

EPIDEMIOLOGICAL INSIGHTS INTO PLASMODIUM SPECIES PREVALENCE AMONG PREGNANT WOMEN: A STUDY FROM MARDAN MEDICAL COMPLEX, KHYBER PAKHTUNKHWA, PAKISTAN

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Abstract

This study aimed to evaluate the prevalence of malaria due to Plasmodium species among pregnant women at Mardan Medical Complex. A total of 250 blood samples were collected from pregnant women presenting with clinical symptoms including fever, chills, vomiting, and headache. Diagnostic assessments utilized both microscopy and rapid diagnostic tests (RDTs) to detect specific Plasmodium species. Out of the samples analyzed, 65 (26%) tested positive for malaria, while 185 (74%) were negative. Among the positive cases, 58 (23.2%) were identified as *P. vivax*, 6 (2.4%) as *P. falciparum*, and 1 (0.4%) showed mixed infection with both *P. vivax* and *P. falciparum*. Statistical analysis indicated a significant correlation between malaria infection and the presence of clinical symptoms, with a p-value of less than 0.005, highlighting the considerable burden of malaria in this demographic. The findings underscore the need for enhanced malaria control strategies to mitigate the risks associated with malaria during pregnancy.



This study contributes to the understanding of malaria prevalence in pregnant populations and emphasizes the importance of targeted interventions.

Keywords: Malaria, Pregnant Women, Mardan, Plasmodium vivax, Plasmodium falciparum, Mixed Infection.

Introduction

Malaria remains a significant global health concern, particularly in underdeveloped regions, with an estimated 3,000 deaths occurring daily due to malaria [18]. The annual incidence of malaria is estimated to range between 400 and 600 million cases [16, 20]. Although the majority of malaria cases are concentrated in Africa, other tropical regions, such as India, Indonesia, Papua New Guinea, and the Amazon basin in Latin America, also experience a substantial burden of the disease. The primary causative agents of human malaria are Plasmodium falciparum and Plasmodium vivax, with P. falciparum being responsible for most severe cases and nearly all mortality associated with malaria [16, 18].

In Pakistan, malaria is hyperendemic, with P. vivax accounting for the majority of infections; however, there has been a notable increase in P. falciparum cases, now constituting approximately 35-40% of total malaria cases. The impact of malaria during pregnancy has been extensively studied, particularly in Sub-Saharan Africa, which accounts for 90% of the global malaria burden and related mortality [14]. Research indicates that pregnant women in malaria-endemic regions exhibit heightened vulnerability to the disease, experiencing both higher incidence and greater severity compared to their non-pregnant counterparts [6-11]. This increased susceptibility is thought to result from a temporary reduction in cell-mediated immunity during pregnancy, which typically improves following the birth of a neonate and diminishes with subsequent pregnancies [13].

Notably, parasite density and the prevalence of anemia are found to be highest in primigravida women, with these parameters decreasing in women with multiple pregnancies. Consequently, complication rates are elevated in primigravida patients relative to those who have experienced multiple pregnancies [5, 7-3]. The complications associated with malaria during pregnancy are severe and include cerebral malaria, maternal anemia, intrauterine growth restriction, preterm



labor, stillbirth, and abortion [5, 9]. Moreover, the antimalarial treatments administered can significantly exacerbate the risk of these complications.

