

EPIDEMIOLOGICAL STUDY ON MALARIA PREVALENCE AND SPECIES DISTRIBUTION IN THE HUMAN POPULATION OF DISTRICT MARDAN, KHYBER PAKHTUNKHWA, PAKISTAN

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ABSTRACT

Malaria, caused by Plasmodium parasites, is a major public health concern in many countries, including Pakistan, where Plasmodium vivax and Plasmodium falciparum are the most common species. The purpose of this study was to determine the prevalence of malaria among the population of District Mardan, Khyber Pakhtunkhwa, Pakistan. A descriptive epidemiological research was undertaken from May to November 2022, involving blood sample analysis from 147,342 individuals at DHQ Hospital and MMC Mardan. The investigation focused on variables such as species identification, gender, age, tehsil, and socioeconomic status.

Methods: All samples underwent microscopic examination to identify specific Plasmodium genotypes. Prevalence rates were calculated, and data were analyzed to discern patterns in malaria infection.

Results: Malaria was detected in 3,886 samples, representing a prevalence rate of 2.63% among the total analyzed. A peak incidence was observed in September with 814 cases (20.94%), while the lowest recorded incidence was in May, with 296 cases (7.61%). Notably, P. vivax was identified in 3,612 samples (92.94%), surpassing the prevalence of P. falciparum. Gender analysis revealed a higher infection rate among males 2,063 cases (53%) compared to females 1,823 cases, (47%). The age distribution indicated that the lowest infection rate occurred in the 0-4 year age group 255 cases, (6.5%), whereas the highest prevalence was observed in individuals aged 15 years and older 2,872 cases, (73.90%). Tehsil-specific analysis showed that malaria prevalence was highest in Tehsil Mardan 1,302 cases, (33.50%) and lower in Tehsil Katlang 722 cases, (18.57%). Furthermore, socioeconomic status significantly influenced infection rates, with 2,248 cases (57.84%) reported among individuals living in poverty, compared to 575 cases (14.79%) among wealthier individuals.

Conclusion: The findings underscore the ongoing challenge of malaria in District Mardan, particularly among vulnerable populations. The high prevalence of P. vivax, coupled with socioeconomic disparities, highlights the need for targeted malaria control interventions and further research to inform public health strategies in the region.

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Keywords: *Malaria, Plasmodium vivax, Plasmodium falciparum, prevalence, epidemiology, socioeconomic factors, Khyber Pakhtunkhwa, Pakistan.*

INTRODUCTION

Malaria is one of the most serious worldwide health issues, with the greatest impact on populations in tropical and subtropical areas. Annually, the disease contributes to over a million deaths, predominantly in low-income countries where healthcare resources are limited [15]. In 2010, malaria was reported in 106 countries, with approximately 216 million infections and 655,000 fatalities, primarily among children under five years old in sub-Saharan Africa [19]. The disease is caused by protozoan parasites of the genus *Plasmodium*, which are responsible for infections in humans, birds, and other animals. Among more than 100 known species, four are particularly relevant to human health: *Plasmodium vivax*, *Plasmodium falciparum*, *Plasmodium ovale*, and *Plasmodium malariae*. Notably, *P. vivax* and *P. falciparum* account for the majority of malaria cases [3].

Plasmodium falciparum is particularly well known for its severity; it is responsible for approximately 80% of malaria infections and accounts for nearly 90% of malaria-related mortality globally [17][4]. Malaria transmission occurs when infected female *Anopheles* mosquitoes bite humans, introducing the parasites through their saliva into the bloodstream. Clinical manifestations of malaria can range from mild symptoms, such as headache and fever, to severe complications that may lead to coma or death.

The occurrence of severe symptoms determines whether a malaria infection is simple or complex [6]. Malaria should thus be explored in the differential diagnosis of any patient who presents with unexplained fever [12]. The microscopic inspection of blood smears, particularly Giemsa-stained thick and thin films, is still the gold standard for malaria diagnosis. While rapid diagnostic tests (RDTs) may identify particular antigens associated with *P. vivax* and *P. falciparum*, their reliability is reduced for other *Plasmodium* species [9]. Furthermore, molecular methods such as polymerase chain reaction (PCR) provide sensitive detection of parasite DNA, but are sometimes prohibitively costly and difficult for everyday usage [8]. Malaria is endemic in some regions of the Americas, much of Asia, and large sections of Africa, with prevalence rates ranging from 85% to 90% [10].

