Abstract

Plasmodium knowlesi is the fifth species of plasmodium infecting humans and the infection was first discovered in Southeast Asia in 2004. The incidence has been increasingly reported from almost all Southeast Asian countries, including Indonesia. Although the global incidence of malaria has decreased around 50% in the last decade, the increase of knowlesi malaria infection which can cause severe malaria is of concern. During the period of 2018 to 2021, there were seven newfound cases of knowlesi malaria infection in patients treated at hospital in Samarinda, East Kalimantan, Indonesia. The clinical manifestations and laboratory examinations of these patients are described here. All patients were male and worked in mining and palm oil plantations in the forest in several districts in East, North, and South Kalimantan. The diagnosis was based on microscopic examination of Giemsa-stained thin blood smear and confirmed by polymerase chain reaction (PCR) test. Antimalarial treatment was artemisinin-based combination therapy (ACT) / dihydroartemisinin-piperaquine (DHP) fixed-dose combination via oral administration for three days with the doses were based on body weight. All knowlesi malaria patients in this report were presented as uncomplicated cases with great response to ACT after 2-3 days of administration without any adverse effects. Besides fever, gastrointestinal symptoms were major symptoms. Anemia was rare, leukocyte count was normal, however thrombocytopenia was found in all patients. P. knowlesi infection has been discovered in East Kalimantan Province and recently the incidence might be higher than the reported cases, making it resemble an iceberg phenomenon. Therefore, we should build awareness of the rapid increasing of knowlesi malaria cases and its prevention.

Keywords

Plasmodium knowlesi, Malaria, East Kalimantan, Indonesia
Introduction
Malaria remains a public health problem in several Indonesia regions, including East Kalimantan Province. The majority of East Kalimantan land is covered by forest with some districts still malaria endemic with either low, middle or high endemcity. National data showed there were 250,664 malaria cases in 2019 in Indonesia, which East Kalimantan having the fourth highest number of cases.1

The incidence of malaria worldwide has decreased in the last decade, with the incidence of malaria in Indonesia is decreasing significantly too.2 The majority of malaria cases in East Kalimantan were caused by Plasmodium falciparum and Plasmodium vivax. However, in 2018 we found a malaria case caused by Plasmodium knowlesi in East Kalimantan. Previously, knowlesi malaria cases had been reported from South Kalimantan and Central Kalimantan Province, the neighbouring provinces in the south and the west in 2013.3 Another region in Indonesia that had also reported P. knowlesi cases was Sumatera Island (North Sumatera and Aceh Province).14 In the period of 2008 to 2015, there were 418 cases of P. knowlesi infection reported from Indonesia.6 The first reported case was from our neighbouring state Sarawak, Malaysian Borneo in 2004 and since that time the incidence has been increasing rapidly.7 Until 2016, P. knowlesi infection in humans had been reported from South China, Myanmar, Thailand, Vietnam, Laos, Cambodia, the Philippines, Singapore, Peninsular Malaysia, Brunei, Indonesia and the highest incidence was reported from Malaysia (18,687 cases from 2010 to 2018).6 There were also increasing numbers of knowlesi malaria cases imported from Southeast Asia to Europe, Asia, America and Oceania.8 P. knowlesi vectors are member of the Anopheles leucosphyrus group that are found in Southeast Asia, associated with dense jungle and forest fringe, rest and feed outdoors (exophagic) typically after dusk.9

Plasmodium knowlesi is known as the fifth Plasmodium species that can cause malaria in human beings, previously it was reported that it could infect the long-tailed macaque (Macaca fascicularis), and pig-tailed macaca (Macaca nemestrina, Macaca leonina).6,10 Plasmodium knowlesi infection can cause severe malaria like P. falciparum and P. vivax and recently its diagnosis should be confirmed by polymerase chain reaction (PCR) test since it is difficult to differentiate between P. knowlesi and P. Malariae morphology by microscopic examination only. The ongoing increase of P. knowlesi cases poses a major challenge in malaria control and elimination program in Southeast Asia, including Indonesia.6

We would like to report the features of P. knowlesi infection cases in Samarinda, East Kalimantan Province, Indonesia from 2018 to 2021. This article was approved by The Ethical Committee for Health Research at Abdul Wahab Sjahranie General Hospital Samarinda, East Kalimantan (approval number 083/KEPK-AWS/V/2022).

