthly positivity, which earlier studies in the area did not. Its limitations follow from its design. First, it is a single-facility study of febrile patients, so the SPR cannot be read as community prevalence or extrapolated to the whole district. Second, we relied on routine microscopy without RDT or PCR confirmation and without documented external quality control. Low-density and mixed infections may therefore have been missed and species occasionally misclassified [11,28]. Third, age was recorded only as child or adult and only for positives, and two positives lacked sex and age. Fourth, the data are for a single, atypical year (2020) and cannot show trends. Fifth, we had no information on travel, previous malaria (relapse versus new infection), treatment or outcome. Finally, the analysis is unadjusted. With only sex recorded at the individual level for negatives, multivariable modelling was not possible.

5. Conclusions

In 2020, 7.84% of febrile patients tested at the DHQ Hospital in Lower Dir had malaria, and almost all of these infections (98.3%) were P. vivax. Positivity was significantly higher in males, and recorded cases were almost all adults. Very high monthly positivity in April and November reflected the small number of patients tested in those months, not transmission peaks. Monthly SPR from hospital laboratories should therefore always be reported with its denominator. Control in Lower Dir should prioritize G6PD-guided radical cure of vivax, consistent species-specific diagnosis, stable year-round surveillance with

complete recording of age and sex, and targeted vector control. Multi-year, multi-site surveillance with molecular confirmation is needed to establish true trends and seasonality in the district.

Declarations

Credit authorship contribution statement. Investigation, Data curation, Formal analysis, Writing original draft.

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Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability. The aggregated monthly data underlying this study are given in full in Tables 1–4. Anonymized line-level data are available from the corresponding author on reasonable request, subject to approval by the hospital administration.

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