In Pakistan, malaria presents a significant public health issue, exacerbated by climatic conditions, irrigation practices, and inadequate waste management, particularly in rural areas [15]. The seasonal patterns of malaria transmission typically decline with the onset of the colder months in November [11]. Accurate laboratory confirmation of *Plasmodium* infection is crucial before initiating treatment for malaria, with blood smears being a fundamental diagnostic tool [8]. Malaria control strategies encompass pharmacological interventions, mosquito population management, and preventative measures to avoid bites. The transmission dynamics of malaria are heavily influenced by both human and *Anopheles* mosquito populations [14].

Despite several epidemiological studies undertaken in various parts of Pakistan, detailed data remains scarce, particularly in certain areas. The purpose of this study is to assess malaria prevalence in District Mardan, Khyber Pakhtunkhwa, Pakistan, and contribute to a better understanding of malaria's impact in this region.

Material and methods

Study area

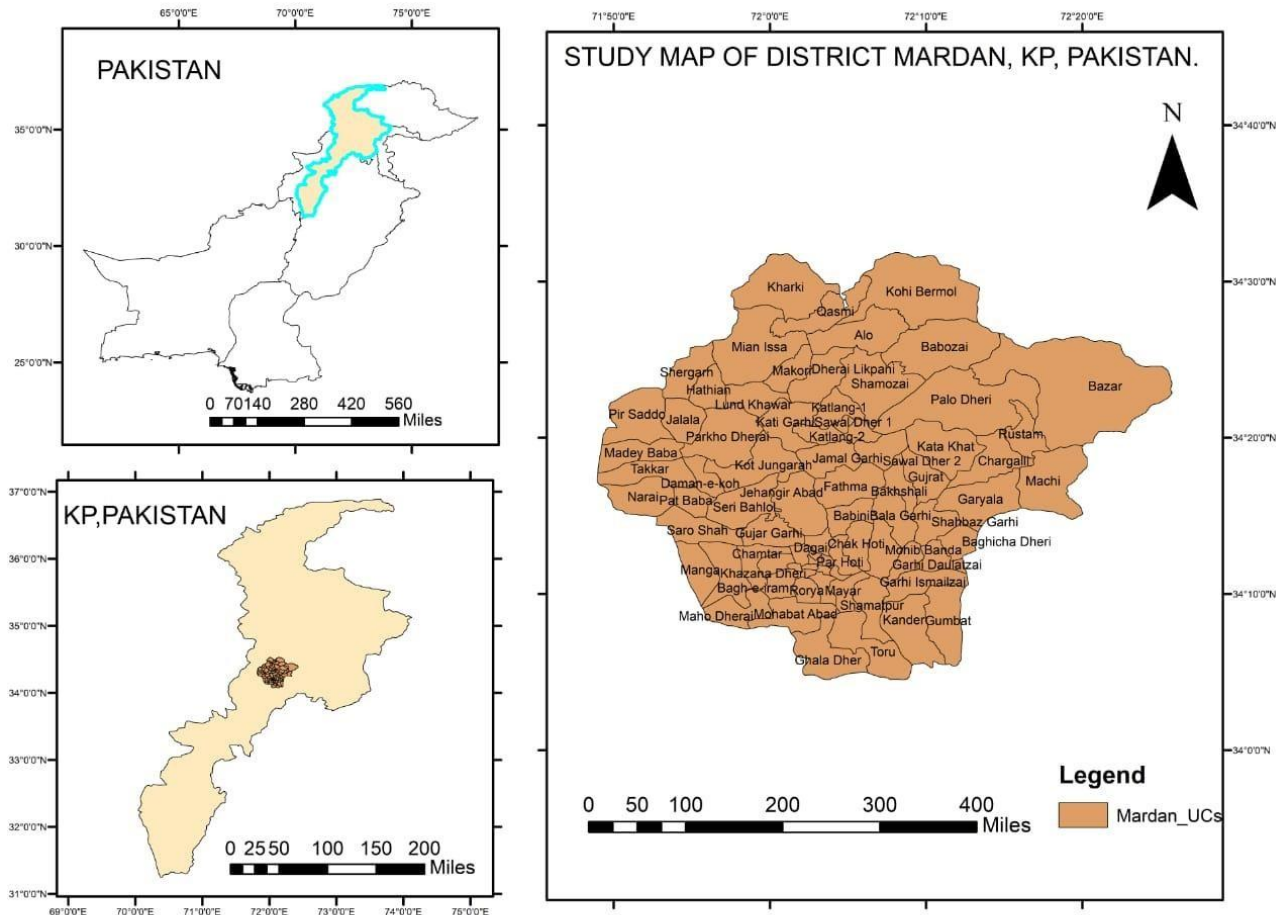
District Mardan, located in the southwest region of Khyber Pakhtunkhwa (KPK), Pakistan, is historically and culturally significant. Geographically, it is situated at coordinates 34°12'44" N and 72°50'33" E, with an elevation of 283 meters (928 feet) above sea level. The district encompasses a total area of 1,632 square kilometers. According to the 2017 census, the population of District Mardan was approximately 2,373,399, comprising 1,201,122 males, 1,172,215 females, and 78 transgender individuals. The literacy rates stand at 71.88% for males and 41.60% for females, with an average annual population growth rate of 2.8%.

