

Rapid-Antigen Test Negative Malaria in a Traveler Returning From Thailand, Molecularly Diagnosed as *Plasmodium knowlesi*

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Plasmodium knowlesi has been identified in the last decade as a fifth species causing malaria in areas of South East Asia. Due to its short erythrocytic cycle, rapid development of high parasitemia and severe manifestations are frequently observed. Therefore, prompt diagnosis of infection is essential to prevent complications, but the low sensitivity of rapid diagnostic tests for *P knowlesi* pose a diagnostic challenge in acute settings. In this study, we report the case of a German traveler to Thailand, who was treated for *P knowlesi* malaria after returning to Germany. Rapid antigen test for malaria was negative on presentation. Diagnosis of a nonfalciparum malaria was made based on microscopy, and species definition was determined using polymerase chain reaction technique.

Keywords. *Plasmodium knowlesi*; Thailand; travel medicine.

CASE PRESENTATION

In February 2015, a 42-year-old German male presented to the emergency department of the University Medical Centre in Hamburg, Germany with a 6-day history of fever up to 40°C, arthralgia, and retro-orbital pain. The day before presentation, he had returned from a 10-week trip to Thailand, where he had spent 2 months on Little Koh Chang, an island in the Andaman Sea in the Ranong Province. At the end of his trip, he spent 5 days in Hua Hin, a town at the Gulf of Thailand in the Prachuap Khiri Khan Province, and 5 days in Bangkok. He had not taken antimalarial chemoprophylaxis while being in Thailand.

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There was no past medical history of note. On clinical examination, a temperature of 39.1°C, blood pressure of 156/80 mmHg, and a pulse of 106/minute were noted. The respiratory rate was recorded at 16 breaths/minute with an oxygen saturation of 95% on room air. The full blood count showed a thrombocytopenia of $81 \times 10^9/L$ and was otherwise normal; the C-reactive protein was 55 mg/L (reference range up to 5 mg/L). The liver enzymes were slightly elevated with an aspartate aminotransferase of 62 U/L (reference range, 10–50 U/L) and an alanine aminotransferase of 106 U/L (reference range, 10–50 U/L). The bilirubin was 0.5 mg/dL (reference range, <1.2 mg/dL). Rapid diagnostic tests for malaria (Alere BinaxNOW, which detects the *Plasmodium falciparum*-specific Histidine-rich Protein II and the panmalarial Aldolase) and dengue fever gave negative results. However, a thick blood film showed plasmodia trophozoites at a density of 920/μL. On thin film, the microscopists on duty were unable to differentiate the *Plasmodium* species in the acute setting. Based on the negative result of the rapid-diagnostic test, a nonfalciparum malaria was suspected. The patient was admitted to the infectious disease ward. Treatment was started with atovaquone/proguanil 250/100 mg 4 tablets daily over 3 days. The patient rapidly recovered and was discharged 4 days after admission. For further parasite differentiation, malaria microscopy was repeated by the head parasitologist. After intense reading of the Giemsa-stained thin blood film, 2 parasite-infected erythrocytes were identified with morphological characteristics compatible with *Plasmodium malariae* or *P knowlesi* infection (Figure 1). Subsequently, species-specific polymerase chain reactions (PCRs) for the 5 human plasmodia species confirmed the presence of a monoinfection with *P knowlesi* [1, 2].

DISCUSSION

Plasmodium knowlesi was first described in detail in 1932 by Biraj Das Gupta and Robert Knowles in Kalkutta as malaria of macaques [3]. *Plasmodium knowlesi* gained recognition as the fifth human malaria parasite in 2004, when Singh et al [4] discovered that 58% of microscopically diagnosed malaria patients in the Kapit Division, Sarawak, Borneo, Malaysia were positive for *P knowlesi* by PCR.

So far, the vast majority of *P knowlesi* patients have been diagnosed in Sabah and Sarawak, Borneo, Malaysia. Increasing incidences of *P knowlesi* malaria have been reported from Sabah and Sarawak in the last few years [5–7]. *Plasmodium knowlesi* infections have also been identified in Thailand, Vietnam, Singapore, Indonesia, Cambodia, Myanmar, and the Philippines [8, 9]. Travelers returning from Southeast Asia with imported *P knowlesi* malaria have occasionally been reported from nonendemic

