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Plasmodium knowlesi Malaria During Pregnancy

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Background. *Plasmodium knowlesi* is the commonest cause of malaria in Malaysia, but little is known regarding infection during pregnancy.

Methods. To investigate comparative risk and consequences of knowlesi malaria during pregnancy, we reviewed (1) Sabah Health Department malaria-notification records created during 2012–2013, (2) prospectively collected data from all females with polymerase chain reaction (PCR)–confirmed malaria who were admitted to a Sabah tertiary care referral hospital during 2011–2014, and (3) malaria microscopy and clinical data recorded at a Sabah tertiary care women and children's hospital during 2010–2014.

Results. During 2012–2013, 774 females with microscopy-diagnosed malaria were notified, including 252 (33%), 172 (20%), 333 (43%), and 17 (2%) with *Plasmodium falciparum* infection, *Plasmodium vivax* infection, *Plasmodium malariae*/*Plasmodium knowlesi* infection, and mixed infection, respectively. Among females aged 15–45 years, pregnancy was reported in 18 of 124 (14.5%), 9 of 93 (9.7%), and 4 of 151 (2.6%) *P. falciparum*, *P. vivax*, and *P. malariae*/*P. knowlesi* notifications respectively ($P = .002$). Three females with knowlesi malaria were confirmed as pregnant: 2 had moderate anemia, and 1 delivered a preterm low-birth-weight infant. There were 17, 7, and 0 pregnant women with falciparum, vivax, and knowlesi malaria, respectively, identified from the 2 referral hospitals.

Conclusions. Although *P. knowlesi* is the commonest malaria species among females in Sabah, *P. knowlesi* infection is relatively rare during pregnancy. It may however be associated with adverse maternal and pregnancy outcomes.

Keywords. malaria; pregnancy; *Plasmodium knowlesi*; maternal anemia; preterm delivery.

Malaria during pregnancy is a major global public health problem, with each of the most prevalent *Plasmodium* species, *Plasmodium falciparum* and *Plasmodium vivax,* causing substantial maternal and infant morbidity. In malaria-endemic areas, pregnant women have an increased risk of infection with both *P. falciparum* and *P. vivax*, and with higher parasitemia, compared with nonpregnant women [1, 2]. Falciparum malaria during pregnancy is associated with maternal anemia [3], increased risk of severe malaria [4, 5], miscarriage [6], low infant birth weight [7, 8], maternal mortality [9, 10], and perinatal mortality [11]. *Plasmodium vivax* infection during pregnancy is also associated with maternal anemia, miscarriage, and low infant birth weight [6, 12–14]. Severe disease and mortality associated with vivax malaria during pregnancy has been reported, although at lower rates than those of falciparum malaria [15].

The simian parasite *Plasmodium knowlesi* is the most common cause of malaria in Malaysia, and the incidence of notified infection is increasing [16, 17]. In Sabah, *P. knowlesi* accounted for 62% of malaria notifications in 2013 [16]. Knowlesi malaria has also been reported in every country in Southeast Asia except Timor Leste and Laos, and it is increasingly reported in travelers returning from these areas [18]. In adults, the proportion with severe clinical disease has been reported to be at least as high as that in falciparum malaria [19], and fatal cases have been reported [20–25].

Little is known, however, about the risk and consequences of knowlesi malaria during pregnancy. In a

retrospective study conducted at Queen Elizabeth Hospital (QEH), an adult tertiary care referral hospital in Sabah, Malaysia, 2 of 11 women (18%) with knowlesi malaria were reported to be pregnant [26]. While one of these had uncomplicated disease, the other had severe disease, with acute kidney injury, shock, and fetal loss. While this study raised the possibility of increased risk and severity of knowlesi malaria among pregnant women, in a subsequent prospective study at the same hospital pregnancy was reported in 0 of 144 adults with polymerase chain reaction (PCR)–confirmed *P. knowlesi* monoinfection [19]. Although this study reported 4 pregnant females with *P. knowlesi* and *P. vivax* mixed infection, the PCR assay used at that time for these patients was known to falsely characterize true *P. vivax* monoinfections as *P. knowlesi* and *P. vivax* mixed infections [27], and therefore these diagnoses are uncertain. In another prospective study of knowlesi malaria, conducted at a district hospital in Sarawak, pregnancy was reported in only 1 of 113 adults with *P. knowlesi* monoinfection, despite approximately half of all *P. knowlesi*–infected patients at this hospital being female [23].

