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 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, Southern India. 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. Given the recent increase in the number of people travelling to areas where P. ovale is endemic, a PCR-based protocol should be included as a complementary tool for malaria reference laboratories, especially in the context of achieving the malaria elimination target of the Sustainable Development Goals.

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 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 out-patient 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. Rapid Diagonistic Test (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 Bhubaneshwar, promptly re-confirmed the slides as *P.ovale [25,26]*. 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

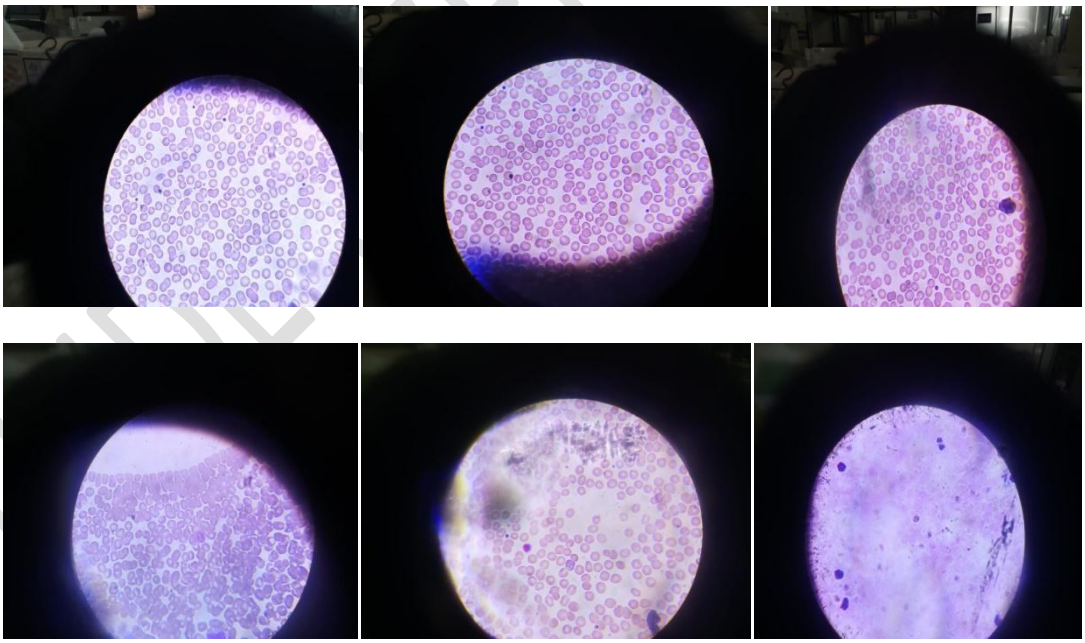
any malaria symptoms during his time in Sudan. On the 15th of February 2020, he arrived in Delhi from Sudan, and on the 29th of February, he visited his hometown in Kannur, Kerala and stayed upto March 11th, 2020. From Delhi, he travelled again to Kannur and stayed there from June 30 to August 13, 2020, 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/µl 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 1st 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