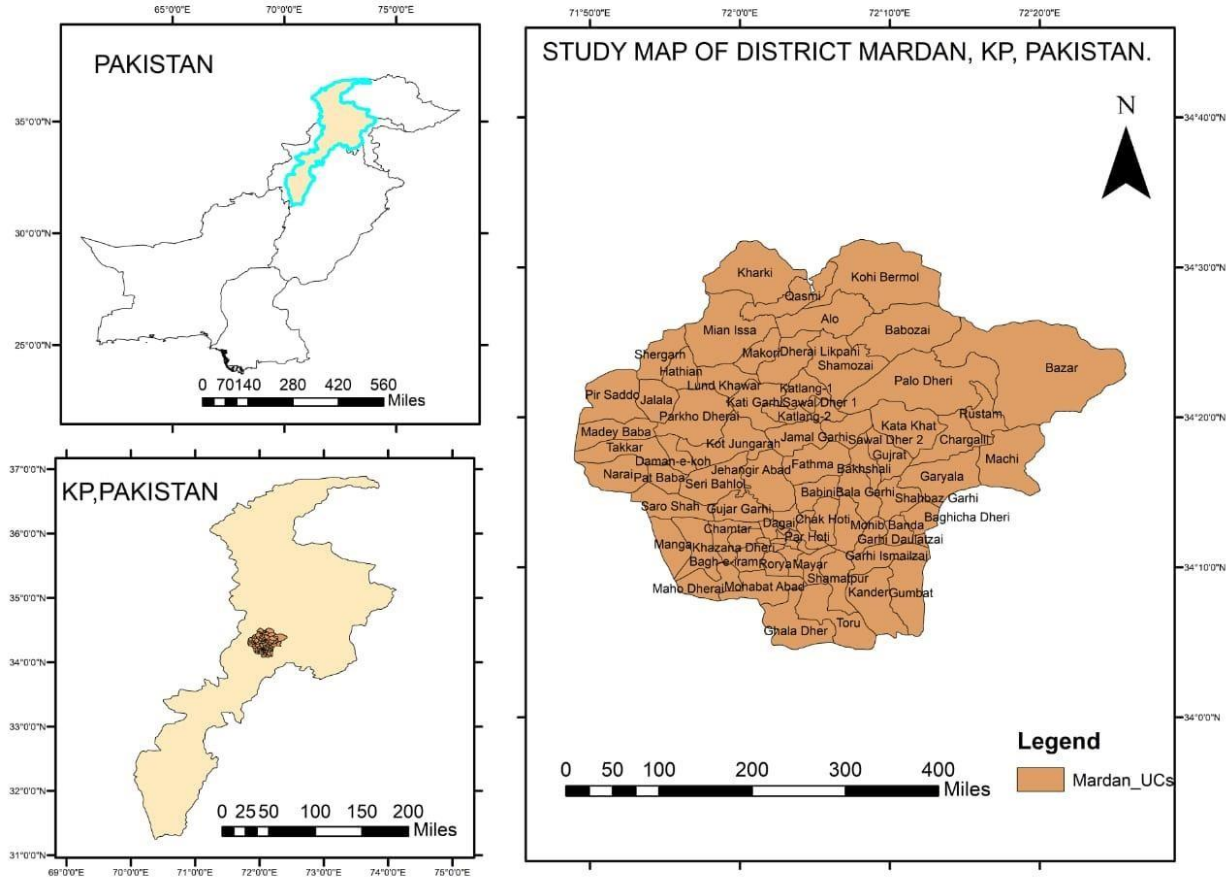
Given that Pakistan is an endemic region for malaria, this study aims to investigate the prevalence of malaria among pregnant women attending Mardan Medical Complex, as well as to identify the associated risk factors.

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



Map of study area

Patient Selection and Sample Collection

This study was conducted at Mardan Medical Complex. Pregnant women presenting with symptoms such as high fever, chills, vomiting, and headache were included in the study. Blood samples were collected after cleaning the finger with spirit-moistened cotton, discarding the first drop of blood, and then collecting 2-3 drops from the subsequent blood flow using a sterile pricker. The collected blood was used to prepare thick smear slides, which were stained with 3% Giemsa's stain for microscopic analysis.

Microscopic Examination and Parasite Density

Prepared slides were examined under a binocular Japan Olympus microscope at 100x oil

immersion magnification. Parasite density per field was calculated, with the trophozoite stage being observed in the majority of cases.

Rapid diagnostic test

The study employed the SD Bioline Malaria Antigen Pf/Pv rapid diagnostic test (RDT) (Catalogue No. 05FK80, Standard Diagnostics Inc., Korea). This test uses a lateral flow immunochromatographic format, consisting of a membrane strip within a plastic cassette. It is pre-coated with monoclonal antibodies that specifically bind to the *P. falciparum* HRP2 protein and the *P. vivax* PLDH enzyme. Results interpretation begins with verifying the control line; the presence of one or two additional test lines indicates the presence of malaria parasites in the blood sample. RDTs provide an effective alternative for malaria diagnosis.

Statistical Analysis

Data was analyzed using chi-square tests to assess the distribution of Plasmodium species across different age groups and localities. The results were considered statistically significant if the p-value was less than 0.05.