The climate in District Mardan is classified as semi-arid, characterized by hot summers and mild winters. The hottest months are June, July, and August, with average temperatures reaching up to 39°C. January is typically the coldest month of the year. The district experiences a favorable spring season, while rainfall patterns vary throughout the year. The majority of precipitation occurs during the summer months,

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contributing to an annual rainfall average of approximately 12 mm, whereas winter rainfall is considerably lower.

Map of study area



Patient selection

This hospital-based study was carried out in District Mardan, Khyber Pakhtunkhwa (KPK), Pakistan. From May to November 2022, 147,342 blood samples were randomly taken at the District Head Quarters (DHQ) Hospital and the Mardan Medical Complex. Blood films were taken from individuals who had signs of malaria, such as fever, chills, and shivering, or who had a relevant medical history.

All individuals suspected of malaria underwent peripheral blood sampling, which was subsequently processed in the laboratory. Concurrently, a data collection form was completed in the field, documenting essential patient information, including name, sex, age, and the presence or absence of malaria parasites, as well as the specific *Plasmodium* species identified.

Patient age was categorized into three groups: infants (0-4 years), children (5-14 years), and adults (15 years and older). A clinical pathologist was tasked with examining the blood slides, counting the parasites, and completing the associated data sheets to ensure accurate reporting and analysis.

Sample Preparation

Preparation of Thick and Thin Blood Smears

Both thick and thin blood smears were prepared using a single slide to optimize resource utilization. Two clean glass slides were utilized: one for the blood film and the other for the preparation of the smear.

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Following sterilization of the lancet, the patient's fingertip was disinfected with methylated spirit. The first droplet of blood was discarded, and following drops were placed at one end of the slide, with another drop at the other end. The tiny smear was fixed with methyl alcohol. The blood films were stained with a 10% Giemsa stain. Microscopic examination was conducted using an oil immersion objective at 100x magnification. All slides were thoroughly reviewed by experienced microscopists at DHQ and MMC hospitals to ensure accuracy in results.

Rapid Diagnostic Test (RDT)

Rapid diagnostic tests (RDTs) serve as a valuable tool for the detection of malaria parasites in blood samples, facilitating confirmation of malaria diagnosis. In scenarios where high-quality microscopy services are unavailable, RDTs can effectively supplement clinical assessments or replace microscopy-based diagnoses. These tests provide timely results, enabling prompt clinical decision-making in the management of malaria cases.