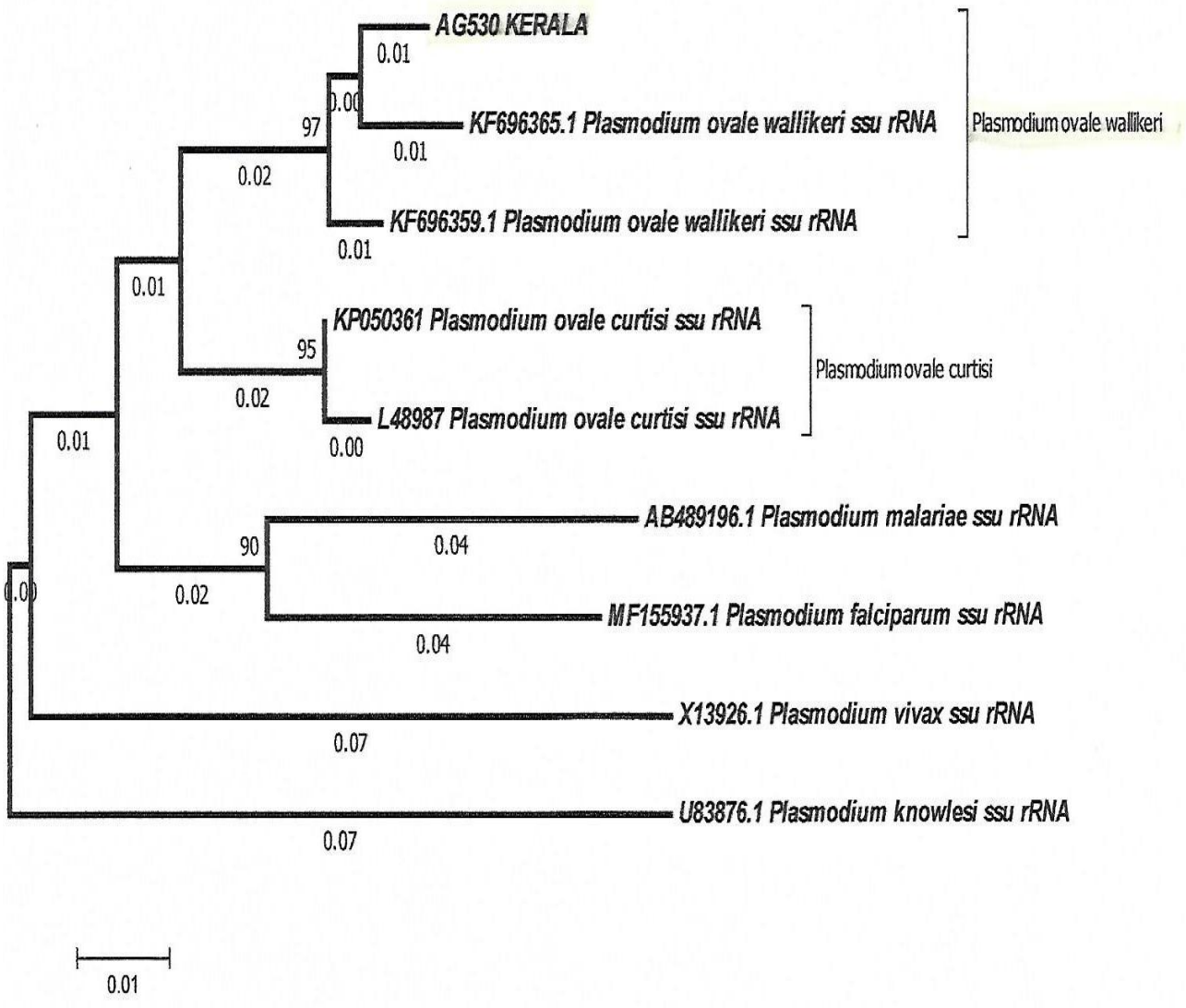


In Geimsa stained thick blood smear examined under oil immersion objective. After examining 100oil immersion field noted the presence of malaria parasite. Examined the feathery edge of Giemsa stained thin film for species identification. It was noted the Trophozoite stages. Infected RBCs were oval shaped 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 duration of 15 days, a reported delayed primary infection presentation on the order of years, and the ability to relapse, this type of ovale malaria can be challenging to diagnose and treat for big groups that move frequently, as well as practitioners in non-endemic areas [16,17]. Visualization on microscopy remains the gold standard for parasitaemia diagnosis and correlation to presentation [4] . Although P. ovale species can be diagnosed with microscopy and species confirmed with PCR, *P. ovale* spp. can be mistaken with P. vivax, necessitating a diagnostic method that balances the available resources, the patient or population impacted, and the lab's skill 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]. Only a few clinical, epidemiological, and therapeutic research with specific data for the P. ovale subspecies have been published thus far. However, based on DNA study, the geographical distribution of P. ovale appears to be larger than previously anticipated.

All the countries are in progress of malaria elimination, but the imported cases of malaria become an increasing risk for re-establishing endemicity [21]. In an era of climate change, where the proportion of imported cases is increasing, migration of people from endemic areas to nonendemic areas where competent vectors exist or expanding their geographic distribution to suitable transmission areas is a major 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.

Ethical Approval:

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

Consent

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

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