IJID Regions 12 (2024) 100418

Contents lists available at ScienceDirect

IJID Regions

journal homepage: www.elsevier.com/locate/ijregi

Moving population is a challenge for malaria elimination in India: A cross-sectional study to assess malaria parasite infections in walking pilgrims in western Rajasthan, India

Suman Lata [1,2], Sanjeev Kumar Gupta [1], Gaurav Kumar [1], Suresh Yadav [3], Suman Sundar Mohanty [3], Pooja Prasad [1], Bharat Singh [1,2], Sangeeta Singh [1,2], Poonam Saroha [1,2], Dinesh Kumar [4], Prem Singh [1,2], Kumar Vikram [5], Deepali Savargaonkar [1], Himmat Singh [1,2,*]

[1] *ICMR-National Institute of Malaria Research, New Delhi, India*
[2] *Academy of Scientific and Innovative Research, Ghaziabad, India*
[3] *National Institute for Implementation Research on Non-Communicable Diseases, Jodhpur, India*
[4] *Department of Botany, Dyal Singh College, University of Delhi, New Delhi, India*
[5] *Indian Council of Medical Research, New Delhi, India*

ARTICLE INFO

Keywords:
Migration
Malaria
Pilgrimage
Transmission
Surveillance

ABSTRACT

Objectives: India is vigorously pursuing malaria elimination by 2030 and one of the key challenges is how to prevent the malaria risk associated with long-distance migrations of populations from endemic to non-endemic areas. Millions of pilgrims walking to the holy Ramdevra temple stay in temporary shelters along the route in district of Jaisalmer, Rajasthan state in western India. The main pilgrimage period coincides with the post-monsoon period with elevated vector densities. We investigated this situation to assess the potential risk posed by migrant pilgrims along the four highways in the temple district in disseminating malaria infections associated with their annual movements.

Methods: A cross-sectional study was conducted for screening malaria in walking pilgrims from neighboring states at selected sites and a follow-up study for screening residents in Rajasthan from 2021 to 2023. The study comprises state entry routes, Ramdevra shrine, and pilgrimage route villages situated in western Rajasthan. Epidemiologic and entomologic surveillance was conducted during the "fair" period (August-September) and in pilgrimage route villages in three different seasons of the year to assess malaria in humans and the vector's infectivity.

Results: Of the 5251 individuals tested for malaria, a total of 76 (1.4%) tested positive for malaria, of whom 40.7% were infected with *Plasmodium vivax* (n = 31), 36.8% with *P. falciparum* (n = 28), and 22.3% with *P. vivax* and *P. falciparum* (n = 17). Anopheles density was highest during the fair season, peaking in August. *An. stephensi* exhibited a higher human blood index (0.65) than *An. culicifacies* (0.50). No mosquitoes were found positive for parasites.

Conclusions: Up to 1.5% of pilgrims carried malaria parasites, posing a risk of spreading malaria to surrounding communities that otherwise would have low or no malaria burden. Moving populations pose the risk of local malaria transmission and reintroduction to the areas undergoing elimination. Sentinel point surveillance at the border of states will be helpful for states to share information on malaria and subsequently for the prevention of malaria transmission.

Introduction

India is committed to eliminating malaria to reach a target of zero incidence by 2027 and elimination by 2030. Presently, 21 states and eight union territories have less than one annual parasite incidence (API) and seven states have more than one API [1]. Among the identified threats of malaria elimination, marginalized populations, for example, populations of remote or border areas, migrant workers, and tribal populations with limited access to health care services, need to be screened at the junction of two states to prevent the introduction of malaria through population movement [2]. Migration is one of the major issues that need to be addressed properly by taking proactive approaches to prevent the reintroduction of malaria at inter-country and inter-state levels. The National Strategic Plan for Malaria Control

* Corresponding author: Tel.: 011-25307342, 8860349351.
E-mail address: hspawar@rediffmail.com (H. Singh).

https://doi.org/10.1016/j.ijregi.2024.100418
Received 29 April 2024; Received in revised form 29 July 2024; Accepted 30 July 2024

in India (2012-2017) placed special emphasis on screening the migrant population in category I (districts having API <1) and II (districts having API >1 but less than 2) areas of the malaria elimination [3].

Malaria has been associated with population migration during the early developmental era across Oriental, Mediterranean, and African regions [4]. In India, malaria is also associated with the mass movement of laborers from endemic areas to non-endemic areas and *vice versa* for mega projects [5–7]. In addition to these conventional migrations, there exists a less-known form of population movement driven by religious motivations.

Rajasthan, along with its western districts, comes under category I of malaria elimination as per the National Framework for Malaria Elimination 2016-2030 [1]. To move from category I to 0, effective measures are required for the prevention of the reintroduction of malaria in the state. Malaria in western Rajasthan is unstable and reported to be reintroduced through migrating populations [8]. However, poor drug adherence and health-seeking behavior are also major confounders [9,10]. Studies have shown that there is a strong correlation between malaria spread and migration in western Rajasthan [11]. Various forms of population movements take place in western Rajasthan, including labor migration, migration to escape unfavorable circumstances, migration for agricultural opportunities, and employment purposes [12]. Furthermore, there are short-duration population movements for devotional purposes locally known as *Yatras*, wherein pilgrims reach the shrine by walking from far-flung areas from different states during specific months August and September. During these *Yatras*, walking pilgrims halt overnight at temporary tent shelters (camps) set up along the routes to accommodate walkers. Each year, a large number of pilgrims pay their homage at the Ramdevra temple since its inception in 1931. Pilgrims visit the temple round the year, and this number swells to over 1-2 million during the post-monsoon period, August-October, when vector densities also increase. This period coincides with the malaria transmission post-monsoon season conducive for mosquito propagation due to favorable temperature and humidity [13]. The pilgrims cover 300-500 km by walking and there is a possibility of malaria transmission if pilgrims are infected and vectors are present or *vice versa*.

