Malaria Journal

CASE REPORT

Open Access

# A case of an asymptomatic *Plasmodium falciparum* infection followed by a symptomatic *Plasmodium ovale* infection in a soldier deployed to South Sudan

Choon Mee Kim[1], Jun-Won Seo[2], Da Young Kim[2], Na Ra Yun[2], Beomgi Lee[1], You Mi Lee[1], Munawir Muhammad[2,3] and Dong-Min Kim[2*]

## Abstract

**Background** Asymptomatic malaria poses a significant challenge to malaria eradication efforts and delays global elimination strategies. Mixed infections are also a major concern, as they frequently relapse, increase the risk of severe malaria, require more accurate diagnosis for appropriate treatment, and contribute to the development of drug resistance.

**Case presentation** A 25-year-old soldier was diagnosed with malaria following deployment in South Sudan. A comprehensive survey identified an asymptomatic *Plasmodium falciparum* infection, confirmed by peripheral blood smear and polymerase chain reaction (PCR). Despite being discharged after treatment, the patient developed fever and other symptoms one month later. Subsequent laboratory tests confirmed *Plasmodium ovale* infection based on peripheral blood smears and PCR.

**Conclusion** This case underscores the importance of molecular detection for surveillance and vigilant follow-up in malaria management, particularly among patients with a history of deployment in endemic regions. The detection of *P. ovale* after treatment for *P. falciparum* highlights the need for increased awareness and testing for mixed infections to ensure effective malaria control strategies.

**Keywords** Malaria, *Plasmodium falciparum*, *Plasmodium ovale*, Asymptomatic infection, South Sudan

## Background

Asymptomatic malaria infection poses risks to human health and malaria elimination efforts [1]. In the Central African Republic, 51.2% of children and 12.2% of adults are asymptomatic [2]. Moreover, patients with mixed infections are at a higher risk of relapse and progression to more severe disease [3, 4]. Furthermore, accurate diagnosis is crucial for providing appropriate and effective therapies [4, 5]. The global prevalence of severe mixed infections is approximately 9% [6]. Traditional methods such as microscopy are less sensitive than molecular methods for diagnosing mixed infections [5, 7].

*Correspondence:
Dong-Min Kim
drongkim@chosun.ac.kr
[1] Premedical Science, College of Medicine, Chosun University, Gwangju, South Korea
[2] Department of Internal Medicine, College of Medicine, Chosun University, Gwangju, South Korea
[3] Department of Pharmacology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia

South Sudan is one of the 22 countries with the highest malaria burden in the world. Between 2010 and 2020, *Plasmodium falciparum* was the most prevalent species, followed by *Plasmodium vivax,* and other *Plasmodium* species [8]. During this period, no mixed infections were reported [8]. Survey data from 1978, before the separation of South Sudan from Sudan, showed that *Plasmodium ovale* was very rarely found in South Sudan [9]. *Plasmodium ovale* is an understudied malaria species prevalent throughout sub-Saharan Africa. In a study of 18,149 adults tested, the researchers detected 143 cases of prevalent *P. ovale* infections, yielding an estimated prevalence of 0.8% [10].

Here, a case of malaria was identified in a soldier following deployment in South Sudan. A comprehensive survey was conducted among soldiers using peripheral blood (PB) smears and PCR. During the survey, three patients with asymptomatic *P. falciparum* infection were identified. Notably, one of these patients, who had previously received treatment for an asymptomatic *P. falciparum* infection was referred to the Infectious Disease Department. One month later, the patient presented with fever, and a symptomatic *P. ovale* infection was confirmed.

## Case presentation

After confirmed malaria cases were reported among soldiers following their deployment to South Sudan, a comprehensive survey was conducted at Chosun University Hospital on March 23, 2023, to assess the malaria infection status among the deployed troops.

