Imported malaria in Qazvin Province, Iran: A retrospective study from 2008 to 2023

***Running Title*:** Imported malaria in Qazvin Province, Iran

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Abstract:

Malaria remains a significant public health challenge worldwide, particularly in tropical and subtropical regions, where it is associated with high morbidity and mortality rates. Although Iran has made substantial progress in malaria elimination, imported cases continue to pose a threat to these achievements. This retrospective study investigates the epidemiology of imported malaria cases in Qazvin Province, Iran, between 2008 and 2023. Blood smears were collected from patients referred to Health Centers and the Vice-Chancellor of Health in Qazvin Province. The samples were stained using Giemsa and examined microscopically for the presence of Plasmodium parasites. Statistical analysis was performed using the Chi-Square ($\chi 2$) test to evaluate data distribution.

A total of 41 malaria cases were recorded during the study period, with a significant predominance of males (97.56%). The highest prevalence of malaria was observed in the 21–30 age group, accounting for 39.02%

of the cases. Among the identified species, *Plasmodium vivax* was the most prevalent, detected in 87.80% of the cases. Passive malaria cases constituted the majority (95.12%), with trophozoites being the most frequently observed stage of the parasite (43.90%). Afghan nationals represented the largest proportion of cases (73.17%), with a significant number of laborers among the affected population (65.85%).

The findings highlight the critical role of migration, particularly among laborers from endemic regions, in sustaining imported malaria cases. These results underscore the need for targeted public health interventions focusing on migrant populations to prevent the re-establishment of malaria in areas where it has been eliminated. Enhancing surveillance systems, strengthening border health measures, and raising awareness among at-risk groups are essential steps to address the challenges posed by imported malaria. Maintaining these efforts is vital to sustaining progress toward malaria elimination in Iran and preventing its re-emergence due to imported cases. This study provides valuable insights into the epidemiological patterns of imported malaria, emphasizing the importance of proactive measures in regions transitioning towards malaria-free status.

Keywords: Imported malaria, *Plasmodium* parasites, Epidemiology, Qazvin Province, Iran

1. Introduction

Malaria, caused by *Plasmodium* parasites, remains a significant issue for public health globally, significantly impacting children and pregnant women residing in tropical and subtropical areas of the world (1). The transmission occurs via the bite of infected female *Anopheles* mosquitoes during their blood-feeding process (2). Further modes of transmission include blood transfusions, congenital transmission (from an infected mother to a fetus during the acute stage of infection), and organ transplantation (3). Five primary *Plasmodium* species, including *P. vivax*, *P. falciparum*, *P. malariae*, *P. ovale*, and *P. knowlesi*, are responsible for human malaria. Zoonotic *Plasmodium* parasites such as *P. inui* and *P. cynomolgi* can also infect humans (4).

Annually, the World Malaria Report provided by the World Health Organization (WHO) offers a detailed and current assessment of malaria control and elimination trends worldwide. According to WHO's 2023 report, malaria constitutes a considerable global health concern, with an estimated 249 million cases and 608,000 deaths reported across 85 endemic countries in 2022 (5).

The clinical presentation of malaria differs between children and adults. Symptoms can vary from mild febrile syndrome in uncomplicated cases to severe, life-threatening complications such as

severe anaemia, acute respiratory distress syndrome, hypoglycaemia, shock, metabolic acidosis, acute kidney injury, or cerebral malaria (6).

Studies conducted in Iran have identified *P. vivax* as the predominant malaria species, while *P. falciparum* is primarily limited to the eastern and southeastern regions of the country (7, 8). Historically, malaria was prevalent in Iran, with an estimated 4 to 5 million out of a population of 13 million approximated to have been affected by the disease by 1924 (9). The anti-malaria campaign launched in 1951 led to the interruption of malaria transmission in many regions. Reports from recent years revealed a decreasing trend in malaria incidence in Iran (10).

In recent years, Iran has experienced a significant decline in malaria incidence rate. The 2009 World Health Organization (WHO) malaria report stated that Iran is now in the pre-elimination phase. The ultimate goal of Iran's malaria elimination program is to terminate local malaria transmission by 2025 (7).

