Detection of *Plasmodium knowlesi* DNA in urine samples Collected from a Japanese macaque (*Macaca fuscata*) over the course of an experimentally induced infection

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Urine from malaria-infected individuals contains trace amounts of *Plasmodium* DNA, and therefore, could be used as a non-invasive alternative to blood sampling for diagnosis. However, it is not known whether the parasite DNA load present in urine during the course of an infection correlates with the parasite load in the peripheral blood, or whether it can be detected following anti-malarial treatment. In the present study, we demonstrate the detection of *Plasmodium knowlesi* DNA (*Pk* DNA) in urine samples collected from a Japanese macaque (*Macacafuscata*) over the course of an experimentally induced infection. The monkey was inoculated intravenously with *P. knowlesi* H strain (ATCC No.30158)-infected blood. After infection, thin blood films were prepared daily from peripheral blood and the parasitemia of the infected monkey was monitored by microscopy. Blood and urine samples were obtained from the monkey during the course of the infection, and frozen at -80^{O}C until use. Detection of *Pk* DNA was based on nested PCR (nPCR) amplification of the mitochondrial cytochrome *b* gene (*cytb*), and the amount of parasite DNA in the urine samples was estimated using real-time quantitative PCR (qPCR).Parasites in the peripheral blood were first detected by microscopy on day 5; parasite densities then increased sharply to around 10% by day 7 post-infection. We were able to detect *Pk* DNA in urine samples by nPCR during the infection. The amount of *Pk* DNA in the urine samples increased markedly following chloroquine treatment, before falling to non-detectable levels four days after cessation of treatment. Our results indicate that PCR of parasite DNA from urine obtained from malaria-infected individuals is possible, and has potential application in longitudinal field survey or endpoint determination in clinical trials of anti-malarial drugs.