

# Original Research Article

## The First Report of *Plasmodium Ovale wallikeri* in Kerala, India

### **Abstract**

The first imported case of *Plasmodium ovale wallikeri* malaria in Kerala, was reported in this article after confirmation using both conventional microscopy and PCR-based protocols. The patient was a 51-year-old Indian male belongs to northern part of Kerala, the Kannur district. He had been working as an army person and as a part of his job in UN security visited Sudan, Africa. Phylogenetic analysis revealed that these isolates showed close homology with West African genotypes. Based on his travel history, it was found that the parasite was dormant for at least one year. The relatively long incubation period of *P. ovale* may obscure the link between exposure and onset of disease. The recent increase in the number of people travelling to regions where *P. ovale* is endemic, suggests that a PCR-based protocol should be included as a complementary tool for malaria reference laboratories particularly in the context of achieving the target of Sustainable Developmental Goals of malaria elimination.

**Key words:** Malaria, Kerala, *Plasmodium ovale*, long incubation period

### **Introduction:**

Malaria is one of the most prevalent causes of fever in travellers returning from the tropics and migrants [1]. Five *Plasmodium* species (*Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale*, and *Plasmodium knowlesi*) are responsible for almost all human cases of malaria. *Plasmodium ovale Curtisi* and *Plasmodium ovale Wallikeri* are two disease-causing sympatric *Plasmodium* species indigenous to Southeast Asia and Central and West Africa, respectively [2,3]. The presence of *P. ovale* in India, particularly in Kerala, has been uncommon.

Human malaria parasites *P. vivax* and *P. ovale* have a dormant liver stage (with hypnozoites) following primary infection if effective treatment is not given. Even months after the first infection, hypnozoites can mature into schizonts and release merozoites into the bloodstream, triggering clinical signs of malaria (relapsing malaria). The treatment for *ovale* infection is the same as for *vivax* malaria.

The first imported case of *P. ovale* species in Kerala has been reported in this article in a Keralite who had travelled from Africa.

### **Case Report:**

After two days of feeling intermittent fever, shivering, and tiredness, a 51-year-old person went to the outpatient department of District Hospital Kannur to see a doctor. After arriving from Delhi, the patient was quarantined at his hometown during the onset of symptoms. He got a Covid 19 test, which turned out negative. RDT and a peripheral blood smear examination for malaria were performed because he had malaria-like symptoms and a history of travel outside of Kerala. Although the RDT for malaria was negative, a peripheral smear confirmed *P. ovale* infection. WHO-trained L2 technician (Malaria microscopy) from Kozhikode, Kerala, as well as L1 technicians at State Headquarters in Thiruvananthapuram and those from Bhubaneswar, promptly re-confirmed the slides as *P. ovale*. Other laboratory tests revealed that Serum Bilirubin was increased to 5.1, Platelet count was 84000 per cubic millimetre, and Hb was 15.2gm/mm.

The patient is an army officer who, as part of his UN Security work, travelled to Sudan (Africa) and lived there for around 8 months between June 14, 2019 and February 15,

