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CO-INFECTION RATE OF MALARIA AND TYPHOID IN NORTHERN AND CENTRAL REGIONS OF KHYBER PAKHTUNKHWA, PAKISTAN

Original Research

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ABSTRACT

Background: Malaria and typhoid fever are significant infectious diseases in tropical and subtropical regions, particularly in areas with poor sanitation and widespread vector breeding grounds. Malaria is caused by protozoan parasites of the *Plasmodium* genus, while typhoid fever is a bacterial infection resulting from *Salmonella enterica* serovar Typhi. Despite their distinct etiologies, co-infection with both pathogens is frequently reported in malaria-endemic regions, complicating diagnosis and treatment. Reliable diagnostic tools are crucial to distinguish these infections, as overlapping symptoms often lead to misdiagnosis and inappropriate treatment.

Objective: This study aimed to determine the prevalence of malaria, typhoid fever, and their co-infection in the Swat, Dir Lower, and Mardan districts of Khyber Pakhtunkhwa, Pakistan, while evaluating diagnostic challenges and public health implications.

Methods: A cross-sectional study was conducted from November 2018 to April 2019 in hospitals across the selected districts. A total of 400 febrile patients, aged 1 to 40 years, were randomly recruited. Blood samples were collected and tested using the malaria parasite (MP) test for *Plasmodium* species and the Widal agglutination test for *Salmonella* Typhi detection. Data were analyzed using SPSS, and chi-square tests were applied to assess statistical significance between demographic variables and infection rates.

Results: Malaria was detected in 168 patients (42.00%), while 126 (31.50%) tested positive for typhoid fever. Co-infection with both pathogens was found in 34 patients (8.50%). The highest prevalence of malaria was recorded in Mardan (44.11%), followed by Swat (41.79%) and Dir Lower (38.80%). Typhoid fever was most prevalent in Mardan (33.82%), with slightly lower rates in Swat (31.34%) and Dir Lower (28.35%). Co-infection rates were highest in Swat (9.70%), followed by Mardan (8.20%) and Dir Lower (7.50%). The incidence was higher in children (12.00% in ages 1–10) and females (9.50%) compared to males (7.50%). Seasonal variation showed co-infection peaking in November (10.28%) and April (10.44%), with the lowest rates in January and February (5.97%). Statistical analysis showed no significant gender ($p = 0.5907$) or age-group ($p = 0.4402$) differences in co-infection prevalence.

Conclusion: The findings indicate a substantial burden of malaria and typhoid fever in the study regions, with notable co-infection rates and diagnostic challenges. The high rate of false positives in Widal testing suggests the need for more reliable diagnostic tools, such as molecular and blood culture techniques, to improve disease identification and management. Public health interventions focusing on improved sanitation, vaccination, and enhanced diagnostic accuracy are essential to reduce the disease burden in endemic areas.

Keywords: Co-infection, Diagnosis, Malaria, Pakistan, Prevalence, *Plasmodium*, *Salmonella typhi.*

INTRODUCTION

Malaria and typhoid fever are two significant infectious diseases that continue to pose major public health challenges, particularly in tropical and subtropical regions. Malaria, caused by protozoan parasites of the *Plasmodium* genus, is primarily transmitted through the bite of an infected *Anopheles* mosquito, with *Plasmodium falciparum* being the most virulent species (1). Globally, malaria accounts for millions of clinical cases and fatalities each year, making it one of the most burdensome parasitic infections (2). Typhoid fever, in contrast, is a bacterial infection caused by *Salmonella enterica* serovar Typhi and is transmitted through the ingestion of contaminated food and water. It remains a major cause of morbidity and mortality, with millions of cases reported annually, particularly in developing countries where sanitation and hygiene practices are inadequate (3). Despite their distinct causative agents and transmission pathways, malaria and typhoid fever share overlapping clinical presentations, including fever, malaise, and gastrointestinal symptoms, which complicates accurate diagnosis and effective treatment. Over the past two decades, co-infections involving *Plasmodium* species and *Salmonella* Typhi have been increasingly reported, particularly in endemic regions. This phenomenon has led to diagnostic challenges and potential mismanagement, as both infections may mimic each other or coexist in an individual (4). In regions with a high prevalence of malaria, the misdiagnosis of typhoid fever—or vice versa—remains a persistent issue due to the reliance on clinical symptoms alone. Furthermore, inadequate laboratory infrastructure in resource-limited settings often exacerbates the difficulty in distinguishing between the two infections (5).