During the survey, a 25-year-old soldier was diagnosed with asymptomatic malaria based on a positive PB smear. An initial examination revealed an asymptomatic *P. falciparum* infection, with the PB smear showing a few infected red blood cells (RBCs: 1/100 high-power field [HPF]) (Fig. 1A). Laboratory findings included white blood cells (WBC): $6.66\times10^3$/μL, haemoglobin (Hgb): 13.1 g/dL, platelets (PLT): $313\times10^3$/μL, C-reactive protein (CRP): 0.30 mg/dL, aspartate aminotransferase (AST): 24 U/L, alanine aminotransferase (ALT): 26 U/L, and glucose: 57 mg/dL.

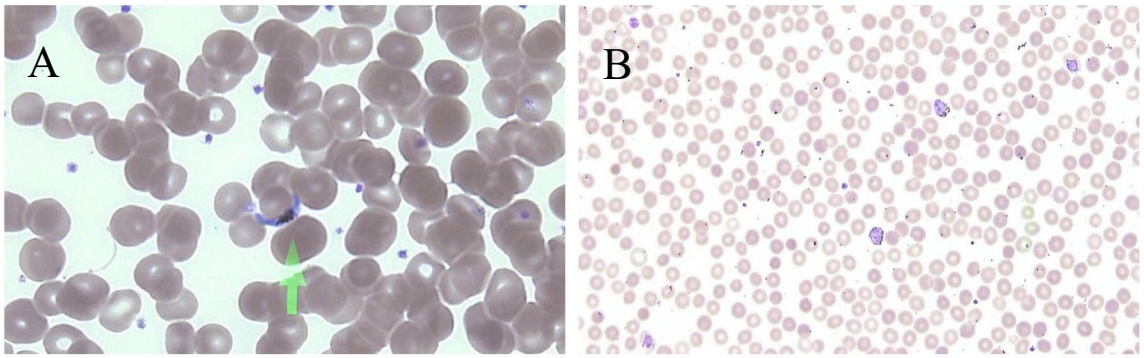
The patient had a history of military service in malaria-endemic regions (Incheon, Gyeonggi-do, and northern Gangwon, South Korea) from June to August 2021, and was deployed to South Sudan from April 7 to December 9, 2022. He stated that he had taken mefloquine (Lariam®) 250 mg (228 mg base), one tablet orally once a week, from March 2022 until January 2023. He began taking it one week before deployment, continued throughout the entire deployment period, and took it for four weeks after returning home.

Upon confirmation of the initial findings, the patient was hospitalized from April 3 to April 6, 2023, for further evaluation. Follow-up PB smears showed no abnormalities. Rapid diagnostic test (RDT) using immunochromatography was performed on blood samples using the Standard Q Malaria Pf/Pan Ag RDT (SD Biosensor, Inc., Suwon, South Korea). This kit was designed to detect histidine-rich protein 2 (HRP-2) specific to *P. falciparum*, and *Plasmodium* lactate dehydrogenase (pLDH), specific to *P. falciparum*, *P. vivax*, *P. ovale*, *Plasmodium malariae* and *Plasmodium knowlesi*. Laboratory tests confirmed a positive rapid antigen test for *P. falciparum* and a confirmatory malaria polymerase chain reaction (PCR) test (Table 1). Blood tests during this admission revealed WBC: $7.54\times10^3$/μL, RBC: $4.57\times10^6$/μL, Hgb: 13.8 g/dL, PLT: $262\times10^3$/μL, AST: 25 U/L, ALT: 22 U/L, creatinine kinase (CK): 743 U/L, CRP: 0.33 mg/dL, and glucose: 42 mg/dL.

The patient exhibited no significant clinical symptoms and was treated with artesunate (180 mg) every 12 h (at 0, 12, and 24) for two days, from April 4 to April 5, 2023. After the treatment, the patient remained asymptomatic and was discharged on April 6, 2023. One week after discharge, on April 12, 2023, a PB smear showed no abnormalities; CK levels decreased from 743 to 238 U/L, and CRP levels were 0.08 mg/dL. Follow-up assessments over the next three weeks in the outpatient clinic revealed no significant issues.