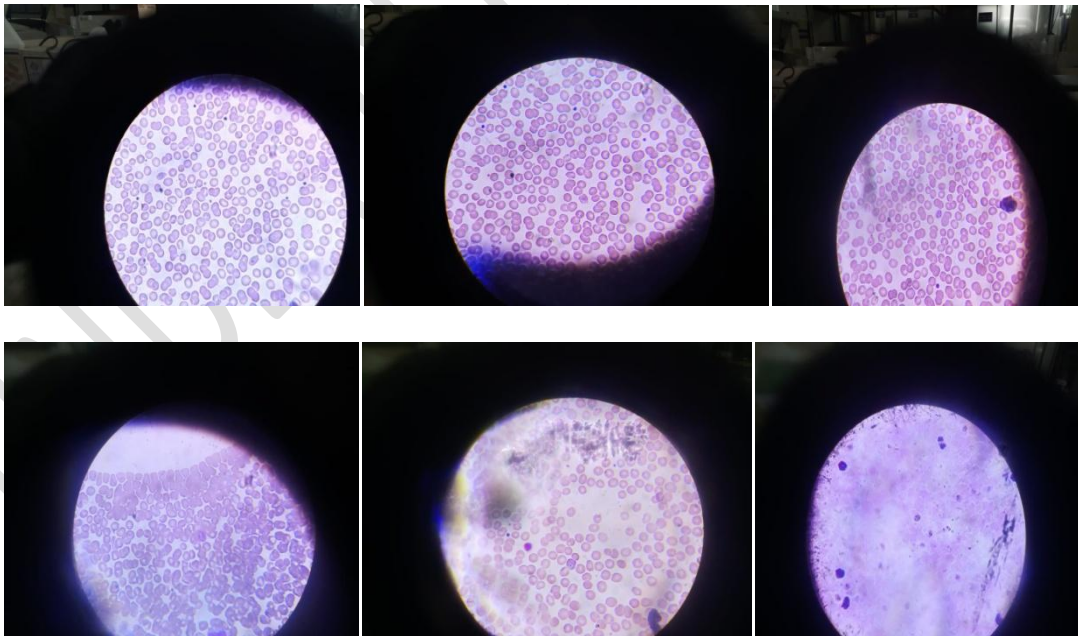
2020. During that time, he also travelled to Uganda and spent a week there. He did not have any malaria symptoms during his time in Sudan. On the 15th of February 2020, he arrived in Delhi from Sudan, and on the 29th of February, he visited his hometown in Kannur, Kerala and stayed upto March 11th, 2020. From Delhi, he travelled again to Kannur and stayed there from June 30 to August 13, 2020, and returned back to Delhi and stayed upto November 14 2020. He was placed under home quarantine for COVID-19 after arriving on November 15th and was tested negative for COVID-19. He had been in various places as part of his job in the last ten years, including Nagaland (2010-2013), Pathankott- Punjab (2013-2015), Leh Ladakh (2016 - 2018), and Jabalpur (2017- 2018), and he had not contracted malaria during that period.

The patient began weekly Mefloquine chemoprophylaxis two weeks before to his visit to Sudan and continued for the duration of his stay in Sudan. He continued to take the medicine for another two weeks after arriving in Delhi.

Thick and thin blood smears were prepared according to WHO recommendations [4]. Using conventional microscopy, in thick film cytoplasm was found to be irregular, slightly fragmented and gametocytes were present and it's highly suggestive of *P. ovale*, where as in thin film the host RBCs were found slightly enlarged, fimbriated ends seen as oval shape, nucleus in red colour and cytoplasm in blue colour. The parasite density was 7862.4 parasites/ $\mu$ l in the thick blood smear.

The patient was treated with chloroquine (600mg each for two days & 300 mg for third day) and primaquine (15 mg daily for 14 days from the 1<sup>st</sup> day onwards) to prevent relapse and Artesunate injection also taken. No *Plasmodium* stages were detected on the follow-up of 60 days of the peripheral blood smear.

