Title: **Is the epidemiology of *Plasmodium knowlesi* changing and what does this mean for malaria control in South East Asia?**

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It had long been clear that the "monkey-malaria" species, *Plasmodium knowlesi*, was capable of infecting humans. Its name comes from Robert Knowles, the British parasitologist who first demonstrated experimental monkey-human transmission and pioneered its use as "malaria therapy" for syphilis and leprosy from as early as 1932.[1] Although there had been occasional isolated cases of naturally acquired *P.knowlesi* infection reported from as early as 1965, [2] Singh and colleagues' 2004 report of a large focus of naturally acquired *P.knowlesi* infection in the Kapit Division of Sarawak, Malaysian Borneo came as a major surprise.[3] This discovery was made possible by the advent of highly specific PCR diagnostics. It is now almost certain that *P.knowlesi* had been a longstanding significant cause of human malaria infection in this region, but not recognized as such because conventional microscopy-based diagnosis had failed to differentiate it from the morphologically similar human parasite, *P.malariae*. [4] Dubbed shortly after as the "fifth human malaria", [5] more widespread application of PCR-based testing demonstrated a wide distribution throughout South-East Asia, including in neighbouring Sabah state, peninsular Malaysia and in Singapore, Thailand, Cambodia, Laos, Vietnam, Vietnam, Myanmar and Indonesia. [6] This geographic distribution reflects that of the definitive primate hosts, macaque monkeys, in which *P.knowlesi* prevalence is almost universal. [6] Significant human-to-human transmission in the absence of macaques has so far been considered unlikely.[7] This is reassuring, as it would make it likely that future human cases will remain restricted to a small ecological niche of people living in close proximity to monkey populations. So we might reasonably hope that its currently modest public health burden (in Sabah's population of 3 million it causes an estimated average of 2 deaths per year) [8,9] will continue to remain low. Nevertheless, there have been approximately 35 *P.knowlesi* clinical articles in the last 5 years, many by Cooper and colleagues, so the report of an ongoing increase in *P.knowlesi* incidence by Cooper et al in the current issue [8] should be carefully considered in a broad context.

Has *P.knowlesi* epidemiology really changed significantly since the seminal discovery reported in 2004? Much of the subsequent "explosion" in reported cases probably reflected increased awareness,

case detection and PCR-enabled diagnosis. Cooper and colleagues' update on the *P.knowlesi* situation in Sabah province, Malaysia, using state-wide hospital surveillance data for the three years 2015-2017,[8] follows a similar previous report by this group that covered the three years 2011-2013. [9] Cases between 2011 and 2017 using standardized methodology utilized in these two reports are summarized in the FIGURE. Although cases increased from 2011 to 2014, they decreased from 2014 to 2016, and only the number of 2017 cases represent a dramatic increase. The interpretation by Cooper et al of an "ongoing increase in incidence" rests on a single data point in 2017**.** At this stage, all we think can be said is that there was a worrying "spike" in cases in Sabah in 2017 and it is not yet clear whether or not this represents a significant ongoing or more widespread trend.

But what if this increase does turn out to be sustained and is also occurring in other regions? What factors could be driving an increase in the incidence of this zoonotic infection? As Cooper *et al.* [8] note, firstly, we should consider factors that bring human populations into closer and more frequent contact with macaque monkeys. Secondly, environmental changes, especially those associated with deforestation may favour *P.knowlesi*'s anopheline mosquito vectors. Thirdly the authors hypothesize that, as the successful control of the human malarias continues apace, populations may now be at greater risk of *P.knowlesi* due to diminishing natural cross-protective immunity. This is especially plausible with *P.vivax* which shares important pathophysiologic and antigenic similarities with *P.knowlesi*. [10-12] The original description of the correlation of Duffy antigen negativity with lack of erythrocyte infection was first described for *P.k*nowlesi [10] before being extended to *P vivax*.[11] *Plasmodium* apical asparagine -rich protein (AARP) may be involved in merozoite invasion of erythrocytes. Recombinant *P.vivax* AARP protein was recognized by sera from *P.knowlesi* malaria patients. Antibody raised against the *P.vivax (*and *P.knowlesi)* AARP N-termini inhibited erythrocyte invasion by *P. knowlesi* in a concentration-dependent manner, thereby suggesting a cross-species nature of anti-*Pv*AARP antibody against *Pk*AARP. [12] However clinical evidence to support significant cross-species protective immunity between *P.vivax* and *P.knowlesi* is very limited. We could only find the statement from 1935 that some syphilitic patients were resistant to infection

with *P.knowlesi*, including "three patients who had previously received a course of *P. vivax* malaria therapy a year before". [13] A fourth and more worrying possibility would be if *P.knowlesi* had managed to "jump" from its narrow zoonotic ecological niche to one where there is sustained and ongoing human to human transmission. This could be potentiated by loss of potential cross-species protective immunity and would result in a very much greater public health impact of this species. Ongoing vigilance is warranted.