Results

Table 1: Age-wise Incidence of Plasmodium Species in Pregnant Women

A total of 250 blood samples from suspected malaria patients were tested, revealing that 65 (26%) were positive for malaria. The highest prevalence of malaria was observed in pregnant women aged 20 to 30 years, with 30 cases (12%). On the other hand, pregnant women over 50 years of age had the lowest prevalence, with only 5 cases (2%). These results indicate that while the difference in prevalence among the age group 20-30 was statistically significant ($p < 0.005$), the differences in prevalence among the other age groups were not statistically significant, as $p > 0.05$.

S. No	Age groups(years)	Total n(%)	Negative n(%)	Positive n(%)	P.v n(%)	P.f n(%)	Pv/Pf n(%)	p-value
1	20-30	80(32%)	50(20%)	30(12%)	28(11.2%)	2(0.8%)	0	0.002
2	31-40	75(30%)	55(22%)	20(8%)	18(7.2%)	2(0.8%)	0	0.015
3	41-50	79(31.6%)	69(27.6%)	10(4%)	9(3.6%)	1(0.4%)	0	0.045
4	>50	16(6.4%)	11(4.4%)	5(2%)	3(1.2%)	1(0.4%)	1	0.250

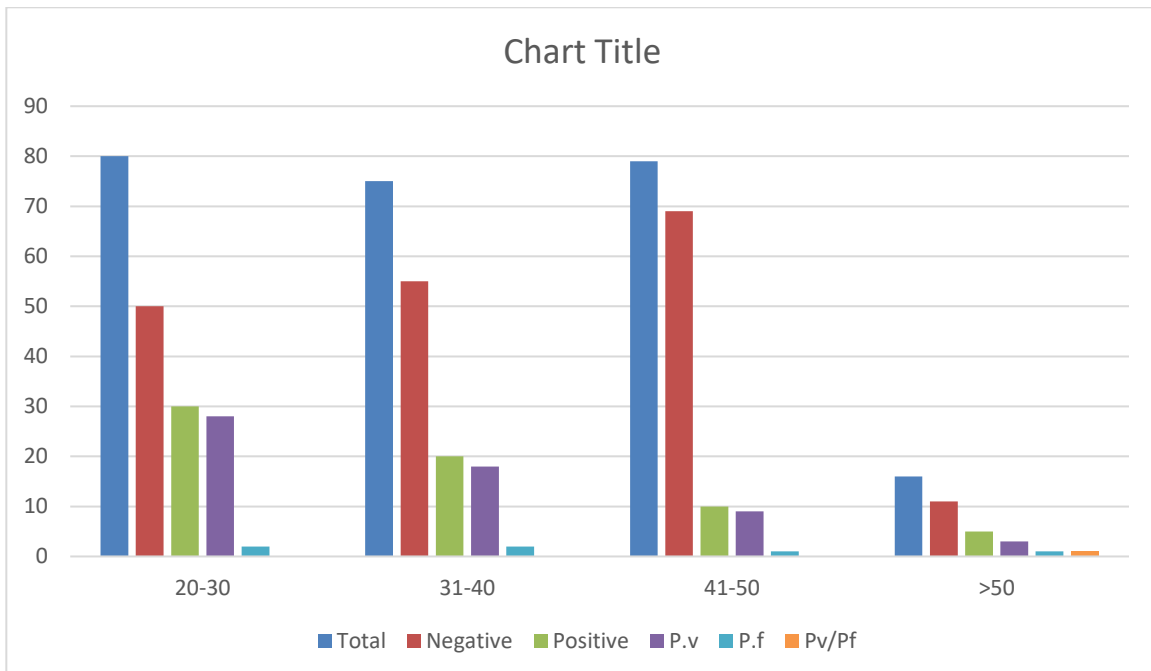


Figure 1: Age-wise Incidence of Plasmodium Species in Pregnant Women

Table 2: Plasmodium Species Densities and Number of Patients

In density level 1, 28 (11.2%) patients tested positive, with 25 (10%) cases of Plasmodium vivax and 3 (1.2%) cases of Plasmodium falciparum. As the density levels increase, the number of

positive cases decreases, with level 4 showing only 5 (2%) positive cases, all of which vivax. The data indicates a significant decline in positivity rates as density levels rise. Overall, the data shows a clear trend of declining positivity rates with increasing density levels, but only the results from density level 1 were statistically significant ($p < 0.005$).