Pakistan is among the countries where both malaria and typhoid fever remain endemic, posing a significant health burden. Malaria is a major vector-borne disease in the country, with approximately 127,825 confirmed cases reported in 2006 alone. However, due to underreporting and lack of comprehensive surveillance, the actual disease burden is expected to be much higher (6). The prevalence of malaria varies across different regions of Pakistan, with higher incidence observed in Baluchistan and the Federally Administered Tribal Areas (FATA), while Khyber Pakhtunkhwa (KP) and Sindh report moderate transmission rates (7). On the other hand, typhoid fever continues to be a leading cause of enteric infections, with an estimated incidence of 412 cases per 100,000 people annually in Pakistan (8). While population-based surveillance data for typhoid fever remain limited, local epidemiological studies indicate a disproportionately high burden among children (9). The historical association between malaria and typhoid fever dates back to the mid-19th century when co-infection was initially described among military personnel. However, it was later established that distinct laboratory-based diagnostic methods were necessary to confirm the presence of both pathogens concurrently (10). Given the shared endemicity and similar clinical manifestations of these diseases, individuals residing in malaria-prone areas are at increased risk of developing concurrent infections. This not only complicates clinical decision-making but also contributes to higher rates of morbidity and mortality, particularly in vulnerable populations with limited access to healthcare (11). Furthermore, Pakistan's tropical climate and extensive irrigation networks, particularly in regions like Khyber Pakhtunkhwa, provide an ideal environment for mosquito breeding, thereby sustaining malaria transmission throughout the year, with peak incidence following monsoon rains (12,13). Agricultural practices and stagnant water sources further exacerbate the risk of mosquito proliferation, heightening malaria exposure in these communities.

The present study aims to assess the rate of malaria, typhoid fever, and their co-infection in the northern and central regions of Khyber Pakhtunkhwa, Pakistan. By evaluating the prevalence and associated consequences of these infections, this research seeks to provide valuable epidemiological insights that can inform diagnostic protocols and public health interventions in endemic areas. Understanding the burden of co-infection is essential for improving disease management strategies, reducing misdiagnosis, and ultimately mitigating the impact of these infectious diseases on affected populations.

METHODS

A cross-sectional study was conducted in the Mardan, Swat, and Dir Lower districts of Khyber Pakhtunkhwa, Pakistan, to assess the co-infection rate of malaria and typhoid fever. These districts were chosen due to their environmental conditions, including extensive irrigation systems and water reserves, which create favorable conditions for both *Plasmodium* species and *Salmonella* Typhi transmission. Mardan, located in the central region of the province, shares borders with Charsadda, Nowshera, Swabi, and Malakand, while Swat and Dir Lower are in the northern region, where vector-borne diseases remain a significant public health concern. The study population comprised febrile patients seeking medical attention at healthcare facilities within the study region. Patients of all ages and genders presenting with fever and symptoms suggestive of either malaria or typhoid fever were eligible for inclusion, provided they had not initiated antimicrobial or antimalarial therapy before sample collection. Exclusion criteria included patients who had already started treatment, immunocompromised individuals (e.g., those with HIV/AIDS or receiving immunosuppressive therapy), pregnant women, and patients with chronic illnesses such as diabetes mellitus or renal disease that could influence immune response and confound diagnostic interpretation. Ethical approval was obtained from the Institutional Review Board (IRB) of Abdul Wali Khan University, Mardan. Written informed consent was obtained from all participants or, in the case of minors, from their parents or legal guardians before sample collection.