During 2021 and 2022, political unrest in Afghanistan and floods in Pakistan caused a surge in malaria outbreaks and a notable influx of migrants and refugees. This situation heightened the risk of imported malaria cases into Iran (11). Factors such as war, violence, drought, insecurity, and unemployment have led to Afghan migration to Iran (12). Especially on August 15, 2021, the Taliban captured Kabul, resulting in the collapse of the Islamic Republic of Afghanistan and prompting a considerable influx of refugees into Iran (13). Many of them carried a range of infectious diseases, including tuberculosis, cholera, Crimean-Congo hemorrhagic fever (CCHF), leishmaniasis, hepatitis B, and malaria (14). Moreover, recent reports indicate that a majority of Afghan migrants (74%) in Iran are individuals aged 24 and younger, frequently arriving to seek employment opportunities. This demographic trend could potentially contribute to the spread of various infectious diseases (15).

Qazvin Province, situated in northern Iran, is home to a considerable population of Afghan refugees residing on the outskirts of Qazvin city (16). As a result of the importance of this issue, we aimed to study the epidemiological features of imported malaria and investigate the cases reported between 2008 and 2023 in Qazvin Province, northwest Iran.

2. Materials and methods

2.1. Study area

Qazvin Province, with a population of approximately 1,273,761, is an area of 15,821 km2 located in the Northwest of Iran. It is situated between longitudes 48°45′ to 50° 50 E and latitudes 35° 37′ and 36° 45′ N. It is surrounded by the Zagros Mountains to the west and the Alborz Mountains to the northeast and southeast. Moreover, it shares borders with Mazandaran and Guilan provinces to the north, Hamedan and Zanjan Provinces to the west, Markazi Province to the south, and Tehran Province to the east (17).

2.2. Cases and data collection

Patients referred to the Health Centers and the Vice-Chancellor of Health in Qazvin Province underwent blood sample collection using a sterile lancet. Subsequently, a drop of blood was placed on a slide to prepare peripheral blood smears, stained with Giemsa and examined under a light microscope to detect and identify parasites. All cases were diagnosed by the lab-confirmed and there was no case of mortality. A questionnaire capturing various demographic factors has been created for each confirmed case. These are: name, age, reporting year and month, forms of malaria (passive/active), gender, occupation, county, nationality, parasite species, parasitic stage in blood, and other pertinent information was filled out.

2.3. Statistical analysis

Microsoft Excel and Stata software (version 18) were used to perform the descriptive analysis. The Chi-Square ($\chi 2$) test was used to compare the data counts. A p-value < 0.05 is regarded as statistically significant.

3. Results

Between 2008 and 2023, 41 imported malaria cases, including 40 (97.56%) males and one (2.44%) female were reported in Qazvin Province (Table 1).

In terms of the age groups, the highest prevalence rate was observed in the 21-30 age group [16 (39.02%)], followed by the 11-20 age group [12 (29.27%)] (Table 1).

The highest prevalence rate of malaria cases [15 (36.59%)] was documented in 2008, followed by the cases reported in 2023 [7 (17.07%)]. Additionally, regarding the seasonal distribution, the highest number of cases was reported in May 2008-2023, when malaria accounted for 9 (21.95%)

cases. The lowest monthly incidence of malaria, 1 (2.44%), occurred in January (Table 1; Figure 1).

Table 1: Demographic and clinical characteristics of imported malaria cases in Qazvin Province reported between 2008 and 2023.

