Siregar *et al. Malar J (2015) 14:404*
DOI 10.1186/s12936-015-0857-2

MALARIA JOURNAL

METHODOLOGY Open Access

Non-invasive surveillance for *Plasmodium* in reservoir macaque species

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Abstract

Background: Primates are important reservoirs for human diseases, but their infection status and disease dynamics are difficult to track in the wild. Within the last decade, a macaque malaria, *Plasmodium knowlesi*, has caused disease in hundreds of humans in Southeast Asia. In order to track cases and understand zoonotic risk, it is imperative to be able to quantify infection status in reservoir macaque species. In this study, protocols for the collection of non-invasive samples and isolation of malaria parasites from naturally infected macaques are optimized.

Methods: Paired faecal and blood samples from 60 *Macaca fascicularis* and four *Macaca nemestrina* were collected. All animals came from Sumatra or Java and were housed in semi-captive breeding colonies around West Java. DNA was extracted from samples using a modified protocol. Nested polymerase chain reactions (PCR) were run to detect *Plasmodium* using primers targeting mitochondrial DNA. Sensitivity of screening faecal samples for *Plasmodium* was compared to other studies using Kruskal Wallis tests and logistic regression models.

Results: The best primer set was 96.7 % (95 % confidence intervals (CI): 83.3–99.4 %) sensitive for detecting *Plasmodium* in faecal samples of naturally infected macaques (n = 30). This is the first study to produce definitive estimates of *Plasmodium* sensitivity and specificity in faecal samples from naturally infected hosts. The sensitivity was significantly higher than some other studies involving wild primates.

Conclusions: Faecal samples can be used for detection of malaria infection in field surveys of macaques, even when there are no parasites visible in thin blood smears. Repeating samples from individuals will improve inferences of the epidemiology of malaria in wild primates.

Keywords: Malaria, *Macaca fascicularis*, *Macaca nemestrina*, Non-invasive sampling, Zoonotic surveillance

Background

Non-human primates (NHPs) are important hosts of zoonotic diseases: they can share pathogens with humans and act as reservoirs for several emerging infectious diseases of pandemic proportions [1–4]. NHPs are also susceptible to human pathogens that can have mild to catastrophic impacts on populations [5–9]. In order to predict zoonotic risk and understand conservation implications of pathogen exchange between humans and NHPs, it is essential to understand infectious disease dynamics within wild primate populations.

Primates are infected with at least thirty *Plasmodium* parasites globally [10, 11]. Spillover of NHP malaria has been suspected in cases in the Amazon [12–14] and a tourist returning from Central Africa [15]. On a much larger scale, a monkey malaria, *Plasmodium knowlesi*, has emerged in human populations across Southeast Asia (Fig. 1, Additional file 1). The parasite species has been recorded, and is presumably endemic, in wild populations of two macaque species (*Macaca fascicularis* and *Macaca nemestrina*) and two leaf monkeys (*Presbytis femoralis* and *Trachypithecus obscurus*) [16–19]. These primates can be co-infected with up to five species of *Plasmodium* parasite [20], but most morphological surveys report moderate (10–30 %) malaria prevalence in long-tailed macaques (Fig. 1, see Additional file 1). While spillover cases have

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