However, on May 8, 2023, the patient developed a fever of up to 39 °C, accompanied by chills, dizziness, myalgia, and diarrhea. A PB smear revealed numerous gametocytes (infected RBCs: 1/1 HPF) (Fig. 1B), and qPCR confirmed positive *P. ovale* infection (Ct: 21.89). The patient was hospitalized for evaluation on May 10, 2023. Upon admission, a PB smear revealed numerous gametocytes (infected RBCs: 3/1 HPF). Blood tests indicated WBC: $7.64\times10^3$/μL, Hgb: 14.0 g/dL, PLT: $48\times10^3$/μL, AST: 31 U/L, ALT: 19 U/L, total bilirubin: 2.93 mg/dL, LDH: 586 U/L, CK: 34 U/L, and CRP: 16.1 mg/dL. The patient was initially treated with atovaquone and proguanil for two days but continued to experience high fever, chills, myalgia, and nausea/vomiting. Consequently, treatment with primaquine and artesunate was initiated on May 12, 2023, after which the patient's fever subsided. Primaquine was administered orally at a dose of 15 mg once daily for 14 consecutive days, from May 11 to May 23, 2023.

On May 12, 2023, qPCR confirmed the presence of only *P. ovale*. By May 14, the patient was free of fever and chills, with CRP levels at 3.39 mg/dL and PLT recovering to $11\times10^3$/μL. However, on May 15, the patient experienced a recurrence of fever (38.7 °C), with the PB smear showing a few gametocytes (infected RBCs: 1/10