In Sabah, notification of all malaria cases is mandatory, with self-reported pregnancy status recorded during follow-up interviews conducted by the Sabah Department of Health. To investigate the incidence of knowlesi malaria during pregnancy in Sabah, relative to the incidence of malaria due to other species during pregnancy in this area, we reviewed the Sabah Department of Health malaria notification database. Because a proportion of malaria cases may not be notified, we also reviewed prospectively collected data for all women with PCR-confirmed malaria who were admitted to QEH during 2011–2013, in addition to retrospectively reviewing the malaria microscopy records and corresponding clinical records at Likas Women and Children's Hospital, the only other tertiary care referral hospital in the same catchment area as QEH.

METHODS

Ethics Statement
The study was approved by the Ethics Committees of the Malaysian Ministry of Health and Menzies School of Health Research, Australia.

Review of Sabah Department of Health Malaria Notification Data
Sabah Department of Health malaria notification records created during 2012–2013 were reviewed, with the start of this period selected on the basis of the commencement of recording of self-reported pregnancy status in the database. Infecting *Plasmodium* species, age, and pregnancy status were recorded. As malaria notifications are based on microscopy results, *P. malariae* and *P. knowlesi* notifications were considered a single group and are referred to hereafter as "*P. malariae/P. knowlesi*" notifications.

PCR-confirmed *P. malariae*, however, is known to be rare in Sabah [19, 28] (accounting for <1% of *P. malariae/P. knowlesi* blood slides referred for PCR testing during 2011–2013 [16]), and therefore the large majority of these notifications can be assumed to be *P. knowlesi*.

For *P. malariae/P. knowlesi*–infected patients recorded as being pregnant, clinical records were sought from the notifying hospitals and reviewed for clinical and epidemiological details.

Enrollment of Patients With Malaria at Queen Elizabeth Hospital
QEH is located in Kota Kinabalu and services the state's West Coast and Kudat divisions, with 6 district hospitals and a population of 1.14 million (Figure 1). Sabah health policy requires all patients with malaria to be hospitalized and all patients with severe malaria or high parasitemia to be transferred to a tertiary care referral hospital. In the QEH catchment area, females with malaria during pregnancy may be referred to either QEH or Likas Hospital, a nearby hospital for women and children. Likas Hospital and QEH are the only 2 tertiary care referral hospitals in Sabah's West Coast and Kudat divisions.

During 2010–2014, all patients with microscopy-diagnosed malaria admitted to QEH were reviewed as part of a prospective epidemiological and clinical study of knowlesi malaria. Sabah state policy requires that these patients would also have been notified to the Sabah Department of Health. Written informed consent was provided by patients or their guardians, and epidemiological and clinical details, including information obtained through routine questioning of females about their pregnancy status, were recorded on standardized forms. Results obtained during September 2010–October 2011 have been reported, but data for pregnant females were excluded [19]. For the current study, we assessed self-reported pregnancy status, *Plasmodium* species distribution, and demographic details for all female patients with malaria admitted during July 2011–March 2014. Species confirmation for these patients was performed by PCR at the Sabah State Public Health Laboratory, with the commencement of the study period chosen to coincide with the introduction at this laboratory of a real-time PCR assay for the detection of *P. knowlesi* [29], which replaced the nested PCR assay that had been reported to cross-react with *P. vivax* DNA [27] and potentially led to overdiagnosis of *P. knowlesi/P. vivax* mixed infections among true *P. vivax* monoinfections [28]. Detection of the other human malaria species was performed using a species-specific real-time PCR assay as previously described [30].

