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 SDG of malaria elimination.

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

Introduction:

Malaria is one of the most common causes of fever in returning travellers and migrants from the tropics [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 *P. ovale wallikeri* are two disease-causing sympatric species endemic to areas in Southeast Asia and particularly in Central and West Africa [2,3]. Occurrence of *P. ovale* has not been very common in India and especially in Kerala.

P. vivax and *P. ovale* are the two species of human malaria parasites that have a dormant liver stage (with hypnozoites) following primary infection. The hypnozoites can develop into mature schizonts and release merozoites into the blood stream causing clinical symptoms of malaria (relapsing malaria) even many months after the primary infection. The treatment of ovale infection is not different from that of vivax malaria. Reported here the first imported case of *Plasmodium ovale* species in Kerala.

Case Report:

A 51-year-old male with intermittent fever, shivering and tiredness consulted the doctor in the out patient department of District Hospital Kannur after two days of beginning of his symptoms. During the onset of symptoms, the patient was on quarantine at his native place after reaching form Delhi. He had undergone Covid 19 test and was found negative. As he was having malaria like symptoms and history of travel outside Kerala, malaria RDT and peripheral blood smear examination were conducted. Malaria RDT turned negative, but peripheral smear revealed *Plasmodium ovale* infection. The slides were immediately re-confirmed as *P.ovale* by WHO trained L2 technician (Malaria microscopy) from Calicut, and also by L1 technician at State headquarters and those from Bhuvaneshwar. The other laboratory investigations like Serum Bilirubin which was elevated to 5.1, Platelet count 84000 per cu mm and Hb – 15.2gm%.

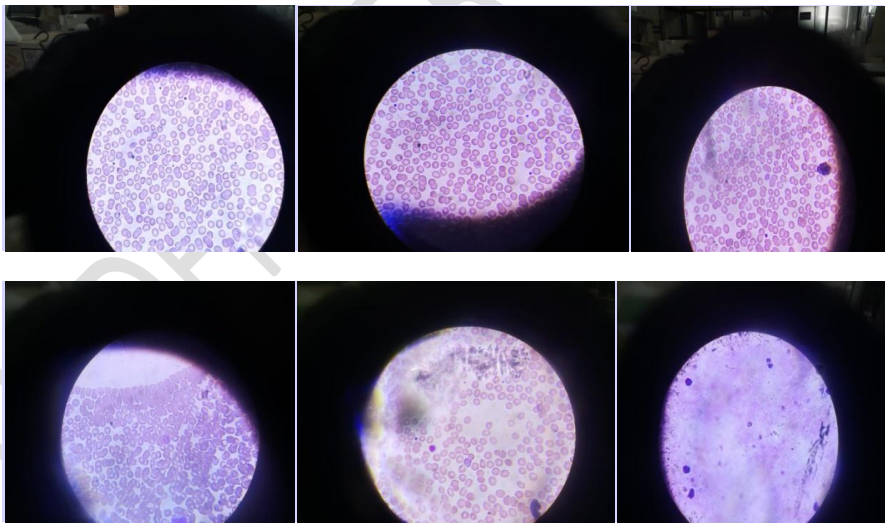
The Patient is an Army Person and as part of his job in UN Security had visited Sudan (Africa) and stayed there for about 8 months from 14th June 2019.While on his stay at Sudan,

he did not have any symptoms of malaria. During that period, he had visited Uganda also and stayed there for one week. From Sudan, he reached Delhion 15th February 2020 and subsequently visited his native place Kannur, Kerala on 29th February. He stayed at Kannur till 11th March 2020before returning to Delhi. Again, he came to Kannur and stayed there from30th June to 13th August 2020. His last visit to Kannur was on 15th November2020. After reaching on 15thNovember, he was under home quarantine for COVID–19 and was tested negative for COVID-19. As part of his job, he had been in many places like Nagaland (2010-2013), Pathankott- Punjab (2013 -2015), Lay Ladak (2016 to 2018), Jabalpur (2017- 2018) in the last ten years and during that time he was not contracted any malaria.

Before starting his journey to Sudan, the patient was on weekly Mefloquine chemoprophylaxis (started two weeks before journey) and continued for the entire period of stay in Sudan. After reaching Delhi, ~~also~~ he continued the drug for two more weeks.

