Research article

Malaria prevalence in Pakistan: A systematic review and meta-analysis (2006–2021)

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ABSTRACT

Malaria is one of the major public health issues globally. Malaria infection spreads through mosquito bites from infected female Anopheles mosquitoes. This study aims to conduct a systematic review and meta-analysis on malaria prevalence in Pakistan from 2006 to 2021. We searched PubMed, Science Direct, EMBASE, EMCare, and Google Scholar to acquire data on the prevalence of malaria infections. We performed a meta-analysis with a random-effects model to obtain the pooled prevalence of malaria, Plasmodium vivax, and Plasmodium falciparum. Meta-analysis was computed using R 4.1.2 Version statistical software. I^2 and time series analysis were performed to identify a possible source of heterogeneity across studies. A funnel plot and the Freeman-Tukey Double Arcsine Transformed Proportion were used to evaluate the presence of publication bias. Out of the 315 studies collected, only 45 full-text articles were screened and included in the final measurable meta-analysis. Pooled malaria prevalence in Pakistan was 23.3%, with *Plasmodium vivax*, *Plasmodium falciparum*, and mixed infection rates of 79.13%, 16.29%, and 3.98%, respectively. Similarly, the analysis revealed that the maximum malaria prevalence was 99.79% in Karachi and the minimum was 1.68% in the Larkana district. Amazingly, this systematic review and meta-analysis detected a wide variation in malaria prevalence in Pakistan. Pakistan's public health department and other competent authorities should pay close attention to the large decrease in mosquito populations to curb the infection rate.

1. Introduction

Malaria is a protozoan disease caused by five *Plasmodium* species and spread by the female Anopheles mosquitoes [1,2]. It is one of

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the leading causes of morbidity and mortality, with enormous medical and economic impact [3,4]. Four species, including *P. vivax* and *P. falciparum*, are known to infect humans in Pakistan and are the most common and widely distributed parasites [5,6]. According to the World Malaria Report for 2021, about half of the world's population lives in 87 nations and regions at risk of malaria transmission. In 2020, it was estimated that Malaria affected 241 million medical episodes and caused about 627,000 deaths [7]. Apart from that, it's a major public health issue in humid and subtropical areas [8,9]. According to the World Health Organization (WHO), Pakistan is one of the seven countries in the Eastern Mediterranean Region that account for 98% of the total malaria burden in the region. Around 217 million people in Pakistan are at moderate risk of malaria, and 63 million are at high risk. Approximately 0.47 million malaria cases and 800 deaths have been reported in 2020 [10].

Environmental factors such as topography, rainfall, climate, and people's socioeconomic status significantly impact malaria transmission. As a result, tropical countries with warm temperatures, heavy rainfall, and high humidity, such as Ethiopia, are ideal for mosquito breeding, lifespan, and parasite sporogony. Malaria is endemic in Pakistan, where it is unstable and spreads seasonally. Transmission peaks twice a year, in September and December and April and May, to coincide with the height of agricultural activity [11]. Fever, high temperature, sweating, shivering, vomiting, and severe headache are unusual malarial antecedents in terms of clinical signs [12]. Several malaria diagnostic tests are available, including microscopy, rapid diagnostic tests, and polymerase chain reaction (PCR) assays. Despite its low sensitivity and the necessity for trained professionals, microscopy is still the gold standard for malaria diagnosis [13]. Early diagnosis, timely treatment, targeted vector control, and epidemic prevention are among the techniques used by the Pakistani government to combat malaria [14].

In the late 1970s, the health departments of all four provinces recorded a progressive increase in annual malaria cases. Thus, malaria frequency surged dramatically in the Punjab province between 1980 and 1990, then declined slightly in the late 1980s before increasing again in the 1990s. Similarly, Malaria spread expanded in Khyber Pakhtunkhwa (KP) and Sindh provinces in 1990, but in 1980, Malaria spread throughout northern and southern Khyber, with cases documented in Balochistan. Malaria is most prevalent in KP and Balochistan, specifically Swat, Malakand, Peshawar, Mardan, Lower Dir, Quetta, and Buner [6,11,15,16].

Pakistan is extremely vulnerable to climate change due to its geographical location, reliance on agricultural and water resources, and weak disaster preparedness infrastructure. Frequent floods in Pakistan have been linked to a significant rise in malaria cases. Climate change, poor vector management, and insufficient health care are most likely to be blamed for malaria cases [17,18].

Accurate epidemiological data on malaria prevalence and species distribution across genders and all age categories, including all provinces—especially those frequently ignored—are crucial for achieving the goals outlined in the global technical strategy against malaria for 2016–2030. To the best of our knowledge, there isn't any comprehensive report from Pakistan on malaria epidemiology in different provinces, species distribution, or disease burden across gender and age groups. This study sought to assess the pooled prevalence and proportion of mixed infection by *Plasmodium* spp. In different regions of Pakistan. This information is necessary to guide the.

progress of research on mixed infections and can guide stack holders to develop successful malaria control and elimination strategies. This systematic review or meta-analysis is the first-ever report estimating pooled malaria incidence in Pakistan.

2. Methods

2.1. Searching strategy. The preferred reporting items for systematic reviews and meta-analyses

(PRISMA) guideline was used to conduct this systematic review and meta-analysis. Utilized the following electronic databases to ascertain malaria prevalence in Pakistan: Pubmed, Science Direct, EMBASE, EMCare, and Google Scholar. We used a keyword procedure to match the search keywords to the Medical Subject Headings (MeSH). The search words were also combined and separated using Boolean operators like "OR" and "AND." We used the MeSH terms "prevalence" or "prevalence" and "malaria" or "malaria" [MeSH terms] and "Pakistan" or "Pakistan" [MeSH terms] to find important articles. All reports and research studies on malaria prevalence in Pakistan published from 2006 to 2021 were included.

2.2. Criteria for inclusion and exclusion

2.2.1. Criteria for inclusion

We considered cross-sectional, descriptive, and retrospective investigations using microscopies, RDTs, and PCR as laboratory procedures in this meta-analysis.

2.2.2. Criteria for exclusion

Studies that reported an unknown sample size were removed simultaneously, and studies that lacked strong data on disease cases were also removed.

2.3. Procedure for searching and evaluating quality

We imported the published research articles into EndNote 20 and deleted duplicates once they were also deleted while searching for them. Each author examined the literature individually for possibly eligible publications that satisfied the stated inclusion criteria by looking at.

titles, abstracts, and full texts. Seventy-five study articles were checked for suitability standards by looking at abstracts and titles.

Sixty-one of them were chosen by all the authors for full-text review.

2.4. Data extraction

All authors of this paper collaborated to design the data extraction form in Microsoft Excel. This information extraction sheet included the first author's name, year of publication, region, study design, age, laboratory method used, gender, sample size, overall malaria prevalence, *P. vivax*, and *P. falciparum*. Finally, the reliability of the eliminated data files was rigorously reviewed, and a detailed discussion between the authors resolved any differences among the removed data.

2.5. Analysis of data

After extracting the data with Microsoft Excel, we utilized the R 4.1.2 version for statistical analysis. Because of the considerable heterogeneity in the study, we adopted random-effects models. Otherwise, it generates study encumbrances, mostly used to distinguish between different research variations. The I^2 statistic, which has a value ranging from 0 to 100%, predicts that the major difference among studies involved in a systematic review and meta-analysis is attributable to heterogeneity instead of chance. Low, medium, and high heterogeneity between trials is indicated by I^2 values of 25%, 50%, and 75% plus. When a p-value was less than 0.05, the presence of heterogeneity was determined. We also investigated the publication's bias.

2.6. Random effect model

A random-effects meta-analysis model (equation (2.1)) considers that empirical estimates of treatment effect may differ across studies due to differences in treatment effect in each study and sampling variability (chance). As a result, even if every research study had an arbitrarily high sample size, the apparent study effects would still differ due to actual variances in treatment effects. Changes in study populations induce treatment effect heterogeneity, treatments received, follow-up time, and other variables. The pooled estimate and confidence interval for the random-effects model relate to the center of the distribution of intervention effects but do not indicate the width of the distribution. It is common to reference the pooled estimate and its confidence interval as an alternative
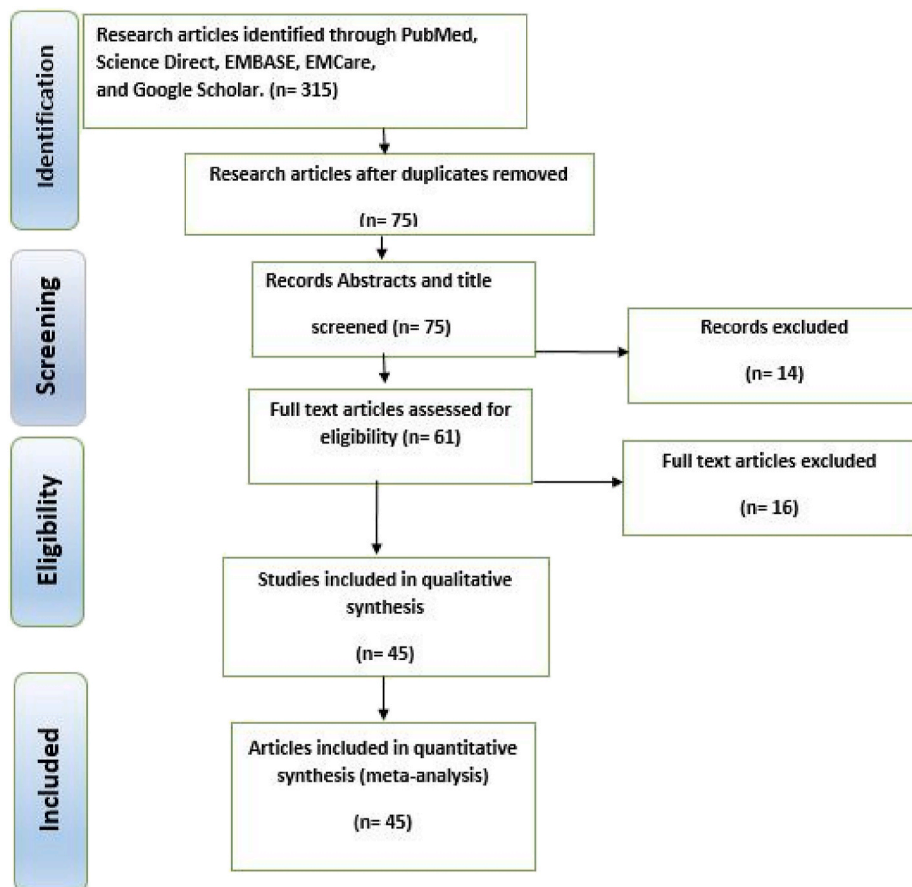

Fig. 1. Collection of studies involved in a Systematic Review and Meta-Analysis.

