CASE REPORT

Plasmodium knowlesi infection: a diagnostic challengeLijia Fan,¹ Shir Ying Lee,² Evelyn Koay,³ Christian Harkensee¹

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SUMMARY

Plasmodium knowlesi malaria is an uncommon, but highly prevalent parasitic infection in parts of Malaysia. This is the case of a 14-year-old Singaporean boy presenting to our emergency department with an 11-day history of fever following a school trip to Malaysia. Hepatosplenomegaly was the only clinical finding; laboratory tests showed thrombocytopenia, lymphopenia, mild anaemia and liver transaminitis. Specific malaria antigen tests were negative, but the peripheral blood film showed plasmodia with atypical features, with a parasite load of 0.5%. PCR confirmed the diagnosis of *P. knowlesi*. The patient was successfully treated with chloroquine. The clinical course of *P. knowlesi* malaria is indistinguishable from that of *Plasmodium falciparum*. This case highlights the importance of taking detailed travel history, careful examination of malaria blood films and judicious use of molecular techniques. Antigen tests alone may have missed a malaria diagnosis altogether, while blood film examination may wrongly identify the species as *Plasmodium malariae* or *P. falciparum*. Third-generation PCR assays can be used to reliably identify *P. knowlesi*.

BACKGROUND

In endemic areas of Malaysia, particularly Western Malaysia and the state of Sarawak, *Plasmodium knowlesi* prevalence of hospitalised malaria cases can be as high as 50%,¹ and the risk for travellers to these regions to contract *P. knowlesi* is substantial. Diagnosis of *P. knowlesi* malaria is difficult with commonly used tests (thin blood film and antigen tests). This could potentially expose patients to either unnecessary investigations and treatment, or if the wrong species is identified, underestimation of the severity of the illness. Unlike *Plasmodium falciparum*, *P. knowlesi* has an animal reservoir in the long-tailed macaque (*Macaca fascicularis*), indicating a shared vector preference for humans as well as this particular monkey species.² Various species of *Anopheles leucosphyrus*, a dusk-active forest-dwelling group of mosquitos, were identified as vectors for *P. knowlesi*.^{1–3} *P. knowlesi* malaria can present with the same life-threatening complications as *P. falciparum* malaria and can potentially be fatal.¹

CASE PRESENTATION

We present a case of 14-year-old boy from Singapore, previously healthy, who had a history of 11 days of daily nocturnal fever of 39–40°C with significant chills and rigours. He was also experiencing nausea and vomiting of 1–2 episodes a day,

associated with loose stools. Five days before the onset of fever, he had returned from a four day school trip to Perak in Western Malaysia. The group stayed in open cabins in a rainforest area and participated in outdoor activities which included swimming, white water rafting, kayaking and cave exploration. No antimalarial prophylaxis was taken and the only protective measure against mosquito bites was the use of repellents.

On physical examination, the patient was pale, miserable and lethargic but fully conscious. His peak fever at presentation was 40.3°C. He had non-tender hepatosplenomegaly. There was no jaundice, conjunctivitis, lymphadenopathy or rash.

INVESTIGATIONS

Initially a full blood count (FBC), C reactive protein (CRP), liver function tests and blood cultures were performed. The FBC showed leukopenia (white blood cells $2.56 \times 10^9/l$ (reference range 3.9–9.6)) and neutropenia (neutrophil count of $0.79 \times 10^9/l$ (1.27–6.20)) with mild anaemia (haemoglobin 10.9 g/dl (12.9–17.0)) and thrombocytopenia (platelet count $61 \times 10^9/l$ (132–372)). Liver function tests showed a mild transaminitis (aspartate aminotransferase 43 U/l (10–40), alanine transaminase 34 (10–35)) and elevated lactate dehydrogenase at 1107 U/l (250–580). Renal function and electrolytes were normal. The CRP was elevated at 149 mg/l (0–10). Three sets of malaria blood films were obtained on admission. The blood culture showed no bacterial growth, the urine microscopy and culture was normal.

DIFFERENTIAL DIAGNOSIS

Given the significant travel history to Perak, Malaysia, and prolonged fever with clinical finding of hepatosplenomegaly, typhoid fever and malaria were the main differential diagnoses. Leptospirosis was also considered because of extensive involvement in water-sport activities. Leptospirosis IgM serology was negative, and blood culture subsequently showed no salmonella bacteraemia. The diagnosis was made on the malaria blood film that reported the presence of both early ring form trophozoites resembling *P. falciparum* and late trophozoites resembling those of *P. malariae* (figures 1 and 2). As the blood film was positive for malaria parasites, a rapid detection test for malaria antigens (BinaxNow) was performed to determine the species and the result was negative. The BinaxNOW@Malaria test is an immunochromatographic assay which targets the histidine-rich protein II antigen specific to *P. falciparum* and a

To cite: Fan L, Lee SY, Koay E, et al. *BMJ Case Rep* Published online: [please include Day Month Year] doi:10.1136/bcr-2013-009558

Figure 1 The early ring form trophozoites occupying one-third to half the diameter of the red cells.

pan-malarial antigen, common to the four more well-known human malaria species, *P falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *P malariae*. The test has not been validated in *P knowlesi* infections.

The finding of malaria parasites resembling both *P falciparum* and *P malariae*, conflicting results of the malaria antigen test, plus travel to a region with known transmission of *P knowlesi*, led to a suspicion of *P knowlesi* infection, which had to be confirmed with PCR.

