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Case Report

Sequential *Plasmodium* Infections in Two Resident Families in Mangalore, India

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Abstract

Eleven members of two resident families in Mangalore city, Southwestern coastal India suffered from sequential malaria infections of *Plasmodium falciparum, Plasmodium vivax*, and mixed infections, despite using regular bed nets. Malarial symptoms of febrile illness started with chills and rigours in children at schools and adults at workplaces. Five members of the first family, residing in the central city, got infected with *P. falciparum* followed by *P. vivax,* while six members of the second family, located ~10 km away from the city, and initially suffered from *P. vivax*, followed by mixed infections and subsequently with *P. falciparum*. Both families reside nearer to the construction sites, indicating that construction activities foster vector mosquito breeding around houses resulting in indoor biting that leads to sustained malaria transmission. This study findings highlight the need for targeted vector control strategies that address closed malaria transmission, escaping bed net usage to counter malaria risk in the urbanized areas. Construction-related activities favour the suitable conditions in vector ecology and associated health risks.

Keywords: *Plasmodium falciparum; Plasmodium vivax*; Mixed Infection; Construction Sites; Close Transmission

Introduction

Malaria is one of the major public health challenges in most of the tropical and sub-tropical world. Severe forms of malaria are usually caused by the malignant *Plasmodium falciparum* malaria [1]. However, in recent years mounting evidence supports the severe form of *Plasmodium vivax* [2], contributing to the global burden of the disease. In 2023, an estimated 263 million cases of malaria occurred worldwide and 597,000 people died from the disease. India contributed 0.8% of total global malaria burden and still contributes almost half of burden in the Southeast Asia region [1]. Now, India has achieved remarkable milestone towards malaria elimination and exited the high burden high incidence countries and moving forward to eliminate this disease by 2030 [3]. To achieve this goal, it is important to understand the local malaria epidemiology for an effective elimination strategy [3]. Malaria infections among the family members represent an important aspect of epidemiology to understand the local transmission dynamics [4]. Mangalore, the administrative headquarters of Dakshina Kannada district in the Southwestern coastal city of Karnataka, India brings a unique understanding in the local malaria epidemiology. The city's tropical climate, characterized by high humidity (78%), heavy monsoon rains (95%) with an average annual rainfall (2721

mm), and an average temperature of 27°C [5], creates optimal conditions for *Anopheles* breeding and contribute to high incidences of malaria. The city is characterized by immigrants, migrant workers, travellers, which are involved in import-export profession, building constructions, roadways, railway track constructions, fishing and tourism contribute to the persistent malaria transmission. All these factors contribute to high incidence of malaria in Mangalore and its surrounding areas. Malaria transmission in Mangalore is perennial and occurs mostly during monsoon and post-monsoon periods. It is associated with building constructions that support breeding grounds for the urban malaria vector *Anopheles stephensi* [6]. Infection occurs mostly among construction workers and their families who live within a close proximity to the construction sites [7]. Despite the progress in malaria control through interventions such as long-lasting insecticide-treated nets (LLINs), changes in the mosquito behaviour [8], including earlier evening and indoor biting [9], allows persistent transmission. In addition, construction sites have the potential to act as significant mosquito breeding grounds, and indoor biting contributes to malaria transmission that poses new challenges in malaria elimination [7]. Collaborative efforts from government bodies, NGOs, and local stakeholders are essential in addressing these challenges ensuring sustained reductions in malaria transmission as a part of India's broader elimination efforts [10].

Case Presentations

Here, we report of two families, who had prior malaria awareness and despite regular use of bed nets contracted malaria on a sequential manner. These families, residing near construction sites, infected with *P. falciparum*, *P. vivax* and mixed *Plasmodium* infections. Adult members of the families worked as daily wage labourers. Malaria was diagnosed and parasitaemia was confirmed by two trained expert microscopists using Giemsa-stained thick and thin blood smears examined under Carl Zeiss Primo Star light microscopes [11,12,15]. Results were validated using rapid diagnostic tests (RDTs) accredited by the national malaria programme [12,15]. Biochemical and haematological parameters were analysed from pre-treated intravenous blood samples [13]. Additionally, serological tests for HIV, HCV and HBsAg were also conducted [14].

All the patients received free treatment at the Wenlock District Government Hospital, following the guidelines of the National Centre Vector Borne Disease Control (NCBDC) guidelines [16]. All the treated patients were discharged on day 4 after confirming negative for *Plasmodium*, with a follow-up up to day 28 for *P. vivax* and day 42 for *P. falciparum* and mixed infections, respectively. Throughout the treatment and follow-up period, no evidences of reinfection or recrudescence or relapse were observed. Tables 1 and 2 summarize the baseline values and follow-up details.