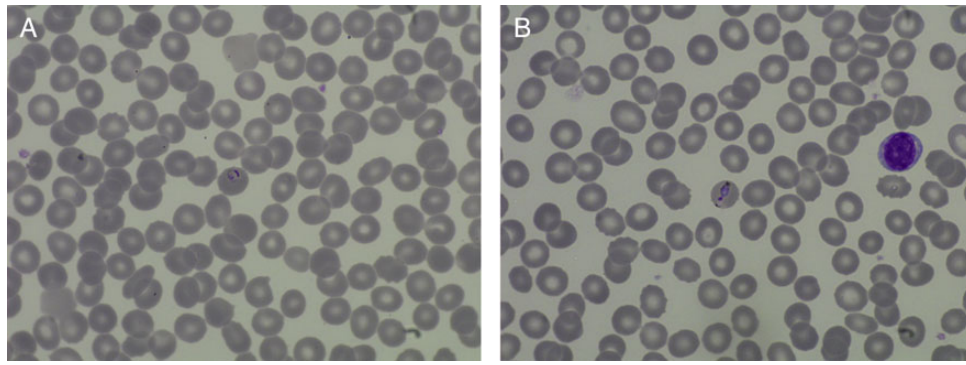


Figure 1. *Plasmodium knowlesi* trophozoites in the thin blood film. Shown are 2 microscopic images of a Giemsa-stained thin blood film of the patient, each containing a parasite-infected erythrocyte: (A) young trophozoite (ring form); (B) late trophozoite (band form).

countries including the United Kingdom, Australia, Japan, Sweden, and Germany [3, 10–13]. Vicinity to natural forest areas is the main risk factor for contracting *P knowlesi* infection. Forests are the natural habitat of the natural hosts of *P knowlesi*, the long- and pig-tailed macaques [14]. Most patients with documented *P knowlesi* malaria lived in or at the fringe of forestry areas [3]. Although our patient had not specifically visited a forest or national park, he stayed close to forestry areas on Little Koh Chang and participated in a rafting tour close to Hua Hin as potential exposures. From both areas, the Ranong and Prachuap Khiri Khan provinces, *P knowlesi* infections in humans have been repeatedly reported [13, 15–18]. The short time duration between the rafting tour and development of fever (4 days) make an infection at Little Koh Chang more likely, but short incubation periods with a minimum of 3 days have been observed with *P knowlesi* [19].

Plasmodium knowlesi presents several diagnostic challenges. *Plasmodium knowlesi* can be detected by rapid diagnostic tests targeting panmalarial *Plasmodium* lactate dehydrogenase (pLDH), *Plasmodium vivax*-specific pLDH, *P falciparum*-specific pLDH, or panmalaria Aldolase, but sensitivities are low [14, 20, 21]. In particular, in infections with low parasitemia, rapid diagnostic tests often fail to diagnose an acute *P knowlesi* malaria, as seen with our patient [20]. Therefore, diagnosis of infection relies primarily on microscopy of blood films, which requires highly trained microscopists. However, even in the hands of an experienced microscopist, *P knowlesi* can be easily misdiagnosed as *Plasmodium malariae* due to morphological similarity of the 2 parasite species. Molecular genetic analysis of malaria patients in Sabah, Borneo determined that up to 83% of malaria patients microscopically diagnosed with *P malariae* malaria were indeed infected with *P knowlesi* [22]. Polymerase chain reaction is the most reliable method to distinguish *P knowlesi* from other species, but it is usually not available in an acute setting. This diagnostic challenge has important consequences for patient management. Although *P malariae* malaria

rarely causes complications, being termed “benign quartan malaria”, *P knowlesi* infection can potentially be fatal because the parasite is able to rapidly develop high parasitemia due to its short erythrocytic cycle of only 24 hours. Severe courses of *P knowlesi* malaria are observed in approximately 10% of hospitalized patients and include respiratory distress syndrome or renal failure [6, 23, 24].

Several treatment options exist for uncomplicated *P knowlesi* infection. To date, chloroquine remains widely used for the treatment of confirmed *P knowlesi* infection [8, 25]. However, due to the increasing chloroquine resistance rates among *P falciparum* in Asia, chloroquine should only be used when a *P falciparum* infection or coinfection can be safely excluded [8]. Alternatively, artemisinin-combination drugs are increasingly used as first choice and offer the advantage to cover all malaria species [8, 26–28]. Furthermore, the first randomized controlled trial for the treatment of *P knowlesi* malaria, which compared treatment with chloroquine to mefloquine-artesunate, showed a faster parasite clearance when using mefloquine-artesunate [29]. Other treatment options for *P knowlesi* include atovaquone/proguanil, which also treats all malaria species [27]. Severe *P knowlesi* infection should be treated with parenteral artesunate [8, 28].

CONCLUSIONS

Our case highlights the need to consider *P knowlesi* malaria in febrile patients residing in or traveling from Southeast Asia in whom rapid antigen test for malaria is negative and microscopic species definition of plasmodia is inconclusive or a nonfalciparum species is suspected. Polymerase chain reaction assays remain an important tool to reliably identify *P knowlesi*.

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