





Prevalence and Association of Malaria With ABO Blood Groups in Bosaso City, Puntland, Somalia: A Cross-Sectional Study

Yahye Isse Hassan¹ | Mohamed Said Hassan²

¹Department of Medical Laboratory, Faculty of Health Science, Red Sea University, Bosaso, Puntland, Somalia | ²College of Health Science, School of Medicine, Amoud University, Borama, Somalia

Correspondence: Mohamed Said Hassan (hassan.ms@amoud.edu.so)

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ABSTRACT

Background and Aims: Malaria remains a global health concern, with an estimated 249 million cases annually and 2.2 billion people at risk of infection. This study aimed to investigate the association between malaria species and ABO blood groups and identify the relationship between ABO blood groups and parasitemia.

Methods: A cross-sectional study was conducted in Bosaso, Puntland, Somalia, from November 2022 to May 2023. Data were collected through simple random sampling involving 201 confirmed malaria cases from the national hospital. Blood samples obtained via finger prick were prepared as thick and thin smears, stained, and examined microscopically. Statistical analysis included descriptive statistics, Chi-square, and ANOVA tests to assess associations between malaria species, blood groups, and parasitemia.

Results: The prevalence of *Plasmodium* species was as follows: *P. falciparum* (48%), *P. vivax* (41%), and others (10%). Older age groups (31–45 and 46–60 years) exhibited higher prevalence rates for *P. falciparum* (50.7%–80.8%), while younger participants demonstrated increased susceptibility to *P. vivax*. Blood groups B (24%) and AB (18.8%) presented lower *P. falciparum* prevalence, whereas blood groups A (55.4%) and O (54.7%) displayed higher prevalence rates. Significant associations were observed between age groups and *Plasmodium* species ($\chi^2 = 14.2$, p = 0.027; F = 4.848, p = 0.030) and between blood groups and *Plasmodium* species ($\chi^2 = 23.9$, p = 0.001; F = 3.583, p = 0.015). A relationship between blood groups and parasitemia was also identified ($\chi^2 = 17.4$, p = 0.008; F = 12.79, p = 0.017).

Conclusion: The findings of this study not only underscore the higher risk of *P. falciparum* in older individuals but also provide crucial insights into the interplay between malaria and ABO blood groups. This knowledge is significant for enhancing community awareness and implementing effective management strategies to reduce the risk of malaria, thereby enlightening the reader about the potential impact of this research.

1 | Introduction

Malaria remains a significant global public health challenge. The World Health Organization (WHO) 2022 World Malaria Report (published in 2023) estimated 249 million malaria cases globally,

resulting in approximately 608,000 deaths in 2022 [1]. This affects 2.2 billion people at risk of infection, primarily through *Anopheles* mosquito transmission [1]. Between 2000 and 2021, 2 billion malaria cases and 11.7 million deaths were prevented worldwide. Malaria is the most significant deadly protozoan disease, accounting

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for at least 627,000 deaths annually, with the majority occurring in young African children [1]. The causative agents are *Plasmodium* species, including *P. falciparum*, *P. vivax*, *P. malaria*, *P. ovale*, and *P. knowlesi*. These obligate intracellular sporozoans infect humans and some primates, exhibiting geographical variations in disease patterns and severity [2–6]. In Africa, malaria causes an estimated \$12 billion in annual economic losses and a 1.3% decline in annual economic growth [7].

The global malaria situation has fluctuated. In 2000, there were 864,000 deaths; this decreased to 586,000 in 2015 and 576,000 in 2019. However, 2020 saw a 10% increase to approximately 631,000 deaths before a decrease to 608,000 in 2022 [1]. The majority of malaria cases (88%) and deaths (90%) in 2015 occurred in Africa [8]. At the start of 2016, malaria was endemic in 91 countries and territories [9], disproportionately impacting the poorest communities in sub-Saharan Africa with limited access to diagnosis, treatment, and prevention [10, 11]. In Somalia, In 2022, 336,840 suspected malaria cases were tested, of which 11,550 were positive. Compared with 2021 and 2020, the favorable cases declined by 11% and 61%, respectively. A total of two malaria-related deaths were also reported in 2022 [12].

The ABO blood group system is a genetic factor that may influence malaria susceptibility and severity. *P. falciparum* infection has exerted selective pressure on human ABO blood group distribution [13]. Studies have shown an association between the ABO blood group and the severity of *P. falciparum* malaria [14–17].

This study aims to determine the prevalence and association of malaria with ABO blood groups in Bosaso City, Puntland, Somalia. What makes this research particularly intriguing is its unique focus on the Somali population, providing a crucial perspective on the interplay between malaria and ABO blood groups in this specific context.