Table 1

The detailed studies have general characteristics in the methodical review (systematic review) and meta-analysis.

S. No	Author/Year	Region	Study Design	Patient Age	Diagnostic Method	Gender (%)	Sample size	Prevalence (%)	PV %	PF %	Mixed %
1	Rahman et al., 2016 [19]	Shangla, Swat, KP	Cross-sectional	1 to 84	Microscopic	Male 65.24 Female 34.76	1336	13.99	98.93	0.07	NA
2	Sumbal et al., 2020 [20]	Quetta, Balochistan	Cross-sectional	3 to 16	Microscopic	Male 76.44 Female 23.55	2213	19.50	84.52	6.01	10.39
3	Khan et al., 2018 [21]	Peshawar, KP	Cross-sectional	1 to 15	Microscopic	Male 23.5 Female 10.5	375	17.60	15.50	1.30	4.54
4	Khatoon et al., 2018 [22]	Karachi, Sindh	Cross-sectional	1 to 60	Microscopic	Male 60.10 Female 39.89	481	480	82.32	17.67	NA
5	Khan et al., 2019 [23]	Malakand, KP	Cross-sectional	3 to 80	Microscopic	Male 28.7 Female 24.2	1123	26.70	98.60	1.30	NA
6	Qureshi et al., 2021 [6]	Bannu, KP	Cross-sectional	All ages	RDT	Male 53.9 Female 46.1	31041	13.80	92.40	4.70	2.86
7	Ajmal and Rehan, 2019 [24]	Bajaur Agency, FATA	Descriptive	All ages	Microscopic	Male 14.59 Female 11.24	9720	13.10	80.80	18.50	0.7
8	Ullah et al., 2019 [25]	Dir Lower, KP	Cross-sectional	1 to 31	Microscopic	Male 11.2 Female 9.81	1750	12.20	90	9.81	
9	Noor and Muhammad, 2017	Killa Saifullah, Balochistan	Cross-sectional	All Ages	Microscopic	NA	4208	20.00	64.70	34.70	0.47
10	Zeb et al., 2015 [26]	Lower Dir, KP	Cross-sectional	11 to 51	Microscopic	Male 13.3 Female 10.7	3760	12.20	94.30	3.90	1.70
11	Majid et al., 2016 [27]	Mardan, KP	Descriptive	All ages	Microscopic	Male 43.73 Female 56.27	11272	6.80	92.56	7.44	NA
12	Imran et al., 2017 [28]	Malakand, KP	Cross-sectional	All ages	Microscopic	Male 28.28 Female 18.81	210	22.38	59.57	40.42	NA
13	Zafar et al., 2019 [29]	Multan, Punjab	Cross-sectional	All ages	Microscopic	Male 57.51 Female 42.48	3770	39.20	61.23	28.75	10.01
14	Mahar et al., 2018 [30]	Taluka Sukkur, Sindh	Cross-sectional	All ages	Microscopic	Male 56.63 Female 43.36	1985	9.87	81.63	18.36	NA
15	Hussain et al., 2015 [31]	Lower Dir, KP	Cross-sectional	All ages	Microscopic	Male 62.77 Female 37.23	2741	10.29	98.94	1.06	NA
16	Shah et al., 2016 [32]	Lower Dir, KP	Cross-sectional	All Ages	Microscopic	Male 24.5 Female 15.0	821	39.50	30.10	9.40	NA

Table 1 (*continued*)

S. No	Author/Year	Region	Study Design	Patient Age	Diagnostic Method	Gender (%)	Sample size	Prevalence (%)	PV %	PF %	Mixed %
17	Farooq et al., 2019 [33]	Pishin, Balochistan	Cross-sectional	1 to 60	Microscopic	NA	3031	18.80	90.70	4.80	4.90
18	Syed et al., 2020 [34]	Swat Valley, KP	Cross-sectional	All Ages	Microscopic	Male 59.87 Female 40.13	9255	10.07	10.00	0.07	NA
19	Ibrahim et al., 2014 [35]	Buner, KP	Descriptive	1 to 60	Microscopic	Male 69.79 Female 30.72	4322	26.80	91.53	7.47	NA
20	Bashir et al., 2019 [36]	D.I. Khan, KP	Cross-sectional	All ages	Microscopic	Male 68.9 Female 31.1	3398	12.20	96.60	3.40	NA
21	Jahan and Sarwar, 2013 [37]	Okara, Punjab	Cross-sectional	1 to 70	Microscopic	Male 18.00 Female 8.00	116	26.00	92.00	2.00	NA
22	Tareen et al., 2012 [38]	Quetta, Balochistan	Cross-sectional	1 to 70	Microscopic	Male 62.13 Female 37.87	1831	18.45	81.66	18.34	NA
23	Yasinzai and Khan, 2008 [39]	Barkhan and Kohlu, Balochistan	Cross-sectional	All ages	Microscopic	NA	3340	32.78	47.12	52.87	NA
24	Yasinzai and Khan, 2012 [40]	Jhal Magsi, Balochistan	Cross-sectional	All ages	Microscopic	Male 65.3 Female 34.6	3402	34.90	65	34.90	NA
25	Awan et al., 2012 [41]	Bannu, KP	Cross-sectional	6 to 15	Microscopic	Male 94.12 Female 5.88	556	3.05	2.69	0.35	NA
26	Yasinzai and Khan, 2008 [42]	Duki, Harnai, And Sibi, Balochistan	Cross-sectional	All ages	Microscopic	Male 62.77 Female 37.23	6730	34.20	42.80	57.10	NA
27	Khan et al., 2013 [43]	Bannu, KP	Cross-sectional	All Ages	Microscopic	Male 30.64 Female 24.95	823	27.10	22.60	3.04	5.38
28	Yasinzai and Khan, 2013 [44]	Panjgur, Balochistan	Cross-sectional	All ages	Microscopic	Male 78.00 Female 22.00	6119	38.30	79.60	20.30	NA
29	Ahmad et al., 2013 [45]	Lower Dir, KP	Descriptive	All ages	Microscopic	Male 58.7 Female 41.3	1091	17.32	99.47	0.53	NA
30	Din et al., 2006 [46]	Mansehra, KP, Pakistan	Cross-sectional	14 days to 15 years	Microscopic	Male 71.25 Female 28.75	160	96	92.21	7.79	NA
31	Kurd et al., 2019 [47]	Khuzdar, Balochistan	Cross-sectional	3 to 16	Microscopic	Male 60.47 Female 39.52	1193	27.90	67	15.80	17.06
32	Yasinzai and Khan, 2009 [48]	Ziarat and Sanjavi, Balochistan	Cross-sectional	11 and above	Microscopic	Male 75.9 Female 24.1	3765	26.80	30.20	69.50	NA
33	Yasinzai and Khan, 2010 [48]	Zhob, Balochistan	Cross-sectional	All ages	Microscopic	Male 77.60 Female 22.40	7748	41.80	51.80	48.10	NA

Table 1 (*continued*)

S. No	Author/Year	Region	Study Design	Patient Age	Diagnostic Method	Gender (%)	Sample size	Prevalence (%)	PV %	PF %	Mixed %
34	Malik et al., 2010 [49]	Karachi, Sindh	Cross-sectional	All ages	Microscopic	Male 58.00 Female 42.00	3344	11.72	52	42.60	5.35
35	Idris et al., 2007 [50]	Abbottabad, KP	Cross-sectional	All ages	Microscopic	Male 55.17 Female 44.83	1994	7.27	72.40	24.13	3.44
36	Pakistan Malaria Annual Report, 2019 [14]	Overall Pakistan	Cross-sectional	All ages	Microscopic	NA	6500000	5.76	84.0	14.9	NA
37	Naqvi, 2019 [51]	D.I.Khan, KP	Cross-sectional	All ages	Microscopic	Male 4.97 Female 3.82	108849	8.79	8.38	0.40	0.009
38	Khattak et al., 2013 [52]	Kohat, KP	Retrospective	NA	Microscopic	Male 50.19 Female 49.80	31128	8.28	86.34	13.65	NA
39	Hussain et al., 2013 [53]	Quetta, Balochistan	Cross-sectional	1 to 18	Microscopic	Male 65.21 Female 34.78	3418	6.72	54.34	44.78	0.86
40	Nadeem et al., 2021 [54]	Bajaur, Mohmand, Khyber, Orakzai agencies, FATA	Descriptive	All ages	Microscopic	Male 69 Female 31	762	89.10	77	17.82	5.15
41	Jahan et al., 2019 [55]	Bannu, KP	Cross-sectional	All ages	Microscopic, RDT, PCR	Male 21.2 Female 20.8	3962	18.17	78.61	10.55	NA
42	Ahmad, 2015 [56]	Lower Dir, KP	Descriptive	15 to 64	Microscopic	Male 64.38 Female 35.62	443	16.48	98.63	1.4	NA
43	Hussain et al., 2013 [57]	Quetta, Balochistan	Cross-sectional	1 to 18	Microscopic	Male 51.33 Female 48.67	3418	6.74	54.34	45.66	NA
44	Khattak et al., 2013 [11]	Overall Pakistan	Cross-sectional	Three months to 75 years	Microscopic	Male 63 Female 37	10782	6.6	5	1.2	0.4
45	Soomro, 2010 [58]	Larkana, Punjab	Descriptive	All ages	Microscopic	Male 65 Female 35	87617	1.68	47.15	52.85	NA