Blood samples were collected over a six-month period, from November 2012 to April 2013. A total of 400 febrile patients were enrolled, consisting of 200 males and 200 females. From each patient, 5 mL of venous blood was drawn using sterile syringes and subjected to laboratory analysis for malaria and typhoid fever. Malaria diagnosis was conducted using microscopy with Giemsa-stained thick and thin blood smears. Parasitemia was quantified per microliter of blood using the thick film, following standard microscopy protocols (13), with an assumed leukocyte count of 5400 per microliter (14). The thin smear was examined to identify malaria parasite stages, including gametocytes, trophozoites, and ring forms. A smear was considered negative if no parasites were detected after evaluating at least 100 microscopic fields under oil immersion. The Widal agglutination test was performed for typhoid fever diagnosis using a commercial antigen suspension (*Cal-Test Diagnostic Inc., Chino, U.S.A.*) for detecting somatic (TO) and flagellar (TH) antigens. The rapid slide titration technique was employed, and a serum antibody titer of ≥1:160 against *Salmonella* Typhi O antigen was considered a positive result (15,16).

Following data collection, results were recorded in Microsoft Excel 2016 and subsequently imported into SPSS Version 22.0 for statistical analysis. Descriptive statistics were used to summarize patient demographics and infection prevalence. Correlation analysis was performed to assess the relationship between Widal test results and malaria microscopy findings, which were considered the gold standard for malaria diagnosis. Additional statistical tests, such as chi-square analysis, were applied where applicable to determine the significance of associations. This study adhered to standard biomedical research methodologies, ensuring methodological rigor and ethical compliance. The findings provide valuable insights into the burden of malaria-typhoid co-infections in Khyber Pakhtunkhwa and contribute to improved diagnostic accuracy and disease management in endemic regions.