Results

Month and species-wise prevalence of malaria infection in the human population of District Mardan:

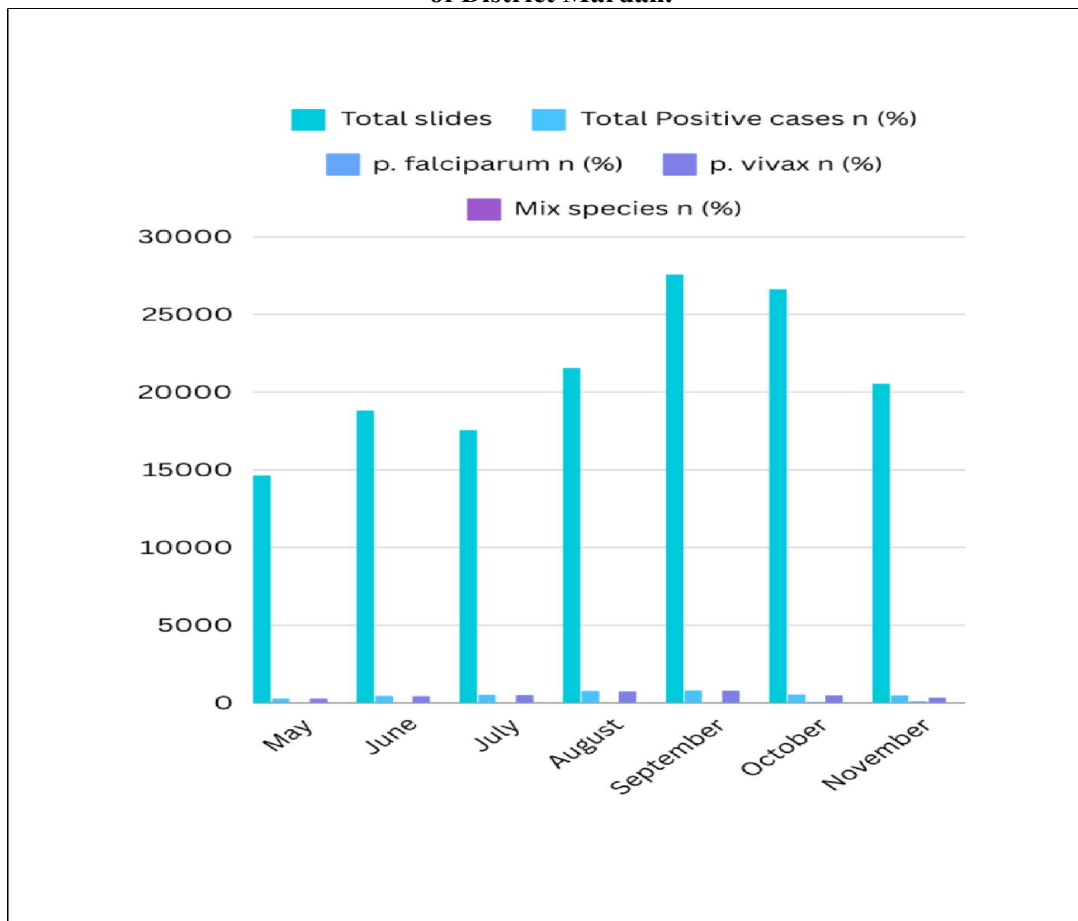
A total of 147,342 blood samples from suspected patients were tested for malaria, with 3886 (2.63%) being positive. *Plasmodium vivax* was responsible for 3612 (72.94%) of the positive cases. 244 were caused by *Plasmodium falciparum*, and 30 by a mixed-species infection. There were no signs of *P. ovale* or *P. malariae*. The greatest monthly prevalence was in September 814 (20.94%) and the lowest in May 296 (7.61%). The difference was not statistically significant, as $P > 0.05$.

Table01: Month and specie-wise prevalence of malaria infection in the human population of District Mardan.