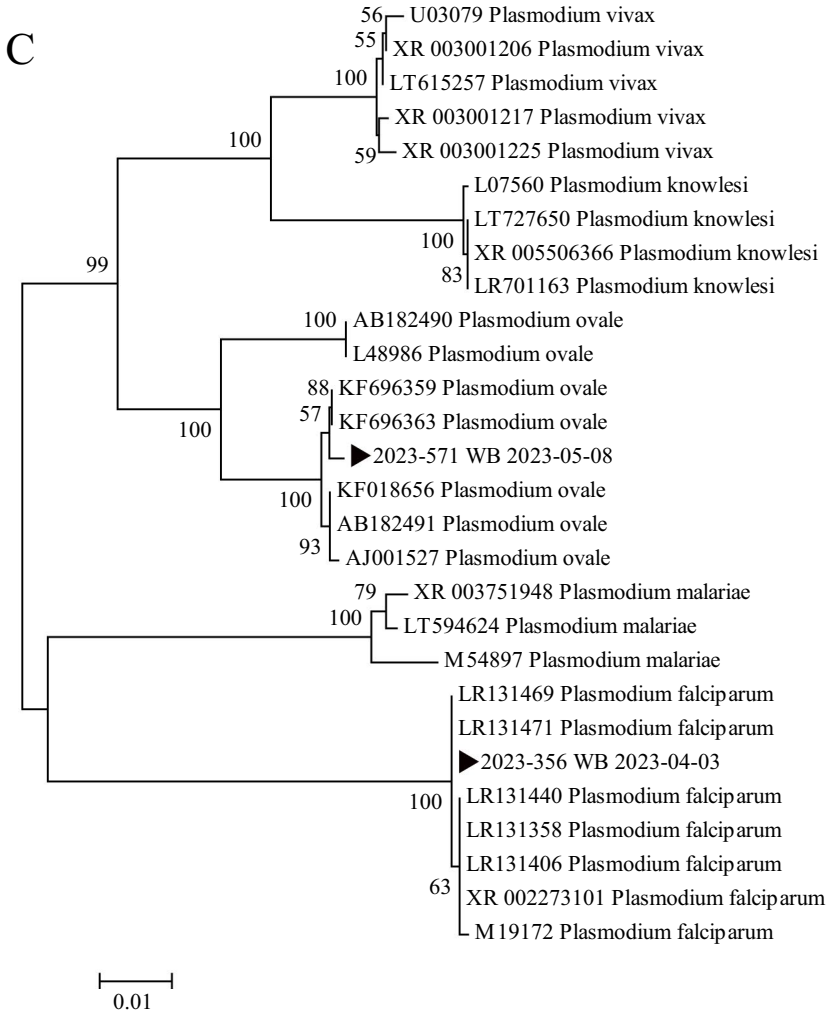




**Fig. 1** A. Peripheral blood smear of a 25-year-old soldier on March 23, 2023, during the first hospitalization. The smear shows low parasitemia with one infected red blood cell per 100 high-power fields, along with a single "banana-shaped" gametocyte. **B**. Peripheral blood smear from the same soldier on May 8, 2023, during the second hospitalization. The smear reveals numerous non-*P. falciparum* gametocytes, indicating a symptomatic *P. ovale* infection. **C**. Phylogenetic tree based on the partial 18S rRNA sequence (875 bp) of *Plasmodium* species. The tree was constructed using the CLUSTAL X software (http://www.clustal.org/clustal2/) with the neighbor-joining method and 1000 bootstrap replicates.

**Table 1** Laboratory test results of a 25-year-old soldier diagnosed with malaria

| Date | Microscopy | Immunochromatography for malaria | | Q-PCR | | C-PCR |
|---|---|---|---|---|---|---|
| | Peripheral blood smear | Pan-*Plasmodium* specific antigen | *P. falciparum* specific antigen | *P. falciparum_msp1* | *P. ovale_msp1* | *18S rRNA* (950 bp) |
| Mar 23rd | Gametocyte of *P. falciparum* | – | + | 35.89 | UD | – |
| Apr 3rd | No abnormalities | + | + | 33.04 | UD | + (*P. falciparum*) |
| Apr 12th | No abnormalities | – | + | UD | UD | |
| Apr 19th | - | – | – | UD | UD | |
| Apr 26th | - | – | + | 37.78 | UD | |
| May 8th | Gametocytes | + | – | UD | 21.89 | + (*P. ovale*) |
| May 10th | Gametocytes | – | – | – | – | – |
| May 15th | Gametocytes | – | – | – | – | – |
| May 12th | – | – | – | UD | 29.86 | + (*P. ovale*) |
| Jun 7th | No abnormalities | – | – | UD | UD | – |
| July 3rd | No abnormalities | – | – | – | – | – |

The table presents the findings from peripheral blood smears, rapid antigen tests using pan-*Plasmodium*-specific antigen and *P. falciparum*-specific antigen, real-time PCR targeting the *msp1* gene for *P. falciparum* and *P. ovale*, conventional PCR targeting the *18S rRNA* gene, and sequencing results

Q-PCR, quantitative polymerase chain reaction; C-PCR, conventional PCR; +, positive; –, negative; UD, undetermined

HPF). By May 16, the PB smear indicated infected RBCs at 1/40 HPF, with the PLT recovering to $258 \times 10^3/\mu L$, and the patient's condition improved, leading to discharge on May 17, 2023.

Follow-up PB smear tests conducted one month later (June 7, 2023) and two months (July 3, 2023) later showed no significant abnormalities.