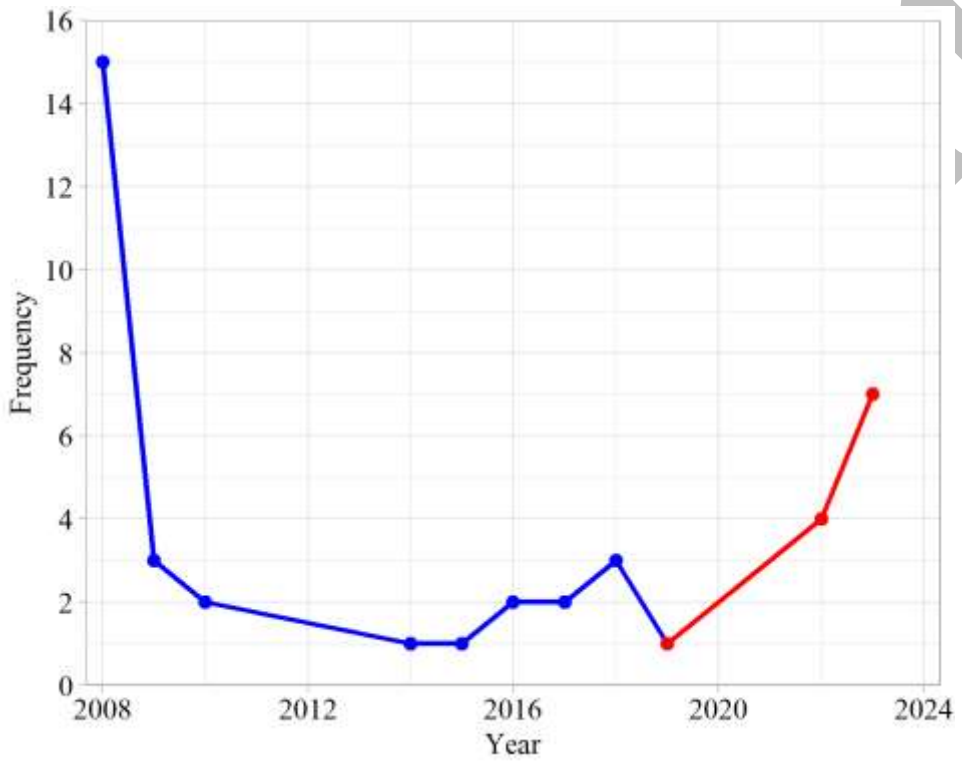

Figure 1: Annual distribution of imported malaria cases in Qazvin province**.**

Plasmodium vivax was the most prevalent species, accounting for 36 cases (87.80%), while *P. falciparum* was the etiological agent diagnosed in 5 cases (12.20%) (Table 1).

Regarding two forms of the disease, passive malaria exhibited the highest prevalence rate, with 39 cases (95.12%). Additionally, among the different stages of the parasite in the blood, trophozoites were the most observed stage, accounting for 18 cases (43.90%) (Table 1).

١٤٤ The nationality-based analysis showed that the sources of the 32 imported malaria cases were
١٤٥ countries outside Iran (Afghanistan and Pakistan), with Afghans constituting the most significant
١٤٦ proportion of reports, accounting for 30 cases (73.17%) (Table 1).

Variables		Number (%)
Age	0-10	3 (7.32)
	11-20	12 (29.27)
	21-30	16 (39.02)
	31-40	7 (17.07)
	>40	3 (7.32)
County	Abyek	1 (2.44)
	Alborz	4 (9.76)
	Buin-Zahra	6 (14.63)
	Qazvin	22 (53.66)
	Takestan	8 (19.51)
Gender	Female	1 (2.44)
	Male	40 (97.56)
Nationality	Iranian	8 (19.51)
	Afghan	30 (73.17)
	Pakistani	2 (4.88)
	N/A	1 (2.44)
Occupation	Laborer	27 (65.85)
	Military/Soldier	3 (7.32)
	Driver	1 (2.44)
	Farmer	1 (2.44)
	Housewife	1 (2.44)
	University student	1 (2.44)
	N/A	7 (17.07)
Year	2008	15 (36.59)
	2009	3 (7.32)
	2010	2 (4.88)
	2014	1 (2.44)
	2015	1 (2.44)
	2016	2 (4.88)
	2017	2 (4.88)
	2018	3 (7.32)
	2019	1 (2.44)
	2022	4 (9.76)
	2023	7 (17.07)
Month	January	1 (2.44)
	March	2 (4.88)
	April	4 (9.76)
	May	9 (21.95)
	June	3 (7.32)
	July	5 (12.20)
	August	4 (9.76)
	September	3 (7.32)
	November	2 (4.88)
	N/A	8 (19.51)
Form of malaria	Passive	39 (95.12)
	Active	1 (2.44)

	N/A	1 (2.44)
Parasite species	*Plasmodium vivax*	36 (87.80)
	Plasmodium falciparum	5 (12.20)
Parasite stages in blood	Trophozoites	18 (43.90)
	Schizonts	7 (17.07)
	Gametocytes	3 (7.32)
	Trophozoites and Schizonts	3 (7.32)
	Trophozoites and Gametocytes	1 (2.44)
	N/A	9 (21.95)
Severity of disease and type of the medical services	Outpatients	5 (12.20)
	No complications	2 (4.88)
	No complications/inpatients	3 (7.32)
	No complications/outpatients	14 (34.15)
	Severe disease / inpatients	7 (17.07)
	Severe disease / outpatients	6 (14.63)
	N/A	4 (9.76)

According to the occupation, labourers had the highest prevalence, accounting for 27 cases (65.85%) (Table 1).

The county-based analysis revealed that the highest and lowest prevalences were documented in Qazvin [22 (53.66%)] and Abyek [1 (2.44%)], respectively (Table 1; Figure 2).