Months	Total slides	Total positive n(%)	<i>P.falciparum</i> n(%)	<i>P.vivax</i> n(%)	Mix species n(%)
May	14659	296(7.61%)	3(0.07%)	292(7.51%)	1(0.02%)
June	18819	456(11.73%)	5(0.12%)	450(11.58%)	1(0.02%)
July	17554	522(13.43%)	3(0.07%)	516(13.27%)	3(0.07%)
August	21554	767(19.73%)	17(0.43%)	743(19.11%)	7(0.18%)
September	27581	814(20.94%)	27(0.69)	783(20.14%)	4(0.10%)
October	26629	556(14.30%)	65(1.67%)	487(12.53%)	4(0.10%)
November	20546	475(12.22%)	124(3.19%)	341(8.77%)	10(0.25%)
		$\chi^2=42.00$ df=36 p-value=0.227	$\chi^2=35.00$ df=30 p-value=0.243	$\chi^2=42.00$ df=36 p-value=0.227	$\chi^2=28.00$ df=24 p-value=0.260

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Figure 1: Showing month and species- wise occurrence of malaria infection in the human population of District Mardan.



Gender-wise prevalence of malaria infection in the human population of District Mardan:

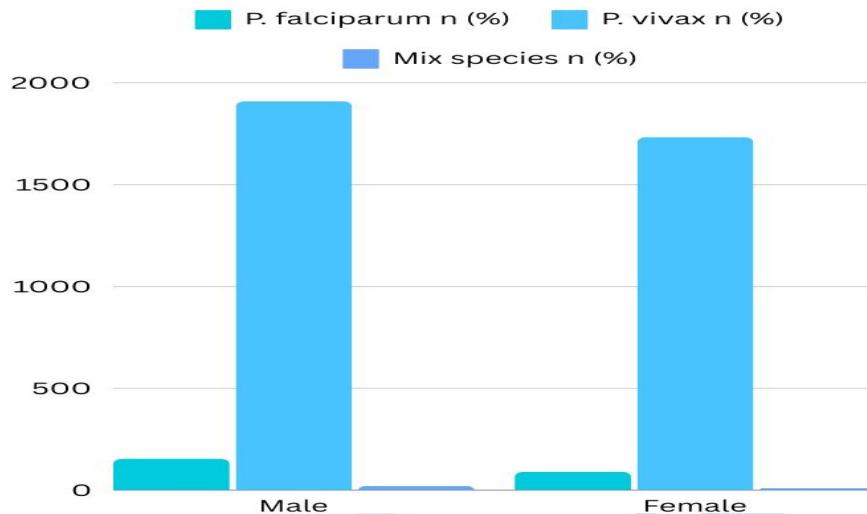
In 3886 positive samples, the high prevalence rate was found in males in 2063 (53.08%) and lowest in females in 1823 (46.92%). The difference is statistically non-significant.

Table02: Gender-wise prevalence of malaria infection in the human population of District Mardan:

Gender	Total slides n(%)	Total Positive n(%)	<i>P. falciparum</i> n(%)	<i>P.vivax</i> n(%)	Mix species n(%)
Male	86671(58.82%)	2063(53.08%)	154(3.96%)	1909(49.12%)	20(0.51%)
Female	60671(41.18%)	1823(46.92%)	90(2.32%)	1733(44.59%)	10(0.25%)
		$\chi^2=2.00$ df=1 p-value=0.157	$\chi^2=2.00$ df=1 p-value=0.157	$\chi^2=2.00$ df=1 p-value=0.157	$\chi^2=2.00$ df=1 p-value=0.157

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Figure-2: Showing gender wise occurrence of malaria in human population of Distric Mardan.