Table 1: Demographic, haematological and biochemical laboratory findings of two families at the time of admission.

	Family - 1					Family - 2						
Clinical Parameter	**Case 1**	**Case 2**	**Case 3**	**Case 4**	**Case 5**	**Case 1**	**Case 2**	**Case 3**	**Case 4**	**Case 5**	**Case 6**	**Ref. Range**
Age, year	40	12	10	14	50	4	6	12	11	32	37	
Sex, male/female	Female	Female	Female	Male	Male	Female	Male	Female	Female	Female	Male	
Weight, Kg	49.3	28.8	26	30.2	62	14.8	18.5	35	32	54	61.5	
Height, Cm	160	114.3	106.6	153	166	63.5	78.7	129.5	124.4	158	162.5	
Hemoglobin levels (g/dL) Packed Cell Volume (%)	12.8 43.7	12.3 47.7	12.4 38.2	12.5 38.7	14 45.2	10.8 32.3	10.4 30.6	12.5 38.3	10.1 30.3	12.8 40.6	15.8 50.5	13-17 M and 12-16 F 35 - 45
Total RBC counts (million/mm3)	5.77	4.97	4.76	4.73	4.23	4.58	3.97	4.46	3.69	4.75	5.54	4.0 - 6.0 million
Platelet counts (per µL)	93000	69000	103000	150000	99000	158000	160000	130000	95000	143000	157000	150000 - 4,10,000
Blood glucose (mg/dL)	48	68	96	70	94	119	91	100	99	99	110	80 - 130
Blood urea (mg/dL)	24	51	25	18.7	13.5	55	20	30	13	28.6	19.6	10 - 45
Serum creatinine (mg/dL)	0.81	1.93	0.7	0.73	0.87	0.4	0.2	0.6	0.5	0.79	1.1	0.4 - 1.4
Serum bilirubin (mg/dL)	1.04	23.7	1.51	0.6	1.3	1.01	1.69	0.83	1.29	1.76	1.54	0.3 -1.2
AST, IU/L	114	47	18	25.6	99	58	28	22	27	37	79	5.0 - 40.0
ALT, IU/L	77.5	68.6	15	22.2	74	19	19.2	13	17	53.5	17	5.0 - 40.0
Alkaline phosphatase (IU/L)	508.2	314	133	593.8	404.5	100	135	138	180	478.8	290.5	40 - 129
Total protein levels (g/dL)	7.59	13.9	7.48	8.26	10.18	7.2	6.7	7.7	7	7.96	6.65	6.0 - 8.3
Albumin levels (g/dL)	4.53	5.03	4.25	4.99	5.04	3.4	4.66	3.9	5	4.41	4.3	3.2 - 5.5
Globulin levels (g/dL)	3.06	8.87	3.23	3.27	5.14	3.5	2.5	2	4.8	3.55	2.35	1.8 - 3.4

Table 2: Clinical follow-up for fever (°C) and parasitaemia (/**µL**)

Family-1	Test Report	Day 0	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Case 1 *P. f.*	Parasitaemia (per µL)	15527	1054	52	0	0	0	0	0	0	0
	Temperature °C	39.1	37.4	37.8	37.4	37	37.2	37.2	36.9	37	36.9
Case 2 *P. f.*	Parasitaemia (per µL)	16786	1880	88	0	0	0	0	0	0	0
	Temperature °C	38.6	37.9	37.6	37	37	36.8	37	37.4	37.2	37
Case 3 *P. f.*	Parasitaemia (per µL)	14116	400	84	0	0	0	0	0	0	0
	Temperature °C	38.8	37.9	36.9	36.8	37	37.2	37.2	36.8	36.8	36.8
Case 4 *P. v.*	Parasitaemia (per µL)	5036	349	16	0	0	0	0	0	Follow-up complete	Follow-up complete
	Temperature °C	38.6	37	37.4	37.6	37	37.2	37	37	nil	nil
Case 5 *P. v.*	Parasitaemia (per µL)	4089	83	0	0	0	0	0	0	Follow-up complete	Follow-up complete
	Temperature °C	38.4	37	37.9	37.2	36.8	37	37.2	37	nil	nil
Family-2	**Test Report**	**Day 0**	**Day 1**	**Day 2**	**Day 3**	**Day 7**	**Day 14**	**Day 21**	**Day 28**	**Day 35**	**Day 42**
Case 1 *P. v.*	Parasitaemia (per µL)	6345	317	73	0	0	0	0	0	Follow-up complete	Follow-up complete
	Temperature °C	38.5	37.6	37.2	37	37.2	37.2	37	37	nil	nil
Case 2 *P. v. & P. f.*	Parasitaemia (per µL)	13988	1389	888	0	0	0	0	0	0	0
	Temperature °C	38.8	37.6	37.4	37.2	37.2	37	37	37.2	37.2	37
Case 3 *P. f.*	Parasitaemia (per µL)	4458	129	0	0	0	0	0	0	0	0
	Temperature °C	38.7	37	38.2	37	36.8	36.9	36.8	37	37.2	37
Case 4 *P. f.*	Parasitaemia (per µL)	4079	267	0	0	0	0	0	0	0	0
	Temperature °C	38.5	37.2	37.2	37	36.8	36.9	37	37	37	37
Case 5 *P. v.*	Parasitaemia (per µL)	5016	117	0	0	0	0	0	0	Follow-up complete	Follow-up complete
	Temperature °C	38.4	37.4	37.2	37	37.1	37.2	37.2	37	nil	nil
Case 6 *P. f.*	Parasitaemia (per µL)	15508	1078	107	0	0	0	0	0	0	0
	Temperature °C	39.1	37.6	37.2	37	37.2	37	37.2	37.5	36.9	36.8