In this study, we propose the hypothesis that the overnight outdoor resting of walking pilgrims in temporary shelters may increase the risk of mosquito bites, particularly, from *Anopheles culicifacies* and *Anopheles stephensi* species. This scenario could potentially lead to the acquisition of parasites to/from an infected individual among the walkers, thus facilitating malaria transmission. We aimed to understand the role of these *Yatras* in the malaria transmission dynamics of Rajasthan and assess the extent to which migrating walking pilgrims contribute to malaria transmission by screening them at the point of entry in the state for the presence of malaria parasite.

Materials and methods

Study design

The present study is a cross-sectional study for screening walking pilgrims and a follow-up study for residents of selected pilgrimage route villages. The study was conducted from September 2021 to May 2023 in Rajasthan, India, focusing on assessing malaria transmission along pilgrimage routes, particularly, around the Ramdevra temple and its surrounding areas.

Study sites

There are two major pilgrims in western Rajasthan: one is Ramdevra temple and the other is Parshuram Mahadev located in Aravalli hills. Because pilgrims coming to Parshuram Mahadev Shrine do not travel by foot and do not halt for night, they were excluded from the present study. Ramdevra temple is located in the Jaisalmer district of Rajasthan, India and a fair is organized every year from August to October at this place. This attracts around 2-2.5 million devotees every year and, among these, a huge number of devotees visit only during the post-monsoon season at an average rate of 5000-10,000 pilgrims per day [13]. Devotees (pilgrims) travel mostly by foot from nearby states, including Gujarat, Madhya Pradesh, Maharashtra, Chhattisgarh, Uttar Pradesh, Haryana, and Rajasthan, and they assemble in Jodhpur for a day to proceed for an onward journey to the temple destination at the Ramdevra shrine [13].

The study focused on tracking walking pilgrims and their night-halting camps for malaria, along with monitoring the surrounding villages from where people are involved in camp management and stay at camp sites. The Ramdevra shrine area and its surrounding villages were also included in the study. Consequently, the parasite infection in the human population and malaria vectors are being assessed throughout the year in selected villages.

The following four sites comprised entry points, pilgrimage route villages, and the shrine:

1. Entry Point NH-68, Sanchore (site 1): This site connects Gujarat and Maharashtra. One entry point camp and two nearby villages were monitored for pilgrim activities and mosquito collections.
2. Entry Point NH-11, Bikaner (site 2): This road connects Punjab, Haryana, and Delhi. Surveillance was conducted at one night-halting camp and two villages along the route.
3. Entry Point NH-52, Jhalawar (site 3): Near the Madhya Pradesh border, this site included one entry point and two villages for epidemiologic and entomologic studies.
4. Ramdevra Shrine, Jaisalmer (site 4): The primary focus of the study, including the shrine and nearby four villages, namely, Viramdevra, Mawa, and Gomat, where pilgrims assemble.

The entry point roads connecting Rajasthan with neighboring states serve as vital thorough fares for normal passengers and pilgrims on foot. Pilgrims were screened for fever at designated night-halting sites, and mosquitoes were collected simultaneously. The State Health Department aided this operation. The two pilgrimage route villages near each entry points were selected by considering the flight range of mosquitoes and the level of interaction between pilgrims and the villagers residing in tents (camps) who played a crucial role in coordinating these temporary camps. A total of two fair seasons and five follow-up studies were done in pilgrimage route villages.

Human participants

The individuals who were staying at night shelter camps at each entry point were included for screening for fever and those with fever were tested for malaria presence with consent.

Environment

The entomologic data were collected from night camps (entry points) and pilgrimage route villages.

Inclusion criteria

For the current study, three entry points and the Ramdevra shrine were included only for screening the population at night-halting camps. The two nearest pilgrimage route villages near each entry point were included for follow-up studies.

Exclusion criteria

Pilgrims traveling by vehicle were omitted from the study because they did not halt at entry points and reached the shrine within 1 or 2 days.

Parasitological survey

Active fever detection was conducted at all sites using a thermal scanner (FLUKE TiS20+ MAX Thermal imager). Our team (consisting of a scientist and two trained technicians for phlebotomy and two assistants for thermal scanning and documentation and other work) maintained safety protocols in line with those implemented during the COVID-19 pandemic while conducting thermal screening and sample collection. At each entry point and their night-halting camps, pilgrims were screened in the early morning hours (6:00-8:00 a.m.), and thereafter, screening was carried out from 7:00 to 10:00 a.m. each day for 3 days during the fair period (August-September) only, whereas the pilgrimage route villages and site 4 were regularly surveyed in all seasons.

The study was conducted for 2 years to understand the case appearance of malaria in the community before and after the fair. All the fever (febrile) cases detected through thermal scanner were tested using the bivalent rapid diagnostic test (RDT) (Falcivax: [*Plasmodium vivax: Pv*] & [*Plasmodium falciparum: Pf*]) for the detection of malaria. In addition, blood smears were prepared for microscopy to confirm RDT positive cases. Blood spots were also collected from every fever case on Whatman filter paper (grade III) strips (size 2×5 cm); each strip was covered with butter paper from both to ensure isolation for polymerase chain reaction (PCR) confirmation. The positive cases detected were referred to the nearest primary health center for treatment with the RDT results.

Molecular detection of parasite was conducted in both samples from patients to confirm and to detect any missed out symptomatic cases and on collected vector mosquitoes from each study site. All the filter paper blood spots were subjected to DNA extraction using the QIAwave DNA Blood & Tissue Kit (Qiagen). Quantitative real-time PCR was performed using HELINI Plasmodium Genotyping, Real-time PCR Kit (HELINI Biomolecules, Chennai, India) using Bio-Rad CXF 96 thermo-cycler for sub-microscopic parasite detection and confirmation of the positive cases [14]. Microscopic slides after examination were kept in labeled slide boxes. The filter paper strips with dried blood spots were also stored in labeled plastic boxes and transported to the laboratory of the Indian Council of Medical Research (ICMR) National Institute of Malaria, Delhi at room temperature (25 ± 2°C). Similarly, the collected microscopic slides were also transported to the National Institute of Malaria within 1 day after the completion of the field tour.

Entomological study

The study involved assessing the presence of vectors and their infectivity in villages along the route during three distinct seasons: pre-monsoon (April-June), monsoon (July-August), and post-monsoon (September-December), in conjunction with fever surveillance.