Age-wise prevalence of malaria infection in the human population of District Mardan:

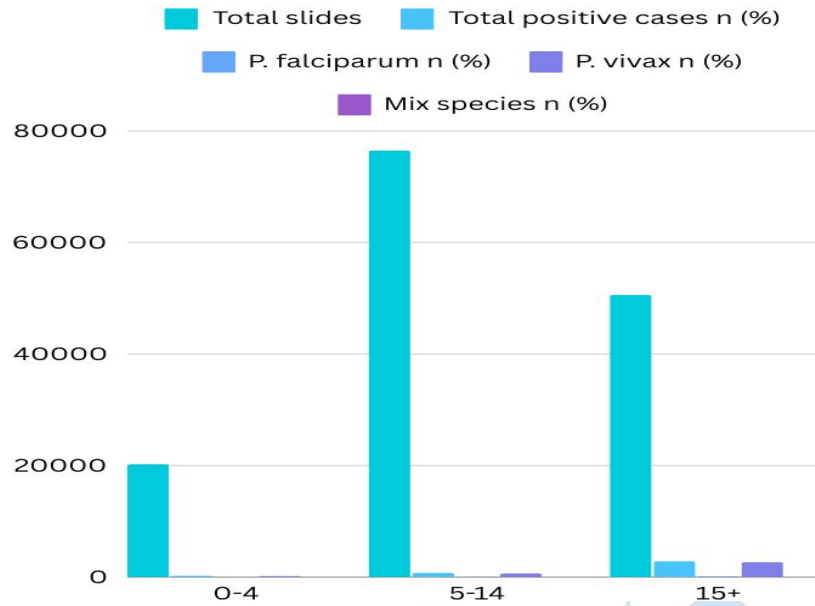
Malaria prevalence was 2872 (73.90%) in people over the age of 15 and 255 (6.56%) among those aged 0 to 4. There was no significant difference ($P > 0.05$) among age groups.

Table 3: Age-wise prevalence of malaria infection in the human population of District Mardan.

Age group	Total slides	Total positive n(%)	<i>P.falciparum</i> n(%)	<i>P.vivax</i> n(%)	Mix Species n(%)
0-4	20250	255(6.56%)	20(0.51%)	232(5.97%)	3(0.07%)
5-14	76500	759(19.53%)	68(1.74%)	679(17.47%)	12(0.30%)
15+	50592	2872(73.90%)	156(4.01%)	270(69.50%)	15(0.38%)
		$\chi^2=6.00$ df=4 p-value=0.199	$\chi^2=6.00$ df=4 p-value=0.199	$\chi^2=6.00$ df=4 p-value=0.199	$\chi^2=6.00$ df=4 p-value=0.199

Figure-3: Showing age wise prevalence of malaria in human population of District Mardan.

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Tehsil-wise prevalence of malaria infection in the human population of District Mardan:

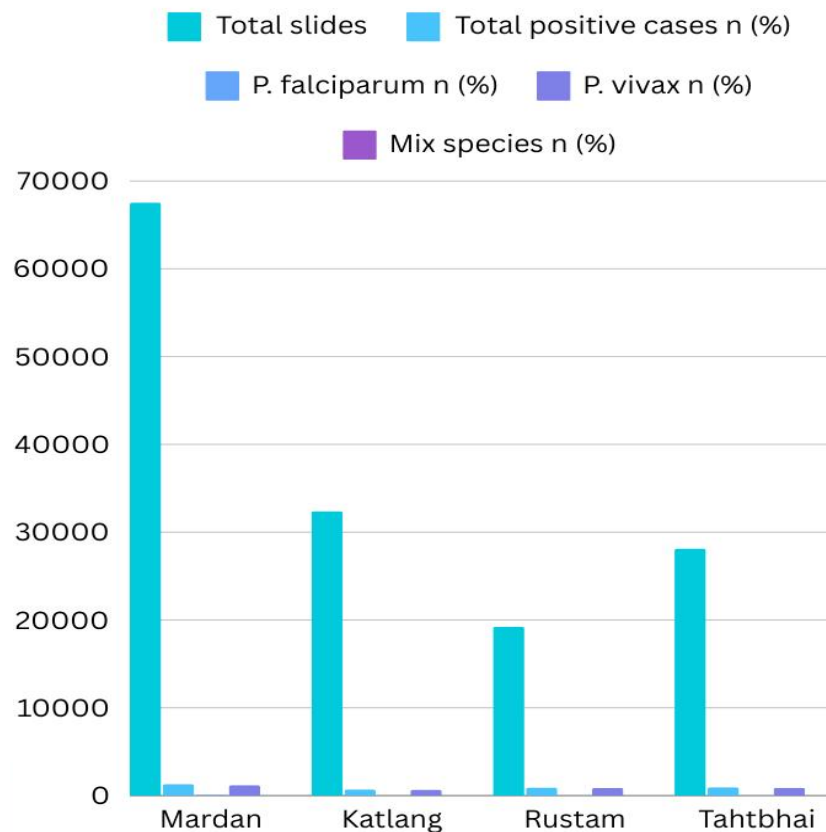
The prevalence of malaria parasites was high in tehsil Mardan, 1302 (33.50%), while the lowest rate was found in tehsil Katlang, 722 (18.57%). The difference was not statistically significant, $P > 0.05$.