PV: Plasmodium vivax; PF: Plasmodium falciparum; Mixed: Plasmodium vivax + Plasmodium falciparum; KP: Khyber Pakhtunkhwa; FATA: Federally Administered Tribal Areas; D.I.Khan: Dera Ismail Khan; KP province 22 studies included; Balochistan province 13 studies included; Sindh province 3 studies included; FATA 2 studies included; Punjab province 3 studies included; Overall Pakistan 2 study included; 37 cross-sectional studies; 7 descriptive studies; 1 retrospective study.

estimate of the quantity examined in a fixed-effect meta-analysis, which is incorrect. A random-effects meta-analysis confidence interval indicates uncertainty in the position of the mean of systematically varied effects in multiple studies. Contrary to popular belief, it does not describe the degree of variability among studies. Mathematically shown as:

$$Y_{it} = \beta_0 + \beta_1 X_{it} + \beta_2 Z_{it} + \alpha_i + \mu_{it} \tag{2.1}$$

Where $\beta_0, \beta_1, and\ \beta_2$ are the coefficients, α_i unobserved heterogeneity and μ_{it} error term

3. Results

3.1. Characteristics of the articles involved

A total of 45 published papers on the prevalence of Malaria in Pakistan were included after examining all inclusion and exclusion criteria. Initially, 315 existing publications were located using a database search engine. From the 315 research papers assessed, 240 duplicates were detected and removed. Then, based on their titles and abstracts, 75 publications were screened. Finally, after thoroughly analyzing the whole text of sixty-one papers, we judged that forty-five were eligible for our systematic review and meta-analysis. The number of research papers picked at each stage and the full screening and eligibility process are depicted in Fig. 1. In terms of study design, all papers considered in this review used cross-sectional, descriptive, and retrospective study designs. With only 116 participants, the research in Okara, Punjab, Pakistan, had the smallest sample size of any study assessed. Another study in Pakistan had the most participants overall, with 6500000. Malaria diagnostic methods used in the studies included microscopic, RDT, and PCR. The majority of the high endemic regions were included in this systematic review and meta-analysis. All articles included in this systematic review were assessed using the JBI quality evaluation technique, and all of them received a satisfactory rating. (Table 1).

3.2. Total prevalence of malaria in Pakistan

We calculated the pooled prevalence of malaria in Pakistan using the selected articles Fig. 2. Thus, there was high heterogeneity
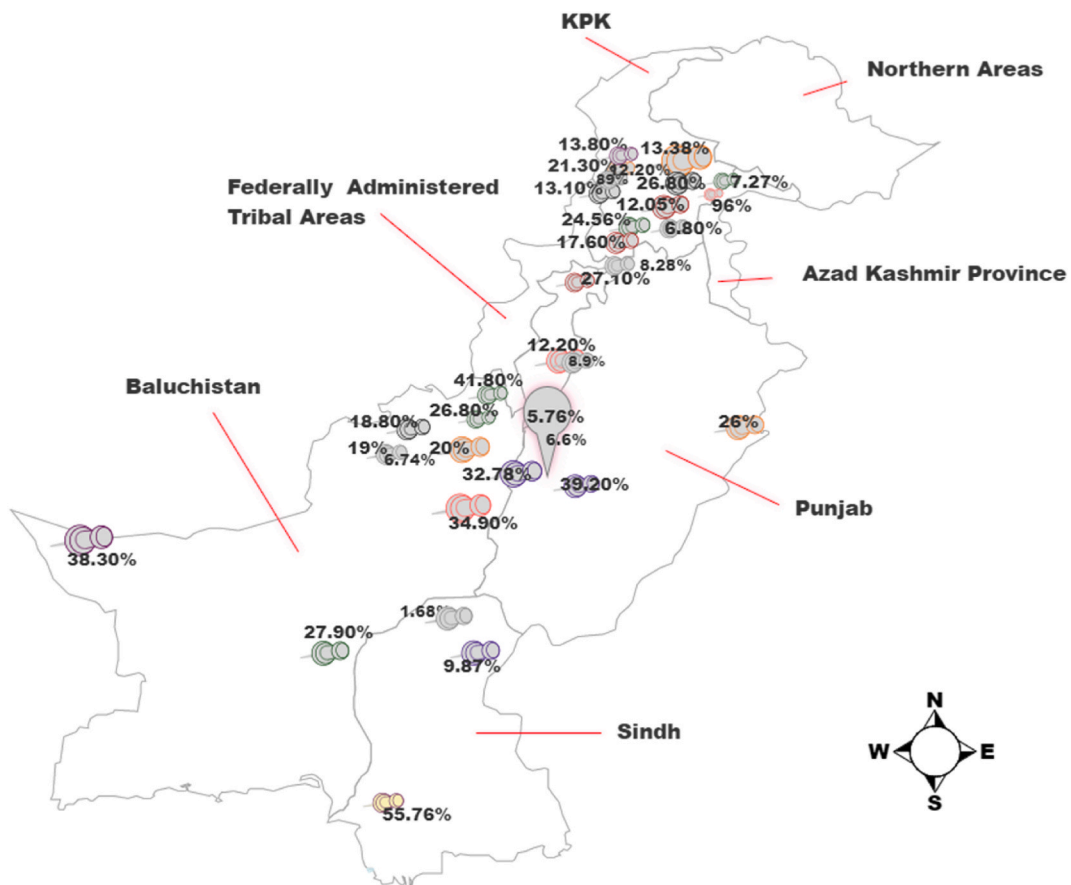

Fig. 2. Prevalence of malaria across Pakistan, 2006 to 2021.

found between the studies ($I^2 = 100\%$, P = 0.0). The random-effects model remained fitted at 95%; the overall estimated pooled prevalence of malaria was 23.25% (95% CI: 16.14–31.21). A forest plot was utilized to depict the malaria incidence in each experiment and the aggregate estimate. The prevalence ranged from 1.68% in Larkana to 99.79% in Karachi (Sindh province) in Pakistan. Each included the study's estimate and incidence, and the consistent CI is shown in Fig. 3.

In this meta-analysis, men had a higher prevalence of malaria than women. The prevalence in males was 61.57% (95% CI: 56.06–66.94, $I^2 = 100\%$, 45 studies), and the prevalence in females was 31.71% (95% CI: 27.24–36.36, $I^2 = 100\%$, 45 studies). Thus, in this meta-analysis, the prevalence of Malaria was higher in people aged 18–30 years. Most of the cases belonged to rural areas.