Mosquito collection was carried out at all sites in the early morning hours, i.e. 6:00-8:00 a.m. for 3 days. At the entry point of each site, mosquito collection was conducted in the fair period (August-September) only, whereas at pilgrimage route villages of each site and site 4, mosquito collection was conducted in all three seasons. In addition, the larval survey was also conducted around these sites for potential breeding habitats. Adult mosquito collection was carried out using flashlights, hand aspirators, light trap, and spray sheet collection method in each village, following World Health Organization 1975 guidelines [15]. This included indoor resting collections from night shelters (camps), human dwellings, cattle sheds, and ruined structures in the villages. The collected mosquitoes were identified using standard taxonomic keys [16]. Blood smear on filter paper was prepared of blood-fed mosquitoes for the presence of human blood using PCR protocol with primers Human741F (ggcttacttctcttcattctctcct, 334 bp) and Cow121F (catcggcacaaatttagtcg, 561 bp), and universal reverse primers ggttgtcctccaattcatgtta after [17]. Human blood index (HBI) was calculated as the number of mosquitoes with human blood/total number analyzed and parasite detection in mosquitoes using PCR. The same protocol was used for parasite detection in mosquitoes [14]. The entomologic team consisted of two insect collectors, one field assistant, and one supervisor for documentation at each study site.

Risk assessment

Based on pilgrims entering from each entry site, the potential risk of malaria importation was calculated. The number of pilgrims passing by was recorded three times a day at 9:00 a.m., 1:00 p.m., and 6:00 p.m. At every point of entry, the counting was carried out for 3 days. The average number of pilgrims passing per entry point per hour was calculated for 12 hours of walking because pilgrims normally walk from 8:00 a.m. to 8:00 p.m. per day and cover about 25-30 km per day as a normal walking capacity. Because the fair duration is approximately 1 month (August-September), the number of passing pilgrims per day were calculated for 1 month. The actual observed malaria-positive cases among the screened population of each entry point was calculated separately and risk was estimated by integrating the risk of malaria importation from all entry sites in 1 month.

$$\text{Expected Imported Cases} = \frac{\text{Total Malaria Positive Pilgrims}}{\text{Total Pilgrims Screened for Malaria}} \times \underset{\text{From all routs per day}}{\text{Average Pi lg rims passing}} \times 30\,\text{days}$$

The approximate estimate was done at 95% confidence interval with SD and minimum and maximum values.

For risk assessment, only the walking pilgrims were considered and the people traveling by vehicle were excluded because they directly reached Ramdevra shrine and did not halt.

Ethical consideration

The study was approved by the ethics committee of ICMR-National Institute of Malaria Research, Delhi *vide* number PHB/NIMR/EC/2020/199.

Limitations

The survey was limited to 3 days at each entry point to sample the migrants coming into the Rajasthan state for the Ramdevra temple visit. Only walking pilgrims and those who rested in the camps for the night were tested for fever. Pilgrims who traveled by vehicle were not stopped at the entrance point camps and were excluded from the current study. For risk, we did not consider the positive residents because we intend to calculate the approximate malaria importation from other states.

Results

Using a thermal scanner, 16,819 people—walking pilgrims (6883) originating from nine neighboring states and inhabitants (10,008)—were screened; 5251 of them had a fever and underwent testing for malaria. Of those tested, a total of 76 (1.4%) individuals were found positive for malaria. Specifically, of the 6883 individuals who were walking pilgrims, 2504 individuals staying at night-halting camps were tested for malaria, with 46 (1.8%) individuals testing positive (Table 1).

During the fair season in September 2021, only 213 pilgrims were screened, of which one malaria case (*Pv*) was detected. There was less sampling due to the call-off of the fair by the State Health Department to avoid COVID-19 transmission risk. Thereafter, the December 2021 and April 2022 surveys did not show any positive cases among the selected surrounding villages of the route. In August 2022 (the second fair period of the study), 2291 walking pilgrims were tested and 45 were found to be positive (18 *Pv*, 19 *Pf*, and eight mixed). During this period pilgrimage route villages did not present any case. In the next surveys, during December 2022 and February 2023 in pilgrimage route villages, positivity was observed, which was reduced during the May 2023 survey (Figure 1).

Table 1
Malaria parasite from September 2021 to May 2023.

Population	Screened (thermal scanner)	Febrile cases tested (%)	Positive by rapid diagnostic test and blood slide examination			
			Pv (%)	*Pf (%)*	Mixed[a] *(%)*	Total
Pilgrims	6883	2504 (36.3)	19 (41.3)	19 (41.3)	8 (17.3)	46
Resident (villagers)	10008	2856 (28.5)	12 (40.0)	9(22.5)	9 (22.5)	30
Total Population	**16891**	**5251 (31.0)**	**31 (40.7)**	**28 (38.6)**	**17 (22.3)**	**76**

Pf: Plasmodium falciparum; Pv: Plasmodium vivax.
[a] Mixed =*Pv* & *Pf* both.

Table 2
Malaria case positivity in the pilgrim enroute the Ramdevra temple.

Site	Months	Screened (thermal scanner)	Febrile cases tested	Species			Total positive	% –ve
				Pv (%)	*Pf (%)*	Mixed[a] *(%)*		
Entry point (Site-1)	Sep-21	213	48	1 (100.0)	0 (0.0)	0 (0.0)	1	2.1
	Aug-22	1600	739	11 (61.1)	4 (22.2)	3 (16.6)	18	2.4
Entry point (Site-2)	Sep-21	520	62	0 (0.0)	0 (0.0)	0 (0.0)	0	0
	Aug-22	1500	639	1 (100)	0 (0.0)	0(0.0)	1	0.2
Entry point (Site-3)	Sep-21	350	47	0(0.0)	0(0.0)	0(0.0)	0	0
	Aug-22	500	290	0(0.0)	7 (100.0)	0(0.0)	7	2.4
Site-4	Sep-21	400	56	0(0.0)	0(0.0)	0(0.0)	0	0
	Aug-22	1800	623	6 (31.5)	8 (42.1)	5 (38.4)	19	3
Total Site-1	**Sep21 + Aug 22**	**1813**	**787**	**12 (63.1)**	**4 (21.0)**	**3 (15.7)**	**19**	**2.4**
Total Site-2	**Sep21 + Aug 22**	**2020**	**701**	**1 (100)**	**0(0.0)**	**0(0.0)**	**1**	**0.1**
Total Site-3	**Sep21 + Aug 22**	**850**	**337**	**0 (0.0)**	**7 (0.0)**	**0(0.0)**	**7**	**2.1**
Total Site-4	**Sep21 + Aug 22**	**2200**	**679**	**6 (31.5)**	**8 (42.1)**	**5 (38.4)**	**19**	**2.8**
Grand total		**6883**	**2504**	**19 (41.3)**	**19 (41.3)**	**8 (17.3)**	**46**	**1.8**