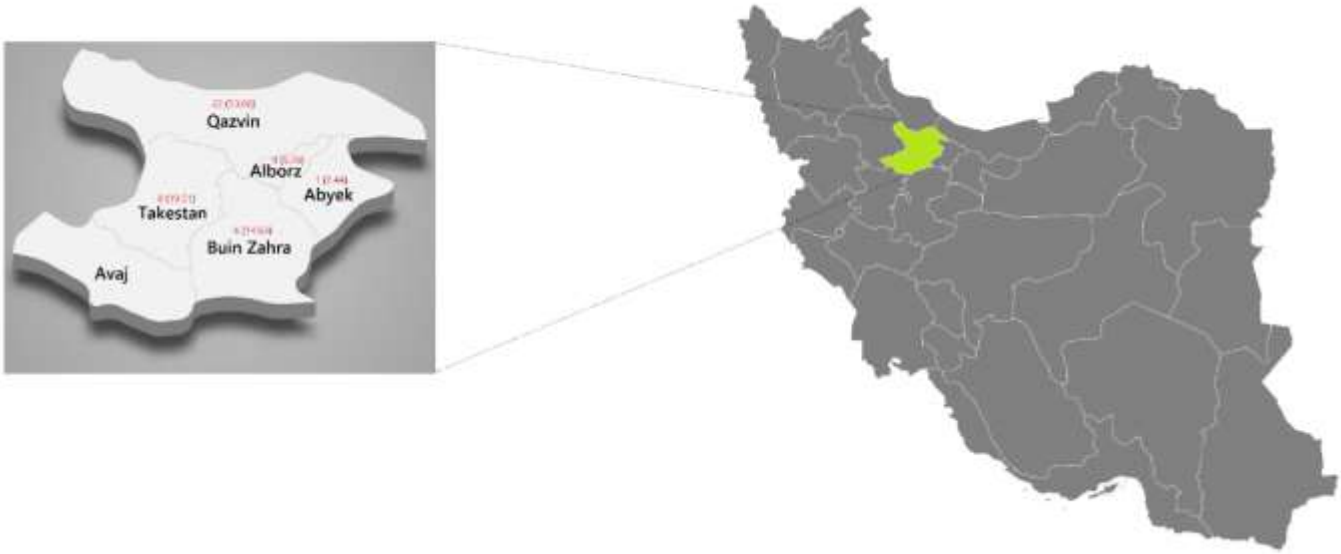

Figure 2: Location of Qazvin province (Northwest of Iran).

The analysis of disease severity and type of medical services indicated that the highest prevalence was observed in the group recorded as no complications/outpatients, comprising 14 cases (34.15%) (Table 1).

According to the Chi-Square ($\chi 2$) test, a statistically significant association was observed for nationality and occupation (P= 0.0001). The highest prevalence was related to Afghan labourers [26 (63.41%)] (Table 2). Additionally, significant differences were not observed in other malaria-related risk factors (Supplementary Table 1-3).

Table 2: The association of occupation and nationality among imported malaria patients in Qazvin province.

		Occupation							
		Driver (%)	**Farmer (%)**	**Housewife (%)**	**Military/Soldier (%)**	**N/A (%)**	**University student (%)**	**Labourer (%)**	**Total (%)**
Nationality	**Afghan (%)**	-	1 (2.44)	1 (2.44)	-	2 (4.88)	-	26 (63.41)	30 (73.17)
	Iranian (%)	1 (2.44)	-	-	3 (7.32)	3 (7.32)	-	1 (2.44)	8 (19.51)
	Pakistani (%)	-	-	-	-	2 (4.88)	-	-	2 (4.88)
	N/A (%)	-	-	-	-	-	1 (2.44)	-	1 (2.44)
	Total (%)	1 (2.44)	1 (2.44)	1 (2.44)	3 (7.32)	7 (17.07)	1 (2.44)	27 (65.85)	41 (100.00)

Supplementary Table 1: The association of county and parasite species among imported malaria patients in Qazvin province.

County	***Plasmodium falciparum***	***Plasmodium vivax***	**Total**
Abyek	-	1 (2.44)	1 (2.44)
Alborz	1 (2.44)	3 (7.32)	4 (9.76)
Buin-Zahra	-	6 (14.63)	6 (14.63)
Qazvin	4 (9.76)	18 (43.90)	22 (53.66)

Takestan	-	8 (19.51)	8 (19.51)
Total	5 (12.20)	36 (87.80)	41 (100.00)

Supplementary Table 2: The association of detection of parasite stages in blood and parasite species among imported malaria patients in Qazvin province.