Case presentation
There were seven knowlesi malaria patients treated at hospital in Samarinda, East Kalimantan, Indonesia from 2018 to 2021. All patients were male and aged 34 to 56 years old (mean age 41.1 years). They worked in mining and palm oil plantations in the forest in several districts in East, North, and South Kalimantan (see Table 1). The patients’ locations were near to Sarawak, Malaysia, where P. knowlesi infection was initially discovered in Southeast Asia (see Figure 1). Four patients visited or worked at districts in East Kalimantan Province that were still malaria endemic areas. Two patients worked in South Kalimantan Province that had reported knowlesi malaria cases since 2013. One patient visited a district in North Kalimantan Province that was also a malaria endemic area. The patients presented to the hospital with three or four days of fever and chills. Besides fever, all patients had gastrointestinal symptoms such as nausea, vomiting, diarrhea, and abdominal pain. There was no patient who showed the clinical manifestations of severe malaria based on World Health Organization (WHO) criteria 2015.11 The patients had no concomitant conditions or a history of inherited or familial illnesses. Their physical examinations were normal, no abnormalities were found.

| Table 1. Distribution of patients treated at hospital in Samarinda (2018–2021). |
|---|---|---|---|---|
| Age | Sex (M/F) | Ethnicity | Occupation | Workplace/place visited |
| 1. | 34 | M | Dayak | Palm oil plantation worker | Kutai Kartanegara, East Kalimantan |
| 2. | 38 | M | Javanese | Palm oil plantation worker | West Kutai, East Kalimantan |
| 3. | 49 | M | Dayak | Miner | Berau, East Kalimantan |
| 4. | 25 | M | Dayak | Miner | Kutai Kartanegara, East Kalimantan |
| 5. | 56 | M | Banjarese | Miner | South Kalimantan |
| 6. | 51 | M | Banjarese | Miner | South Kalimantan |
| 7. | 35 | M | Banjarese | Forestry civil servant | Malinau, North Kalimantan |
Diagnosis of *P. knowlesi* infection was based on microscopic examination of Giemsa-stained thin blood smear and confirmed by PCR test performed at the National Institute of Health Research and Development, Ministry of Health, Indonesia. We used Olympus CX21 binocular microscope to identify the morphology. Blood smear tests of the patients showed erythrocytes with early trophozoite/ring form stage and band form trophozoite of *P. knowlesi* (see Figure 2 and Figure 3). All patients have been confirmed by PCR test and the results were *P. knowlesi* positive.

Antimalarial treatment used was a fixed-dose combination of dihydroartemisinin-piperaquine (DHP) (40/320 mg) via oral administration once daily (3 tablets for body weight 41–60 kg, 4 tablets for body weight 61–80 kg, 5 tablets for body weight > 80 kg) for three days and primaquine 15 mg single dose on day one. Patients were treated at hospital for 3–5 days.
Parasite counts ranged from 3,194 parasites/μL blood to 12,981 parasites/μL blood with a mean of 6,538 parasites/μL blood. No one had the criteria of severe malaria with hyperparasitemia (parasite count > 20,000/μL blood) according to WHO criteria 2015. Two patients had co-infection with dengue virus confirmed by positive anti-dengue IgG. There was no difference in malaria treatment between patients with dengue co-infection and other patients who did not. Hemoglobin levels ranged from 9.8 g/dL to 16.1 g/dL (co-infection with dengue virus) with mean Hb level was 13.6 g/dL. There was only one patient who showed moderate anemia (9.8 g/dL). Leukocyte counts ranged from 5,300/μL to 8,900/μL with a mean of 7,010/μL. This meant all patients had normal leukocyte counts. Platelet counts ranged from 16,000/μL to 108,000/μL with a mean of 69,714/μL. This showed that all patients had moderate to severe thrombocytopenia. The lowest platelet count (16,000/μL) was found in a patient co-infected with dengue virus. Ureum levels ranged from 24.0 mg/dL to 47.9 mg/dL with mean ureum level was 32.8 mg/dL. Creatinine levels ranged from 0.8 mg/dL to 1.4 mg/dL with mean creatinine level was 1.0 mg/dL. No patients had renal dysfunction.