Review of Patients With Malaria Who Were Admitted to Likas Women and Children's Hospital
Malaria microscopy records created during January 2010–March 2014 were reviewed at Likas Women and Children's Hospital to identify all female patients aged >14 years with a
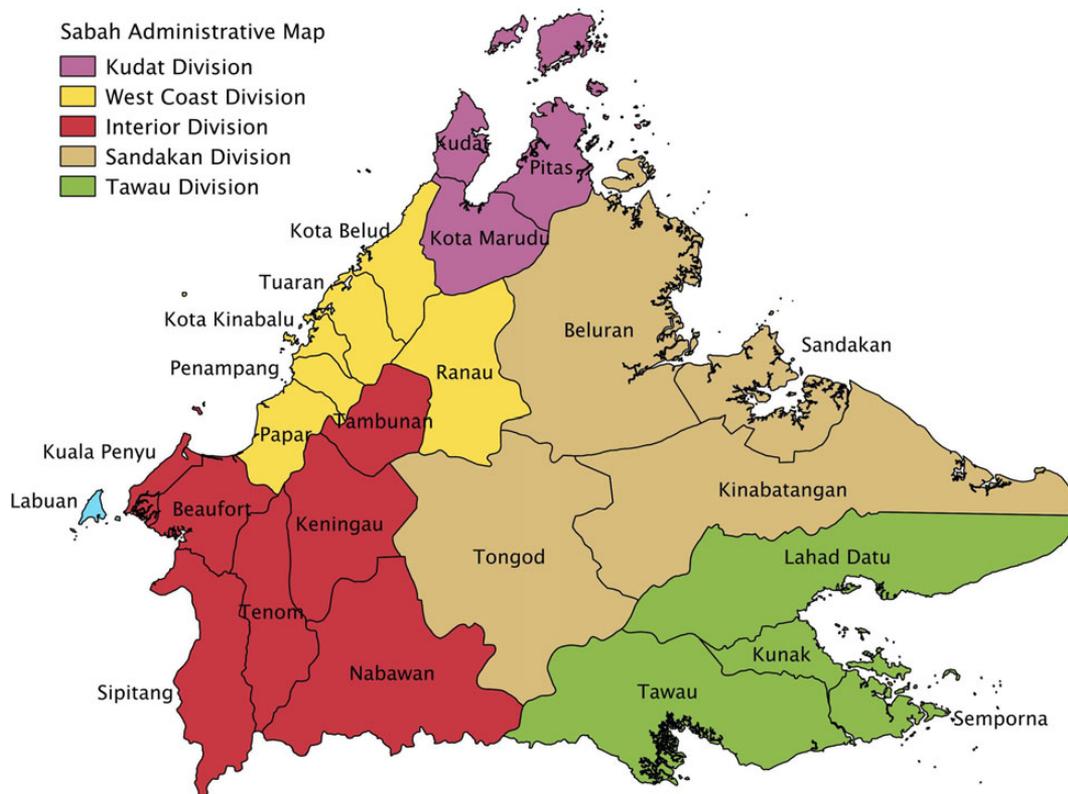
Figure 1. Sabah administrative map.

blood slide positive for malaria parasites. For patients with malaria, pregnancy status was determined from hospital clinical records or, if clinical records could not be located, from the Sabah Department of Health malaria notification database.

Statistical Analysis
Data were analyzed using Stata software. Median ages were compared using the Kruskal–Wallis test, and proportions were compared using the χ^2 or Fisher exact test.

RESULTS

Notifications of Malaria During Pregnancy to the Sabah Department of Health, 2012–2013
From January 2012 to December 2013, 774 females with microscopy-diagnosed malaria were notified to the Sabah Department of Health, including 252 (33%) with *P. falciparum* infection, 172 (20%) with *P. vivax* infection, 333 (43%) with *P. malariae/P. knowlesi* infection, and 17 (2%) with mixed infection. Median ages were 23 years (interquartile range [IQR], 11–36 years) for *P. falciparum* notifications, 23 years (IQR, 11–38 years) for *P. vivax* notifications, and 40 years (IQR, 21–53 years) for *P. malariae/P. knowlesi* notifications ($P = .0001$), with females aged 15–45 years accounting for 124 *P. falciparum* notifications (49%), 93 *P. vivax* notifications (54%), and 151

P. malariae/P. knowlesi notifications (45%). Among females aged 15–45 years, *P. falciparum*, *P. vivax*, and *P. malariae/P. knowlesi* therefore accounted for 33%, 22%, and 43% of malaria notifications, respectively.

During this time, 34 cases of malaria during pregnancy were notified, including 20 females (59%) with *P. falciparum* infection, 9 (26%) with *P. vivax* infection, and 4 (12%) with *P. malariae/P. knowlesi* infection; 1 (3%) had mixed but otherwise unspecified infection and was excluded from further analysis. Females recorded as being pregnant therefore accounted for 8.1%, 5.3%, and 1.2% of all females notified with *P. falciparum*, *P. vivax*, and *P. malariae/P. knowlesi* infection, respectively ($P < .0001$). Among females aged 15–45 years, those notified as being pregnant accounted for 18 of 124 *P. falciparum* notifications (14.5%), 9 of 93 *P. vivax* notifications (9.7%), and 4 of 151 *P. malariae/P. knowlesi* notifications (2.6%; $P = .002$).