Table 4: Prevalence of malaria infection in different tehsil of District Mardan:

Tehsil	Total slides	Total positive n(%)	<i>P.falciparum</i> n(%)	<i>P.vivax</i> n(%)	Mix species n(%)
Mardan	67542	1302(33.50%)	105(2.70%)	1187(30.54%)	10(0.25%)
Katlang	32400	722(18.57%)	43(1.10%)	670(17.24%)	9(0.23%)
Rustam	19250	922(23.72%)	51(1.31%)	864(22.23%)	7(0.18%)
Tahtbhai	28150	940(24.18%)	45(1.15%)	891(22.92%)	4(0.10%)
		$\chi^2=12.00$ df=9 p-value=0.213	$\chi^2=12.00$ df=9 p-value=0.213	$\chi^2=12.00$ df=9 p-value=0.213	$\chi^2=12.00$ df=9 p-value=0.213

Figure-4: Showing tehsil wise prevalence of malaria in human population of District Mardan.

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Socioeconomic status-wise prevalence of malaria infection in the human population of District Mardan:

According to the prevalence of malaria by socioeconomic status, the highest rate was found in poor people, 2248 (57.84%), and the lowest was found in good people, 575 (14.79%). The difference was statistically non-significant, $P > 0.05$.

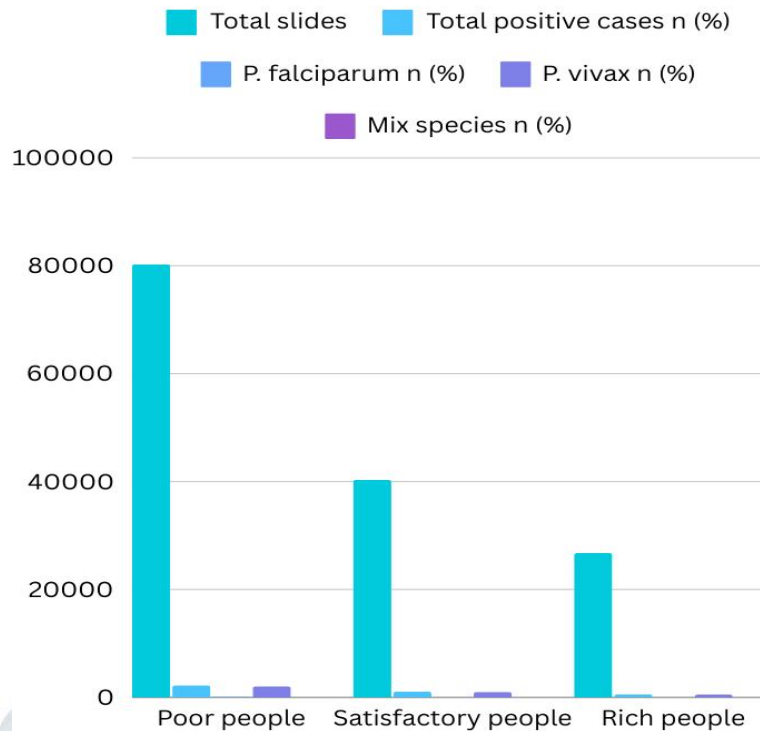
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Table 5: Socioeconomic status-wise occurrence of malaria infection in the human population of District Mardan:

