

# A large focus of naturally acquired *Plasmodium knowlesi* infections in human beings

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## Summary

## Background

About a fifth of malaria cases in 1999 for the Kapit division of Malaysian Borneo had routinely been identified by microscopy as *Plasmodium malariae*, although these infections appeared atypical and a nested PCR assay failed to identify *P. malariae* DNA. We aimed to investigate whether such infections could be attributable to a variant form of *P. malariae* or a newly emergent *Plasmodium* species.

## Methods

We took blood samples from 208 people with malaria in the Kapit division between March, 2000, and November, 2002. The small subunit ribosomal RNA and the circumsporozoite protein genes were sequenced for eight isolates that had been microscopically identified as *P. malariae*. All blood samples were characterised with a genus-specific and species-specific nested PCR assay together with newly designed *P. knowlesi*-specific primers.

## Findings

All DNA sequences were phylogenetically indistinguishable from those of *P. knowlesi*, a malaria parasite of long-tailed macaque monkeys, but were significantly different from other malaria parasite species. By PCR assay, 120 (58%) of 208 people with malaria tested positive for *P. knowlesi*, whereas none was positive for *P. malariae*. *P. knowlesi* parasites in human erythrocytes were difficult to distinguish from *P. malariae* by microscopy. Most of the *P. knowlesi* infections were in adults and we did not note any clustering of cases within communities. *P. knowlesi* infections were successfully treated with chloroquine and primaquine.

## Interpretation

Naturally acquired *P. knowlesi* infections, misdiagnosed by microscopy mainly as *P. malariae*, accounted for over half of all malaria cases in our study. Morphological similarities between *P. knowlesi* and *P. malariae* necessitate the use of molecular methods for correct identification. Further work is needed to determine whether human *P. knowlesi* infections in the Kapit division are acquired from macaque monkeys or whether a host switch to human beings has occurred.