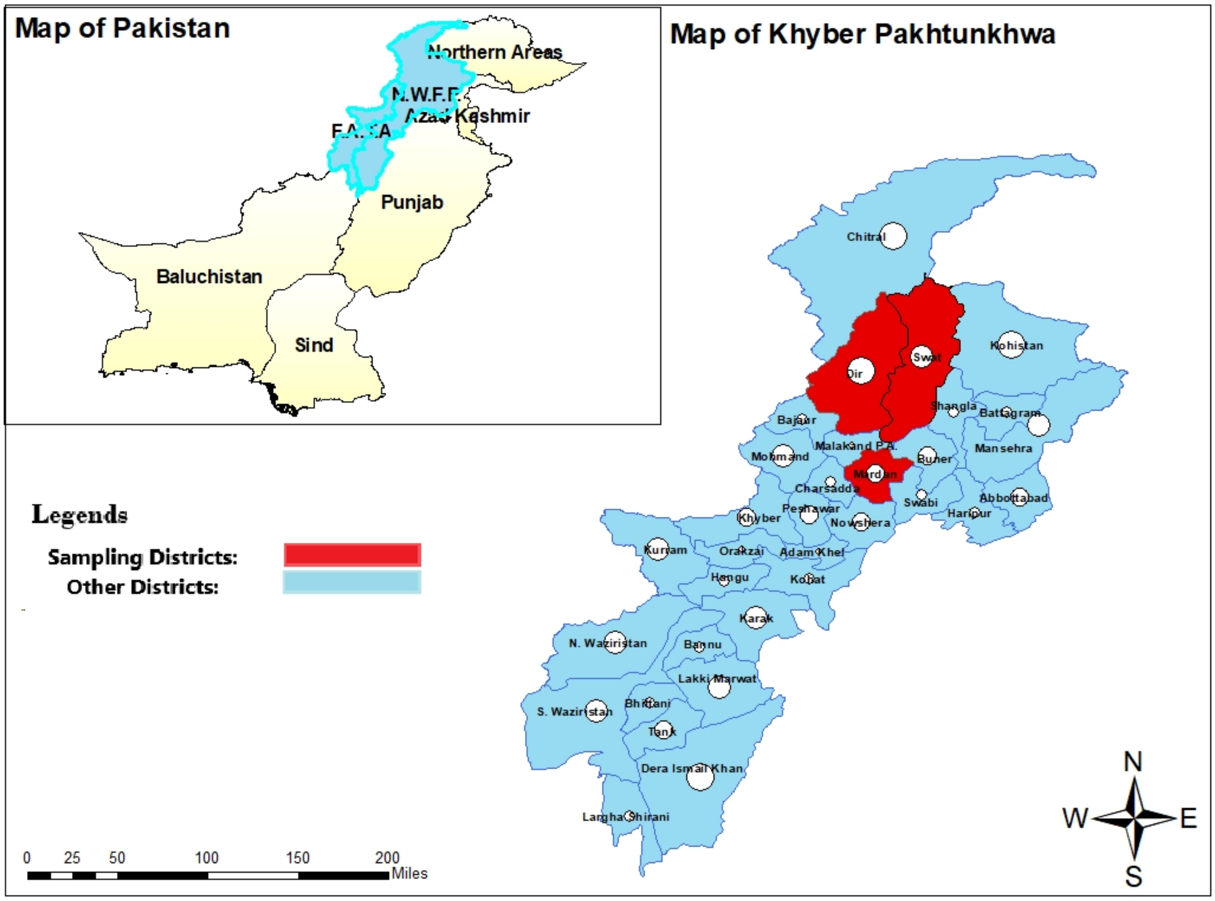

Fig No. 2.1: Study area map showing sampling districts

RESULTS

The study assessed the prevalence of malaria, typhoid fever, and their co-infection among 400 febrile patients who visited hospitals in Mardan, Swat, and Dir Lower over a six-month period between November 2018 and April 2019. Diagnostic assessments included serological and bacteriological tests for typhoid fever and parasitological analysis for malaria. Among the participants, 200 were tested for malaria parasites, while the remaining 200 underwent Widal agglutination testing for typhoid diagnosis. Malaria was detected in 168 of the 200 tested patients, yielding an overall prevalence of 42.00%. The highest prevalence was recorded in Mardan (44.11%), followed by Swat (41.79%), while Dir Lower had the lowest frequency (38.80%). Malaria prevalence was slightly higher in females (45.00%) compared to males (39.00%), though the difference was not statistically significant ($p = 0.2651$). Typhoid fever was identified in 126 of 200 patients tested, corresponding to a prevalence of 31.50%. Mardan exhibited the highest prevalence (33.82%), followed by Swat (31.34%) and Dir Lower (28.35%). Typhoid was slightly more prevalent in females (33.00%) than males (30.00%), but this difference was not statistically significant ($p = 0.5904$).

A total of 34 patients (8.50%) were found to have concurrent malaria and typhoid infections. The highest co-infection rate was observed in Swat (9.70%), followed by Mardan (8.20%) and Dir Lower (7.50%). Age-wise distribution revealed that co-infection was most prevalent in younger age groups, with 12.00% of cases occurring in patients aged 1–10 years. The prevalence decreased progressively with age, reaching 9.00% in individuals aged 11–20 years, 7.00% in the 21–30 age group, and 6.00% among those aged 31–40 years. However, no statistically significant difference in co-infection prevalence was observed across age groups ($p = 0.4402$). Gender-wise, co-infection was slightly higher among females (9.50%) compared to males (7.50%), but the difference was not statistically significant (p = 0.5907). Temporal variations in co-infection prevalence were noted, with the highest rates recorded in November (10.28%) and April (10.44%), whereas the lowest was observed in January and February (5.97%). Despite these fluctuations, the variation across study months was not statistically significant (p = 0.8825). Notably, when typhoid diagnosis was confirmed using blood culture instead of the Widal test, the co-infection rate dropped drastically to 0.50%, suggesting a high rate of false-positive results in Widal-based diagnoses.