2 | Methodology

2.1 | Study Design

This study employed a comprehensive cross-sectional design, meticulously assessing the prevalence and association of malaria with ABO blood groups among patients in Bosaso City, Puntland, Somalia. The thoroughness of our approach should instill confidence in the reader about the validity of our research.

2.2 | Study Period

The research was conducted over 6 months, from November 2022 to April 2023.

2.3 | Study Population

The target population included confirmed malaria cases diagnosed through thick and thin blood film microscopy at a national hospital in Bosaso City.

2.4 | Sample Size Determination

The sample size was calculated using Slovene's formula to ensure adequate representation:

Slovene's formula:

$$n = \frac{N}{1 + N(e)^2} = \frac{405}{1 + N(0.05)^2} = \frac{405}{1 + 405(0.05)^2} = 201,$$

where

- n = required sample size,
- N = total population (405 confirmed malaria cases),
- e = margin of error (0.05).

2.5 | Sampling

Blood samples were collected from 201 individuals under the study, including 405 patients with confirmed malaria cases. Collections were taken randomly by using a simple random sampling method. The participants were chosen randomly and entirely by chance. Blood samples were examined by stained thick blood films to detect malaria parasites and stained thin blood films to identify species. Also, samples were analyzed for ABO blood groups.

2.6 | Sampling Technique

A simple random sampling method was used to select participants. This approach ensured that each individual had an equal chance of being included in the study, minimizing selection bias.

2.7 | Inclusion and Exclusion Criteria

The study included adult and pediatric patients who met all of the following criteria:

- Confirmed Malaria Diagnosis: A positive malaria diagnosis was confirmed by microscopic examination of thick and thin blood films, showing the presence of malaria parasites.
- 2. Presentation at Bosaso National Hospital: Presentation at the national hospital in Bosaso City, Puntland, Somalia, during the study period (November 2022–April 2023).

The study excluded participants who met any of the following criteria:

- Prior Malaria Intervention Studies: Participation in any malaria vaccine trial, antimalarial drug trial, or other intervention study that could potentially influence malaria susceptibility or ABO blood group distribution within the past.
- 2. Insufficient Diagnostic Data: Incomplete or missing data regarding malaria diagnosis (microscopic examination

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results) or ABO blood grouping. Specifically, insufficient blood sample volume to allow for both tests.

2.8 | Data Collection Instruments

The data collection instrument in the study primarily involved laboratory-based diagnostic tools and techniques.

2.9 | Data Collection Procedure

2.9.1 | Collection of Capillary Blood

The tip of the third finger was cleaned with cotton wool dipped in 70% alcohol, and the finger was pricked firmly and rapidly with a sterile lancet. The first drop of blood was wiped.

2.9.2 | Preparation of Thick Blood Smears

Three drops of collected blood were placed in a clean and dry slide (about 2 cm from the edge) and then stirred by a corner of another clean and dry slide until an appropriate thick smear was obtained. The smear was left to dry.

2.9.3 | Preparation of Thin Blood Smears

A drop of blood (approximately $1-2\,\mu L$) was placed in the middle of a clean and dry slide approximately 1 cm from one end and by the edge of another slide (called a spreader) placed just in front of it. The spreader turned until it touched the drop of blood, and then blood was allowed to run along the edge of the spreader. The spreader was pushed forward to the end of the slide at a suitable speed. The smear was left to dry.

2.9.4 | Staining of Blood Films

All thick and thin blood films were stained using Giemsa stain (10%):

- Only thin blood smears were fixed with absolute methanol for 1–2 min before staining.
- The slides were covered with 10% Giemsa solution for 10 min. All slides were washed using buffered water and allowed to air dry.

2.9.5 | Examination of Blood Films

The slides were examined using a light microscope (Olympus x100 oil immersion lenses).

The number of parasites was counted and reported by using the following grading:

l- 10 parasites per 100 thick film fields +

11- 100 parasites per 100 thick film fields ++

Ten parasites per thick film field +++

11- 100 parasites per thick film field ++++

2.9.6 | Interpretation of ABO Blood Groups

The interpretation of ABO blood was grouped as follows as described by Dietze [18].

Agglutination on the (A) circle and no agglutination on the (B) circle mean the ABO blood group is A.

Agglutination on the (B) circle and no agglutination on the (A) circle mean the ABO blood group is B.

Agglutination on Both the (A) circle and (B) circle means the ABO blood group is AB.

No agglutination on both (A) circle and (B) circle mean the ABO blood group is O.