Pregnancy Status of Females Admitted to QEH With Malaria
During July 2011–March 2014, 101 female patients had 102 malaria-associated admissions at QEH, with PCR confirming *P. falciparum* infection in 42 (41%), *P. vivax* infection in 10 (10%), *P. knowlesi* infection in 46 (45%), *P. malariae* infection in 2 (2%), and *P. falciparum/P. malariae* infection in 1 (1%). Coinciding with the adoption of non–cross-reacting PCR primers, there were no patients identified as having mixed *P. vivax/P.*

knowlesi infection. One patient, who had microscopy-diagnosed *P. falciparum* infection but was PCR positive only for organisms from the *Plasmodium* genus, was excluded from analysis. Median ages were 37 years (IQR, 27–50 years) for patients with *P. falciparum* infection, 36 years (IQR, 24–46 years) for those with *P. vivax* infection, 54 years (IQR, 38–65 years) for those with *P. knowlesi* infection, and 21 years (range, 16–25 years) for those with *P. malariae* infection (*P* = .002), with females aged 15–45 years accounting for 29 *P. falciparum* malaria admissions (54%), 7 *P. vivax* admissions (13%), 16 *P. knowlesi* admissions (30%), and 2 *P. malariae* infections (3.7%).

Five females were pregnant, all with nonsevere falciparum malaria. One of these patients, from a known *P. falciparum*–endemic area, was readmitted with nonsevere falciparum malaria 2 months after the initial admission following treatment with quinine and clindamycin. Among females aged 15–45 years, pregnancy was therefore reported in 6 of 29 admissions (26%) for falciparum malaria, compared with none of 16, 7, and 2 admissions for knowlesi, vivax, and malariae malaria, respectively (*P* = .165, or *P* = .075 for *P. falciparum* vs *P. knowlesi*).

Plasmodium Species Distribution Among Pregnant Patients With Malaria at Likas Women and Children's Hospital

From January 2010 to March 2014, 38 female patients aged >14 years were identified from Likas Hospital malaria microscopy records as having blood slides positive for malaria parasites. This included 26 patients (68%) with *P. falciparum* infection, 12 (32%) with *P. vivax* infection, and none with *P. malariae/ P. knowlesi* infection. Twenty of these patients were identified as being pregnant (either from case note review or by correlation with data from the Sabah Department of Health malaria notification database), including 13 (65%) with *P. falciparum* and 7 (35%) with *P. vivax*. Two patients with falciparum malaria were recorded as nonpregnant (from the Sabah Department of Health database), while pregnancy status was unable to be ascertained for the remaining 19 patients.

Clinical Severity and Outcomes of Patients Notified with *P. malariae/P. knowlesi* Malaria in Pregnancy

Clinical records for all 4 females identified from the Sabah Department of Health malaria notification database as having *P. malariae/P. knowlesi* malaria during pregnancy were retrieved from admitting hospitals. One patient was not pregnant, with a documented negative pregnancy test. Pregnancy was confirmed in the other 3 cases. The first was a 22-year-old woman from Kudat Division, northeast Sabah. She had known β-thalassemia minor and was 14 weeks pregnant (G3P2) when she presented with a 2-day history of fever and rigors, headache, dizziness, and loss of appetite. She had a temperature 38.3°C, a heart rate of 103 beats/minute, and a blood pressure of 101/ 60 mm Hg. Examination findings were otherwise normal. Results of blood film analysis at admission were reported as

"*P. malariae* 1+," her hemoglobin level was 9.4 g/dL, and her platelet count was 199×10^3 platelets/µL. Electrolyte levels were within normal ranges, and liver function tests were not performed. She was treated with chloroquine, had cleared her parasites within 1 day, and was discharged on day 3. On the day of discharge, her platelets count was 157×10^3 platelets/ µL (nadir, 128×10^3 platelets/µL on day 2), and her hemoglobin level was 7.8 g/dL. PCR confirmed the presence of *P. knowlesi*. Her infant was born by spontaneous vaginal delivery at 38 weeks, with a birth weight of 2.6 kg.

The second patient was a 35-year-old woman from Sabah's West Coast division. She was 7 weeks pregnant (G5P3) and presented with a 1-day history of fever on a background of 2 weeks of nausea, vomiting, and epigastric pain. On admission, her temperature was 37.5°C, and examination findings were unremarkable. Results of blood slide analysis were reported as "*P. malariae* 4+," her hemoglobin level was 13.3 g/dL, her platelet count was 295×10^3 platelets/µL, and her bilirubin and creatinine levels were within the normal range. PCR was not performed. She was treated with intravenous (and subsequently oral) quinine and oral clindamycin, had negative results of a blood film by day 1, and was discharged on day 3. Her hemoglobin level and platelet count were not repeated prior to discharge. Her pregnancy was further complicated by diet-controlled gestational diabetes and hypertension, requiring 1 additional hospital admission at 33 weeks, during which she received antihypertensive therapy. Her infant was born by spontaneous vaginal delivery at 35 weeks and 4 days, with a weight of 2.1 kg.