3.3. Prevalence of Plasmodium falciparum

According to the primary studies, *P. falciparum* prevalence ranged from 0.11% in the Swat KP region to 70.08% in the Zyarat and Sanjavi regions. *P. falciparum* was the second most common parasite, accounting for 16.29% of the total prevalence estimate with a 95% confidence interval of (11.33–21.92) and $I^2 = 100\%$ between-study heterogeneity (Fig. 4).
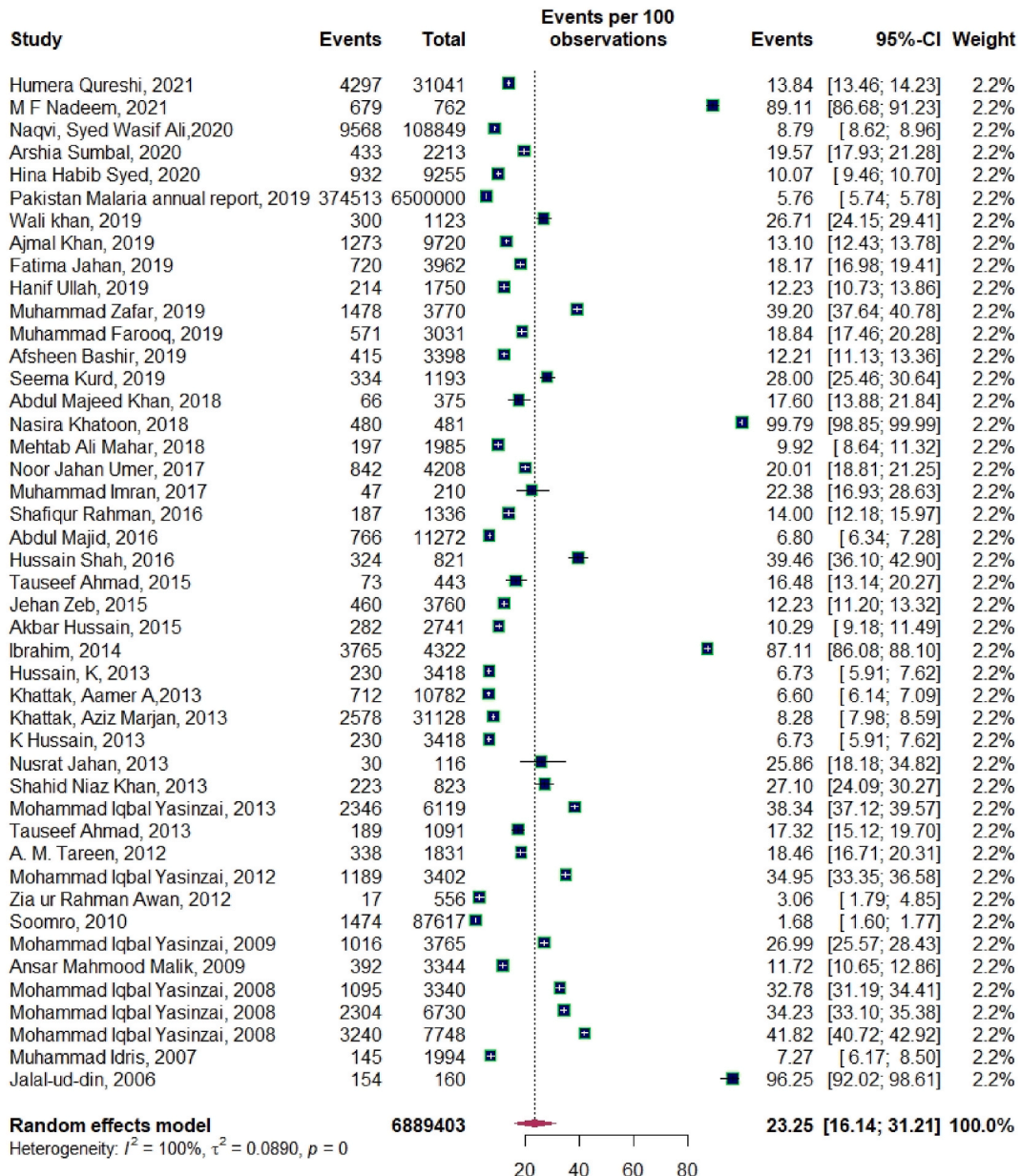
Study	Events	Total	Events per 100 observations	Events	95%-CI	Weight
Humera Qureshi, 2021	4297	31041		13.84	[13.46; 14.23]	2.2%
M F Nadeem, 2021	679	762		89.11	[86.68; 91.23]	2.2%
Naqvi, Syed Wasif Ali,2020	9568	108849		8.79	[8.62; 8.96]	2.2%
Arshia Sumbal, 2020	433	2213		19.57	[17.93; 21.28]	2.2%
Hina Habib Syed, 2020	932	9255		10.07	[9.46; 10.70]	2.2%
Pakistan Malaria annual report, 2019	374513	6500000		5.76	[5.74; 5.78]	2.2%
Wali khan, 2019	300	1123		26.71	[24.15; 29.41]	2.2%
Ajmal Khan, 2019	1273	9720		13.10	[12.43; 13.78]	2.2%
Fatima Jahan, 2019	720	3962		18.17	[16.98; 19.41]	2.2%
Hanif Ullah, 2019	214	1750		12.23	[10.73; 13.86]	2.2%
Muhammad Zafar, 2019	1478	3770		39.20	[37.64; 40.78]	2.2%
Muhammad Farooq, 2019	571	3031		18.84	[17.46; 20.28]	2.2%
Afsheen Bashir, 2019	415	3398		12.21	[11.13; 13.36]	2.2%
Seema Kurd, 2019	334	1193		28.00	[25.46; 30.64]	2.2%
Abdul Majeed Khan, 2018	66	375		17.60	[13.88; 21.84]	2.2%
Nasira Khatoon, 2018	480	481		99.79	[98.85; 99.99]	2.2%
Mehtab Ali Mahar, 2018	197	1985		9.92	[8.64; 11.32]	2.2%
Noor Jahan Umer, 2017	842	4208		20.01	[18.81; 21.25]	2.2%
Muhammad Imran, 2017	47	210		22.38	[16.93; 28.63]	2.2%
Shafiqur Rahman, 2016	187	1336		14.00	[12.18; 15.97]	2.2%
Abdul Majid, 2016	766	11272		6.80	[6.34; 7.28]	2.2%
Hussain Shah, 2016	324	821		39.46	[36.10; 42.90]	2.2%
Tauseef Ahmad, 2015	73	443		16.48	[13.14; 20.27]	2.2%
Jehan Zeb, 2015	460	3760		12.23	[11.20; 13.32]	2.2%
Akbar Hussain, 2015	282	2741		10.29	[9.18; 11.49]	2.2%
Ibrahim, 2014	3765	4322		87.11	[86.08; 88.10]	2.2%
Hussain, K, 2013	230	3418		6.73	[5.91; 7.62]	2.2%
Khattak, Aamer A,2013	712	10782		6.60	[6.14; 7.09]	2.2%
Khattak, Aziz Marjan, 2013	2578	31128		8.28	[7.98; 8.59]	2.2%
K Hussain, 2013	230	3418		6.73	[5.91; 7.62]	2.2%
Nusrat Jahan, 2013	30	116		25.86	[18.18; 34.82]	2.2%
Shahid Niaz Khan, 2013	223	823		27.10	[24.09; 30.27]	2.2%
Mohammad Iqbal Yasinzai, 2013	2346	6119		38.34	[37.12; 39.57]	2.2%
Tauseef Ahmad, 2013	189	1091		17.32	[15.12; 19.70]	2.2%
A. M. Tareen, 2012	338	1831		18.46	[16.71; 20.31]	2.2%
Mohammad Iqbal Yasinzai, 2012	1189	3402		34.95	[33.35; 36.58]	2.2%
Zia ur Rahman Awan, 2012	17	556		3.06	[1.79; 4.85]	2.2%
Soomro, 2010	1474	87617		1.68	[1.60; 1.77]	2.2%
Mohammad Iqbal Yasinzai, 2009	1016	3765		26.99	[25.57; 28.43]	2.2%
Ansar Mahmood Malik, 2009	392	3344		11.72	[10.65; 12.86]	2.2%
Mohammad Iqbal Yasinzai, 2008	1095	3340		32.78	[31.19; 34.41]	2.2%
Mohammad Iqbal Yasinzai, 2008	2304	6730		34.23	[33.10; 35.38]	2.2%
Mohammad Iqbal Yasinzai, 2008	3240	7748		41.82	[40.72; 42.92]	2.2%
Muhammad Idris, 2007	145	1994		7.27	[6.17; 8.50]	2.2%
Jalal-ud-din, 2006	154	160		96.25	[92.02; 98.61]	2.2%
Random effects model		**6889403**		**23.25**	**[16.14; 31.21]**	**100.0%**

Heterogeneity: $I^2 = 100\%$, $\tau^2 = 0.0890$, $p = 0$

20 40 60 80

Fig. 3. Overall prevalence of Malaria in Pakistan from the random-effects model (2006–2021).

3.4. Prevalence of Plasmodium vivax

This systematic review and meta-analysis discovered *P. vivax* prevalence estimates of 29.92% and 99.89% in Zairat, Sanjavi, and Swat, respectively, in the KP province. However, in this systematic review and meta-analysis, *P. vivax* is presently the most prevalent malaria parasite in Pakistan, accounting for 79.13% with a 95% confidence interval of (72.60–84.99) and $I^2 = 100\%$ between-study heterogeneity (Fig. 5).