Regardless of whether *P.knowlesi* incidence is genuinely increasing or not, what is abundantly clear is that it is constituting an ever increasing proportion of the local malaria burden (currently 98% of all cases in Sabah). However, this overwhelmingly reflects the *decrease* in *P.falciparum* and *P.vivax* rather than any *increase* in *P.knowlesi*. It underlines the stunning successes in overall malaria control over the last 2 decades that now make imminent regional elimination of these malaria species tantalizingly possible. This success is owed to the transmission-reducing effectiveness of well-applied public health interventions, including insecticide-treated bed nets, rapid access to effective treatment with artemisinin-combination treatments and indoor residual spraying of insecticides. By contrast, *P.knowlesi*, due to its large non-human population reservoir, is likely to be much less susceptible to these "human-focussed" interventions. It seems likely it will be the "stone in the shoe" of malaria control in this part of the world, persisting long after *falciparum* and *vivax* malaria have been vanquished.

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References

1.Knowles R, Gupta BMD. A Study of Monkey-Malaria, and Its Experimental Transmission to Man. Ind Med Gaz **1932**; 67: 301-20.

2.Chin W, Contacos PG, Coatney GR, Kimball HR. A naturally acquired quotidian-type malaria in man transferable to monkeys. Science. **1965**;149:865

3.Singh B, Kim Sung L, Matusop A, et al. A large focus of naturally acquired Plasmodium knowlesi infections in human beings. Lancet **2004**; 363: 1017-24.

4.Lee KS, Cox-Singh J, Brooke J, Singh B. Plasmodium knowlesi from archival blood films: further evidence that human infections are widely distributed and not newly emergent in Malaysian Borneo. Int J Parasitol **2009**; 39: 1125-8.

[5] White NJ. Plasmodium knowlesi: The fifth human malaria parasite. Clin Infect Dis. **2008**; 46:172-3.

6. Shearer FM, Huang Z, Weiss DJ et al**.** Estimating Geographical Variation in the Risk of Zoonotic Plasmodium knowlesi Infetion in Countries Eliminating Malaria . PLoS Negl Trop Dis. **2016**; 10(8):e0004915.

7. Imai N, White MT, Ghani AC, Drakeley CJ.Transmission and control of Plasmodium knowlesi: a mathematical modelling study. PLoS Negl Trop Dis. **2014**;8:e2978.

8.Cooper D, Rajahram GS, William T, et al. Plasmodium knowlesi malaria in Sabah, Malaysia, 2015-2017: ongoing increase in incidence despite near-elimination of the human-only Plasmodium species. Clin Infect Dis **2019**.

9.William T, Jelip J, Menon J, et al. Changing epidemiology of malaria in Sabah, Malaysia: increasing incidence of Plasmodium knowlesi. Malar J **2014**; 13: 390.

10.Miller LH, Hanson SJ, Dvorak JA, McGinniss MH, Rothman IK. Ertythrocyte receptors for (Plasmodium knowlesi) malaria: Duffy blood group determinants. Science **1975**; 189: 561-3.

11.Miller LH, Mason SJ, Clyde DF, McGinniss MH. The resistance factor to Plasmodium vivax in blacks. The duffy-blood-group genotype, FyFy. N Engl J Med **1976**; 295: 302-4.

12.Muh F, Ahmed MA, Han JH, et al. Cross-species analysis of apical asparagine-rich protein of Plasmodium vivax and Plasmodium knowlesi. Sci Rep **2018**; 8: 5781.

13. Van Rooyen CE, Pile GR. Observations on infection by Plasmodium knowlesi (ape malaria) in the treatment of general paralysis of the insane. Br Med J. **193;** 12(2):662-6.

Figure Legend:

Figure 1: *P knowlesi* case notifications in Sabah

Data from references 8 and 9.

Figure 1