Fig 1. Microscopic Pictures of parasite

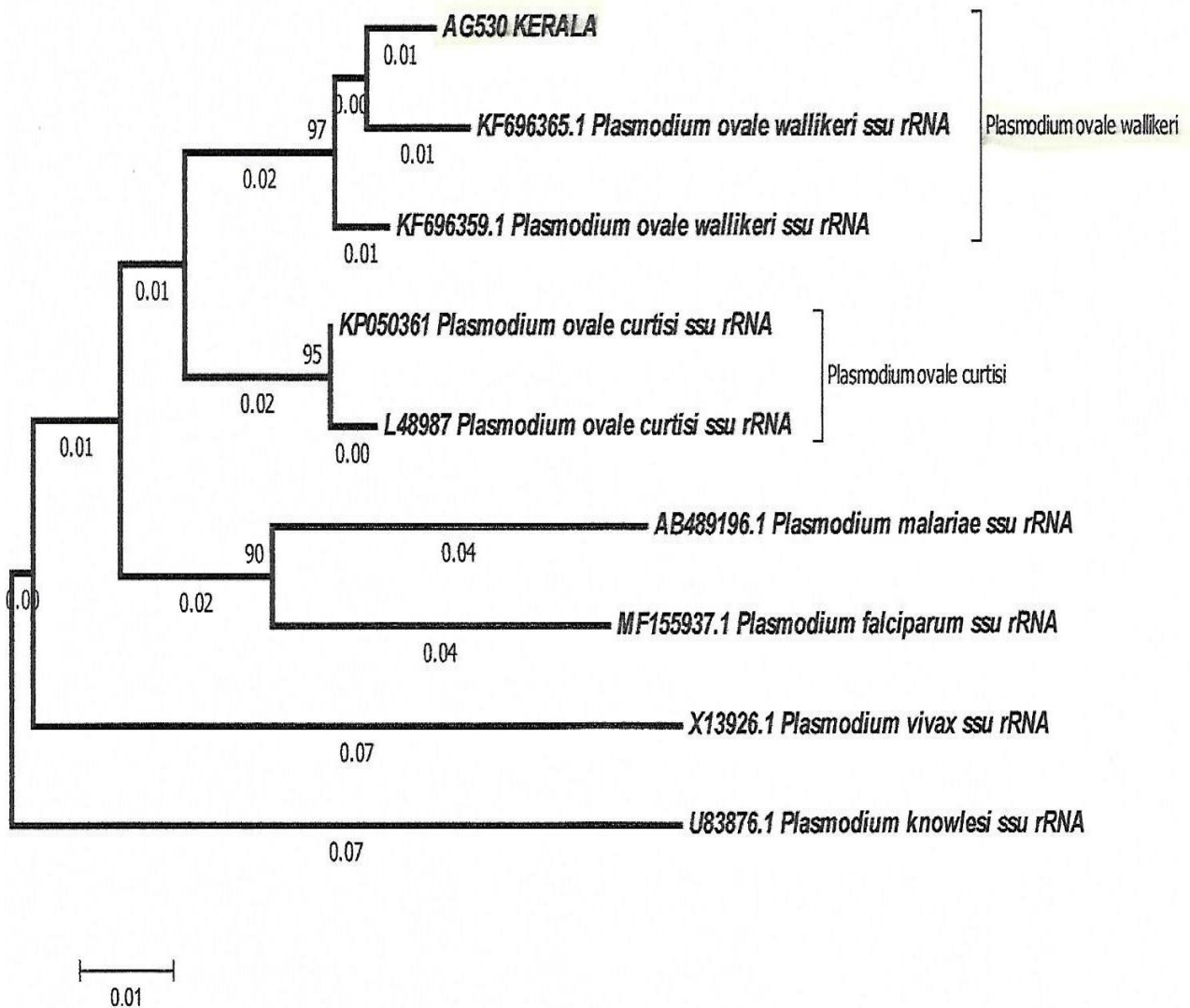


In Giemsa stained thick blood smear examined under oil immersion objective. After examining 100oil immersion field noted the presence of malaria parasite. Examined the feathery edge of Giemsa stained thin film for species identification. It was noted the Trophozoite stages. Infected RBCs were oval shaped with fimbriation and distorted in shape. Thin film host red blood cells were slightly enlarged, irregular and slightly fragmented. Fimbriated end seen as oval. Nucleus red in colour and cytoplasm blue. Gametocytes stages

seen more. Gametocytes having oval shape with scattered brown pigment and some fimbriation. Overload of parasites not observed. Procedures done as per world Health Organization standard operating procedure and basic malaria microscopy part 1 [4].

To confirm the type of *Plasmodium*, nested polymerase chain reaction (PCR) was performed. The PCR assay was carried out at Vector Control Research Centre (VCRC ICMR) Field Unit Kottayam, Kerala. The amplified fragment was custom sequenced. The sequences were blasted with NCBI GenBank and were subjected to genetic analysis. The NCBI blast as well as genetic analysis of the amplified fragment sequence of the sample (AG530) confirmed that the parasite species is *Plasmodium ovale* Wallikeri. This Hierarchical cluster analysis is shown in the figure 2.

