Plasmodium knowlesi infection imported to Germany, January 2013

H Orth[1], B O Jensen[1], M C Holtfreter[1], S J Kocheril[1], S Mallach[1], C MacKenzie[2], I Müller-Stöver[1], B Henrich[2], M Imwong[3], N J White[4], D Häussinger[1], J Richter (Joachim.Richter@med.uni-duesseldorf.de)[1]

1. Department of Gastroenterology, Hepatology and Infectious Diseases, Heinrich-Heine-University, Düsseldorf, Germany
2. Department of Medical Microbiology and Hospital Hygiene, Heinrich-Heine University, Düsseldorf, Germany
3. Department of Molecular Tropical Medicine and Genetics, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand
4. Mahidol-Oxford Tropical Medicine Research Unit; Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

Citation style for this article:
Orth H, Jensen BO, Holtfreter MC, Kocheril SJ, Mallach S, MacKenzie C, Müller-Stöver I, Henrich B, Imwong M, White NJ, Häussinger D, Richter J. Plasmodium knowlesi infection imported to Germany, January 2013. Euro Surveill. 2013;18(40):pii=20603. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20603

Article submitted on 02 October 2013 / published on 03 October 2013

Plasmodium knowlesi **was known as a plasmodium of macaques until *P. knowlesi* transmission to humans was recognised in Borneo and later throughout South-East Asia. We describe here a case of a *P. knowlesi* infection imported to Germany from Thailand. The patient had not taken antimalarial chemoprophylaxis and suffered from daily fever attacks. Microscopy revealed trophozoites and gametocytes resembling *P. malariae*. *P. knowlesi* malaria was confirmed by PCR.**

In January 2013, a 55 year-old German woman presented to her practitioner because of fever, nausea and vomiting ten days after a holiday in Thailand. She had not taken antimalarial chemoprophylaxis. She was referred to hospital, where laboratory abnormalities included a decreased platelet count (27,000x10^9/L), elevated aspartate aminotransferase (AST) (237 U/L; normal value (nv): ‹35 U/L), alanine aminotransferase (ALT) (277 U/L; nv: ‹45 U/L), gamma-glutamyltransferase (gamma-GT) (480 U/L; nv: ‹55 U/L), lactate dehydrogenase (LDH) (419 U/L; nv: ‹248 U/L) and C-reactive protein (CRP) levels (102 mg/L; nv: ‹5 mg/L). Red blood cell count, white blood cell count (7.360x10^9/L), electrolytes, urea, creatinine and CK were normal. Electrocardiography showed no abnormalities except for sinus tachycardia with a heart rate of 105 beats per minute Suspecting a bacterial infection, empirical antibiotic treatment with piperacillin and tazobactam was initiated. However, the patient continued to suffer from daily fever attacks. The serum creatinine rose to 3.45 mg/dL, while she became oliguric. The procalcitonine level rose to 3.71 ng/mL, interleukin-6 to 66.8 pg/mL, haematocrit fell to 29.7%, and microscopy of stained blood films revealed malaria parasites, but the hospital's microbiologist reported that he felt unable to identify a specific *Plasmodium* species.

With a diagnosis of malaria and acute renal failure, the patient was referred to our Tropical and Infectious Diseases service. Rapid immunochromatography test (Malaria now Binax, United States) showed a negative result for *P. falciparum*-specific histidine-rich protein-2 but was positive for pan-plasmodial aldolase. In stained thin and thick blood films, plasmodia resembling *P. malariae* were present with 0.2% trophozoite parasitaemia and numerous gametocytes. *P. knowlesi* malaria was suspected because of the disease severity and the patient's recent stay in Khao Sok National Park in southern Thailand. A multiplex real-time PCR for the species *P. falciparum*, *P. ovale*, *P. vivax* and *P. malariae* detected in addition the presence of plasmodial DNA on genus level. The sequence of the amplified genus-specific DNA was homologous with the species *P. knowlesi*. Infection by this parasite was confirmed by a specific PCR in the Faculty of Tropical Medicine, Mahidol University Bangkok, Thailand. DNA was extracted from a dry blood spot using QIAamp blood mini kit (Qiagen, Germany). *Plasmodium spp*. infection were tested using three PCR protocols based on 18sRNA [1-2], mitochondrial DNA [3], the linker region of dihydrofolate reductase and thymidylate synthase [4]. Monoinfection of *P. knowlesi* was confirmed by all three techniques. Direct sequencing from PCR products were also performed and showed more than 98% identity to reference *P. knowlesi*.