Density level	Total n(%)	Negative n(%)	Positive n(%)	P.v n(%)	P.f n(%)	Pv/Pf	p-value
1	93(37.2%)	65(26%)	28(11.2%)	25(10%)	3(1.2)	0	0.001
2	70(28%)	49(19.6%)	21(8.4%)	18(7.2%)	2(0.8%)	1(1.2)	0.020
3	59(23.6%)	49(19.6%)	10(4%)	10(4%)	0	0	0.090
4	28(11.2%)	23(9.2%)	5(2%)	5(2%)	0	0	0.300

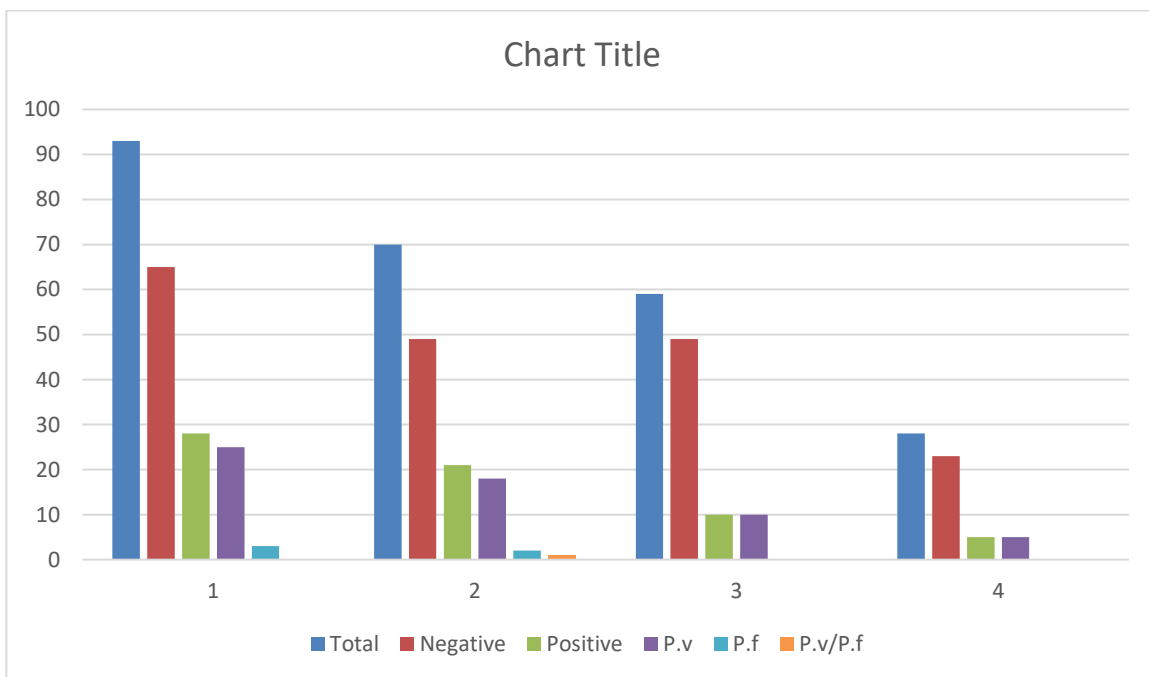


Figure 2: Plasmodium Species Densities and Number of Patients

Table 3: Incidence of Plasmodium Species Based on Locality

The analysis revealed a higher prevalence of malaria parasites among rural pregnant women, with 35 cases (14%), compared to 30 cases (12%) among urban pregnant women. The statistical

significance for urban areas was $p = 0.150$, indicating no significant difference. Conversely, the rural population showed a significant association, with a p-value of 0.005.

S. No	Locality	Total n(%)	Negative n(%)	Positive n(%)	P.v n(%)	P.f n(%)	Pv/Pf n(%)	p-value
1	Urban	110(44%)	80(32%)	30(12%)	28(11.2%)	20.8%	0	0.150
2	Rural	140(46%)	105(42%)	35(14%)	30(12%)	4(1.6%)	1(0.4%)	0.005