Fig 2. Hierarchical Cluster analysis



**Discussion:**

Out of the total four main *Plasmodium* species, *P. ovale* and *P. malariae* were relatively less investigated and reported. *P. ovale* was first described in 1922 [5]. *P. ovale* spp. has historically been described as endemic to sub-Saharan and West Africa [6,7] and Asia and clinically as a less severe form of malaria with lower parasitaemia. *P. ovale* has been given relatively little attention compared with the other species due to its low parasitaemia and low prevalence in limited areas [8, 9,10] and its similar morphology with *P. vivax* and mixed infections with other *Plasmodium* species. By using molecular assays, the presence of *P. ovale* in most of Africa, India, and Southeast Asia [3,11,12,13] were confirmed, and its prevalence has reached as high as 15% in Papua New Guinea [14] and rural Nigeria [15].

With a prepatent period estimated as 15 days, a reported delayed primary infection presentation on the order of years and possessing the ability for relapse, this species of *ovale* malaria can cause difficult diagnosis and management for larger groups who often travel and also for clinicians in non-endemic regions [16,17]. The gold standard for diagnosis and correlation of parasitaemia to presentation remains visualization on microscopy [4]. Although *P. ovale* species may be diagnosed with microscopy and species confirmed with PCR, *P. ovale* spp. may be confused for *P. vivax*, making the diagnostic approach balanced to the resources available, the patient or population affected, and the expertise of the lab assisting in confirming diagnosis [18,19]. According to sequence analysis, *P. ovale* is considered to be comprised of two different subspecies, which were primarily named as classic and variant *P. ovale* and later named as *P. ovale curtisi* and *P. ovale wallikeri* [20]. So far, only a few clinical, epidemiological, and therapeutic studies have been reported with specific data for *P. ovale* subspecies. However, the geographical distribution of *P. ovale* seems larger than previously thought based on molecular analysis.

All the countries are in progress of malaria elimination, but the imported cases of malaria become an increasing risk for re-establishing endemicity [21]. Migration of population from endemic areas to nonendemic areas where competent vectors exist or expanding their geographic distribution to suitable areas of transmission in the era of climate change and where the proportion of imported cases are found increasing is a special concern for the malaria eradication programme [22,23,24].

#### Conclusion:

In India, *P. ovale* is a rare Plasmodium species with little investigation. Through regular microscopy, it appears that the quantum of *P. ovale* is understated. In addition, misidentification of the malaria parasite can cause anaemia and treatment resistance by prolonging parasite clearance time. Because of its sensitivity and specificity at low levels of parasitaemia, PCR-based detection is a better diagnostic technique. However, due to the high cost of equipment, skilled staff, and a reputable laboratory, the application of PCR is restricted. The subject in this study was a relapsed instance who became infected while travelling through Africa. As we focus on malaria elimination measures, screening of travellers from malaria-endemic areas will help with early detection.

#### Disclaimer regarding Consent and Ethical Approval:

As per university standard guideline, participant consent and ethical approval have been collected and preserved by the authors