Pf: Plasmodium falciparum; Pv: Plasmodium vivax.
[a] Mixed =*Pv* & *Pf* both.

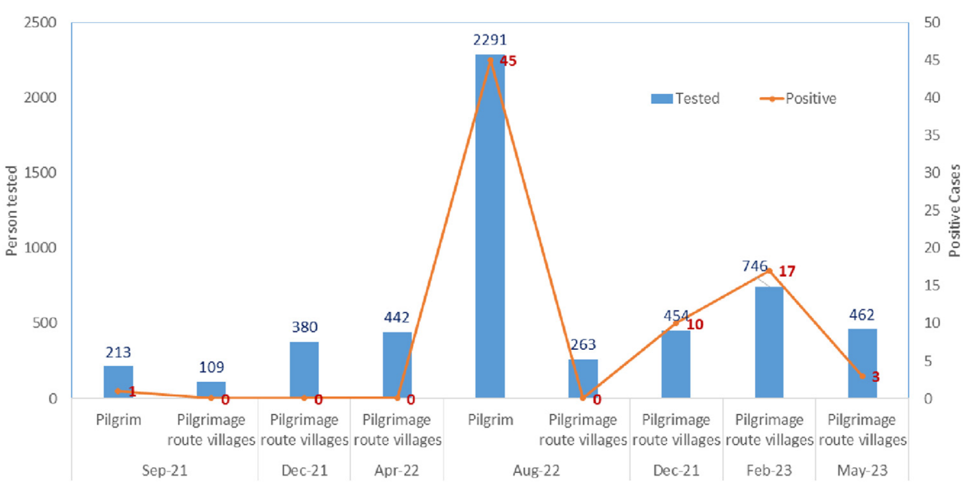

Figure 1. Malaria positivity among the tested pilgrims at different entry points and Ramdevra shrine area.

Positivity at different entry points

At entry point (site 1), the malaria positivity among pilgrims was 2.4% (19 of 787) and *Plasmodium vivax* (79% of the total of 19 cases, including three mixed for *Pv* and *Pf*) was dominant. Although, at the entry point (site 3), positivity was 2.1% (seven of 337) and all the cases were *Pf*. At the entry point (site 2), 701 pilgrims were tested and only one *Pv* was reported. Site 4, the main Ramdevra shrine, reported 19 positive cases (six *Pv*, eight *Pf*, and five mixed) of 679 tested pilgrims (Table 2).

Anopheles density (*An. stephensi* and *An. culicifacies*) was available throughout the year (man-hour density ranged from 0.75 to 42.47), and the density was found to be highest during September and August months, which is also the fair duration. Site 3 (entry point, NH-52 and its pilgrimage routes villages) located at the southeastern entry point in the Jhalawar district of Rajasthan near Madhya Pradesh had the highest density during September and the lowest density during February to May (Figure 2). In addition to anophelines, *Aedes agpypti, Culex quinquefasciatus,* and *Armigeris subalbatus* species were also collected. *C. quinquefasciatus* was found in nearly all villages in varying densities, ranging from 14 to 603 (Supplementary Table 1). Adult mosquitoes were collected using hand catch, light traps, and spray sheet collection methods. Details of the mosquitoes collected by each method are provided in Supplementary Table 1. The average density man-hour density and range of anopheline mosquitoes collected from all four pilgrimage route villages are shown in Supplementary Table 2.

HBI was found higher in *An. stephensi* (HBI = 0.65) as compared to *An. culicifacies* (HBI = 0.5). Both species were also found breeding and demonstrated a mixed feeding behavior by feeding on cows and humans. It is important to note that both species have a notable number of unfed mosquitoes (*An. stephensi* = 22% of 41 tested and *Anopheles culicifacies* = 27% of 44 tested). This suggests that there are opportunities for further feeding, potentially leading to increased disease transmission (Table 3).

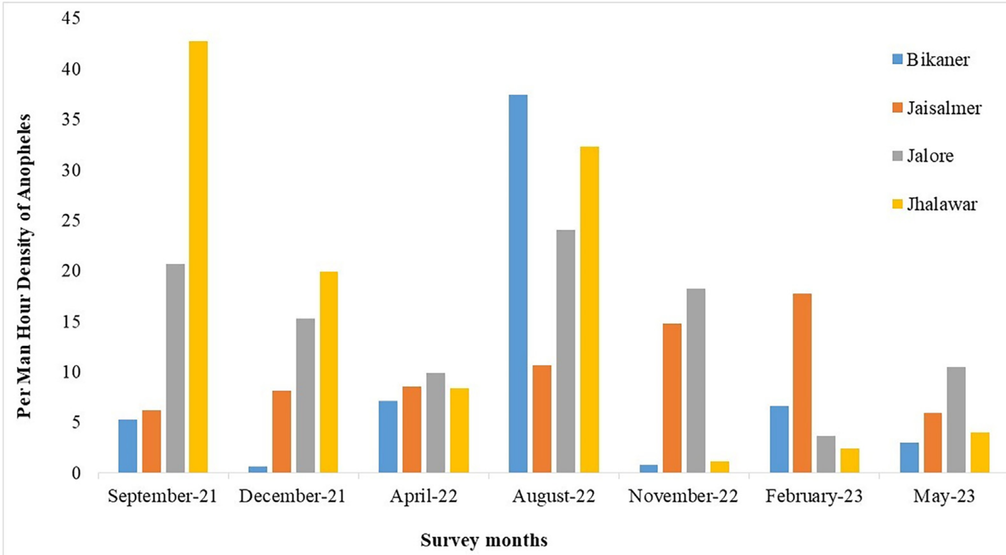

Figure 2. Density of two vector species (*An. stephensi* and *An. Culicifacies*) during the seasonal survey during the study period.