All patients showed great response to ACT given (dihydroartemisinin-piperaquine for three days) without any adverse effects occurred and free of fever on day 2 or 3 after treatment. The patients were discharged from the hospital after they were recovered.

**Discussion**

Southeast Asia and Southern China are regions with natural distribution of long-tail macaques and mosquitoes of the *Anopheles leucosphyrus* group. *Anopheles balabacensis* is known as the most efficient vector, capable of transmitting *P. knowlesi* from monkey-to-human, human-to-human, and human-to-monkey. In Sabah, Malaysian Borneo *Anopheles balabacensis* is the primary vector of *P. knowlesi* and found in village, forest and farming sites. Although human *P. knowlesi* is largely a zoonosis, human-to-human transmission could increase with time and parasite adaptation.

The western and central parts of Indonesia (Sumatera, Java, Kalimantan/Borneo Islands) are included in the region where long–tail macaques and *Anopheles leucosphyrus* group are found. The first *knowlesi* malaria case in human being was reported from Sarawak, Malaysian Borneo in 2004 and the number of cases has been increasing rapidly. Epidemiology studies from Malaysia in the beginning of 2010 showed that 50–60% of malaria cases were caused by *P. knowlesi*. A hospital surveillance study from Sabah, Malaysian Borneo in 2015–2017 showed that of a total of 3,876 malaria cases recorded *P. knowlesi* accounted for 80%, 88%, 98% malaria cases in 2015, 2016, 2017, and the rest were caused by *P. falciparum and P. vivax*. This study also showed that the majority of *P. knowlesi* cases occurred in adults, while children < 13 years accounted for only 5.8% of cases. In 2016 WHO World Malaria Report documented substantial progress toward control and elimination of malaria, however the emergence of *P. knowlesi* as an important cause of human malaria in Southeast Asia should be considered as a major challenge in malaria control and elimination program in this region. Outside of Malaysia, *P. knowlesi* is frequently misdiagnosed by microscopic examination as *P. falciparum or P. vivax*, therefore *P. knowlesi* may be underdiagnosed and its true incidence is underestimated. There are five provinces in Kalimantan (part of Indonesia) and South Kalimantan province reported their first case in 2013, then...
followed by Central Kalimantan Province. First confirmed *P. knowlesi* case in East Kalimantan Province was found in 2018 in Samarinda, five years after the first confirmed case in our neighbouring province, South Kalimantan. It means that four of five provinces in Kalimantan Island have reported knowlesi malaria cases. It is possible that the cases occurred in East Kalimantan Province long before the first confirmed case in 2018 caused by microscopic misdiagnosis as *P. falciparum* or *P. vivax*, the two major *Plasmodium* species found in this region. Data from the East Kalimantan Provincial Health Office from 2019 to 2021 showed that there were 191 suspected malaria knowlesi cases, while *P. vivax* and *P. falciparum* accounted for 55.2% and 38.1%, 50.2% and 45.8%, 50.5% and 48.1% of total malaria cases reported in 2019, 2020, 2021, respectively (unpublished). Nowadays, the true incidence of *P. knowlesi* cases in East Kalimantan Province may be much higher than the confirmed cases found.