The PCR was performed using pathogen-specific primers. Real-time PCR using a LightCycler® TaqMan® Master (Roche, Switzerland) targeted the *msp1* genes of *P. falciparum* and *P. ovale*. Meanwhile, conventional PCR (C-PCR) was designed to amplify all *Plasmodium* species by targeting the *18S rRNA* gene. C-PCR was performed using AccuPower® PCR PreMix (Bioneer, South Korea), and PCR was performed at 94 °C for 10 min, [94 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s] for 35 cycles, and 72 °C for 7 min. The PCR products were electrophoresed on a 1.5% agarose gel (Seakem LE agarose). For C-PCR positivity, the PCR products were purified using the QIAquick PCR Purification Kit, followed by direct sequencing to analyze the nucleotide sequences. Subsequently, NCBI BLAST searches were performed to identify the pathogens. A phylogenetic tree was constructed from the sequences of the positive samples using CLUSTAL X and DNASTAR software). According to NCBI BLASTN results, the sample from April 3 matched *P. falciparum* 3D7 (XR_002273101) with 99.78% identity (891/893), whereas the sample from May 8 matched *P. ovale* (KF219559, KF219560, KF696363, and KF696359) with 99.77% identity (866/868) (Fig. 1C). In the 18S rRNA phylogenetic tree, each sequence clustered with *P. falciparum* and *P. ovale*.

## Discussion

Globally, the high mortality and morbidity rates associated with malaria infections are primarily caused by *P. falciparum* [11]. This is due to its virulence factors, which enable cyto-adhesion, allowing infected erythrocytes to adhere to endothelial cells [12]. However, *P. ovale* generally causes milder disease with lower mortality but can lead to relapse several months or even years after the initial infection due to its ability to form dormant liver stages known as hypnozoites [13–15]. Systemic parasitic and bacterial infections-but not viral infections- can activate *P. vivax* hypnozoites [16]. A study in Papua New Guinea demonstrated that relapses contribute significantly to *P. vivax* and *P. ovale* infections, with approximately 80% of *P. vivax* and 60% of *P. ovale* infections attributed to relapses [17].

Of the 5,261 identified studies conducted between 1996 and 2016, data on triple mixed infections were available for 35 studies involving 601 patients from 22 countries. The overall pooled prevalence of triple-mixed infections was 4% (95% CI: 3–5%; $I^2 = 92.5\%$). The pooled proportion of triple-mixed infections compared to double-mixed infections was 12% (95% CI: 9–18; $I^2 = 91\%$). Most of the included studies (29 of 35, or 82.9%) reported a lower proportion of triple-mixed infections than double-mixed infections. Overall, 12,023 PCR-confirmed malaria cases and 3,059 double mixed infections were reported,

including 188 cases (1.56%) of *P. falciparum* and *P. ovale* across 21 studies [6].

Based on data from the Korea National Infectious Disease Surveillance System, 601 imported malaria cases were recorded in the Republic of Korea between 2009 and 2018. Overall, 76.5% of these cases involved Korean citizens returning from endemic regions, particularly from countries in Asia and Africa, where *P. falciparum* (55.7%) and *P. vivax* (30.3%) were the predominant species [18]. Several case reports on imported malaria have been published, including a case of *P. ovale* imported from Ghana, West Africa, in 2004 and three cases of mixed infections, including *P. falciparum*/*P. ovale* from Cameroon and the Democratic Republic of the Congo in 2016, *P. falciparum/P. vivax* from Angola in 2012, and *P. falciparum/P. vivax* from Bangladesh in 2001 [19–22].

In this patient, the *P. falciparum* infection may have reactivated the *P. ovale* infection, highlighting the need for further research on this possibility.

According to Data from India and Ethiopia, traditional methods such as microscopy have lower sensitivity than PCR in diagnosing mixed infections [5]. This suggests that cryptic mixed infections are more commonly detected through PCR testing. In this patient, only *P. falciparum* PCR was positive during the asymptomatic phase, whereas *P. ovale* was confirmed only after symptoms developed, suggesting that hyponozoites of *P. ovale* may be difficult to detect by PCR. Nevertheless, PCR was positive for *P. falciparum* during the asymptomatic phase, and the patient received treatment for *P. falciparum*. It is crucial to consider the possibility of a mixed infection and to use primaquine for radical cure and preventing relapse from hypnozoites in the liver [23].