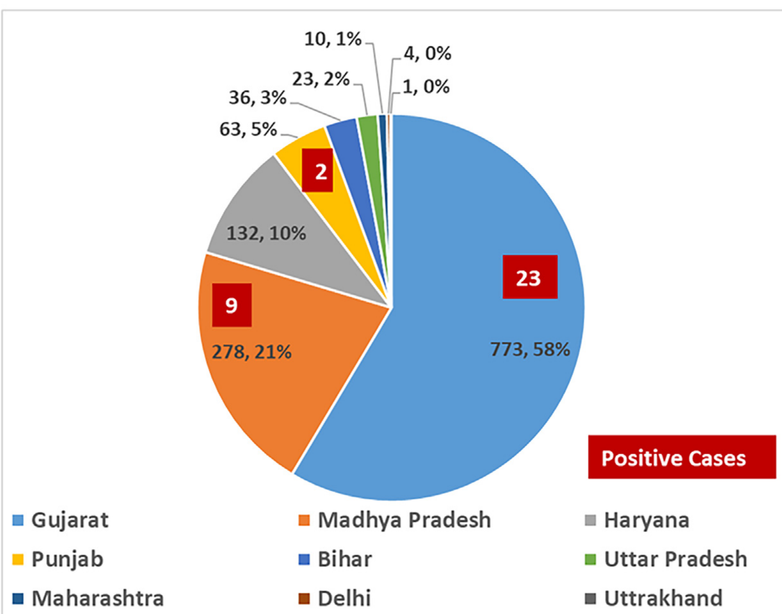

Figure 3. Showing the percentage of pilgrims coming to Rajasthan from different states (September 2021 and August 2022).

Table 3
Human blood index of collected *Anopheles* mosquitoes.

Species	Cow	Human	Both	UI	Human blood index
An. stephensi **(n = 41)**	5	21	6	9	0.65
An. culicifacies **(n = 44)**	12	13	9	12	0.50

It is evident from Figure 3 that most of the pilgrims came from Gujarat (58.5%), followed by Madhya Pradesh, whereas the least reached from Delhi and Uttarakhand. Cases were also found more from Gujarat and Madhya Pradesh.

Of 2504 pilgrims tested for malaria, a total of 46 malaria cases among pilgrims and 30 malaria cases were found in the follow-up studies in pilgrims route villages during post-monsoon season. Figure 4 shows that most pilgrims were from the surrounding states of Rajasthan except Uttarakhand, Maharashtra, and Bihar. Pilgrim walkers coming from far states usually use rail routes and reach directly to Ramdevra (Figure 4).

The risk of malaria case importation was calculated based on the proportion of the population found positive and the expected population crossing the entry points and from the Ramdevra shrine area among migrant pilgrims. The overall risk including all routes and possibility of malaria importation in a month was 3390, which is about 8.3 malaria case per thousand populations (Table 4). The Ramdevra shrine showed 1.06% positivity among daily reaching pilgrims and was at risk of caseload (expected cases: 3230). Followed by entry point NH-68 (site 1) in southern Rajasthan with the bordering state Gujarat, with a high risk of case importation (729 cases), the NH-11 (site 2) in northwestern Rajasthan showed the least risk (16 cases). These results highlight varying levels of malaria risk across different entry points, emphasizing the need for tailored control measures to mitigate transmission. The Ramdevra Shrine entry point stands out as particularly

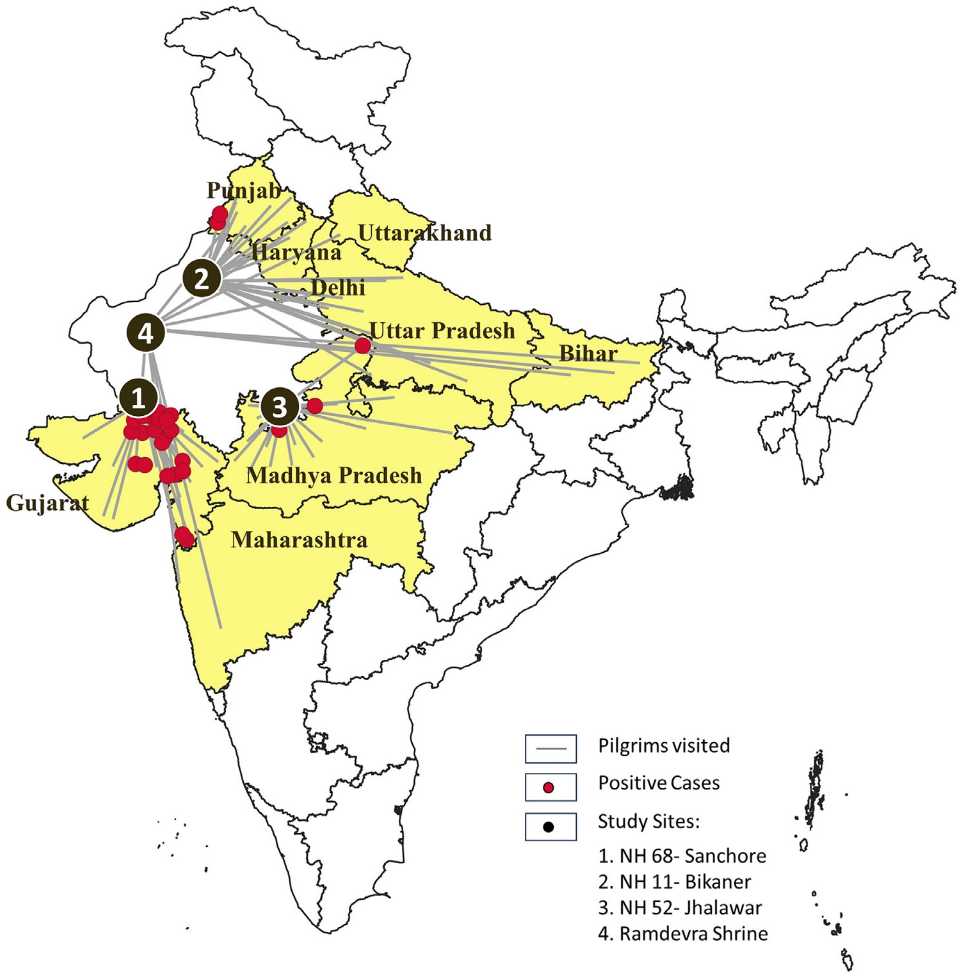

Figure 4. Pilgrims coming to Ramdevra shrine from different states of India (September 2021 and August 2022).