Detection of parasite stages in blood	*Plasmodium falciparum*	*Plasmodium vivax*	Total
Trophozoites	1 (2.44)	17 (41.46)	18 (43.90)
Schizonts	1 (2.44)	6 (14.63)	7 (17.07)
Gametocytes	2 (4.88)	1 (2.44)	3 (7.32)
Trophozoites and Schizonts	-	3 (7.32)	3 (7.32)
Trophozoites and Gametocytes	-	1 (2.44)	1 (2.44)
N/A	1 (2.44)	8 (19.51)	9 (21.95)
Total	5 (12.20)	36 (87.80)	41 (100.00)

Supplementary Table 3: The association of severity of disease and medical and age among imported malaria patients in Qazvin province.

Severity of disease and medical services	0-10	11-20	21-30	31-40	> 40	Total
No complications	-	-	2 (4.88)	-	-	2 (4.88)
No complications/inpatients	-	-	3 (7.32)	-	-	3 (7.32)
No complications/outpatients	-	4 (9.76)	5 (12.20)	3 (7.32)	2 (4.88)	14 (34.15)
Outpatients	-	3 (7.32)	2 (4.88)	-	-	5 (12.20)
Severe disease / inpatients	2 (4.88)	1 (2.44)	-	3 (7.32)	1 (2.44)	7 (17.07)

Severe disease / outpatients	1 (2.44)	3 (7.32)	1 (2.44)	1 (2.44)	-	6 (14.63)
N/A	-	1 (2.44)	3 (7.32)	-	-	4 (9.76)
Total	3 (7.32)	12 (29.27)	16 (39.02)	7 (17.07)	3 (7.32)	41 (100.00)

4. Discussion

To our knowledge, this is the first report on imported malaria cases in Qazvin, Iran. In the current study, we analyzed the longitudinal surveillance data of imported malaria between 2008 and 2023 to explore the epidemiological characteristics of the disease. Four principal epidemiological features were identified through our analyses. First, *P. vivax* was the predominant species among imported malaria cases, with the majority originating from Afghanistan, which accounted for the highest number of cases. Second, most imported malaria cases were concentrated in Qazvin, the province's capital. Third, most cases involved labourers, mainly adult men aged 21-30. Fourth, seasonal variations were observed in imported malaria cases; however, it is noteworthy that in 19.51% of cases, the month variable was not recorded.

In Asia, particularly in Pakistan and Afghanistan, malaria remains a significant public health concern, with ongoing transmission of both *P. falciparum* and *P. vivax* (18, 19). Afghanistan and Pakistan that share borders with Iran are malaria-endemic countries. The significant human migration from these countries to Iran facilitates malaria transmission and presents substantial political, social, operational, and technical challenges to the elimination of malaria in Iran (20, 21). Despite efforts to control malaria, it remains a significant health issue in the southern regions of Iran. Most local malaria transmission occurs in Sistan va Baluchestan, Hormozgan, and Kerman provinces (22, 23).

A comparison of our findings with data from other Iranian provinces reveals distinct epidemiological patterns shaped by regional factors. In southern provinces such as Sistan va Baluchestan, Hormozgan, and Kerman, malaria transmission remains a mix of indigenous and imported cases, driven by proximity to endemic countries like Pakistan and Afghanistan, as well as favorable ecological conditions for *Anopheles* vectors (22, 23). For instance, Sistan va Baluchestan reports the highest burden of *P. vivax* and *P. falciparum* due to cross-border movement and local transmission. The province accounted for 53% of all malaria cases in Iran in

2007. Its borders with Pakistan and Afghanistan facilitate considerable cross-border population movement, contributing to the malaria burden (24). In contrast, Qazvin Province, located in the northwest, has no indigenous transmission and relies entirely on imported cases, primarily from Afghan migrants.

Notably, provinces bordering Afghanistan, such as Khorasan Razavi, report higher imported cases but also face sporadic local outbreaks due to vector presence. Accordingly, 61% of the cases were transmitted from outside the province, including immigrants from Afghanistan and Pakistan, as well as 34.6% of the cases were due to local transmission, particularly in Sarakhs city, where conditions support sporadic local outbreaks (25). Unlike these regions, Qazvin's colder climate and mountainous geography limit vector activity, reducing the risk of re-establishment despite imported cases.