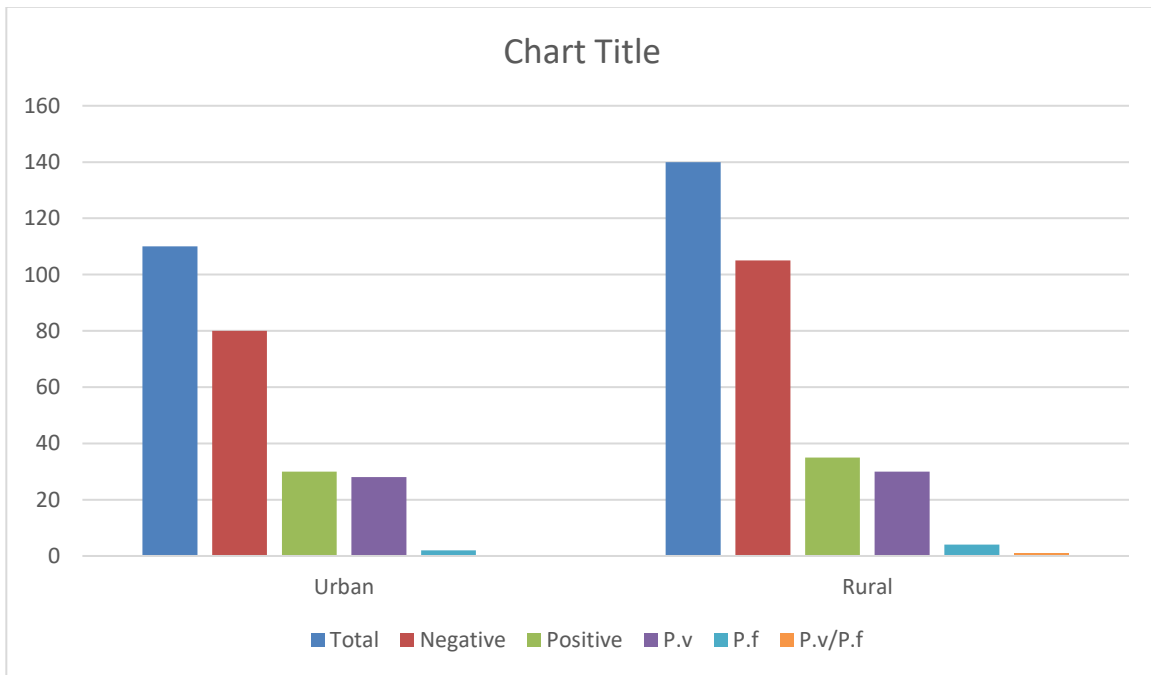


Figure 3: Incidence of Plasmodium Species Based on Locality

Table 4: Laboratory method wise incidence of Plasmodium Species

In this study, rapid diagnostic tests (RDTs) identified 43 (66.15%) cases of malaria, with Plasmodium vivax responsible for 40 (16%) of these cases and Plasmodium falciparum for 3 (1.2%). In comparison, microscopy detected a total of 65 (26%) positive cases, which included 58 (23.2%) instances of P. vivax, 6 (2.4%) of P. falciparum, and 1 (0.4%) case of mixed infection involving both species. These findings underscore a notable disparity in the diagnostic effectiveness between the two methods. The statistical significance for RDTs was $p > 0.005$,

indicating no significant difference. Conversely, the microscopy showed a significant association, with a p-value of 0.005.

S.No	Detection method	Total samples	Negative n(%)	Positive n(%)	P.v n(%)	P.f n(%)	Pv/Pf	p-value
1	RDTs	250	203(81.2%)	43(17.2%)	40(16%)	3(1.2%)	0	0.040
2	Microscopy	250	185(74%)	65(26%)	58(23.2%)	6(2.4%)	1(0.4%)	0.003