Table 4
Estimate of malaria cases importation from entry points and at the Ramdevra shrine.

Entry points	*Total screened using the thermal scanner*	*Tested for malaria*	*No. positive through rapid diagnostic test and microscopy*	*Positive/total screened*	Average pilgrims passing /hour from entry route ± SD (lower-upper limit at 95% confidence interval)	*Approximate population passing in a month (30 days)*	Estimated imported cases during the fair period/route
Site-1	1600	739	18[a]	0.0113	180 ± 33.7 (157.9-202.0)	64800	729
Site-2	1500	639	1	0.0007	65 ± 15.6 (54.9-75.3)	23400	15
Site-3	500	290	7	0.0140	35 ± 13.0 (26.7-43.8)	12600	176
Site-4	1800	623	19	0.0106	850 ± 241.9 (692.2-1008.3)	306000	3230
Total	**5400**	**2291**	**45**	0.0083	**1130 ± 286.9 (972.7-288.8)**	**406800**	**3390**

[a] One *Plasmodium vivax* case was found at site 1 during the 1st year of study (2021) when the fair was called off; therefore, for risk assessment, we have removed one case from the total.

high risk due to its large pilgrim influx and moderate positive rate (Table 4).

Discussion

Migrating populations may pose the risk of vector-borne disease transmission; therefore, understanding the dynamics of human migration is essential to prevent the spread of malaria to new areas [17]. The migrating populations are mostly laborers, farmers, shepherds, nomads, and forest dwellers in different states of India and it is evident this population movement has a role in the transmission of malaria [6,18,19]. The global report on the mobility of humans has shown that across the world 214 million migrations occur internationally and about 740 million people migrate in their own country as internal migration [20].

It is evident from past studies that population migration is associated with malaria transmission in India as well [6,7,21,22]. The inter-state movement of the Indian population is due to several factors, such as labor activities (mining, construction, agriculture, etc.), and sometimes, under stress such as famines and conflicts of borders [23] and natural calamities such as floods, earthquakes, and recent during lockdowns due to COVID-19 [24]. Studies also have shown that forest dwellers move into deep forests for Jhum cultivation and change residence according to the cultivation and harvesting period [25]. People also move for tourism or recreation. These movements may be for changing permanent addresses or temporary movement for work where migrants come back to their original place after the task is over. Rajasthan also follows the migration types occurring across India. In addition, the western arid region has a few more additional movements of the population during the transmission season post-monsoon and winters (August-December), such as cattle movement to avoid harsh climatic conditions, and usually a form of out-migration of shepherd community during February-April when they return on the information of first rain start in their homelands in Rajasthan (July-August).

During September 2021, the pilgrimage was restricted by the State Government considering transmission threat of COVID-19 [26]. As a result, the study was limited to only a few days in 2021 as the team commenced their work. However, during this season, limited pilgrims (213) could be tested and only one was found positive for *Pv*. In the following months (December 2021 and April 2022), no malaria case was found in the pilgrimage route villages, whereas, in the second year of the study during the fair season (August 2022), about 45 (18 *Pv*, 19 *Pf*, and eight mixed) cases were detected in a small window period of the survey duration, i.e. 3 days each at different routes, from 2291 persons were tested; this shows that there was an importation of active cases from the selected sites in Rajasthan state. In the following months' surveys (December 2022 to February 2023), about 30 local cases were detected from the pilgrimage route villages; this suggests that the traveling population might have some role in local transmission of malaria because the concurrent survey in pilgrimage route villages during the fair season (August 2022) did not show any positivity and support our hypothesis. A study conducted in western Rajasthan by Joshi *et al.* [8] also suggests that the inward migration of diseased populations is a primary risk that should be treated to prevent future malaria transmission. It was interesting to note that the case contributions from different states were not uniform in terms of plasmodium species. Among the all three entry points, the maximum *Pf* cases were observed from the entry point NH-52 (site 3) at Jhalawar, which was the entry point from Madhya Pradesh, whereas the maximum *Pv* cases were observed from the entry point near Gujarat state (site 1, NH-68).

Considering the population reaching the Ramdevra shrine in a month duration for a holy visit to the shrine campus, about 729 cases were estimated to enter from a single entry point (NH-68; site 1) in Jalore district of Rajasthan adjoining Gujarat during August 2022, which is very high. However, it is perplexing to note that Gujarat is also progressing toward elimination; still, caseload was found to be high. One such explanation for the cases may be avoiding health facilities or giving less priority to their health conditions etc. and introduced cases during the receptive environmental conditions when vectors are available for biting; they may accidentally pick up parasites from such cases and transmit them locally. The entomologic concurrent surveys suggested that two species of Anopheles, *An. stephensi* and *An. Culicifacies,* were available during the fair season in the night camps and the villages. It was evident through entomologic collections that malaria vectors (anopheles) were available. Despite the high mosquito density at the Bikaner entry point (NH-11, site 2), transmission was less due to negligible caseload among the pilgrims coming from Haryana and Punjab but a similar mosquito density at entry point NH-68 near Gujarat the transmission in the subsequent months in pilgrimage route villages was observed. The presence of an efficient vector is crucial for the local transmission potential of an area [27]. The HBI was high, about 0.65 for *Anopheles stephensi,* which is anthropophagic; on the other hand, *An. culicifacies* which is zoophagic [27], also had high HBI (0.50); this might be because of less availability of cattle than the humans staying in these shelters during the nights. A past study conducted by Joshi *et al.* (2005) also suggested that once a case is introduced in western Rajasthan, it is modulated by various factors, such as vector density types of vector rainfall and cattle density of the village [8].