P. v. – Plasmodium vivax; P. f. - Plasmodium

Family 1 Case History

This family has been living in the city at Bunts Hostel, Mangalore for three decades, which was close to the Ponni Delta construction site. The family has five members including two males and three females (father, mother, son and two daughters). The family depends on parent's daily wage, and all three children attend school in nearby. The family was exposed sequential malaria infections, sustained *Anopheles* breeding in the vicinity, despite regular bed net used and were diagnosed with malaria over a period of nine months.

- **Case 1**: The 40-year-old mother presented with fever (39.1°C), headache, vomiting, anorexia and fatigue on 24 September, 2015. Physical examination revealed elevated blood pressure (136/92 mmHg) and pulse rate (86 beats/min). Blood smear diagnosis confirmed *P. falciparum* infection.
- **Case 2**: The 12-year-old eldest daughter presented with fever (38.6°C), shivering, nausea and myalgia on 15 December, 2015. Blood pressure (116/78 mmHg) and pulse rate (75 beats/min) were recorded and *P. falciparum* malaria was confirmed through microscopy blood smear examination.
- **Case 3**: The 10-year-old youngest daughter was admitted with a fever (38.8°C), headache, and chills with rigours on 18 February, 2016. Physical examination revealed normal blood pressure (112/74 mmHg) and pulse rate (73 beats/min). Laboratory tests confirmed *P. falciparum* infection.
- **Case 4**: The 14-year-old eldest son presented with fever (38.6°C), headache, nausea and myalgia on 24 April, 2016. Blood pressure (122/82 mmHg), pulse rate (78 beats/min). Blood tests confirmed the diagnosis of *P. vivax* infection.

- **Case 5**: On 20 June, 2016, the 50-year-old father presented with chills, severe headache and fever (38.4°C), along with elevated blood pressure (142/90 mmHg) and pulse rate (86 beats/min). Blood smear analysis revealed *P. vivax* infection.

Family 2 Case History

The family had resided in Thokottu under Ullal PHC ~10 km from main city for two decades, which is close to the Palma Citius construction site. The family has six members with two males and four females (father, mother, son and three daughters). The parents were only source of income on daily wage, while two of four children attend school in nearby. Despite regular preventive measures, the family's exposure high-risk malaria infections over a period of 11 months due to sustained *Anopheles* breeding.

- **Case 1**: The 4-year-old youngest daughter presented with overnight cold, sweating and fever (38.5°C) for 2 days on 18 April, 2016. Physical examination showed blood pressure of 104/64 mmHg and pulse rate of 86 beats/min. Blood smear confirmed *P. vivax* infection.
- **Case 2**: The 6-year-old youngest son presented with fever (38.8°C), headache, chills and cough for 1 day on 21 April 2016. His blood pressure was 108/68 mmHg, pulse rate 92 beats/min, microscopy examination revealed mixed *P. falciparum* and *P. vivax* infections.
- **Case 3**: The 12-year-old eldest daughter returned from school with a fever (38.7°C) and underwent blood examination on 22 April 2016. Her initial microscopy was negative for parasites, but upon cross-examination, *P. falciparum* was detected. The RDT confirmed *P. falciparum* after 20 min with a distinct dark band. Her blood pressure and pulse rate were 118/78 mmHg and 74 beats/min, respectively.
- **Case 4**: On 12 November 2016, the 11-year-old second daughter presented with acute illness, fever (38.5°C), blood pressure of 114/76 mmHg, and a pulse rate of 72 beats/min. Blood smear examinations confirmed *P. falciparum*.
- **Case 5**: The 32-year-old mother of the family presented with general weakness and headache along with fever 38.4°C for one day on 12 February 2017. Her blood pressure was of 132/90 mmHg and pulse rate of 86 beats/min. Examination of blood smear confirmed *P. vivax* infection.
- **Case 6**: On 20 March 2017 the 37-year-old father was admitted in the hospital with severe headache, fatigue, vomiting, anorexia and high-grade fever 39.1°C. He had a blood pressure of 144/92 mmHg and pulse rate of 84 beats/minute. Blood smear examinations revealed of *P. falciparum* infection.