2.10 | Ethical Approval and Consent

This study received ethical approval from the Research Ethical Committee of Red Sea University (Ref: RSUIRI/2024/005), ensuring compliance with ethical research standards. Informed consent was obtained from all participants, who were fully informed about the study's objectives, methods, potential risks and benefits, and their right to withdraw. Participant confidentiality and anonymity were maintained throughout the study, adhering to the Declaration of Helsinki and relevant guidelines.

2.11 | Data Analysis

Descriptive statistics (frequencies and percentages) were used to summarize the prevalence of *Plasmodium* species, age group distribution, and ABO blood group distribution. Chi-square tests were used to assess the associations between (1) the ABO blood group and *Plasmodium* infection; (2) the age group and *Plasmodium* infection; and (3) the ABO blood group and *Plasmodium* species.

3 | Results

3.1 | Plasmodium Species Distribution

Among 201 malaria cases, *P. falciparum* was the most prevalent species, accounting for 48% of infections. *P. vivax* infections comprised 41% of the cases, while the remaining 11% were attributed to other *Plasmodium* species. This indicates the dominance of *P. falciparum* and *P. vivax* in this study population, with *P. falciparum* showing a slightly higher prevalence (Figure 1).

3.2 | Distribution of *Plasmodium* Species by ABO Blood Group

In a study of 201 malaria cases, this chart illustrates the distribution of *Plasmodium* species (*P. falciparum*, *P. vivax*, and others) across ABO blood groups. A higher prevalence of *P. falciparum* malaria was observed in blood groups O (48.5%) and A (42.3%), suggesting a possible association between blood type and infection with this species. Conversely, *P. falciparum* prevalence was significantly lower in blood groups B (6.2%) and AB (3.1%) (Figure 2).

3.3 | Association of ABO Blood Groups With Demographic and Parasitological Factors in Malaria Patients (N = [201]

This table presents laboratory results showing the distribution of ABO blood groups (A, B, AB, and O) across various demographic and parasitological factors in a malaria study. A highly significant association (p > 0.0001) is found between age group and ABO blood group distribution. Sex shows no significant association with blood group distribution (p = 0.667). There's a significant association (p = 0.001) between blood group and the presence of P. falciparum in thin blood smears, and a similar significant association (p > 0.0001) exists between blood group and parasite density (as measured in thick blood smears). The table details the frequency and percentage of each blood group within different age groups, sexes, and Plasmodium species detected (both thin and thick blood film results) (Table 1).

3.4 | Prevalence of *Plasmodium* Species on ABO Blood Groups (N = [201]

This study of malaria prevalence found *Plasmodium falciparum* to be the most common species (48%), followed by *P. vivax* (41%) and other species (10%). A significant association was found between age and *Plasmodium* species infection ($\chi^2 = 14.2$, p = 0.027; F = 4.848, p = 0.030), with older age groups (31–45 and 46–60 years) exhibiting higher overall infection rates (50.7%–80.8%). Younger age groups showed a greater susceptibility to *P. vivax*. ABO blood group also showed a significant association with both *Plasmodium* species ($\chi^2 = 23.9$, p = 0.001; F = 3.583, p = 0.015) and parasitemia levels ($\chi^2 = 17.4$,

p = 0.008; F = 12.79, p = 0.017). Specifically, P. falciparum prevalence was significantly higher in blood groups A (55.4%) and O (54.7%) compared to blood groups B (24%) and AB (18.8%). Based on chi-square and ANOVA analyses, these findings highlight the influence of age and blood group on malaria infection patterns and parasite density (Table 2).

4 | Discussion

This study found that P. falciparum, P. vivax, and other Plasmodium species accounted for 48.3%, 41.8%, and 10% of infections, respectively. Significant associations were observed between malaria prevalence and both age and ABO blood groups. Females experienced higher malaria prevalence than males across all Plasmodium species. A and O showed higher infection rates among blood groups than B and AB. The higher prevalence of P. falciparum in older age groups could be attributed to several factors. Over time, acquiring immunity to P. vivax may shift susceptibility towards P. falciparum, as older individuals may have experienced more significant exposure to different strains. Occupational and social factors may also contribute to higher exposure in older groups. The potential role of ABO antigens on the surface of erythrocytes, which is believed to play a role in interactions with parasites, may explain the higher infection rates observed in blood groups A and O. The discrepancy between sex might be associated with socioeconomic and behavioral factors.