Long-term mefloquine chemoprophylaxis showed a high level of compliance and was well tolerated, as evidenced by questionnaire results and medical history [24]. Additionally, this was supported by blood concentration measurements, which indicated sufficient levels for prophylactic therapy with minimal side effects. However, mefloquine is not entirely effective, as cases of malaria infection caused by mefloquine-resistant *Plasmodium* species have been reported. The compliance rate for mefloquine was significantly higher than that for doxycycline [24]. This is also reflected in the current case, in which the patient adhered to mefloquine prophylaxis without experiencing any side effects. However, drug concentration was not measured, and resistance testing was not conducted. Therefore, infection in this case may have been due to mefloquine resistance, necessitating further research to explore this possibility. A case of *P. ovale* infection was also reported in a young soldier from Indonesia who received 250 mg/week mefloquine prophylaxis for one year [25]. The patient developed fever and chills two weeks after returning from a mission in the Congo, Central West Africa. It is suspected that the dormant stage of *P. ovale*, quinoline drug resistance, and the efficacy of mefloquine contributed to this phenomenon [25].

In conclusion, this is a case of mixed malaria infection with *P. falciparum* and *P. ovale* originating in South Sudan. This case highlights the challenges in diagnosing and managing asymptomatic malarial infections, particularly mixed infections. It is crucial to conduct surveillance for malaria infections and monitor resistance to prophylactic drugs in high-risk groups, such as individuals returning from malaria-endemic areas. Furthermore, the detection of *P. ovale* after treatment for *P. falciparum* underscores the importance of vigilant follow-up in malaria management.

**Acknowledgements**
None

**Author contributions**
DM Kim designed and coordinated the study, drafted the manuscript, and revised the manuscript for submission. CM Kim contributed to the investigation and methodology and drafted the manuscript. BG Lee, YM Lee drafted the manuscript, and evaluated pathological findings. DY Kim, JW Seo, and NR Yun collected data and performed the analyses. M Muhammad revised the manuscript. All the authors have read and approved the final manuscript.

**Funding**
This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number: RS-2022-KH126458).

**Data availability**
No datasets were generated or analysed during the current study.

## Declarations

**Competing interests**
The authors declare no competing interests.

Received: 22 November 2024 Accepted: 8 March 2025
Published online: 10 April 2025

**References**

1. Prusty D, Gupta N, Upadhyay A, Dar A, Naik B, Kumar N, et al. Asymptomatic malaria infection prevailing risks for human health and malaria elimination. Infect Genet Evol. 2021;93: 104987.
2. Bylicka-Szczepanowska E, Korzeniewski K. Asymptomatic malaria Infections in the time of COVID-19 pandemic: experience from the Central African Republic. Int J Environ Res Public Health. 2022;19:3544.
3. Mahittikorn A, Masangkay FR, Kotepui KU, Milanez GDJ, Kotepui M. The high risk of malarial recurrence in patients with *Plasmodium*-mixed infection after treatment with antimalarial drugs: a systematic review and meta-analysis. Parasit Vectors. 2021;14:280.