#### References:

1. Wilson ME, Weid LH, Boggild A, et al. Fever in returned travelers: results from the GeoSentinel Surveillance Network. *Clin Infect Dis* 2007; 44:1560–1568.
2. Collins WE, Jeffery GM. *Plasmodium ovale*: parasite and disease. *Clin Microbiol Rev.* 2005; 18:570–81.
3. Sutherland CJ, Tanomsing N, Nolder D, Oguike M, Jennison C, Pukrittayakamee S, et al. Two nonrecombining sympatric forms of the human malaria parasite *Plasmodium ovale* occur globally. *J Infect Dis.* 2010; 201: 1544–50.
4. WHO. Universal access to malaria diagnostic testing: an operational manual. Geneva: World Health Organization; 2011.
5. Stephens, J. W. W. A new malaria parasite of man. *Ann Trop Med Parasitol* 1922;16, 383–388.
6. de Laval F, Oliver M, Rapp C, Pommier de Santi V, Mendibil A, Deparis X, et al. The challenge of diagnosing *Plasmodium ovale* malaria in travellers: report of six clustered cases in French soldiers returning from West Africa. *Malar J.* 2010; 9:358.
7. Ruas R, Pinto A, Nuak J, Sarmento A, Abreu C. Non-falciparum malaria imported mainly from Africa: a review from a Portuguese hospital. *Malar J.* 2017;16:298.
8. Lysenko, A. J. & Beljaev, A. E. An analysis of the geographical distribution of *Plasmodium ovale*. *Bull World Health Organ* 1969; 40,383–394.
9. Collins, W. E. & Jeffery, G. M. *Plasmodium ovale*: parasite and disease. *Clin Microb Rev* 2005; 18, 570–581.
10. Smith, A. D. *et al.* Imported malaria and high risk groups: observational study using UK surveillance data 1987–2006. *BMJ* 2008; 337.
11. Tachibana, M. *et al.* Two types of *Plasmodium ovale* defined by SSU rRNA have distinct sequences for ookinete surface proteins. *Mol Biochem Parasitol* 2002; 122, 223–226.
12. Win, T. T. *et al.* Molecular analysis of *Plasmodium ovale* variants. *Emerg Infect Dis* 2004;10, 1235–1240.
13. Fuehrer, H. P. *et al.* *Plasmodium ovale* in Bangladesh: genetic diversity and the first known evidence of the sympatric distribution of *Plasmodium ovale Curtisi* and *Plasmodium ovale Wallikeri* in southern Asia. *Int J Parasitol* 2012;42, 693–699.
14. Mehlotra, R. K. *et al.* Random distribution of mixed species malaria infections in Papua New Guinea. *Am J Trop Med Hyg* 2000; 62,225–231.
15. May, J. *et al.* High rate of mixed and subpatent malarial infections in southwest Nigeria. *Am J Trop Med Hyg* 1999; 61, 339–343.
16. Groger M, Fischer HS, Veletzky L, Lalremruata A, Ramharter M. A systematic review of the clinical presentation, treatment and relapse characteristics of human *Plasmodium ovale* malaria. *Malar J.* 2017; 16:112.
17. Mellon G, Ficko C, Thellier M, Kendjo E, Aoun O, Adriamanantena D, et al. Two cases of late *Plasmodium ovale* presentation in military personnel. *J Travel Med.* 2014; 21:52–4.
18. Castellanos ME, Díaz S, Parsons E, Peruski LF, Enríquez F, Ramírez JL, et al. First imported *Plasmodium ovale* malaria in Central America: case report of a Guatemalan soldier and a call to improve its accurate diagnosis. *Mil Med Res.* 2015; 2:3.
19. Rojo-Marcos G, Rubio-Muñoz JM, Ramírez-Olivencia G, García-Bujalance S, Elcuaz-Romano R, Díaz-Menéndez M, et al. Comparison of imported *Plasmodium ovale curtisi* and *P. ovale wallikeri* infections among patients in Spain, 2005–2011. *Emerg Infect Dis.* 2014; 20:409–16.
20. Calderaro, A. *et al.* Genetic polymorphisms influence *Plasmodium ovale* PCR detection accuracy. *J Clin Microbiol* 2007; 45, 1624–1627.

21. WHO. World malaria report 2018. Geneva: World Health Organization; 2018. <https://www.who.int/malaria/publications/world-malaria-report-2018/report/en/> Accessed 7 May 2019.
22. Dong X, Yang J, Lou L, Zhu L, Feng X, Yao L. Once malaria is eliminated, more attention should be paid to imported malaria: data from five years of surveillance in the City of Yiwu in eastern China. *Biosci Trends* 2017; 11:360–2.
23. Van Eer ED, Bretas G, Hiwat H. Decreased endemic malaria in Suriname: moving towards elimination. *Malar J* 2018;17:56.
24. Hundessa S, Li S, Liu DL, Guo J, Guo Y, Zhang W, *et al.* Projecting environmental suitable areas for malaria transmission in China under climate change scenarios. *Environ Res* 2018; 162: 203–10.

UNDER PEER REVIEW