Seven species of *Anopheles* mosquitoes, including *Anopheles stephensi*, *An. culicifacies s.l.*, *An. superpictus s.l.*, *An. fluviatilis s.l.*, and *An. maculipennis* complex (*An. sacharovi* and *An. dthali*), are malaria vectors in Iran (26). Imported malaria constitutes most of the disease cases in Iran (references to all imported reports in Iran). Our study showed that imported malaria was the reason for all cases in Qazvin Province during the study period. Imported malaria disseminates malaria parasites to a region and contributes to local transmission. This occurs when local *Anopheles* mosquitoes feed on individuals who have travelled to the area and are infected with malaria (27).

Malaria is endemic in Pakistan and is still one of the leading causes of illness and death in the region. The most significant increase in malaria cases from 2021 to 2022 was observed in Pakistan, with approximately 2.6 million cases reported in 2022 compared to 500,000 cases in 2021 (5). More than 170,000 cases were laboratory confirmed, with 77% attributed to *P. vivax* and 23% to *P. falciparum* (28). The surge in cases in Pakistan in 2022 followed devastating floods in the country between June and October, resulting in a five-fold increase in the caseload. Additionally, countries such as Iran, which had not reported indigenous cases for several years, recorded more than 1000 cases in 2022 (5).

Extreme malaria transmission primarily occurs in districts situated along the borders with Iran and Afghanistan and along the coastal belt in the Sindh and Balochistan provinces. Moreover, there is a significant population movement between Pakistan and its neighbouring countries, particularly

Afghanistan and Iran. This movement is exceptionally high in Khyber Pakhtunkhwa Province, which hosts over 1 million Afghan refugees (28).

Since 2002, Iran has launched strict measures limiting the movement of refugees and has stated "no-go areas" for refugees. Initially limited to border provinces, these restrictions now extend to most of Iran's 31 provinces, with Mazandaran province being the most recent addition. Sixteen provinces have completely banned Afghans residing within their boundaries, while twelve provinces have restrictions on specific areas and cities. Tehran, Alborz, and Qom are the only provinces where Afghan refugees are not subject to residential restrictions (29). It is noteworthy that all eight individuals of Iranian nationality included in our study had a history of traveling to the southern regions of Iran.

Our research has certain limitations. First, the study's reliance solely on microscopic diagnostic techniques could constrain the precision of the findings. Further diagnostic methods, such as molecular or serological testing, may offer a deeper comprehension of the disease. Second, in some cases, patient's history, such as occupation, month, severity of disease and medical services, and forms of malaria diseases (passive/active), was not applicable or was not documented in detail. However, the present paper provides the most valuable information on imported malaria in Qazvin province, which can offer new and significant insights for researchers in infectious and tropical diseases. This can help in further planning comprehensive and precise health programs to decrease the incidence of this infection in Qazvin Province.

This study provides valuable insights into the epidemiological characteristics of imported malaria cases in Qazvin Province between 2008 and 2023. The challenge of imported malaria, particularly *P. vivax*, poses a significant obstacle to malaria elimination efforts in Iran. Although no case of mortality was reported in our study and *P. vivax* represented a higher prevalence than *P. falciparum*, it is still recommended to diagnose and treat imported *P. falciparum* infections promptly to prevent fatal cases. We observed that most cases were Afghans, with labourers as the most affected group. Thus, to address this challenge, malaria prevention activities should mostly focus on imported Afghan labourers. Furthermore, to sustain progress in the elimination of malaria in Iran, it is necessary to prevent the re-establishment of the disease caused by imported cases. Enhanced surveillance and preventive measures are imperative to mitigate the burden of imported malaria and prevent its spread in Qazvin Province. Finally, our findings emphasize the importance

of targeted interventions and effective malaria prevention strategies, especially, it must focus on populations involved in cross-border movements.

Authors contributions:

AVE, LM, FN, MB and GS contributed to the study design. BN, HB, and AK contributed to the sample collection. AVE, MO, and MB analyzed the data and designed the figures and tables. AVE and MB drafted the manuscript. AVE and MB supervised the writing and editing of the manuscript. All authors read and approved the final manuscript.

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Ethical Approval: The experimental research reported in this study was approved by the ethics committee of Qazvin University of Medical Sciences, Iran (code: IR.QUMS.REC.1402.429). The requirement for consent was waived by the mentioned ethics committee.

Data availability statement: Not applicable.

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