3.5. Prevalence of mixed infection

Only 16 out of 45 studies had mixed infection in this systematic review and meta-analysis. Mixed infection estimates of 0.10% and 17.07% in D.I. Khan and Khudzar. However, in this study Mixed infection is presently the lowest prevalent malaria parasite in Pakistan, accounting for 3.98% with a 95% confidence interval of (2.04–6.49) and $I^2 = 99\%$ between-study heterogeneity (Fig. 6).
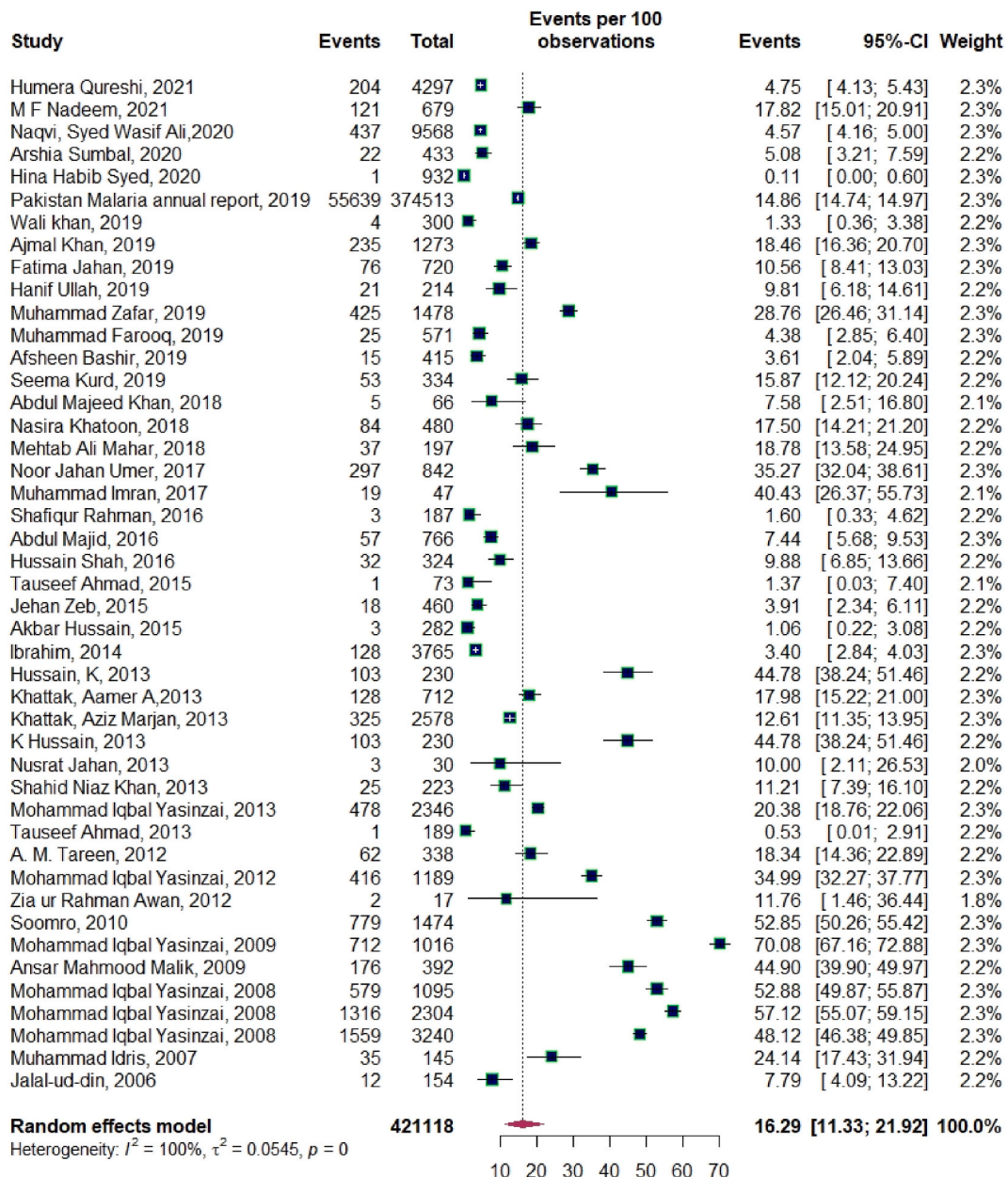
Study	Events	Total	Events per 100 observations	Events	95%-CI	Weight
Humera Qureshi, 2021	204	4297		4.75	[4.13; 5.43]	2.3%
M F Nadeem, 2021	121	679		17.82	[15.01; 20.91]	2.3%
Naqvi, Syed Wasif Ali,2020	437	9568		4.57	[4.16; 5.00]	2.3%
Arshia Sumbal, 2020	22	433		5.08	[3.21; 7.59]	2.2%
Hina Habib Syed, 2020	1	932		0.11	[0.00; 0.60]	2.3%
Pakistan Malaria annual report, 2019	55639	374513		14.86	[14.74; 14.97]	2.3%
Wali khan, 2019	4	300		1.33	[0.36; 3.38]	2.2%
Ajmal Khan, 2019	235	1273		18.46	[16.36; 20.70]	2.3%
Fatima Jahan, 2019	76	720		10.56	[8.41; 13.03]	2.3%
Hanif Ullah, 2019	21	214		9.81	[6.18; 14.61]	2.2%
Muhammad Zafar, 2019	425	1478		28.76	[26.46; 31.14]	2.3%
Muhammad Farooq, 2019	25	571		4.38	[2.85; 6.40]	2.3%
Afsheen Bashir, 2019	15	415		3.61	[2.04; 5.89]	2.2%
Seema Kurd, 2019	53	334		15.87	[12.12; 20.24]	2.2%
Abdul Majeed Khan, 2018	5	66		7.58	[2.51; 16.80]	2.1%
Nasira Khatoon, 2018	84	480		17.50	[14.21; 21.20]	2.2%
Mehtab Ali Mahar, 2018	37	197		18.78	[13.58; 24.95]	2.2%
Noor Jahan Umer, 2017	297	842		35.27	[32.04; 38.61]	2.3%
Muhammad Imran, 2017	19	47		40.43	[26.37; 55.73]	2.1%
Shafiqur Rahman, 2016	3	187		1.60	[0.33; 4.62]	2.2%
Abdul Majid, 2016	57	766		7.44	[5.68; 9.53]	2.3%
Hussain Shah, 2016	32	324		9.88	[6.85; 13.66]	2.2%
Tauseef Ahmad, 2015	1	73		1.37	[0.03; 7.40]	2.1%
Jehan Zeb, 2015	18	460		3.91	[2.34; 6.11]	2.2%
Akbar Hussain, 2015	3	282		1.06	[0.22; 3.08]	2.2%
Ibrahim, 2014	128	3765		3.40	[2.84; 4.03]	2.3%
Hussain, K, 2013	103	230		44.78	[38.24; 51.46]	2.2%
Khattak, Aamer A,2013	128	712		17.98	[15.22; 21.00]	2.3%
Khattak, Aziz Marjan, 2013	325	2578		12.61	[11.35; 13.95]	2.3%
K Hussain, 2013	103	230		44.78	[38.24; 51.46]	2.2%
Nusrat Jahan, 2013	3	30		10.00	[2.11; 26.53]	2.0%
Shahid Niaz Khan, 2013	25	223		11.21	[7.39; 16.10]	2.2%
Mohammad Iqbal Yasinzai, 2013	478	2346		20.38	[18.76; 22.06]	2.3%
Tauseef Ahmad, 2013	1	189		0.53	[0.01; 2.91]	2.2%
A. M. Tareen, 2012	62	338		18.34	[14.36; 22.89]	2.2%
Mohammad Iqbal Yasinzai, 2012	416	1189		34.99	[32.27; 37.77]	2.3%
Zia ur Rahman Awan, 2012	2	17		11.76	[1.46; 36.44]	1.8%
Soomro, 2010	779	1474		52.85	[50.26; 55.42]	2.3%
Mohammad Iqbal Yasinzai, 2009	712	1016		70.08	[67.16; 72.88]	2.3%
Ansar Mahmood Malik, 2009	176	392		44.90	[39.90; 49.97]	2.2%
Mohammad Iqbal Yasinzai, 2008	579	1095		52.88	[49.87; 55.87]	2.3%
Mohammad Iqbal Yasinzai, 2008	1316	2304		57.12	[55.07; 59.15]	2.3%
Mohammad Iqbal Yasinzai, 2008	1559	3240		48.12	[46.38; 49.85]	2.3%
Muhammad Idris, 2007	35	145		24.14	[17.43; 31.94]	2.2%
Jalal-ud-din, 2006	12	154		7.79	[4.09; 13.22]	2.2%
Random effects model		**421118**		**16.29**	**[11.33; 21.92]**	**100.0%**

Heterogeneity: $I^2 = 100\%$, $\tau^2 = 0.0545$, $p = 0$

10 20 30 40 50 60 70

Fig. 4. Pooled prevalence of *P. falciparum* malaria in Pakistan using the random-effects model.

Study	Events	Total	Events per 100 observations	Events	95%-CI	Weight
Humera Qureshi, 2021	3970	4297		92.39	[91.56; 93.17]	2.3%
M F Nadeem, 2021	523	679		77.03	[73.67; 80.14]	2.3%
Naqvi, Syed Wasif Ali,2020	9121	9568		95.33	[94.89; 95.74]	2.3%
Arshia Sumbal, 2020	366	433		84.53	[80.77; 87.80]	2.2%
Hina Habib Syed, 2020	931	932		99.89	[99.40; 100.00]	2.3%
Pakistan Malaria annual report, 2019	314574	374513		84.00	[83.88; 84.11]	2.3%
Wali khan, 2019	296	300		98.67	[96.62; 99.64]	2.2%
Ajmal Khan, 2019	1029	1273		80.83	[78.56; 82.96]	2.3%
Fatima Jahan, 2019	566	720		78.61	[75.43; 81.55]	2.3%
Hanif Ullah, 2019	193	214		90.19	[85.39; 93.82]	2.2%
Muhammad Zafar, 2019	905	1478		61.23	[58.69; 63.72]	2.3%
Muhammad Farooq, 2019	518	571		90.72	[88.03; 92.97]	2.2%
Afsheen Bashir, 2019	401	415		96.63	[94.40; 98.14]	2.2%
Seema Kurd, 2019	224	334		67.07	[61.74; 72.08]	2.2%
Abdul Majeed Khan, 2018	58	66		87.88	[77.51; 94.62]	2.1%
Nasira Khatoon, 2018	396	480		82.50	[78.80; 85.79]	2.2%
Mehtab Ali Mahar, 2018	161	197		81.73	[75.61; 86.86]	2.2%
Noor Jahan Umer, 2017	545	842		64.73	[61.39; 67.96]	2.3%
Muhammad Imran, 2017	28	47		59.57	[44.27; 73.63]	2.1%
Shafiqur Rahman, 2016	184	187		98.40	[95.38; 99.67]	2.2%
Abdul Majid, 2016	709	766		92.56	[90.47; 94.32]	2.3%
Hussain Shah, 2016	97	324		29.94	[25.00; 35.25]	2.2%
Tauseef Ahmad, 2015	72	73		98.63	[92.60; 99.97]	2.1%
Jehan Zeb, 2015	434	460		94.35	[91.83; 96.27]	2.2%
Akbar Hussain, 2015	279	282		98.94	[96.92; 99.78]	2.2%
Ibrahim, 2014	1567	3765		41.62	[40.04; 43.21]	2.3%
Hussain, K, 2013	125	230		54.35	[47.67; 60.91]	2.2%
Khattak, Aamer A,2013	536	712		75.28	[71.94; 78.41]	2.3%
Khattak, Aziz Marjan, 2013	2226	2578		86.35	[84.96; 87.65]	2.3%
K Hussain, 2013	125	230		54.35	[47.67; 60.91]	2.2%
Nusrat Jahan, 2013	27	30		90.00	[73.47; 97.89]	2.0%
Shahid Niaz Khan, 2013	186	223		83.41	[77.86; 88.04]	2.2%
Mohammad Iqbal Yasinzai, 2013	1868	2346		79.62	[77.94; 81.24]	2.3%
Tauseef Ahmad, 2013	188	189		99.47	[97.09; 99.99]	2.2%
A. M. Tareen, 2012	272	338		80.47	[75.84; 84.56]	2.2%
Mohammad Iqbal Yasinzai, 2012	773	1189		65.01	[62.23; 67.73]	2.3%
Zia ur Rahman Awan, 2012	15	17		88.24	[63.56; 98.54]	1.8%
Soomro, 2010	695	1474		47.15	[44.58; 49.74]	2.3%
Mohammad Iqbal Yasinzai, 2009	304	1016		29.92	[27.12; 32.84]	2.3%
Ansar Mahmood Malik, 2009	204	392		52.04	[46.97; 57.08]	2.2%
Mohammad Iqbal Yasinzai, 2008	516	1095		47.12	[44.13; 50.13]	2.3%
Mohammad Iqbal Yasinzai, 2008	988	2304		42.88	[40.85; 44.93]	2.3%
Mohammad Iqbal Yasinzai, 2008	1681	3240		51.88	[50.15; 53.62]	2.3%
Muhammad Idris, 2007	105	145		72.41	[64.38; 79.50]	2.2%
Jalal-ud-din, 2006	142	154		92.21	[86.78; 95.91]	2.2%
Random effects model		**421118**		**79.13**	**[72.60; 84.99]**	**100.0%**

Heterogeneity: $I^2 = 100\%$, $\tau^2 = 0.0622$, $p = 0$

30 40 50 60 70 80 90

Fig. 5. Pooled prevalence of *Plasmodium vivax* Malaria in Pakistan using the random-effects model.