A review of risk factors associated with malaria and typhoid fever in the study population highlighted environmental and socio-economic determinants. The selected districts have extensive irrigation networks and standing water bodies, facilitating mosquito breeding and malaria transmission. Additionally, poor sanitation and contaminated drinking water contribute to the endemicity of typhoid fever. Individuals from rural areas with inadequate access to clean water and proper hygiene were found to be at a higher risk of both infections. The reliability of the Widal test in diagnosing typhoid fever was critically examined. While the Widal test remains a common diagnostic tool in resource-limited settings, its high false-positive rate is a major limitation. The drop in co-infection rates from 8.50% to 0.50% following blood culture confirmation suggests that serological testing alone may overestimate typhoid prevalence. The Widal test is known to cross-react with non-*Salmonella* antigens and may yield misleading results in areas where other enteric infections are prevalent. Consequently, blood culture remains the gold standard for confirming typhoid fever, though its availability is often restricted in low-resource settings. These findings indicate that malaria and typhoid fever remain significant public health concerns in the study region, with a notable burden of co-infection. While geographical, gender-based, and age-related variations were observed, no statistically significant differences were found across these factors. The study highlights the critical need for improved diagnostic accuracy, particularly in differentiating typhoid fever from other febrile illnesses, to prevent misdiagnosis and inappropriate treatment.

Table 1: Gender-wise Prevalence of Malaria, Typhoid, and Co-infection

Gender	Observed Samples	Malaria Infected	Malaria Prevalence (%)	Typhoid Infected	Typhoid Prevalence (%)	Co-infected	Co-infection Prevalence (%)
Males	200	78	39	60	30	15	7.5
Females	200	90	45	66	33	19	9.5
Total	400	168	42	126	31.5	34	8.5

Table 2: Age-wise Prevalence of Malaria and Typhoid Co-infection

Age Group (Years)	Observed Samples	Co-infected	Co-infection Prevalence (%)
10-Jan	100	12	12
20-Nov	100	9	9
21-30	100	7	7
31-40	100	6	6
Total	400	34	8.5

Table 3: Monthly Variation in Co-infection Prevalence

Month	Observed Samples	Co-infected	Co-infection Prevalence (%)
November	68	7	10.28
December	68	6	8.82
January	67	4	5.97
February	67	4	5.97
March	67	6	8.95
April	67	7	10.44
Total	400	34	8.5

Table 4: Area wise percentage of malaria and typhoid co-infection

Factor (Areas)	Observed samples	Infected samples	% Prevalence
Mardan	134	13	9.7%
Swat	133	11	8.2 %
Dir Lower	133	10	7.5 %
Total	400	34	8.5 %