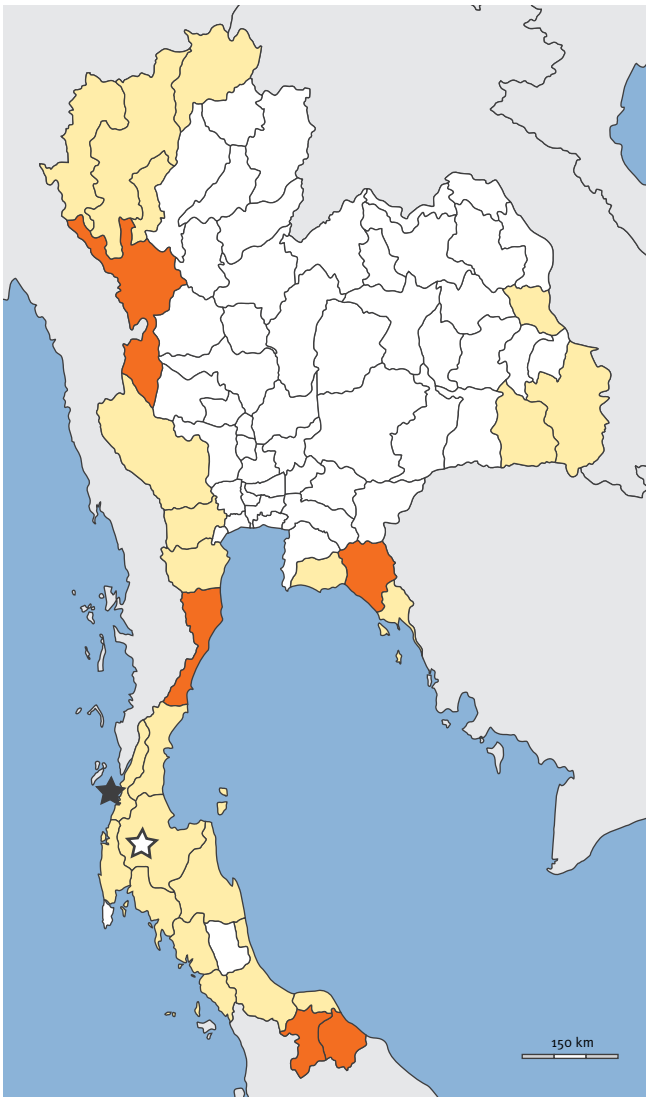
Intravenous treatment with artesunate (2.4 mg/kg) was started on Day 3 after admission and switched to oral treatment with artemether/lumefantrine the following day. The patient recovered promptly and malaria parasites cleared within two days. With intravenous isotonic saline administration, urine output was restored and serum creatinine fell to 1.3 mg/dL on Day 4.

Discussion

P. knowlesi was first described in 1931 as a plasmodium of long tailed and pig-tailed macaques and one year later was shown to be transmissible to humans [5]. A naturally acquired human infection was reported 1965 in a citizen of the United States returning from Malaysia [6]. Thereafter, human *P. knowlesi* infections were reported only occasionally until 2004, when a study by Singh et al. revealed that *P. knowlesi* accounted for more than 50% of endemic human

Figure

Presumable geographical site of acquisition of *Plasmodium knowlesi* infections, Thailand, 2010 and 2013



Orange: Provinces with *P. knowlesi* transmission [3];
yellow: provinces with stable malaria transmission;
black star: presumable site of infection of a French tourist [9];
white star: presumable site of infection of our patient.

malaria cases in Kapit division of the Malaysian state of Sarawak, located on the island of Borneo [7].

With increasing awareness of this pathogen, *P. knowlesi* has been diagnosed frequently in human malaria cases on the island of Borneo, and has also been reported from Indonesia, the Malay peninsula, Myanmar, the Philippines, Singapore, Thailand and Vietnam [8].

As a cause for imported malaria, *P. knowlesi* has been identified only occasionally: only five cases of *P. knowlesi* malaria imported to Europe have been published so far. The first case was a Swedish traveller to Malaysian Borneo in 2006, the second a Finnish traveller to peninsular Malaysia in 2007, the third was a Spanish traveller in 2009 who had spent six month in several south east Asian countries including Indonesia, Malaysia, Thailand and Vietnam, and the fourth was a French tourist who presumably acquired the infection on the island of Kho Phayam (Thailand) in 2010 [9-12] (Figure). The most recent case was reported in August 2013 and occurred in a German traveller with human immunodeficiency virus (HIV) co-infection who presumably acquired the infection in Ranong province in Thailand [13].

The retrospective analysis of blood samples from Thailand suggests that the prevalence of *P. knowlesi* infections has not changed significantly over time during the period from 1996 to 2008 [3]. Therefore, it is most likely that the increasing number of cases recognised is due to the awareness of the possibility of human *P. knowlesi* malaria and to the application of diagnostic molecular biology techniques to differentiate this parasite from other malaria parasites.

The prevalence of *P. knowlesi* infections in Thailand (1%) is very low compared with the highly endemic Kapit division (50%) in Borneo, Malaysia Thailand [3,7]. Therefore, large numbers of imported cases from Thailand are not to be expected in the near future. However, changing tourism patterns like the trend towards eco-tourism might increase the risk of infection with *P. knowlesi* even in low prevalence countries. In the present case for instance, the infection was most likely acquired during a stay in the forested Khao Sok National Park inhabited by the natural monkey host.

It is important to recognise *P. knowlesi* infections, especially in the late stage when the parasites resemble *P. malariae*, because *P. knowlesi* infections can sometimes be associated with complications and may be fatal. A study on 107 patients reported severe malaria in 6.5% of *P. knowlesi* infections, among these three cases presenting with acute renal impairment. Whereas these severe *P. knowlesi* cases reported were associated with hyperparasitaemia, acute kidney failure occurred in our case despite a low parasitaemia of 0.2% [14]. Because of the possibility of a severe course of *P. knowlesi* infections, physicians must be increasingly aware of this possibility and contact specialised centres as soon as possible to ensure early appropriate diagnosis and timely treatment.

Conflict of interest

None declared.

Authors' contributions

HM O and JR treated the patient and wrote the paper. MC H, C MkC, B H and M I did the parasitological and molecular investigations and contributed to writing the manuscript. BEO J, SJ K, S M, I MS, and D H managed the patient and contributed to the manuscript. All authors participated in writing the manuscript and approved the final version.

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