The incubation period of *P. knowlesi* infection is 3–14 days (mostly > 8 days). Besides fever, other non-specific symptoms such as headache, muscle pain, joint pain, nausea, abdominal pain, lost of appetite are frequently found. A study in Borneo showed that thrombocytopenia was the most common laboratory abnormality in *P. knowlesi* infection, while anemia appeared to be mild. Some studies in Borneo showed that the risk of severe malaria in adults caused by *P. knowlesi* appeared at least as high as that of *P. falciparum* (severe malaria in patients with *P. knowlesi* 29%, in *P. falciparum* 11%, in *P. vivax* 16%). *Plasmodium knowlesi* has the shortest asexual replication cycle of all *Plasmodium* species leading to rapidly increased parasitemia levels. *Plasmodium knowlesi* has lower threshold of parasitemia (> 20,000 parasites/µL blood) than *P. falciparum* (> 500,000 parasites/µL blood) to be classified as severe malaria with hyperparasitemia. A hospital study in Sabah, Malaysia showed that the risk of severe knowlesi malaria increased 11-fold with parasitemia > 20,000/µL and 28-fold with parasitemia > 100,000/µL. A hospital surveillance study from Sabah, Malaysian Borneo from 2015–2017 showed that case fatality rate of *P. knowlesi* cases was 1.7 per 1,000 cases.

Microscopic examination of thin blood smear can not differentiate *P. knowlesi* and *P. malariae*, therefore PCR test is used to confirm the species. However, a case report from Sarawak, Malaysian Borneo showed that rapid diagnostic test by OptiMAL and BinaxNOW could detect *P. knowlesi* infection (pan-malarial lactate dehydrogenase (LDH) and pan-malarial aldolase) although it was not specific and should be confirmed by PCR. Recently, *P. knowlesi*-specific rapid diagnostic tests (RDTs) have demonstrated low sensitivity. A systematic review of 40 studies showed that the sensitivities of RDTs in detecting *Plasmodium knowlesi* infections ranged from 2% to 48%.

Antimalarial used to treat uncomplicated knowlesi malaria case is artemisinin-based combination therapy. Currently, based on National Guidelines, we use a fixed-dose combination of dihydroartemisinin-piperaquine (40/320 mg) for 3 days (3–5 tablets once daily based on body weight) and primaquine 15 mg single dose. A study in Malaysia using another ACT, a fixed-dose combination of artemether-lumefantrine showed parasite clearance time (PCT)_90 was 13.7 hours and microscopy negative at 48 hours reached 100%. Artesunate intravenous injection is used in the management of severe knowlesi malaria like other severe malaria cases caused by *P. falciparum* or *P. vivax*. In Indonesia nowadays artesinin derivatives remain show good efficacy in treating uncomplicated and severe malaria.

Overall, the discovery of *P. knowlesi* infection cases in East Kalimantan, where cases had already been reported in the neighboring country, is particularly noteworthy for this study. However, there were not enough *P. knowlesi* instances to characterize in general due to the small number of reported cases would be the limitation of this study. The cause of the limitation was the difficulty to distinguish the morphology of *P. knowlesi* from the others by microscopic examination.

**Conclusion**

*Plasmodium knowlesi* infection in human beings, previously reported from other regions of Kalimantan, Indonesia had also been found in East Kalimantan Province. All cases reported here were adult males working in mining and oil palm plantations in the forest and presented as uncomplicated malaria. Besides fever, gastrointestinal symptoms were major symptoms. Anemia was rare, leukocyte count was normal, but thrombocytopenia was found in all patients. All patients showed great response to ACT given (dihydroartemisinin-piperaquine for three days) without any adverse effects occurred and free of fever on day 2 or 3 after treatment. *P. knowlesi* infection that can cause severe malaria has been discovered in East Kalimantan Province and recently the incidence might be higher than the reported cases, making it resemble an iceberg phenomenon. Therefore, we should build awareness of the rapid increasing of knowlesi malaria case and its prevention.
Data availability
All data underlying the results are available as part of the article and no additional source data are required.

Consent
Written informed consent for publication of their clinical details and clinical images was obtained from the patients.

References

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