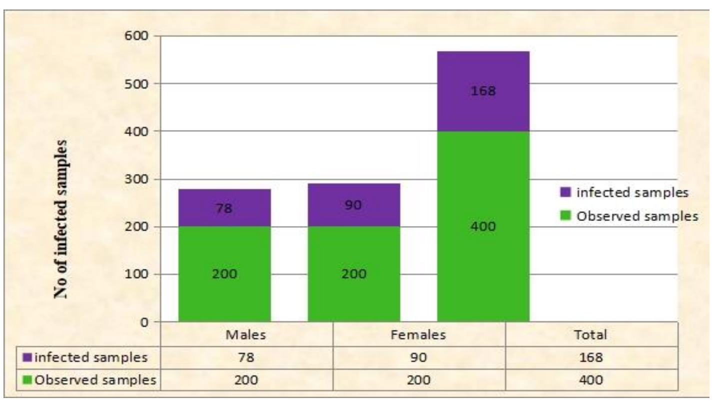

Figure 1 Gender wise chart of Malaria

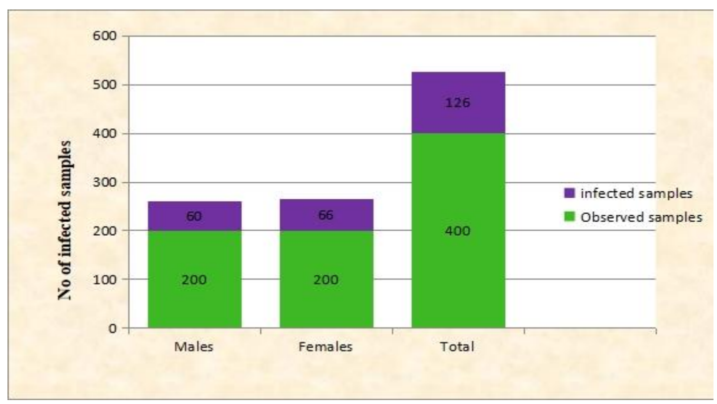

Figure 2 Gender wise prevalence of typhoid

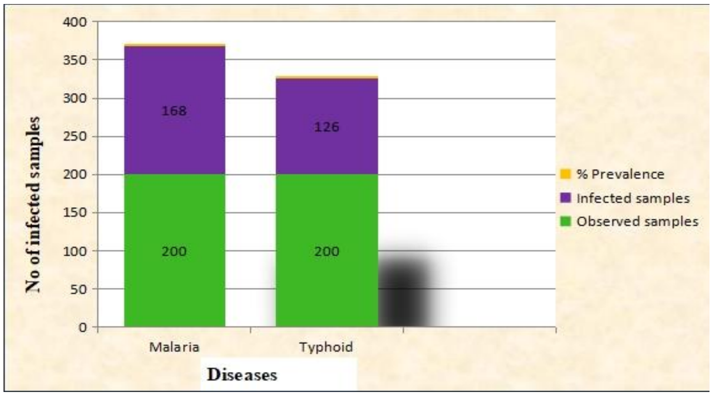

Figure 3 Graph of Overall prevalence of Malaria and typhoid

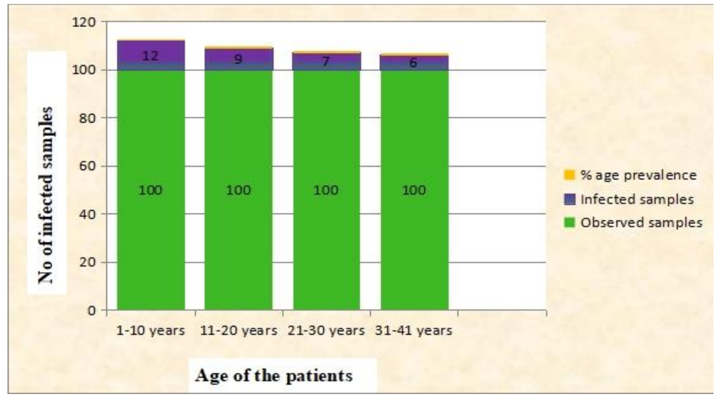

Figure 4 Age wise chart of Malaria and typhoid co-infection

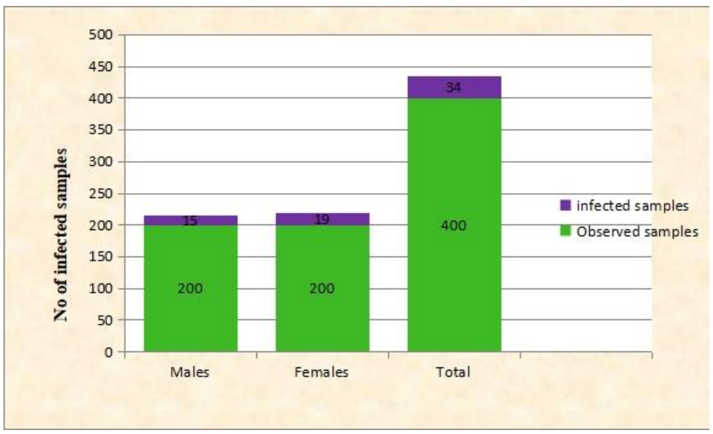

Figure 5 Gender wise graph of Malaria and typhoid co-infection

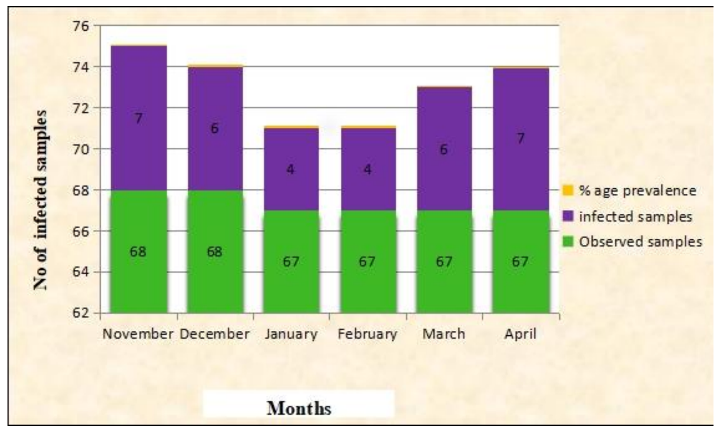

Figure 6 Month wise chart of Malaria and typhoid co-infection

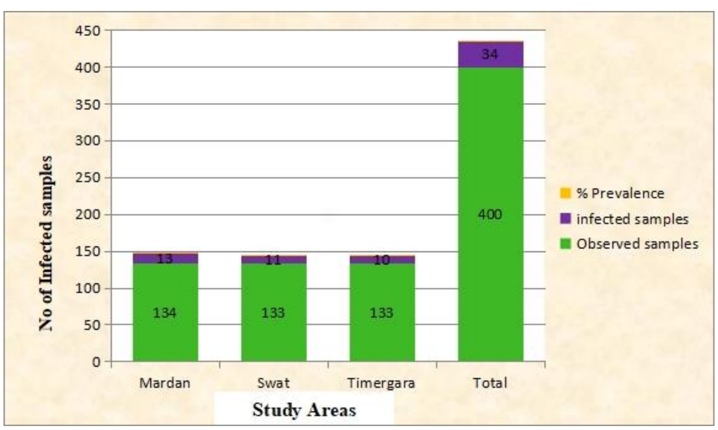

Figure 7 Area wise prevalence percentage of Malaria and typhoid co-infection

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DISCUSSION

The findings of this study demonstrated an overall co-infection rate of 8.5% for *Salmonella typhi* and malaria parasites among febrile patients in Mardan, Swat, and Dir Lower. Malaria prevalence exceeded that of typhoid fever, aligning with observations from previous research conducted in endemic regions. The relatively low co-infection rate observed is consistent with findings from other studies, suggesting that diagnostic methodologies often rely on presumptive approaches rather than definitive laboratory confirmation. A significant concern remains the tendency of clinicians to prescribe empirical antimalarial or antityphoid treatments without confirmatory diagnostics, potentially leading to an overestimation of co-infection prevalence based on clinical suspicion alone rather than microbiological evidence (15). Geographical variation in co-infection prevalence was evident, with the highest rates in Mardan, possibly attributable to climatic conditions, warmer temperatures, and increased human migration from other endemic regions. Enhanced diagnostic facilities in this region may have also contributed to improved detection rates. Conversely, Dir Lower exhibited the lowest prevalence, which may be associated with its relatively cooler climate and potential underdiagnosis due to limited laboratory capacity. Swat demonstrated an intermediate prevalence, likely influenced by its mixed climatic conditions and moderate healthcare infrastructure. These findings align with previous research highlighting the role of climate and environmental factors in the transmission dynamics of malaria and typhoid fever (17,18). Similar studies conducted in other endemic regions, including Nigeria, India, and urban centers of Pakistan such as Peshawar and Islamabad, have reported comparable co-infection rates, further validating these observations.

