

The Manifestation of Forest Malaria in Khanh Phu

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Before 1997 malaria was holo-endemic among the 2000 inhabitants of Khanh Phu, a commune about 30 km West of Nha Trang city, and this was transmitted by two vectors: *Anopheles minimus* and *An. dirus*. Right after the introduction of full scale coverage with permethrin impregnated bed nets from August 1998, *An. minimus*, till that time the most common vector in and around the villages, virtually disappeared and malaria prevalence and incidence were reduced by about 80%. From then on all malaria transmission was and still is maintained by *An. dirus* only and most infections occurred in the surrounding forests where part of the population regularly overnights. Although the prevalence of malaria gametocytes among humans entering the forest had decreased over time, the sporozoite infection rate among *An. dirus* caught in the forest remained equally high (1 – 5%, by dissection and microscopic examination). PCR analysis of the DNA and RNA in sporozoite positive mosquito salivary glands in 2009 revealed a regular occurrence of *P. knowlesi* DNA next to that of *P. falciparum*, *P. vivax* and *P. malariae*. Subsequent analysis of blood samples of humans in Khanh Phu also demonstrated *P. knowlesi*, in frequencies up to 26% of the infected cases. *P. knowlesi* DNA was sometimes found as a single infections in the mosquitoes but in human blood it always manifested as a co-infection, mostly with *P. vivax*, or with *P. vivax* and *P. falciparum*. The combination of *P. knowlesi* with only *P. falciparum* was rare. *P. knowlesi* did not lead to additional fever or other symptoms in humans and was more frequent in the younger age groups. *P. knowlesi* parasites have not yet been confirmed by microscopic examination, possibly due to their occurrence in mixed infections in which *P. vivax* was always dominant. Naïve macaque monkeys held in a cage in the forest where *An. dirus* is common did develop malaria parasitaemia after four months. Morphologically these parasites looked like *P. simiovale* but they were later identified with molecular methods as *P. cynomolgi*. Another macaque introduced in the cage in 2011 became infected with a malaria parasite that looked like *P. inui*, certainly not *P. knowlesi*, but these samples have not yet been analysed by PCR.

Over the last three years all malaria infections in Khanh Phu were incurred by just about 230 inhabitants, which forms less than 8% of the present population in the commune. Almost all these people have received a radical malaria treatment (Artekin + Primaquine), irrespective of actual parasitaemia, in December 2011. This is an attempt to reduce the reservoir of parasites in the forest just before the start of the dry season, when most people start to go to the forest and *An. dirus* populations usually start to climb to their peak densities. The results of this

intervention on malaria infections among humans and the mosquitoes are routinely monitored but not yet known. If the usual human malaria parasites would become less frequent it might be expected to increase the chance of finding pure *P. knowlesi* infections in humans.

The possible implications of these findings for the control of forest malaria and the priorities for further research will need to be discussed during this Symposium.