3.6. Publication bias Tests for Heterogeneity

The funnel plot screened the publication bias between studies on the prevalence of Malaria and objectively using the Freeman-Tukey Double Arcsine Transformed Proportion. Based on a visual examination of the funnel plot, we found no evidence of publishing bias (Fig. 7).

3.7. Time series analysis

The prevalence of Malaria has not changed uniformly in studies. From 2006 to 2007, the prevalence of Malaria decreased dramatically. However, from 2007 to 2021, the rest of the spread increased and sometimes decreased Fig. 8.

Tests for Heterogeneity.
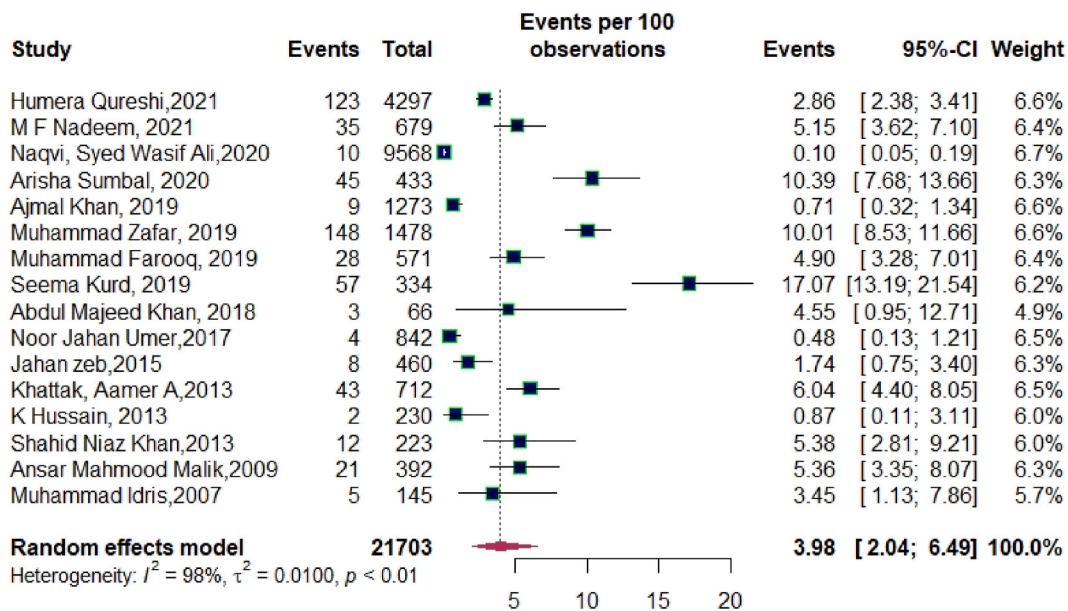
Fig. 6. Shows the pooled prevalence of *mixed infection* malaria in Pakistan using the random-effects model.
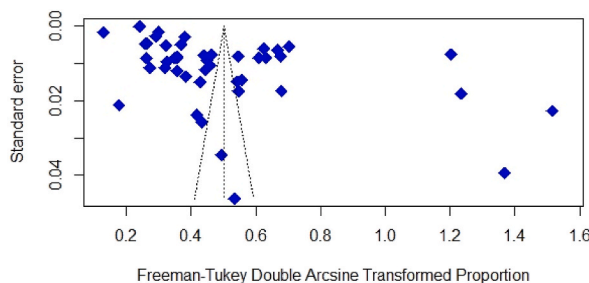
Fig. 7. The publication bias among the included studies, as demonstrated by the funnel plot.

Significant heterogeneity was identified among the 45 studies included in the Meta-analysis, as evidenced by Q statistics (p-value 0). In addition, the studies had a significantly larger degree of heterogeneity.

Quantifying heterogeneity:

$$tau^2 = 0.0890\ [0.0606;\ 0.1418];\ tau = 0.2982\ [0.2463;\ 0.3765] \tag{3.1}$$

$$I^2 = 99.9\%;\ H = 37.13\ [36.36;\ 37.91] \tag{3.2}$$

Test of heterogeneity: Q d. f. p-value.
60646.14 44 0.00.

In a meta-analysis, the I^2 index measures (equation (3.2)) the dispersion of effect sizes, and tau^2 estimates (equation (3.1)) the variance of the underlying distribution of true effect sizes. It seemed reasonable to apply the random effect model based on these statistics. The combined estimate from the random effect model showed a 23.25% prevalence.

4. Discussion

Malaria is the fifth leading cause of mortality worldwide, killing hundreds of thousands of people, especially children under five and pregnant women [10]. According to the WHO, Pakistan has 1.5 million malaria cases each year. Pakistan has been placed in the Eastern Mediterranean Region's Group 3 countries, accounting for 95% of the overall regional malaria burden [59]. Pakistan reduced case incidence by 40% or more in 2020 compared with 2015, according to the WHO 2021 report. However, Pakistan remained one of the top four countries responsible for the most anticipated malaria cases in the region (56%) in 2020 [10].

In 2018, KP was the most malaria-affected province in Pakistan, followed by Balochistan (17%), the FATA region (19%), and

Punjab (0.2%) [14]. Malaria cases differ substantially between regions and cannot be correctly analyzed due to a lack of data. Because Pakistan experiences all four seasons with harsh climatic conditions, malaria parasitic species have an unequal distribution throughout the country, and their occurrence varies with the seasons [5]. The examination of microscopic slides is routinely used for malaria diagnosis. The rapid diagnostic test is usually used in remote areas among migrant outbreaks and when microscopic detection is impossible. Based on the national malaria treatment guidelines, chloroquine plus primaquine is used as a treatment regimen for *P. vivax* [60]. Together with sulfadoxine/pyrimethamine, artesunate is recommended as the first-line treatment for *P. falciparum* after the withdrawal of chloroquine in 2007. However, from 2017 onward, artesunate lumefantrine has been recommended by the WHO as the drug of choice [61].

Malaria control in Pakistan is difficult due to a lack of experienced health personnel, laboratory facilities, and appropriate drugs [62]. Active and passive diagnostics can substantially reduce malaria incidence and prevalence [63]. Accurate malaria infection surveillance and control initiatives in Pakistan may also be scaled up with accurate data [64]. The current systematic review included 45 studies with a total of 6889403 malaria infections in Pakistan [65]. This systematic review and meta-analysis derived basic information regarding Pakistan's estimated pooled malaria prevalence. It used 45 full-text publications to determine the prevalence of two common *Plasmodium* species in Pakistan: *P. falciparum* and *P. vivax* infections. The estimated overall pooled prevalence of Malaria in Pakistan was 23.25% (95% CI: 16.14–31.21) in this systematic review and meta-analysis, with considerable heterogeneity among the studies ($I^2 = 100\%$, P = 0.0), the pooled prevalence estimates of *P. falciparum* were 16.29% (95% CI 11.33–21.92), and the pooled prevalence estimates of *P. vivax* were 79.13% with 95% CI (72.60–84.99). In this study, the *P. falciparum*.

studies ($I^2 = 100\%$, P = 0.0), *P. vivax* studies ($I^2 = 100\%$, P = 0.0) and mixed studies ($I^2 = 99\%$, P = 0.01) with a lot of heterogeneity. Malaria is more common in Pakistan than in other South Asian countries due to differences in insecticide-treated bed nets, test types, environmental conditions, presumptive diagnosis, and the lack of appropriate treatment [5]. *P. vivax* was the most prevalent species in Pakistan, followed by *P. falciparum*, and there were no cases of *P. malariae* or *P. ovale*. *P. vivax* has been reported worldwide and can grow in both temperate and tropical settings. Its larger geographical distribution has been attributed to its early emergence of gametocytes, efficient transmission by Anopheles vectors at lower parasite numbers, faster development of sporozoites within the mosquito, and wider viable temperature ranges than *P. falciparum* [66].

The majority of cases in this study were seen in men and adults over the age of 15, especially in rural areas. The higher proportion of malaria cases among adult men has been a prominent epidemiological characteristic in malarial countries. This high percentage is due to professional and behavioral characteristics that expose men to more infective vectors than women. Due to the prevalence of asymptomatic infections with low parasite concentrations among adult men, these so-called "hot groups" can play a crucial role in outbreaks [67].

In Pakistan, continuous insecticidal netting and indoor spraying, early detection and treatment, and environmental control should be routinely encountered. The current study discovered substantial heterogeneity in malaria prevalence in Pakistan compared to other studies done in South Asia. The use of Insecticide-Treated Nets (ITNs), the local environment, age, gender, socioeconomic status, health care facilities, geographic location, household crowding, comorbidities (sickle cell anemia and G6PD deficiency), diagnosis, and treatment facilities are all important determinants of malaria incidence heterogeneity [68,69].

A meta-analysis was conducted in 2021, which showed the spread of Malaria in India and Thailand was 8% (95% CI: 4–13%, I^2: 85.87%, nine studies with 59/794 cases) and 35% (95% CI: 7–64%, $I2$: 98.9%, four studies with 262/624 cases) [70]. And so meta-analysis was done also in Afghanistan, Iran, and China, which showed the spread of Malaria which was 0.15 (95%CI: 0.10–0.21; I2 = 83.3%) [71], 63% (95% CI 51–74%; seven studies) [72] and 9% (2006/35,768, 95% CI 7.0–12.0%) [73]. The publications included in this systematic review and meta-analysis demonstrated considerable diversity. Even though all researchers included in this research attempted to explore probable sources of heterogeneity, the reasons for inconsistency remained unknown. Some of the studies were conducted during the high malaria transmission season, while the remainder were conducted during the low malaria transmission season. The seasons in which the studies were done may have contributed to the variability. An improved epidemiological survey and improvement of the surveillance system are two of the most important activities that should be done by public health to control and eliminate Malaria in Pakistan. The two most significant success factors are abundant political commitment to Malaria and good inter-sectoral coordination.