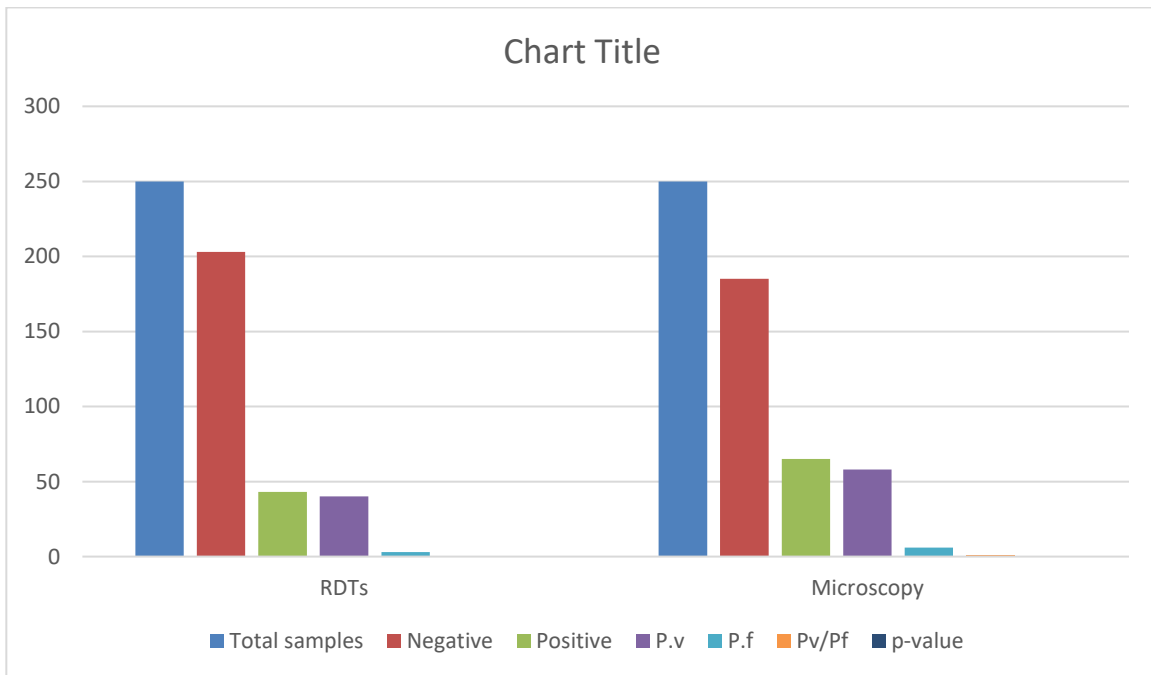


Figure 4: Laboratory method wise incidence of Plasmodium Species

Discussion



The present study investigates the incidence of malaria, specifically focusing on Plasmodium species, among pregnant women in the Mardan district of Khyber Pakhtunkhwa, Pakistan. With a total of 250 blood samples analyzed, the overall malaria prevalence was found to be 26%, with notable variations across age groups, locality, and diagnostic methods.

Analysis of the age distribution of malaria cases revealed that pregnant women aged 20 to 30 years had the highest prevalence, accounting for 12% of the total cases. This finding is consistent with studies conducted in other regions, which indicate that younger populations are often more susceptible to malaria due to a combination of factors, including lower immunity and increased exposure to vector habitats [19]. Conversely, the lower prevalence observed in women over 50 years, with only 2% positivity, suggests that age-related immunity could play a role in reducing malaria risk among older women [2].

The study further indicated a higher prevalence of malaria among rural pregnant women (14%) compared to their urban counterparts (12%). This disparity aligns with existing literature that highlights the increased malaria risk in rural areas due to factors such as poor healthcare access, higher mosquito breeding sites, and inadequate preventive measures [4]. Rural communities often lack the resources for effective vector control and have less access to healthcare facilities, leading to higher transmission rates [24].

In terms of species distribution, Plasmodium vivax was predominant, accounting for a significant majority of cases detected. The findings revealed that P. vivax comprised 13.2% of malaria infections identified through microscopy, which is in line with previous studies in Pakistan where P. vivax has been noted as the most common malaria species [27]. The notable presence of P. falciparum, though smaller in proportion, should not be overlooked, as it is associated with more severe disease and complications [26].

The diagnostic comparison between rapid diagnostic tests (RDTs) and microscopy showed that while RDTs detected 17.2% of malaria cases, microscopy was able to identify all 65 positive cases, including mixed infections. This disparity emphasizes the need for reliance on microscopy in endemic regions for accurate diagnosis, particularly in cases where mixed infections might occur [17]. The limitations of RDTs, especially in detecting lower densities of malaria parasites, can lead to underreporting and mismanagement of the disease, further stressing the importance of robust diagnostic methods [23].



Conclusion

This study highlights the continued burden of malaria in pregnant women, particularly in rural areas and younger age groups. The results emphasize the need for targeted malaria control strategies in these populations, including the use of insecticide-treated nets, intermittent preventive treatment, and improved healthcare access. The findings contribute valuable insights into the epidemiology of malaria in pregnancy in Mardan, providing a basis for further research and intervention development.

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