The higher prevalence of malaria relative to typhoid fever is consistent with findings from regions such as Owerri and Imo State in Nigeria, where malaria remains the dominant febrile illness. Differences in co-infection rates across studies may be influenced by diagnostic techniques, as demonstrated by bacteriological culture studies that reported slightly higher co-infection rates of 10.1% in certain endemic areas (15). The notable decline in co-infection prevalence when typhoid fever was diagnosed using blood culture instead of the Widal test highlights the potential for misclassification. The Widal test is prone to false-positive results due to cross-reactivity with other bacterial antigens, previous infections, or vaccinations. This limitation has been widely acknowledged, necessitating cautious interpretation of serological findings. The observed discrepancy between Widal-based diagnosis and culture-confirmed typhoid cases underscores the critical need for more reliable diagnostic tools to accurately determine true co-infection rates (19,20). Socio-environmental determinants, including inadequate sanitation, consumption of contaminated water, and insufficient healthcare access, continue to contribute to the endemicity of both malaria and typhoid fever. The presence of stagnant water bodies in irrigation-intensive regions facilitates mosquito breeding, while poor waste disposal and hygiene practices sustain *Salmonella typhi* transmission. Individuals residing in such conditions are at increased risk of either concurrent infections or acute infections superimposed on chronic carriers. Addressing these factors requires the implementation of targeted public health measures, including improved sanitation, vaccination programs, and comprehensive community health education to reduce the disease burden (22).

Clinical observations from this study suggest that individuals with malaria-typhoid co-infection often exhibit distinct symptomatology compared to those with single infections. Participants with dual infections presented with more pronounced gastrointestinal symptoms, including nausea, vomiting, and abdominal pain, which were more consistent with enteric fever. Persistent fever, as opposed to the intermittent fever characteristic of malaria, was also frequently reported. A notable delay in fever resolution following antimalarial treatment further indicated potential diagnostic confusion in endemic settings. These findings reinforce the need for heightened clinical suspicion in regions where malaria and typhoid fever co-exist, emphasizing the importance of laboratory confirmation to avoid mismanagement and delayed recovery (8,9,23). The study's strengths lie in its robust data collection from diverse geographical regions, enabling a comparative analysis of disease prevalence across different environmental settings. The use of both parasitological and serological diagnostic techniques provided valuable insights into the limitations of commonly used diagnostic tools. However, certain limitations must be acknowledged. The reliance on the Widal test for typhoid diagnosis may have led to an overestimation of true infection rates, as reflected in the substantial reduction in co-infection prevalence when blood culture was employed. The cross-sectional study design limited the ability to assess disease progression over time, and the absence of molecular diagnostic techniques constrained the ability to confirm co-infection with higher specificity. Additionally, potential recall bias in symptom reporting may have influenced clinical correlations. Future research should incorporate molecular-based diagnostics, longitudinal study designs, and broader epidemiological surveillance to enhance the accuracy of co-infection assessments. These findings highlight the critical need for improved diagnostic accuracy, particularly in endemic regions where misdiagnosis can lead to inappropriate treatment strategies. Strengthening laboratory capacity, integrating advanced diagnostic tools, and promoting evidence-based treatment protocols will be essential to reducing the burden of malaria and typhoid fever co-infections. Expanding access to clean water, reinforcing vector control measures, and implementing comprehensive disease prevention strategies will further contribute to mitigating the impact of these infections in vulnerable populations.

CONCLUSION

The study highlights the substantial burden of malaria, typhoid fever, and their co-infection in the selected regions, emphasizing the influence of demographic and geographical factors on disease prevalence. Findings suggest that children and women were more frequently affected, while geographical variations indicated differing transmission dynamics across districts. The observed correlation between *Salmonella typhi* and malaria underscores the diagnostic challenges posed by overlapping symptoms, reinforcing the need for more reliable diagnostic tools. The results stress the importance of targeted public health interventions, improved sanitation, and enhanced laboratory capacities to ensure accurate disease management. Strengthening surveillance systems and integrating comprehensive prevention strategies will be crucial in mitigating the impact of these infections and reducing their burden on affected communities.

Author Contribution

Author	Contribution
Wasia Ullah*	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Abdul Nasir	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Muhammad Izaz	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Muhsin Khan	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Rabia Rabia	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Ihsan Ullah	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published

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