5. Strength and limitations

To the best of our knowledge, this is the first comprehensive systematic review and meta-analysis of malaria prevalence and species distribution across Pakistan's provinces. The current study investigated the pooled prevalence of malaria in Pakistan. The highest infection rate, gender ratio, age group, and peak period during our meta-analysis will all be determined by this study. The evidence could help primary care providers and policymakers create effective malaria management and, eventually, elimination policies.

In Pakistan, malaria is a severe public health concern. For decision-makers, this report summarized Pakistan's malaria prevalence. When interpreting the significant findings, it is necessary to keep in mind certain essential limitations that the current review possesses. All of the publications under investigation were cross-sectional, descriptive, and retrospective; as a result, other confounding variables might have impacted the outcome variable. Studies included different malaria diagnosis techniques, such as microscopy, RDTs, and PCR, which might have influenced the incidence and species distribution in the study areas. Although it's crucial to take surveillance data and country-level reporting constraints into account (e.g., over or underreporting; missing districts), continued monitoring includes active and passive surveillance and ensures reported data is accurate and consistent across districts. Most of the research has been carried out in the provinces of KP and Baluchistan, while Sindh, FATA, and Punjab only had a small number. The uneven distribution of publications around the country may impact this study's findings. The results of this systematic review and
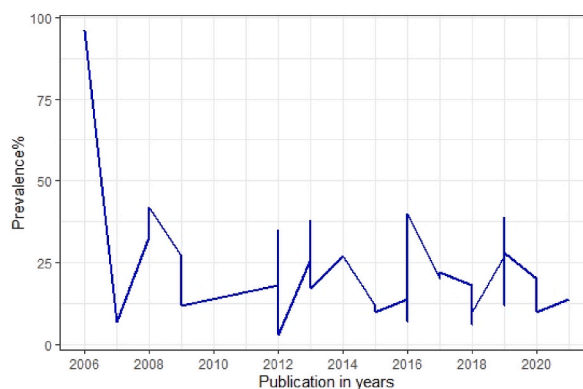
Fig. 8. Malaria prevalence in Pakistan from 2006 to 2021: a time trend analysis.

meta-analysis, reporting the pooled proportion of malaria positivity rate among the population screened based on the articles selected, might be impacted by the limitations mentioned.

6. Conclusion

In this comprehensive review and meta-analysis, a substantial range in malaria prevalence in Pakistan was discovered. According to this comprehensive review and meta-analysis, the total incidence of Malaria in Pakistan has increased and decreased over time. This malarial spread was higher in KP and Balochistan provinces than in other parts of Pakistan. Early diagnosis, effective treatment of the disease, integrated mosquito control programs, and regular monitoring for drug resistance in *Plasmodium* spp. should be prioritized and implemented by Pakistan's government. The two most significant malaria control and elimination variables are substantial political commitment and excellent inter-sectoral coordination.

This review studies covered all four provinces of Pakistan; however, there are no published data from the fifth province of Gilgit-Baltistan and the autonomous state of Azad Jammu and Kashmir (AJK). Further research in these locations would be interesting to understand Pakistan's malaria epidemiology comprehensively. Plasmodium epidemiology studies must be carried out regularly in Pakistan's 169 districts to get a clear epidemiological picture. As there is a shortage of antimalarial resistance data in Pakistan, antimalarial efficacy trials must be done at least once every 24 months, according to WHO guidelines. To control and eventually eradicate malaria, emphasize the urgent need for researchers, research funding organizations, government agencies, and health authorities (Malaria Control Program, Ministry of Health, and WHO) to step up their efforts.

Author contribution statement

Muhammad Imran Khan; Humera Qureshi: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Suk Joo Bae: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Shahid Anwar: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Sadique Ahmad; Fazal Hassan; Shabir Ahmad: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Aamer Ali Khattak: Conceived and designed the experiments; Wrote the paper.

Funding statement

Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no conflict of interest.

References

[1] F.E. Cox, History of the discovery of the malaria parasites and their vectors, Parasites Vectors (3) (2010) 1–9.
[2] R.W. Snow, C.A. G, A.M. Noor, H.Y. Myint, S.I. Hay, The global distribution of clinical episodes of Plasmodium falciparum malaria, Nature 434 (2005) 214–217.
[3] WHO, World Malaria Report, Britain and Bill Gates Fight Malaria, Wor. Health Organ., Geneva, Switzerland, 2015.
[4] WHO, World Malaria Report, Wor. Health Organ., Geneva, Switzerland, 2017.
[5] N.A. Qureshi, H. Fatima, M. Afzal, et al., Occurrence and seasonal variation of human Plasmodium infection in Punjab Province, Pakistan, BMC Infect. Dis. 19 (1) (2019) 1–13.
[6] H. Qureshi, M.I. Khan, H. Ambachew, et al., Baseline survey for malaria prevalence in khyber Pakhtunkhwa province, Pakistan, eastern med, Health J (2021) 453–460.
[7] J.I. Nankabirwa, A. Y, E. Arinaitwe, et al., Estimating malaria parasite prevalence from community surveys in Uganda: a comparison of microscopy, rapid diagnostic tests and polymerase chain reaction, Malar. J. 14 (2015) 1–11.
[8] K. Raghavendra, T.K. B, B.P.N. Reddy, P. Sharma, A.P. Dash, Malaria vector control: from past to future, Parasitol. Res. 108 (2011) 757–779.
[9] WHO, World Malaria Report, Wor. Health Organ., Geneva, Switzerland, 2020.
[10] Organization, W.H., World Malaria Report, 2021.
[11] A.A. Khattak, M. Venkatesan, M.F. Nadeem, et al., Prevalence and distribution of human Plasmodium infection in Pakistan, Malar. J. 297 (12) (2013) 2–8.
[12] D.A. Milner, Malaria pathogenesis, Cold Spri. Harb. persp. in med. 8 (1) (2018) a025569.
[13] M.F. Nadeem, A.A. Khattak, N. Zeeshan, et al., Assessment of microscopic detection of Malaria with nested polymerase chain reaction in war-torn federally administered tribal areas of Pakistan, Acta Parasitol. 66 (4) (2021) 1186–1192.
[14] Malaria report of Pakistan, Direct. of Malar. Con. Pak. (2019) 1–40.
[15] I. Hussain, N.A. Qureshi, M. Afzal, et al., Prevalence and distribution of human Plasmodium infection in federally administrative tribal areas of Pakistan, Acta Parasitol. 61 (3) (2016) 537–543.
[16] A.M. Karim, I. Hussain, S.K. Malik, et al., Epidemiology and clinical burden of Malaria in the war-torn area, Orakzai Agency in Pakistan, PLoS Neglected Trop. Dis. 10 (1) (2016), e0004399.
[17] S.M. Malik, H. Awan, N. Khan, Mapping vulnerability to climate change and its repercussions on human health in Pakistan, Glob. Health 8 (1) (2012) 1–10.
[18] Ministry of Health Pakistan, Epidemiology of Malaria in Pakistan. Ministry of Health Pakistan, 2010.
[19] S. Rahman, F. Jalil, H. Khan, et al., Prevalence of malaria in district shangla, khyber Pakhtunkhwa, J. Ento. Zool. St. (2017) 678–682.
[20] A. Sumbal, N. Khan, M. Naseem, et al., Prevalence of pediatric malaria in Quetta (city) balochistan, Pakistan, Int. J. Ent. Res. 5 (4) (2020) 20–23.
[21] A.M. Khan, A. Hassan, I. Ullah, et al., The frequency of Malaria in patients visiting selected hospitals in Peshawar, Khyber Pakhtunkhwa, Pakistan, J. Ent. Zoo. St. 105 (15) (2018) 2279–2283.
[22] N. Khatoon, R. Malik, A. Khan, et al., Incidence of malaria in the population of korangi creek area, Karachi, Pakistan, Pak. J. Pharm. Sci. 31 (6) (2018) 2575–2578.
[23] W. Khan, A. Rahman, W. Khan, et al., Malaria prevalence in Malakand district, the north western region of Pakistan, J.Pak. Med. Asso. 69 (946) (2019).
[24] A. Khan, R. Ullah, A report on prevalence of malaria infection in general population of Bajaur Agency, FATA, Pakistan, Pakistan J. Zool. 51 (1) (2019) 367–370.
[25] H. Ullah, M.I.U. Khan, Suleman et al. Prevalence of malaria infection in district Dir lower, Pakistan, Punjab Univ. J. Zool. 34 (2) (2019) 137–141.
[26] J. Zeb, M.S. Khan, N. Ullah, et al., Epidemiology of Plasmodium species and prevalence of Malaria on the basis of age, sex, area, seasonality and clinical manifestation in population of District Lower Dir, Khyber Pakhtunkhwa, Pakistan, World J. Zool. 10 (2) (2015) 147–152.
[27] A. Majid, M. Rehman, T. Ahmad, et al., Prevalence of malaria in human population of district mardan, Pakistan, World J. Zool. 11 (1) (2016) 63–66.
[28] M. Imran, I. Ahmad, M. Kalim, Identification of Plasmodium vivax and Plasmodium falciparum in the northern areas (district Malakand) of khyber Pakhtunkhwa, Pakistan, PSM Mircrob 2 (3) (2017) 59–62.
[29] M. Zafar, I. Mushtaq, S. Masud, et al., Prevalence and distribution of human malarial infection in district Multan, Punjab, Pakistan, Pure and App. Bio. 8 (1) (2019) 873–881.
[30] M.A. Mahar, A.M. Sheikh, N.A. Birmani, Prevalence of malarial parasites Plasmodium in human population of taluka Sukkur, Sindh, Pakistan, J. Ent. Zoo. Stud. 6 (2) (2018) 112–115.
[31] A. Hussain, T. Ahmad, S.G. Jamal, et al., Prevalence of human malaria infection in lal qilla Pakistan, Ameri. J. Biom. Sci. 7 (1) (2015) 9–14.
[32] H. Shah, R. Khan, F. Naz, et al., Prevalence and distribution of malaria parasites in general population of district Dir Lower, Khyber Pakhtunkhwa, Pak, J. Ento. Zoo. Stud. 4 (4) (2016) 1211–1215.
[33] M. Farooq, M.I. Yasinzai, N. Khan, et al., Current status of malaria in district pishin, balochistan, Intern. J. Mosq. Res. 7 (1) (2019) 33–36.
[34] H.H. Syed, M. Shah, S. Sherzada, et al., Occurrence of malaria in the local population of Swat valley, Pakistan, Pakistan J. Zool. 52 (4) (2020) 1615–1618.
[35] K. Saeed Ibrahim, S. Khan, et al., Epidemiological finding of malaria in district buner khyber Pakhtunkhwa, Pakistan, World J. Med. Sci. 11 (4) (2014) 478–482.
[36] A. Bashir, S. Arif, R. Bano, et al., Frequency and risk factors of malaria infection in Dera Ismail khan, khyber Pakhtunkhwa, Pakistan, Intern. J. Mosq. Res. 6 (5) (2019) 37–40.
[37] N. Jahan, M. Sarwar, Malaria prevalence in district Okara, Punjab, Pakistan, Biologia 59 (2) (2013) 191–195.
[38] A.M. Tareen, M. Rafique, A. Wadood, et al., Malaria burden in human population of Quetta, Pakistan, Europ. J. Microb. Immun. 2 (3) (2012) 201–204.
[39] M.I. Yasinzai, J.K. Kakarsulemankhel, Incidence of human malaria infection in Barkhan and Kohlu, bordering areas of East Balochistan, Pakistan J. Med. Sci. 24 (2) (2008).
[40] M.I. Yasinzai, J.K. Kakarsulemankhel, Prevalence of human malaria infection in district Jhal Magsi of Balochistan, Rawal Med. J. 37 (2012) 9–12.
[41] Z. Awan, A.K. Khan, A.H. Shah, et al., Assessment of malaria prevalence among school children in RuralAreas of bannu district khyber Pakhtunkhwa, Pakistan, Pakistan J. Zool. 44 (2) (2012) 321–326.
[42] M.I. Yasinzai, J.K. Kakarsulemankhel, Frequency of various human malaria infections in hottest areas of Central Balochistan, Pakistan: duki, harnai, and Sibi. Pak. Armed for, Med. J. 58 (3) (2008) 276–285.
[43] S.N. Khan, S. Ayaz, S. Khan, et al., Malaria still a health problem in the general population of Bannu District, Khyber Pakhtunkhwa, Pakistan, Annu. Res. Rev. Biofeedback 3 (4) (2013) 835–845.
[44] M.I. Yasinzai, J.K. Kakarsulemankhel, Prevalence of human malaria infection in Pakistani areas bordering with Iran, J. Pakistan Med. Assoc. 63 (3) (2013) 313–316.
[45] T. Ahmad, A. Hussain, S. Ahmad, Epidemiology of malaria in lal qilla, Intern. J. Sci. Tech. Res. 11 (2) (2013) 199–202.
[46] S.A. Khan, S.H. Ally, Malaria in children: study of 160 cases at a private clinic in Mansehra, J. Ayub Med. Coll. Abbottabad 18 (3) (2006) 44–45.
[47] S. Kurd, M.I. Yasinzai, M. Naseem, et al., Prevalence of human malarial infection in school going children of district Khuzdar (city), Balochistan, Int. J. Mosquito Res. 6 (6) (2019) 120–123.
[48] M.I. Yasinzai, J.K. Kakarsulemankhel, Prevalence of human malaria infection in district ziarat and Sanjavi, Pakistan, Pakistan J. Zool. 41 (6) (2009) 475–482.
[49] A.M. Malik, N. Zaffar, N. Ali, et al., Haematological findings and endemicity of Malaria in Gadap region, J. Coll. Physi. Surg. Pak. 20 (2) (2010) 112–116.
[50] M. Idris, J. Sarwar, J. Fareed, Pattern of malarial infection diagnosed at ayub teaching hospital abbottabad, J. Ayub Med. Coll. Abbottabad 19 (2) (2007) 35–36.
[51] S.W.A. Naqvi, S. Saeed, A. Rafique, et al., Prevalence and distribution of Malaria by sex, age groups and species in year 2019 in suspected malarial population of district DI Khan, Pakistan, Gomal J. Med. Sci. 18 (4) (2020) 164–173.
[52] A.A. Khattak, M. Venkatesan, M.F. Nadeem, et al., Prevalence and distribution of human Plasmodium infection in Pakistan, Malar. J. 12 (1) (2013) 1–8.
[53] K. Hussain, M. Shafee, N. Khan, et al., Seroprevalence of pediatric malaria in Quetta, balochistan, Pakistan, Iran. J. Parasitol. 8 (2) (2013) 342–347.
[54] M.F. Nadeem, A.A. Khattak, N. Zeeshan, et al., Molecular epidemiology of Plasmodium species in conflicted federally administered tribal area (FATA) Pakistan, Braz. J. Biol. 83 (2021).

