

## Identifying the risks for human transmission of *Plasmodium knowlesi*



*Plasmodium knowlesi* is principally a primate malaria parasite that affects long-tailed and pig-tailed macaques, transmitted by the exophagic *Anopheles leucosphyrus* group. Robert Knowles and Das Gupta provided the first extensive description of the parasite, including the ability of this primate malaria to infect humans in 1932. Soon after, *P knowlesi* was used to infect patients with neurosyphilis to ameliorate symptoms in the pre-antibiotic era, but clinicians soon stopped using it when it became too pathogenic. In recent years *P knowlesi* has emerged as an important human pathogen and now poses a substantial challenge to malaria elimination in the Asia Pacific Region. This is especially the case in Malaysia, where *P knowlesi* has become the predominant cause of human malaria, but is also likely to impact on elimination efforts in Indonesia, Philippines, Vietnam, Cambodia, Laos, Thailand, and Myanmar.

This threat to elimination is largely due to the large parasite reservoir that infected macaques provide for ongoing transmission and the challenges that this poses in the prevention of sporadic human infection. Accurately quantifying this risk has been challenging for various reasons. Among these is the lack of adequate diagnostics. The light microscopy appearance of *P knowlesi* is indistinguishable from *Plasmodium malariae* and is frequently confused with *Plasmodium falciparum* and *Plasmodium vivax*. There are no *P knowlesi*-specific antigen detection rapid diagnostic tests (RDTs) and those with pan-malaria antigen detection suffer from low sensitivity, low specificity, and cross-reactivity with other *Plasmodium* species.<sup>1</sup> Indeed, PCR remains the most reliable way to diagnose *P knowlesi* malaria with high sensitivity and specificity, although there are reports of loop-mediated isothermal amplification (LAMP) being used.<sup>2</sup> Poor diagnostics partly contribute to inadequate surveillance for *P knowlesi* infection and calls have been made to include specific *P knowlesi* reporting to WHO to encourage improved regional surveillance.<sup>3</sup>

Perhaps the greatest challenge however, relates to the epidemiology of this disease and understanding the interactions that occur between the reservoir hosts (ie, monkeys), vectors, and human activities that enable transmission. Understanding these complex behavioral

and ecological interactions will provide insights into how this disease is evolving from a forest-located entity, to one that is found at the forest fringe and is moving into peri-urban areas.<sup>4</sup> Within these interactions is the need to examine specific risk factors that drive disease transmission to humans. Such details are crucial to identify those most at risk and to implement appropriate prevention strategies.

In *The Lancet Planetary Health*, Matthew Grigg and colleagues provide important insights into human activities that affect the risk of *P knowlesi* infection.<sup>5</sup> This case-control study of *P knowlesi* infection was done in Sabah, Malaysia, which has had the highest recorded rates of *P knowlesi* infection globally. The study recruited 229 cases with *P knowlesi* infection over a 2 year period and 91 cases with infection due to other *Plasmodium* species. This study is the first published work of its kind and seeks to quantify the risk of infection for a wide range of potential risk factors, including demographics, medical history, work or school activities, sleeping or bathing patterns, travel activities, malaria prevention measures, travel history, interactions with monkeys, and features of the residences of participants. This study design and the wide range of risk factors assessed is a strength of this work and affords helpful insights into the risks most strongly associated with clinical episodes of *P knowlesi*.

There are several findings from this study that are especially noteworthy. First, the study finds significant heterogeneity in co-transmission of *P knowlesi* and other *Plasmodium* species. The investigators found that *P knowlesi* infection was present widely throughout both districts of Kota Marudu and Kudat, whereas other *Plasmodium* species (ie, non-*P knowlesi* species) were largely confined to inland areas of Kota Marudu, with few cases on Kudat. This distribution suggests that current strategies are successfully targeting non-*P knowlesi* species and that residual cases are largely confined to remote regions, possibly those with lower coverage of control interventions. Grigg and colleagues also show that these strategies are having less impact on *P knowlesi* infections and that specific interventions need to be identified for successful *P knowlesi* elimination. It is important to note that the study design accounted for this geographic

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heterogeneity with regionally matched controls, enabling an assessment of specific risk factors.