Our findings are broadly consistent with several previous studies. A Kenyan study similarly reported a higher P. falciparum prevalence in blood group O, although it observed higher parasitemia in this group, contrasting our results [19]. We propose the variation in findings between our results and that of the Kenyan study, which may be associated with the methodologies used for the study, such as different parasite densities and parasite species. A Tanzanian study also noted a high proportion of blood group O participants, suggesting potential susceptibility to malaria [13]. An Ethiopian study found the highest malaria prevalence in blood group A, consistent with our observation of blood group A as the second most affected, but this contrasts with our blood group O findings [20]. Variations in the findings of our study compared to those of the Ethiopian studies may arise from the genetic diversities of the population under study. Another Ethiopian study found a

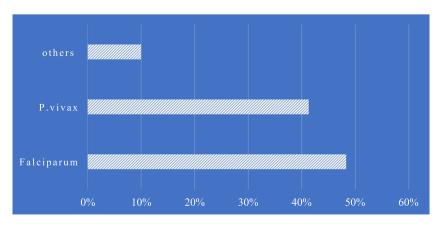


FIGURE 1 | Plasmodium distribution.

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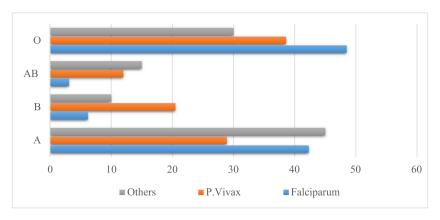


FIGURE 2 | Distribution of *Plasmodium* species by ABO blood group.

TABLE 1 Association of ABO blood groups with demographic and parasitological factors in malaria patients (n = [201].

	Laboratory results of ABO blood groups						
	A Frequency (%)	B Frequency (%)	AB Frequency (%)	O Frequency (%)	Chi square p value		
Participants age (y	vears)						
1–15	22 (62.9)	0 (0)	3 (60)	6 (13.3)	> 0.0001		
16-30	8 (22.9)	13 (86.7)	1 (20)	16 (35.6)			
31–45	5 (14.3)	2 (13.3)	1 (20)	12 (26.7)			
46-60	0 (0)	0 (0)	0 (0)	11 (24.4)			
Sex							
Male	18 (51.4)	8 (53.3)	4 (80)	26 (57.8)	0.667		
Female	17 (46.8)	7 (46.7)	1 (20)	19 (42.2)			
Identified Plasmoo	dium species in thin						
P. falciparum	22 (62.9)	2 (13.3)	1 (20)	30 (66.7)	0.001		
P. vivax	8 (22.9)	12 (80)	2 (40)	12 (26.7)			
Others	5 (14.3)	1 (6.7)	2 (40)	3 (6.7)			
Identified Plasmoo	dium species in thick						
+	18 (51.4)	1 (6.7)	2 (40)	8 (17.8)	> 0.0001		
++	1 (2.9)	13 (86.7)	0 (0)	26 (57.8)			
+++	16 (45.7)	1 (6.7)	3 (60)	8 (17.8)			
++++	0 (0)	0 (0)	0 (0)	3 (6.7)			

significant association between blood group A and *P. falciparum* infection (AOR = 2.75, 95% CI: 1.20–6.31), supporting our observations regarding blood group A susceptibility [21].

Further supporting our conclusions, a Nigerian study reported the highest malaria prevalence in blood group O and a significant association between age and *Plasmodium* species infection [22]. A cross-sectional study in Iran also found a significant association between *P. falciparum* and blood group O [23]. Furthermore, a study in Ghana showed a significant association between blood group A and severe malaria (p = 0.042), partially consistent with our results [18]. Additional studies have reported similar findings [24–26].

In summary, this study and existing literature suggest a complex relationship between ABO blood group, age, sex, and

malaria susceptibility. However, the discrepancies among studies emphasize the need for further research to elucidate the mechanisms underlying these associations.

4.1 | Strength of the Study

Despite its limitations, this study possesses several strengths. First, it provides valuable data on the prevalence of different *Plasmodium* species and their distribution across ABO blood groups and age groups in Bosaso City, Somalia. This information contributes to the understanding of malaria epidemiology in the region. Second, the study employed appropriate statistical methods (chi-square and ANOVA tests) to analyze the associations between variables, enhancing the rigor of the findings. Thirdly, the findings are discussed in the context of existing

TABLE 2 | Prevalence of *Plasmodium* species on ABO blood groups (n = [201].