Socioeconomic status	Total slides	Total positive cases n (%)	<i>P. falciparum</i> n (%)	<i>P. vivax</i> n (%)	Mix species n (%)
Poor people	80250	2248(57.84%)	156(4.01%)	2074(53.37%)	18(0.46%)
Satisfactory people	40345	1066(27.43%)	52(1.33%)	1005(25.86%)	9(0.23%)
Rich people	26747	575(14.79%)	36(0.92%)	536(13.79%)	3(0.07%)
		$X^2=6.00$ df=4 p-value=0.199	$X^2=6.00$ df=4 p-value=0.199	$X^2=6.00$ df=4 p-value=0.199	$X^2=6.00$ df=4 p-value=0.199

Figure-5: Showing socioeconomic status wise prevalence of malaria in human population of District Mardan.

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Discussion

Malaria represents a significant public health challenge in Pakistan, characterized by high incidence rates, substantial morbidity, and a notable mortality burden. This disease is endemic in 91 countries and affects approximately 40% of the global population. Annually, malaria results in over one million deaths, impacting an estimated 500 million individuals worldwide. The disease poses particularly severe risks to vulnerable groups, including children, pregnant women, and individuals lacking prior immunity. The escalating issue of malaria is compounded by the development of resistance in mosquito populations.

Pakistan's geographic and climatic conditions, particularly its extensive irrigation systems and the prevalence of standing water due to seasonal rainfall, facilitate mosquito proliferation. The country exhibits moderate endemicity for malaria, particularly within its rural populations and in regions characterized by tropical and subtropical climates. The endemic malaria belt encompasses Pakistan, as supported by previous studies.

In 1990, global assessments highlighted factors such as inadequate sewage systems, the presence of standing water, and poor waste management as critical facilitators for malaria transmission in both urban and rural environments. The two predominant malaria species identified in Pakistan are *Plasmodium vivax* and *Plasmodium falciparum*, which are responsible for the majority of infections. Prevalence rates exhibit regional variability across provinces and demographic groups, including neonates.

In our investigation, data were collected from the District Headquarters Hospital of Mardan and Mardan Medical Complex, revealing that the Punjab province reported less than 10% of malaria cases, while Sindh contributed approximately 30%. Notably, Baluchistan, despite representing only 5% of the national population, accounted for 30% (38.6%) of reported cases. The findings indicate that *P. vivax* is the most prevalent species, consistent with prior research in the region.

Our analysis identified a seasonal pattern in malaria cases, with a notable decrease in May (296 cases, 7.51% frequency) and a peak in September (814 cases, 20.94%). This increase in cases exceeds the previously recorded human prevalence rate of 13.9% for the same region five years prior, suggesting a rising trend in malaria incidence in District Mardan.

Gender-wise analysis indicated a higher prevalence of malaria in males (53%) compared to females (47%). This disparity may be attributed to increased outdoor exposure among males, who typically engage in

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outdoor labor more frequently than females. Similar trends have been observed in other districts of Khyber Pakhtunkhwa, such as District Buner and District Charsadda.

Age-related prevalence data revealed that individuals over 15 years of age accounted for 73.90% of malaria cases, while those in the 0-4 year age group represented only 6.5%. This pattern likely reflects greater outdoor exposure and mosquito interactions among older individuals in the local context. Tehsil-wise analysis showed the highest prevalence in Tehsil Mardan (33.50%) and the lowest in Tehsil Katlang (18.57%).

Moreover, socioeconomic status appeared to significantly influence malaria prevalence, with 57.84% of cases occurring in individuals from lower socioeconomic backgrounds compared to 14.79% among those with higher socioeconomic status.

Conclusion

Our study underscores the elevated prevalence of malaria in District Mardan, particularly among individuals of lower socioeconomic status. The data reveal a higher incidence of malaria in adults compared to children, with males being disproportionately affected due to their increased outdoor activities. These findings highlight the urgent need for targeted interventions to mitigate malaria transmission, particularly in vulnerable populations.

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