Second, the authors successfully identify several features of individuals with high-risk for *P knowlesi* disease. This includes a strong risk bias towards individuals over the age of 15 years (adjusted odds ratio [aOR] 4.16, 95% CI 2.09–8.29) and a strong bias towards men, who made up 76% (174 of 229 cases) of *P knowlesi* cases. Notably, the results showed that infection with other *Plasmodium* species was largely confined to individuals younger than 15 years but that men were also at higher risk. Similar findings were identified among the 19 *P knowlesi* cases and 1 subpatent infection described in a cross-sectional study in Indonesia.<sup>6</sup> The study results also mirror gender-biased findings widely observed across southeast Asia for other *Plasmodium* species. These results show that interventions need to be strongly targeted towards men and that programmes centred on maternal and child health will be inadequate for malaria elimination, especially for *P knowlesi*. The observation that *P knowlesi* infection occurs in older individuals has several possible explanations that need further investigation. One possibility is that, in areas of co-transmission with other *Plasmodium* species, the acquisition of natural immunity to *P falciparum* and *P vivax* early in life, is not providing substantial protection against *P knowlesi* infection. Further investigation is required to understand cross-protective immunity between these species, and the maintenance of natural immunity and memory responses over many years. The observation of higher risk in older individuals might also relate to occupational risk. The study identifies farming and palm oil plantation work as particularly high-risk occupations. Despite being quite different jobs, it is likely that there are many other shared risk factors, but these are not clearly disentangled in this study. A further subgroup analysis would be informative. Notably, the prevalence of G6PD deficiency was very low among cases compared with controls, and this was especially so for *P knowlesi* infections compared with other *Plasmodium* species.

Significant uncertainty remains about the precise vectors, the plasticity of vector host-selection (ie, monkey vs human) and the exact location of vector exposure that results in human transmission of *P knowlesi*, especially across different parts of Asia.<sup>7,8</sup> In particular, uncertainty exists as to whether transmission is occurring predominately in the forest, farms at the forest-fringe,

or villages, and the degree of transmission that is peri-domestic or domestic. Grigg and colleagues noted that having long grass around the house, and having open eaves or gaps in walls are associated with increased risk of *P knowlesi*. Similarly the use of indoor residual spraying, which was only implemented in 94 (41%) of the 229 cases, was associated with a significant reduction in risk. They also found that the use of bednets by 177 (78%) cases, seemed to have only a marginal effect on risk. These findings are generally supportive of those of Manin and colleagues<sup>7</sup> who identified peri-domestic transmission as being a more greater problem than domestic transmission. Together these studies show that greater benefits might result from improving housing and cutting back peri-domestic vegetation than from intensifying indoor residual spraying and bednet distribution.

Wong and colleagues<sup>9</sup> have described higher densities of *Anopheles balabacensis* in villages than in farming areas and forests, but that mosquito longevity, which is crucial for *Plasmodium* development, was higher in farming areas and forests. Brant and colleagues<sup>10</sup> have also described a preference of *A balabacensis* to bite humans at ground level rather than high in the forest canopy. Thus, the likelihood that the main place of transmission occurs outdoors at ground level in forested areas was strongly supported by the findings of Grigg and colleagues, who showed that recent clearing of vegetation was a high-risk activity, as was sleeping outside the house, even though this was relatively infrequent; only 33 (15%) of cases. Recent awareness of the presence of monkeys was also a strong predictor of risk, further suggesting a high likelihood of monkey-to-human transmission as the predominant transmission pathway, rather than human-to-human transmission.

This study by Grigg and colleagues provides quantifiable risk estimates for a wide range of variables relevant to the transmission of *P knowlesi* to humans. There are now a series of studies shedding light on the transmission dynamics of *P knowlesi* in Sabah, but further research is needed in this area and in other parts of southeast Asia where humans live in the forest or on the forest fringe, sharing their habitat with *P knowlesi*-infected macaques and their Anopheline vectors. In particular, further research is needed to better understand the ecological interactions that place people at risk of *P knowlesi* and the measures that can be implemented to minimise these risks. This will also be greatly aided by improving

diagnostics and surveillance for this infection so that hot spots of transmission can be rapidly and precisely identified.

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We declare no competing interests.

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