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 was7862.4 parasites/µl in the thick blood smear. The patient was treated with chloroquine (600 mg 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

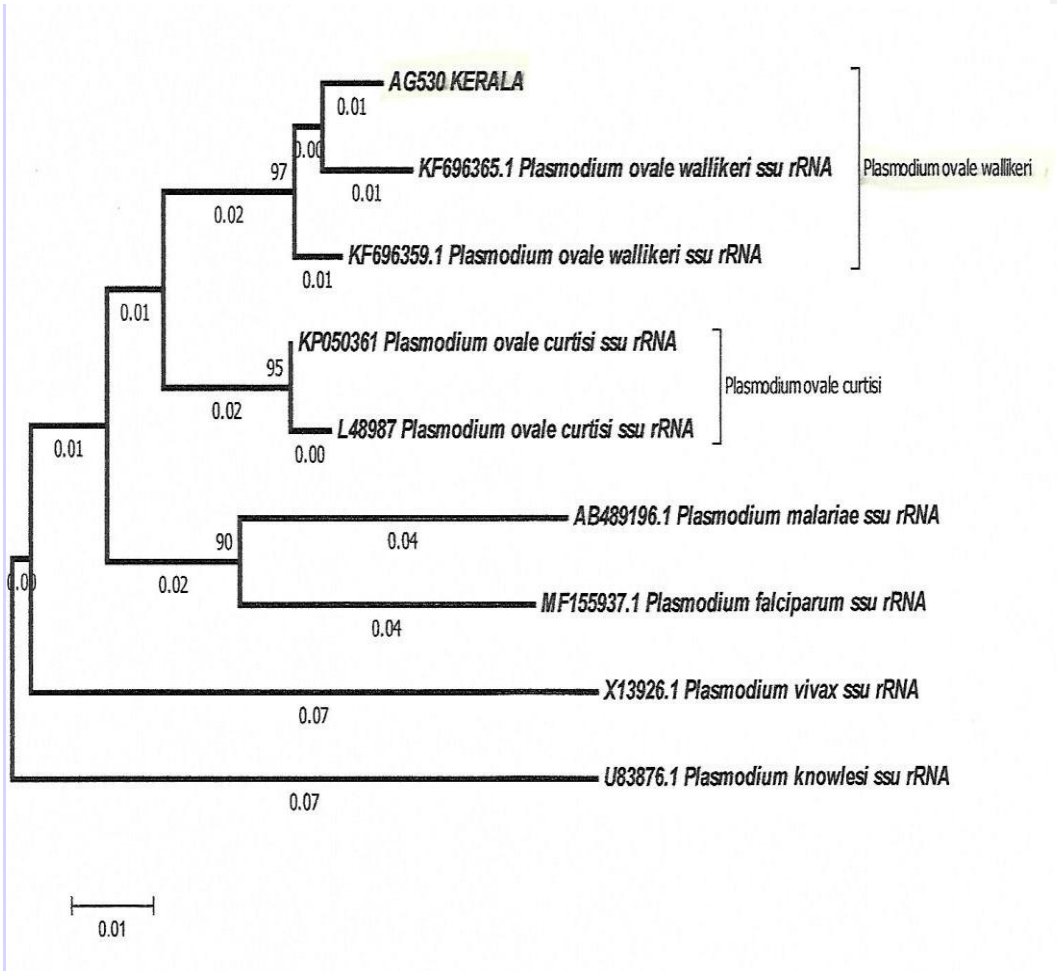


In Geimsa thick film 100 o~~i~~f takes 10 mts for sensitivity. But in thin film approximately taken 30 mints 800 thin oifs for species identification. Thin film host red blood cells slightly enlarged, irregular and slightly fragmented. Fimbriated end seen as oval in shape. Nucleus red in colour and cytoplasm blue. Gametocytes stages seen more. Overload parasite not seen. Procedures done as per world Health Organization standard operating procedure and also basic malaria microscopy part 1 [4].

Comment [ST1]: Define initials for the first time

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 to be *Plasmodium ovale wallikeri*.

Fig 2. Hierarchical Cluster analysis



Discussion:

Out of the total four main Plasmodium species, *P. ovale* was reported lastly 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 parasitemia. *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].

Comment [ST2]:

Comment [ST3]: This manuscript did not follow guidelines and poorly written. Though the science was good.
Authors need to do very major revision of the whole manuscripts following the guidelines.

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