The third patient was a 24-year-old woman from Kudat Division, northeast Sabah, who was 35 weeks pregnant (G2P1) when she presented with a 1-week history of fever and rigors. She had a temperature of 37.7°C and a heart rate of 110 beats/minute. Examination findings were otherwise unremarkable. Results of blood film analysis were reported as "*P. malariae*" 1026 parasites/µL, her hemoglobin level was 7.8 g/dL (having decreased from 8.7 g/dL 1 week previously), her platelet count was 134×10^3 platelets/µL, and her bilirubin and creatinine levels were within normal ranges. PCR was not performed. She received artemether-lumefantrine and cleared her parasites by day 3. Her platelet count had normalized by day 3, but her hemoglobin level decreased to 7.2 g/dL, for which she received a transfusion prior to discharge on day 5. She delivered a 3.2-kg infant by spontaneous vaginal delivery at 39 weeks.

DISCUSSION

Our review of the Sabah Department of Health malaria records found that although *P. knowlesi* was the commonest cause of malaria among females of reproductive age, accounting for 43% of notifications, it was the least common cause of malaria during pregnancy, accounting for only 12% of cases. In contrast,

P. falciparum, accounting for 33% of malaria notifications among females of reproductive age, accounted for 59% of cases of malaria during pregnancy. The ratio of *P. falciparum* to *P. knowlesi* among all females was therefore 0.8:1, compared with 5:1 during pregnancy. Our review of the malaria notification database was further supported by our prospectively collected data at QEH, where all 6 cases of PCR-confirmed malaria during pregnancy were caused by *P. falciparum,* with no case of knowlesi malaria during pregnancy, and by our retrospective review of malaria microscopy records at Likas Women and Children's Hospital, which revealed 13 cases of *P. falciparum* infection during pregnancy, 7 cases of *P. vivax* infection during pregnancy, and no case of *P. knowlesi* infection during pregnancy.

As our studies included only women with malaria, we were unable to compare the risk of malaria infection, from any species, in pregnant versus nonpregnant women. The relative rarity of *P. knowlesi* infection, compared with *P. falciparum* infection and *P. vivax* infection, during pregnancy may therefore be due to either reduced susceptibility of pregnant women to knowlesi malaria or increased susceptibility of pregnant women to falciparum and vivax malaria.

Reduced susceptibility of pregnant women to *P. knowlesi* may be due to decreased exposure to this parasite during pregnancy. *Plasmodium knowlesi* is a simian parasite that is highly prevalent in long-tailed and pig-tailed macaques in Malaysian Borneo [31] and is transmitted via the forest-associated *Anopheles leucosphyrus* group of mosquitoes. Acquisition of *P. knowlesi* is thought to be related to forest exposure, with infection common among plantation workers and farmers [19, 23]. During pregnancy, females may modify their behavior so as to spend less time undertaking forest-related activities, therefore reducing their exposure to mosquitoes infected with *P. knowlesi*. In contrast, *P. falciparum* and *P. vivax,* although possibly sharing the same vector as *P. knowlesi* in Sabah [32, 33], are more likely to be transmitted in and around homes, because of the human reservoir of infection, and pregnancy may therefore be less likely to modify risk of exposure.

Increased susceptibility of pregnant women to *P. falciparum* and *P. vivax* has been well documented, with pregnant women infected more frequently and with higher parasitemia than nonpregnant women [2, 4, 13, 34–37]. In Africa, the main vector for *P. falciparum*, *Anopheles gambiae*, has been shown to bite pregnant women more frequently than nonpregnant women [38, 39], and this may contribute in part to increased frequency of infection among pregnant women in this region. However, the increased susceptibility of pregnant women to *P. falciparum* is also thought to be related to immunological and hormonal changes that occur during pregnancy, together with the ability of infected erythrocytes to adhere to and sequester in the placenta [40]. In falciparum malaria, placental-infected erythrocytes are immunologically distinct from infected erythrocytes

found in nonpregnant women and bind to unique receptors, such as chondroitin sulphate A [41]. Protective antibodies against these placental erythrocytes are found in multigravid women in areas of intense transmission and are thought to account for the reduced risk of infection in later pregnancies [42–44]. In areas of low transmission, women acquire little immunity and remain at increased risk of falciparum malaria throughout every pregnancy [45]. In *P. knowlesi* malaria, placental histology has not been examined, but cytoadherence is not thought to occur to the same extent as observed for *P. falciparum* [22], and it is possible that this may contribute to a differential susceptibility to infection during pregnancy. A study from Brazil has previously reported an overrepresentation of falciparum malaria, compared with vivax malaria, during pregnancy, with a paucity of cytoadherence of *P. vivax* in the placenta hypothesized to account for this finding [46].