[55] F. Jahan, N.H. Khan, S. Wahid, et al., Malaria epidemiology and comparative reliability of diagnostic tools in Bannu; an endemic malaria focus in south of Khyber Pakhtunkhwa, Pakistan. Path. Glob. heal. 113 (2) (2019) 75–85.
[56] T.A.A. Hussain, S. Ahmad, A descriptive study of malaria in lal qilla, khyber Pakhtunkhwa, Pakistan, Amer. J. Life Sci. Res. 3 (2) (2015).
[57] K. Hussain, M. Shafee, N. Khan, et al., Seroprevalence of pediatric malaria in Quetta, balochistan, Pakistan, Iran. J. Parasitol. 8 (2) (2013) 342–347.
[58] F.R. Soomro, G.M. Pathan, A.L. Gurbakhshani, et al., Prevalence of malarial parasites in Larkano district, Sindh, Pakistan, Gomal J. Med. Sci. 8 (2) (2010).
[59] W.H. Organization, World malaria report 2013, Wor.Heal. Organ. (2013) 1–255.
[60] M.F. Nadeem, A.A. Khattak, N. Zeeshan, et al., Surveillance of molecular markers of antimalarial drug resistance in Plasmodium falciparum and Plasmodium vivax in Federally Administered Tribal Area (FATA), Pakistan, Revista do Inst. de Med. Trop. de São Paulo 63 (2021).
[61] A.A. Khattak, U.A. Awan, M.F. Nadeem, et al., Chloroquine-resistant Plasmodium falciparum in Pakistan, Lancet Infect. Dis. 21 (12) (2021) 1632.
[62] S. Kurd, M.I. Yasinzai, M. Naseem, et al., Prevalence of human malarial infection in school going children of district Khuzdar (city), Balochistan. Intern. J. Mosq. Res. 6 (2019) 120–123.
[63] S. Zoghi, A.A. Mehrizi, A. Raeisi, et al., Survey for asymptomatic malaria cases in low transmission settings of Iran under elimination programme, Malar. J. 11 (1) (2012) 1–10.
[64] M.I. Yasinzai, J.K. Kakarsulemankhel, Incidence of human infection in northern hilly region of Balochistan, adjoining with NWFP, Pakistan: district Zhob, Pakistan J. Biol. Sci. 11 (12) (2008) 1620–1624.
[65] Uk Najeeb, Z. Ali, W. Muhammad, et al., Incidence of malaria in khyber Pakhtunkhwa Pakistan – a meta-analysis, Annals of Reviews and Research 3 (4) (2018) 1–7.
[66] K.E. Battle, P.W. Gething, I.R.F. Elyazar, et al., The global public health significance of Plasmodium vivax, Adv. Parasitol. 80 (2012) 1–111.
[67] C. Cotter, H.J.W. Sturrock, M.S. Hsiang, et al., The changing epidemiology of malaria elimination: new strategies for new challenges, Lancet 382 (9895) (2013) 900–911.
[68] T.D. Clark, B. Greenhouse, D. Njama-Meya, et al., Factors determining the heterogeneity of malaria incidence in children in Kampala, Uganda, The J. inf. dis. 198 (3) (2008) 393–400.
[69] T.N. Williams, T.W. Mwangi, S. Wambua, et al., Sickle cell trait and the risk of Plasmodium falciparum malaria and other childhood diseases, J. Infect. Dis. 192 (1) (2005) 178–186.
[70] P. Wilairatana, S. Kuraeiad, P. Rattaprasert, et al., Prevalence of Malaria and scrub typhus co-infection in febrile patients: a systematic review and meta-analysis, Paras. & vect. 14 (1) (2021) 1–13.
[71] R.J. Commons, J.A. Simpson, J. Watson, et al., Estimating the proportion of Plasmodium vivax recurrences caused by relapse: a systematic review and meta-analysis, The Amer. J. Trop. Med. Hygiene 103 (3) (2020) 1094–1099.
[72] H. Azizi, R. Majdzadeh, A. Ahmadi, et al., Health workers readiness and practice in malaria case detection and appropriate treatment: a meta-analysis and meta-regression, Malar. J. 20 (1) (2021) 1–12.
[73] M. Kotepui, K.U. Kotepui, G.D.J. Milanez, et al., Plasmodium spp. mixed infection leading to severe Malaria: a systematic review and meta-analysis, Sci. Rep. 10 (1) (2020) 1–12.