Laboratory results of Plasmodium species in thick on ABO blood groups						
	P. falciparum n (%)	P. vivax n (%)	Others n (%)	ANOVA F (p value)	Chi-square χ^2 (p value)	
Age (years)						
1–15	19 (34.5)	7 (20.6)	5 (45.5)	4.848 (0.030)	21.7 (0.001)	
16-30	13 (23.6)	22 (64.7)	3 (27.3)			
31-45	12 (21.8)	5 (14.7)	3 (27.3)			
46-60	11 (20)	0(0)	0(0)			
Sex						
Female	36 (65.5)	16 (47.1)	4 (36.4)	3.539 (0.018)	4.82 (0.090)	
Male	19 (34.5)	18 (52.9)	7 (63.6)			
Blood groups						
A	22 (40)	8 (23.5)	5 (45.5)	4.158 (0.008)	23.9 (0.001)	
В	2 (3.6)	12 (35.3)	1 (9.1)			
AB	1 (2.8)	2 (5.9)	2 (18.2)			
O	30 (54.5)	12 (35.3)	3 (27.3)			

Laboratory results of <i>Plasmodium</i> species in thick on ABO blood groups from shafici clinic and kaafi hospital						
	+ n (%)	++ n (%)	+++ n (%)	++++ n (%)	ANOVA F (p value)	Chi-square χ^2 (p value)
Age (years)						
1–15	17 (58.6)	1 (2.5)	13 (46.4)	0 (0)	2.146 (0.071)	55.6 (0.00)
16-30	7 (11)	22 (55)	9 (32.1)	0 (0)		
31-45	5 (17.2)	10 (25)	5 (17.9)	0 (0)		
46-60	0 (0)	7 (17.5)	1 (3.6)	3 (100)		
Sex						
Female	16 (55.2)	21 (52.5)	17 (60.7)	2 (66.7)	0.281 (0.597)	0.598 (0.897)
Male	13 (44.8)	19 (47.5)	11 (39.3)	1 (33.3)		
Blood groups						
A	18 (62.1)	1 (2.5)	16 (57.1)	0 (0)	0.407 (0.748)	50 (0.00)
В	1 (3.4)	13 (32.5)	1 (3.6)	0 (0)		
AB	2 (6.9)	0 (0)	3 (10.7)	0 (0)		
O	8 (28.6)	26 (65)	8 (28.6)	3 (100)		

Abbreviation: ANOVA, analysis of variance.

literature on the relationship between ABO blood groups and malaria, allowing for a meaningful comparison and interpretation of the results. Finally, the study highlights the significant associations between ABO blood groups and malaria infection, particularly the higher prevalence of *P. falciparum* in blood groups A and O and the association between age and malaria prevalence, providing essential insights into risk factors for malaria in the study population. This information can potentially contribute to targeted interventions and improved regional malaria control strategies.

4.2 | Limitations of the Study

This study has several limitations that should be considered when interpreting the results. First, the cross-sectional design limits the ability to establish causality; it only demonstrates associations, not cause-and-effect relationships, between the ABO blood group, age, sex, and malaria infection. Second, the study was conducted at a single hospital in Bosaso City, limiting the generalizability of the findings to other regions or populations in Somalia. The reliance on a convenience sample from a single hospital might introduce selection bias, potentially affecting the representativeness of the study population. Third, the specific methodology used for identifying and quantifying parasitemia (parasite density) is not explicitly described, making it difficult to assess the reliability and comparability of these data. The lack of detailed information on the diagnostic methods used and the criteria for determining the species of Plasmodium and parasite density may impact the accuracy and reproducibility of the results. Furthermore, the absence of information regarding potential confounding variables, such as socioeconomic status, access to healthcare, and exposure to malaria

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vectors, limits the ability to fully control factors influencing malaria risk. Finally, the relatively small sample size (201 cases) may limit the statistical power to detect more minor but potentially meaningful associations.

5 | Conclusion

According to the study, the prevalence of *Plasmodium* species, including *Falciparum*, *P. vivax*, and others, was 48%, 41%, and 10%, respectively. The study identified that age and ABO blood groups had a strong association with *Plasmodium* species. The study discovered that females had higher malaria prevalence in each *Plasmodium* species than males, while Blood Group A and O had higher rates than the other two groups. According to the study, children under 15 are more likely to have *Falciparum*. As a result, particular consideration should be paid to prevention and control measures to raise local community awareness of malaria.

Author Contributions

Yahye Isse Hassan: conceptualization, methodology, investigation, data curation, writing – original draft, resources. **Mohamed Said Hassan:** writing – review and editing, visualization, validation, methodology, data curation, supervision.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The authors confirm the data used in the article to support the study's conclusions, and raw data can be obtained from the first author upon justifiable request.

Transparency Statement

The lead author Mohamed Said Hassan affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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