The PCR test used in this child was a laboratory-designed qualitative PCR, based on the amplified product band size (number of base pairs (bp)) specific for each of the five known species of malaria. The test showed a positive band (426 bp), which corresponded to that of the *P knowlesi* positive control, and an absence of for the 440 bp-sized band, which ruled out *P falciparum* infection.

TREATMENT

As typhoid fever was initially suspected, the patient was started on intravenous ceftriaxone at a dose of 50 mg/kg/day, which was

Figure 2 Schizonts containing multiple chromatin masses and abundant pigment granules.

stopped when blood cultures were negative after 48 h. When the malaria blood film reported the presence of possible *P falciparum*, oral quinine was started at 30 mg/kg/day as for uncomplicated *P falciparum* malaria. When *P knowlesi* was confirmed and *P falciparum* was ruled out by PCR testing, treatment was changed to oral chloroquine at 10 mg/kg/dose for the first dose, 5 mg/kg/dose at the 6th, 24th and 48th hour.

OUTCOME AND FOLLOW-UP

The patient initially remained febrile and unwell while on intravenous ceftriaxone, however, upon starting on the antimalarial treatment, fever, nausea and vomiting resolved. He was discharged home after 5 days of hospital stay and followed up in the outpatient clinic after 1 week by which time he had fully recovered clinically. A full blood count showed improvement to the initial anaemia (Hb of 11.1×10^9 g/dl (12.9–17.0) and thrombocytopenia (platelets of 283×10^9 /l (132–372).

DISCUSSION

This traveller to Malaysia had uncomplicated *P knowlesi* infection and had responded well to a standard course of oral chloroquine. In the coastal provinces of the Malaysian peninsula, where *P falciparum* has largely been eliminated, *P knowlesi* is as common as *P vivax*.⁴ With increasing tourism to the forested areas of Malaysia, travellers are now at higher risk of contracting *P knowlesi* infection. In areas where *P falciparum* and *P malariae* are not endemic, diagnosis of *P knowlesi* is made largely on blood films—presence of parasite that resembles *P falciparum* at the early trophozoite stage or *P malariae* at the late trophozoites stage would be classified as *P knowlesi* (figures 3 and 4). This situation is more complex in North and East Borneo where *P knowlesi* occurs concurrently with *P falciparum* and *P malariae* (University of Oxford⁵). Medical centres that rely on antigen tests alone for diagnosis of the four human malaria parasites may fail to diagnose malaria altogether and microscopic diagnosis using thin blood films can be confusing. Patients diagnosed with having *P falciparum* may receive unnecessary treatment with quinine, artesunate or combination drugs with all their associated

Figure 3 The late trophozoites, which had denser and more amoeboid cytoplasm which was frequently irregular in shape with wispy edges.

Figure 4 The mature trophozoites which had more solid and dense cytoplasm and presence of malaria pigment and small inconspicuous vacuoles, lying within non-enlarged, stippled red cells.

side effects, whereas a diagnosis of *P malariae* would underestimate the more serious complications of *P knowlesi* infection. Moreover, *P malariae* infection can lead to episodes of recrudescence for up to several decades with repeated drug treatment or exclusion from blood donation, a feature not seen in *P knowlesi* infection. *P knowlesi* belongs to the group of simian malaria parasites, which include *Plasmodium cynomolgi*, *Plasmodium brazilianum*, *Plasmodium inui* and *Plasmodium simium*, all of which have different species of monkeys as their respective hosts. *P knowlesi* was first identified in a long-tailed macaque monkey imported to the USA from Singapore. The first natural transmission of *P knowlesi* infection was reported in the 1960s in a USA surveyor who travelled to the Western part of Malaysia.^{1 3} His blood film had initially identified the parasite as *P malariae*, similar to the previous two reported cases of *P knowlesi* from Singapore.^{6 7} Clinical manifestation of *P malariae* infection, however, differs from that of *P knowlesi*. In *P malariae*, the fever pattern after synchronisation is quartan, whereas that for *P knowlesi* is daily.^{2 3} *P malariae* has a mild, chronic, recrudescence course; in contrast *P knowlesi* infection may resemble *P falciparum* malaria including its complications such as cerebral malaria, hypoglycaemia and pulmonary oedema.² Coinfection with both *P knowlesi* and *P falciparum* is also possible.⁸ Aside

from the fever pattern and clinical symptoms, *P malariae* infection usually has a parasite load of less than 5000 copies/ml of blood, while *P knowlesi* parasite load can be as similarly high as that in *P falciparum* infection.¹

Although the history and examination of blood films can provide some clues, only the molecular approach has so far been able to clearly distinguish *P knowlesi* from *P falciparum*.

Learning points

- Clinicians should suspect *Plasmodium knowlesi* infection in patients presenting with a clinical picture of *Plasmodium falciparum* malaria and a travel history to endemic areas of the Malaysian peninsula and Borneo.
- Early ring forms of *P knowlesi* resemble trophozoites of *P falciparum*, whereas late trophozoite stages may appear similar to those of *Plasmodium malariae*.
- PCR is the method of choice to identify *P knowlesi* and mixed infections with *P falciparum* and/or *P malariae*.

Contributors LF was part of the team that diagnosed and managed the patient during his admission and drafted and revised the manuscript. LSY obtained the malaria blood films from the haematology laboratory, digitalising the blood films for uploading and drafted the captions for the blood films. E K provided details of molecular testing to identify the various species of the malaria parasite. C H paediatrics infectious disease physician, diagnosed and treated the patient, as well as helped with drafting and editing the manuscript.

Competing interests None.

Patient consent Obtained.

Provenance and peer review Not commissioned; externally peer reviewed.

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