In falciparum malaria, pregnant women living in areas of unstable transmission are at increased risk of developing complications of severe disease, including hyperparasitemia, severe anemia, hypoglycemia, and acute pulmonary edema [5, 13, 47, 48]. Infection also results in adverse pregnancy outcomes, including miscarriage, low infant birth weight, and increased perinatal mortality [6, 11]. In our study, only 3 women had knowlesi malaria during pregnancy. Although 2 of 3 had moderate anemia, other features of severity were not reported. However, the small number does not allow us to comment on the risk of severe knowlesi malaria in pregnant versus nonpregnant women. Severe knowlesi malaria during pregnancy with fetal loss has been reported [26], and ongoing surveillance will be important to determine whether pregnancy increases the risk of complications from knowlesi malaria. This will include monitoring for bleeding complications in the event of infection with *P. knowlesi* at the time of delivery, given that thrombocytopenia is nearly universal in knowlesi malaria and more severe than in falciparum or vivax malaria [19, 23]. It is notable that the second patient with knowlesi malaria during pregnancy in this study did not have thrombocytopenia. However, her platelet count was measured only on admission, and development of thrombocytopenia may have been missed. In addition, PCR was not performed, and therefore we cannot exclude misdiagnosis by microscopy. However, this patient was referred from a hospital where, in 2013, 46 of all 53 notified malaria cases (87%) were reported as *P. malariae/P. knowlesi* monoinfection, with *P. knowlesi* monoinfection confirmed in 32 of 40 malaria blood slides (80%) referred from this hospital for PCR testing (Sabah Department of Health and State Public Health Laboratory, unpublished data).

Plasmodium falciparum and *P. vivax* infections during pregnancy are also associated with adverse pregnancy outcomes, including miscarriage, low infant birth weight, and increased perinatal mortality. In this study, one woman delivered a preterm infant with a low birth weight of 2.1 kg. Although

gestational diabetes and hypertension may have contributed to prematurity [49], *P. knowlesi* infection during pregnancy may be associated with preterm delivery and/or low infant birth weight. Further studies are required to determine the risk and magnitude of adverse pregnancy outcomes.

Our study was associated with several limitations. Because of possible underreporting, the Sabah malaria notification database may underestimate the absolute number of patients with malaria, as well as the proportion of these patients who are pregnant. It is possible that there were additional women with knowlesi malaria during pregnancy who were not identified in this study. However, any lack of reporting should not affect the overall species distribution among pregnant women with malaria. Furthermore, the state notification data are supported by the underrepresentation of *P. knowlesi* during pregnancy relative to *P. falciparum* at the 2 referral hospitals. Second, malaria notifications in Sabah are based on microscopy results, and with the known difficulties of diagnosing *P. knowlesi* by microscopy [50], it is possible that this may have led to inaccuracies in the estimate of species distribution among pregnant women with malaria. However, it is unlikely that misdiagnosis by microscopy would have led to such a highly significant difference in the proportion of pregnant women with falciparum malaria, compared with those with knowlesi malaria. Furthermore, our findings were supported by prospectively collected data at QEH, which involved PCR-based confirmation of all malaria cases.

Finally, our data did not allow us to compare the risk of malarial parasite infection, of any species, in pregnant versus nonpregnant women, and population-based studies will be required to answer this question. In addition, longitudinal studies involving regular follow-up of pregnant women, preferably using molecular-based diagnostic methods, will be required to determine the true burden of *P. knowlesi* infection during pregnancy, including the prevalence and consequences of asymptomatic parasitemia.

In conclusion, we found that knowlesi malaria during pregnancy was relatively rare, despite *P. knowlesi* being the most common cause of malaria among females of reproductive age. With the ongoing increase in incidence of knowlesi malaria, however, and with the potential for human transmission of the species, continued surveillance is required to monitor the burden and clinical consequences of *P. knowlesi* during pregnancy.

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