Discussion

Despite regular use of bed nets by both the families, malaria transmission persisted, suggesting that bed nets alone were not sufficient to prevent infection. This could be due to feeding behaviour changes of *An. stephensi*, the primary malaria vector in urban Mangalore [6]. Studies suggest that *An. stephensi* may shift their feeding times, biting outside of bed net protection hours. Only 30.4% of the local population uses bed nets, and while the local health department distributes LLINs to high-risk groups, such as hotel staff and school children, this coverage appears inadequate for full protection against malaria [17]. The case presentations in the present study reveal that each case of the two families was from different locations of the city, and the malaria infections happened at different times. This indicates that the infective bites were at different time resulting in continued malaria transmission.

Mangalore has been a malaria hotspot for over three decades, contributing 71.4% of the state's malaria cases [17]. Rapid urbanization and vigorous construction activities involving migrant labourers from malaria-endemic states like Odisha, Jharkhand, West Bengal and Northeast states contribute to sustained malaria transmission [18]. Migrant workers pose a risk for malaria resurgence, complicating control efforts. Recent initiatives, including "smart surveillance" systems using GPS-tagged mobile devices for real-time tracking of symptomatic individuals, show promise for enhancing malaria surveillance in the city [10]. However, persistent infections in these two families highlight significant gaps in local malaria control strategies.

A key challenge is the irregular availability of family members during routine visits by health workers or auxiliary nurse midwives (ANMs). Parents are often absent from home during the day due to work at construction sites, while children are at school, creating opportunities for cases to go unreported. To address this, a more dynamic and frequent surveillance policy is required. The Man-

galore City Antimalarial Campaign (MCAC) could extend surveillance efforts to schools, construction sites, and residential areas with regular follow-up visits to high-risk households. This would ensure more comprehensive case detection, minimizing missed opportunities for early diagnosis and treatment.

Innovative approaches like involving school teachers, construction supervisors, and volunteers to conduct primary screenings using RDTs, could enhance the existing health system. RDTs require minimal training and resources, making them ideal for early detection of both symptomatic and asymptomatic malaria cases. Asymptomatic carriers are often missed by passive surveillance, remain infectious and act as reservoirs for sustain malaria transmission [19]. Targeting these hidden reservoirs could significantly reduce the malaria burden and help close the gap in achieving elimination by 2030.

While digital surveillance tools represent a key advancement, integrating community-based interventions will be essential. By creating a broader network of health workers, volunteers, and local stakeholders, Mangalore can adopt a more comprehensive strategy to address symptomatic cases and capture asymptomatic carriers, advancing towards malaria elimination. The digital surveillance in the city since 2015 has shown effective cases-based surveillance resulting in reduction of malaria cases [20]. In 2024, only 162 malaria cases have been reported compared to 11021 cases in 2015 [21].

Conclusion

We present two families who lived in a close proximity to the construction sites and were found consecutively infected with *P. falciparum, P. vivax* and mixed *Plasmodium* infections despite regular bed nets use, which signifies the role of mosquito breeding sites and indoor biting in ongoing malaria transmission in the city. The proximity to such sites and potential adaptive feeding behaviour of *An. stephensi* suggest bed nets alone may be insufficient. Need to improve diagnosis, suspected malaria cases should undergo cross-examination of blood smears by at least two expert microscopists to reduce diagnostic errors and unnecessary treatments.

Ethical Approval

Written prior informed consent was obtained from all the study participant patients. The institutional review board of ICMR-National Institute of Malaria Research, New Delhi, India reviewed and approved the study (Ref. No-ECR/NIMR/EC/2012/39). The research and ethics committee of the Kasturba Medical College (KMC) under Manipal Academy of Higher Education, Mangalore, Karnataka, India, approved the study (Ref. No- IEC KMC MLR 03 16/49). All essential regulatory procedures were followed strictly.

Informed Consent

Informed consent was obtained from all study participants.

Author's Contributions

- B.M: Conception, design the work, analysis, drafting the article.
- H.G: Literature search, interpretation of the data, critically review.
- C.G: Manuscript review and editing.
- A.R.A: Manuscript review, final approval.
- S.K.G: Generated resources, critically review, final drafting the article, supervision, submission and final versions approval.

Conflict of Interest

The authors declare no conflict of interest.

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