The risk of importing malaria cases was estimated based on the proportion of the population found positive and the expected population crossing the entry points and from the Ramdevra shrine area among migrant pilgrims. The overall risk of case importation in about 30 days was approximately 3390 cases. The Ramdevra shrine (site 4) showed 1.05% positivity among daily reaching pilgrims, followed by the entry point located on NH-52 (site 3; 1.4% positivity) and NH-68 (site 1; 1.1% positivity) in southern Rajasthan with the bordering state Gujarat, with a high risk of case importation (729 cases). The risk was found to be very low (16 cases), despite the high vector density in NH-11 (site 2; 0.07% positivity) in the fair season of a month. Every unnoticed case among the pilgrim walkers may pose a high risk because they take at least 10-15 days to reach the shrine. During the nights, they halt and stay in temporary roadside shelters (camps). These camps are vulnerable spots for mosquito bites. Every night, any malaria case among the pilgrims gets the risk of mosquito biting. In these unnoticed cases, the further increases by 10-15 times, depending on the reaching time to the shrine. The risk to the state may increase due to the importation of resistant plasmodium species [28]; some studies already suggest that states have been reporting severe *Pv* malaria [29,30]. Our study also found high *Pv* cases coming from Gujarat (site 1 at NH-68), which might be a concern for malaria elimination because severity and deaths are associated with vivax malaria. In co-existing areas of *P. vivax* and *P. falciparum, Pv* decreases less rapidly than *Pf* in the community. In addition, *Pv* can also remain in the liver as hypnozoites and may cause malaria relapse [31,32]. The health-seeking behavior in western Rajasthan was reported to be poor [33] and the moving population also has lesser health-seeking behavior, which might make them a vulnerable carrier of the malaria parasite [34].

It is evident from our study that most of the pilgrims come from Gujarat (58.5%), followed by Madhya Pradesh (21.0%), whereas the least were from Punjab and Delhi, and the out-migration of the shepherd community also occurs mostly in these two states. Our study has shown that the risk of malaria transmission through case importation is from the adjoining large states, Madhya Pradesh and Gujarat (55%, during the fair season), and the possibility of transmission can be *vice versa* if cases are aggravated in Rajasthan due to the population movement.

Conclusion

The present study showed that tracking population movement may prevent invasion of new cases in the state, which may amplify in the presence of mosquito vectors; because of low API, the state is least under vector interventions. To prevent new cases in low-endemic communities along the pilgrim route, setting up sentinel surveillance clinics is recommended. This approach allows a better enumeration of risks and implementation of mitigation strategies at both ends. As the malaria elimination goal (2030) is pursued, it is essential to track the mobile population for malaria during transmission season has important epidemiological significance. Therefore, setting up sentinel surveillance clinics in identified junction points of states will be helpful for each state because shared information on malaria cases will help in preventing the introduction of malaria in these states. This extra effort, along with the existing ongoing elimination strategies, will be very helpful for the achievement of malaria elimination on time (2030) and will help check reintroduction at inter-state borders.

Declarations of competing interest

The authors have no competing interests to declare.

Funding

This work was supported by the Indian Council of Medical Research in New Delhi, India.

Ethical consideration

The study was approved by the ICMR-National Institute of Malaria Research; Delhi Ethics Committee *vide* number PHB/NIMR/EC/2020/199.

Acknowledgment

The authors thank the Director of NIMR for allowing this work, Malaria Elimination Research Alliance, India for financial support State Health Department Rajasthan, Director of National Institute of Implementation Research for his kind support in the conduction of the study. The technical help provided by the NIMR technical staff is duly acknowledged. This work was supported by the Indian Council of Medical Research in New Delhi, India (*vide* letter no. MERA/7/2020-ECD-II).

Author contributions

SL: writing-original draft; data collection and curation, formal analysis: SKG; writing-original draft, writing-review & editing, data curation, formal analysis, GK; data curation, SY, SSM, coordinated study in Rajasthan, verified data and edited the article. PP, BK, SS, PS, DK, PS, VK & DS: data curation, writing-review and editing. HS: conceptualization, formal analysis, funding acquisition, writing-original draft, and project administration.

Availability of data and materials

The data sets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijregi.2024.100418.