4. Kotepui M, Kotepui KU, De Jesus MG, Masangkay FR. Plasmodium spp. mixed infection leading to severe malaria: a systematic review and meta-analysis. Sci Rep. 2020;10:11068.
5. Nema S, Singh A, Krishna S, Poriya R, Dubey S, Ali NA, et al. Unreported mixed *Plasmodium* species infection may increase vivax malaria in India: a challenge for malaria elimination. Trans R Soc Trop Med Hyg. 2022;116:600–3.
6. Kotepui M, Kotepui KU, Milanez GD, Masangkay FR. Prevalence and proportion of Plasmodium spp triple mixed infections compared with double mixed infections: a systematic review and meta-analysis. Malar J. 2020;19:224.
7. Tajebe A, Magoma G, Aemero M, Kimani F. Detection of mixed infection level of *Plasmodium falciparum* and *Plasmodium vivax* by SYBR Green I-based real-Time PCR in North Gondar, north-west Ethiopia. Malar J. 2014;13:411.
8. WHO. World Malaria Report 2021. Geneva: World Health Organization; 2021.
9. Omer AH. Species prevalence of malaria in Northern and Southern Sudan, and control by mass chemoprophylaxis. Am J Trop Med Hyg. 1978;27:858–63.
10. Mitchell CL, Brazeau NF, Keeler C, Mwandagalirwa MK, Tshefu AK, Juliano JJ, et al. Under the radar: epidemiology of *Plasmodium ovale* in the Democratic Republic of the Congo. J Infect Dis. 2021;223:1005–14.
11. Yohanna J, Oti V, Amuta E, Akpu P, Anizoba L. *Plasmodium falciparum* infection among febrile patients attending a tertiary healthcare facility in Central Nigeria: prevalence, hematologic and sociodemographic factors. Int J Trop Med. 2019;2:1–6.
12. Wiser MF. Knobs, adhesion, and severe falciparum malaria. Trop Med Infect Dis. 2023;8:353.
13. Kotepui M, Kotepui KU, Milanez GD, Masangkay FR. Severity and mortality of severe *Plasmodium ovale* infection: a systematic review and meta-analysis. PLoS ONE. 2020;15: e0235014.
14. Merrick CJ. Hypnozoites in *Plasmodium*: do parasites parallel plants? Trends Parasitol. 2021;37:273–82.
15. Zanghi G, Vaughan AM. Plasmodium vivax pre-erythrocytic stages and the latent hypnozoite. Parasitol Int. 2021;1:85.
16. Shanks GD, White NJ. The activation of vivax malaria hypnozoites by infectious diseases. Lancet Infect Dis. 2013;123:900–6.
17. Robinson LJ, Wampfler R, Betuela I, Karl S, White MT, Li Wai Suen CSN, et al. Strategies for understanding and reducing the *Plasmodium vivax* and *Plasmodium ovale* hypnozoite reservoir in Papua New Guinean children: a randomised placebo-controlled trial and mathematical model. PLoS Med. 2015;12:e1101891.
18. Infectious Disease Policy Bureau, Korea Disease Control and Prevention Agency, Division of Zoonoses and Emerging Infectious Diseases. Characteristics of malaria occurrence in the country in 2020 [Internet]. Public Health Weekly Report. 2021; 14:1023–35. www.kdca.go.kr1023
19. Kang Y, Yang J. A case of *Plasmodium ovale* malaria imported from West Africa. Korean J Parasitol. 2013;51:213–8.
20. Kim G, Hong HL, Kim SY, Lee HR, Kim DG, Park S, et al. Mixed infection with *Plasmodium falciparum* and *Plasmodium ovale* in a returned traveller: the first case in Korea. J Korean Med Sci. 2019;34: e23.
21. Shin SY, Yu JH, Kim JY, Kim YJ, Woo HY, Kwon MJ, et al. A case of mixed malaria infection with severe hemolytic anemia after travel to Angola. Infect Chemother. 2012;44:386–90.
22. Shin KS, Kim S, Ryu SW, Suh IB, Lim CS. A case of *Plasmodium vivax* infection diagnosed after treatment of imported falciparum malaria. Korean J Clin Pathol. 2001;21:360–4.
23. WHO. Guidelines for the treatment of malaria. Geneva: World Health Organization; 2015.
24. Hopperus Burna AP, Van Thiel PP, Lobel HO, Ohrt C, Van Ameijden EJC, Veltink RL, et al. Long-term malaria chemoprophylaxis with mefloquine in Dutch Marines in Cambodia. J Infect Dis. 1996;173:1506–9.
25. Liwang F, Ratih DM, Lardo S. *Plasmodium ovale* infection after one year mefloquine prophylaxis in a young Indonesian soldier: a case report. Acta Med Indones. 2019;51:59–62.


## Publisher's Note