References

[1] Directorate General of Health Services (DGHS) *National framework for malaria elimination in India. Directorate of National Vector Borne Diseases Control Program. Ministry of Health and Family Welfare, Government of India, 2016–2030 (NVBDCP)*. New Delhi: Directorate General of Health Services (DGHS); 2016.
[2] World Health Organization. *Global strategic plan 2016–2030*; 2016. https://www.who.int/docs/default-source/documents/global-technical-strategy-for-malaria-2016-2030.pdf; [accessed 16 September 2023].
[3] Ministry of Health and Family Welfare. Strategic plan for malaria control in India, 2012–2017. A five-year strategic plan, https://ncvbdc.mohfw.gov.in/Doc/Strategic-Action-Plan-Malaria-2012-17-Co.pdf; 2012 [accessed 16 September 2023].
[4] Bruce-Chwatt LJ. Movements of populations in relation to communicable disease in Africa. *East Afr Med J* 1968;**45**:266–75.
[5] Kumar A, Sharma VP, Thavaselvam D. Malaria related to constructions in Panaji, Goa. *Indian J Malariol* 1991;**28**:219–25.
[6] Kumar A. Some considerable issues concerning malaria elimination in India. *J Vector Borne Dis* 2019;**56**:25–31. doi:10.4103/0972-9062.257770.
[7] Rajagopalan PK, Jambulingam P, Sabesan S, Krishnamoorthy K, Rajendran S, Gunasekaran K, et al. Population movement and malaria persistence in Rameswaram Island. *Soc Sci Med* 1986;**22**:879–86. doi:10.1016/0277-9536(86)90242-x.
[8] Joshi V, Adha S, Singh H, Singhi M, Dam PK. Introduction, transmission and aggravation of malaria in desert ecosystem of Rajasthan, India. *J Vector Borne Dis* 2006;**43**:179–85.
[9] Yadav SP, Kalundha RK, Sharma RC. Sociocultural factors and malaria in the desert part of Rajasthan, India. *J Vector Borne Dis* 2007;**44**:205–12.
[10] Yadav SP, Sharma RC, Joshi V. Study of social determinants of malaria in desert part of Rajasthan, India. *J Vector Borne Dis* 2005;**42**:141–6.
[11] Dash AP, Valecha N, Anvikar AR, Kumar A. Malaria in India: challenges and opportunities. *J Biosci* 2008;**33**:583–92. doi:10.1007/s12038-008-0076-x.
[12] Mosse D, Gupta S, Shah V. On the margins in the city: Adivasi seasonal labour migration in Western India. *Econ Pol Wkly* 2005;**40**:3025–38.
[13] Government of Rajasthan. Ramdevra final report on development and management plan of temple town, https://devasthan.rajasthan.gov.in/DPR_Reports/Ramdevra_Final_Report.pdf; 2016 [accessed 24 March 2024].
[14] HELINI. Plasmodium genotyping realtime PCR Kit, https://www.helini.in/uploads/3/0/9/5/30951267/helini_plasmodium_genotyping_real-time_pcr_kit.pdf; 2023 [accessed 16 September 2023].
[15] World Health Organization *Manual on practical entomology in malaria part II. Methods and techniques*. Geneva: World Health Organization; 1975.
[16] Gupta SK, Nagpal BN, Singh H, Vikram K, Nayak A, Chalga MS, et al. Mobile app based pictorial identification key for Indian anophelines. *J Vector Borne Dis* 2021;**58**:306–10. doi:10.4103/0972-9062.325634.
[17] Kent RJ, Norris DE. Identification of mammalian blood meals in mosquitoes by a multiplexed polymerase chain reaction targeting cytochrome B. *Am J Trop Med Hyg* 2005;**73**:336–42. doi:10.4269/ajtmh.2005.73.336.
[18] Saxena VK, Devadethan MA. Impact of the seasonal migration of labour forces on the spread of malaria. *Ann Trop Med Parasitol* 1998;**92**:821–2. doi:10.1080/00034989859078.
[19] Prothero RM. Disease and mobility: a neglected factor in epidemiology. *Int J Epidemiol* 1977;**6**:259–67. doi:10.1093/ije/6.3.259.
[20] International Organization for Migration (IOM). A global report on population mobility and malaria, https://www.iom.int/sites/g/files/tmzbdl486/files/201807/MMP_GMS_Legal%20Frameworks_2017.pdf; 2014 [accessed 16 September 2023].
[21] Singh N, Chand SK, Mishra AK, Nagpal AC. Migration malaria associated with forest economy in central India. *Curr Sci* 2014;**87**:1696–9.
[22] Sharma RC, Sharma VP. Epidemiological implications of population migration: Part I. Imported malaria in Kheda district. *Gujarat. Indian J Malariol* 1988;**25**:113–16 PMID: 3077367.
[23] Tyagi BK. Trans-border migration and malaria in desert populations. In: *Desert Malaria*. Singapore: Springer; 2023. p. 333–44.
[24] Simonovic SP, Kundzewicz ZW, Wright N. Floods and the COVID-19 pandemic-A new double hazard problem. *WIREs Water* 2021;**8**:e1509. doi:10.1002/wat2.1509.
[25] Galagan SR, Prue CS, Khyang J, Khan WA, Ahmed S, Ram M, et al. The practice of jhum cultivation and its relationship to *Plasmodium falciparum* infection in the Chittagong Hill Districts of Bangladesh. *Am J Trop Med Hyg* 2014;**91**:374–83. doi:10.4269/ajtmh.13-0592.
[26] Ministry of Home Affairs. Order, https://www.mha.gov.in/sites/default/files/MHAOrderdt_28092021.pdf; 2021 [accessed 16 September 2023].
[27] Subbarao SK, Nanda N, Rahi M, Raghavendra K. Biology and bionomics of malaria vectors in India: existing information and what more needs to be known for strategizing elimination of malaria. *Malar J* 2019;**18**:396. doi:10.1186/s12936-019-3011-8.
[28] Asare KK, Africa J, Mbata J, Opoku YK. The emergence of chloroquine-sensitive *Plasmodium falciparum* is influenced by selected communities in some parts of the Central Region of Ghana. *Malar J* 2021;**20**:447. doi:10.1186/s12936-021-03985-8.
[29] Kochar D, Kumawat BL, Karan S, Kochar SK, Agarwal RP. Severe and complicated malaria in Bikaner (Rajasthan), western India. *Southeast Asian J Trop Med Public Health* 1997;**28**:259–67.
[30] Kochar DK, Das A, Kochar SK, Saxena V, Sirohi P, Garg S, et al. Severe Plasmodium vivax malaria: a report on serial cases from Bikaner in northwestern India. *Am J Trop Med Hyg* 2009;**80**:194–8. doi:10.4269/ajtmh.2009.80.194.
[31] Garnham PCC. Malaria parasites of man: life-cycles and morphology (excluding ultrastructure). In: Wernsdorfer WH, McGregor I, editors. *Malaria parasites of man: life–cycles and morphology (excluding ultrastructure)*. Edinburgh: Churchill Livingstone; 1988. p. 61–96.
[32] World Health Organization *Control and Elimination of Plasmodium vivax malaria A technical brief –WHO 2015*; 2015. https://iris.who.int/bitstream/handle/10665/181162/9789241509244_eng.pdf; [accessed 24 March 2024].
[33] Yadav SP. A study of treatment seeking behaviour for malaria and its management in febrile children in rural part of desert, Rajasthan, India. *J Vector Borne Dis* 2010;**47**:235–42.
[34] Kusuma YS, Babu BV. Migration and health: A systematic review on health and health care of internal migrants in India. *Int J Health Plann Manage* 2018;**33**:775–93. doi:10.1002/hpm.2570.