

Falling *Plasmodium knowlesi* Malaria Death Rate among Adults despite Rising Incidence, Sabah, Malaysia, 2010–2014

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Learning Objectives

Upon completion of this activity, participants will be able to:

- Describe notification-fatality rates of fatal *P. knowlesi* cases in Sabah during 2010–2014, based on a surveillance study using the Sabah Department of Health malaria notification database
- Identify clinical characteristics of fatal *P. knowlesi* cases in Sabah during 2012–2014
- Discuss management details of fatal *P. knowlesi* cases in Sabah during 2012–2014

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Deaths from *Plasmodium knowlesi* malaria have been linked to delayed parenteral treatment. In Malaysia, early intravenous artesunate is now recommended for all severe malaria cases. We describe *P. knowlesi* fatalities in Sabah, Malaysia, during 2012–2014 and report species-specific fatality rates based on 2010–2014 case notifications. Sixteen malaria-associated deaths (caused by PCR-confirmed *P. knowlesi* [7], *P. falciparum* [7], and *P. vivax* [1] and microscopy-diagnosed “*P. malariae*” [1]) were reported during 2012–2014. Six patients with severe *P. knowlesi* malaria received intravenous artesunate at hospital admission. For persons ≥ 15 years of age, overall fatality rates during 2010–2014 were 3.4, 4.2, and 1.0 deaths/1,000 *P. knowlesi*, *P. falciparum*, and *P. vivax* notifications, respectively; *P. knowlesi*-associated fatality rates fell from 9.2 to 1.6 deaths/1,000 notifications. No *P. knowlesi*-associated deaths occurred among children, despite 373 notified cases. Although *P. knowlesi* malaria incidence is rising, the notification-fatality rate has decreased, likely due to improved use of intravenous artesunate.

Plasmodium knowlesi is the most common cause of malaria in East Malaysia, and the incidence of disease is increasing despite intensive control efforts that have substantially reduced the incidence of *P. falciparum* and *P. vivax* malaria in Malaysia (1–3). Although the greatest number of *P. knowlesi* cases has been reported in East Malaysia, the infection is also the predominant cause of malaria in Peninsular Malaysia (4) and is increasingly reported in other Southeast Asia countries and in travelers returning from these countries (5).

P. knowlesi infection can be associated with high parasitemia and is at least as likely as *P. falciparum* to cause severe malaria in adults (6). Age is strongly associated with parasitemia and, thus, a key risk factor for severe and fatal disease (6,7), neither of which has been reported in children with PCR-confirmed *P. knowlesi* malaria (5,8,9). In a tertiary referral hospital in Sabah, northeastern Malaysia, the rate of *P. knowlesi* malaria-associated deaths was low among persons ≥ 12 years of age who were promptly treated (including before hospital referral) with artesunate (6); however, *P. knowlesi* continues to cause fatal malaria among adults in Sabah (10,11). During 2010–2011, *P. knowlesi* was responsible for 6 of 14 fatal malaria cases in Sabah. Microscopy-based misdiagnosis of *P. knowlesi* malaria as the nearly identical, but more benign, *P. malariae* malaria was common, and fatal outcome was associated with delayed or lack of parenteral therapy: 2 of 6 patients with fatal *P. knowlesi* malaria received parenteral therapy (1 each with quinine and artesunate); the other 4 received chloroquine or sulfadoxine/pyrimethamine (10).

In the time since that study was conducted, recognition of *P. knowlesi* and its ability to cause severe disease has increased. The 2013 Management Guidelines of

Malaria in Malaysia recommend that results for blood films with parasites resembling *P. malariae* be reported as *P. knowlesi*/*P. malariae* (12). The guidelines emphasize that PCR-confirmed *P. malariae* is rare in Sabah and that patients with a microscopy-based diagnosis of *P. malariae* infection should be assumed to have *P. knowlesi* malaria. Moreover, like recent World Health Organization (WHO) global guidelines (13–15), Malaysian guidelines now recommend intravenous artesunate for all patients with severe malaria caused by any *Plasmodium* spp. (12), and in western Sabah, the drug is being used earlier and more frequently for all malarial infections (6). In addition, oral artemisinin combination treatment is now recommended for uncomplicated *P. knowlesi* malaria (12).

We assessed clinical features and management of fatal *P. knowlesi* malaria cases in Sabah during 2012–2014. We also determined age-stratified death rates among patients with *Plasmodium* spp. malaria and assessed trends in fatality rates for *P. knowlesi* malaria during 2010–2014.

Methods

In Sabah, which has an area of 73,600 km² and population of 3.7 million (16), reporting of all malaria cases and associated deaths to the Sabah Department of Health (DoH) is mandatory; species are reported according to microscopy results. We obtained details of reported malaria-associated deaths during 2012–2014 from the Sabah DoH and reviewed district hospital case notes for clinical details. The study was approved by the ethics committees of the Malaysian Ministry of Health and Menzies School of Health Research.

We reviewed the Sabah DoH malaria notification database for the total number of microscopy-based *P. knowlesi*/*P. malariae*, *P. falciparum*, and *P. vivax* malaria case notifications during 2010–2014. These data were used to determine case-fatality rates (CFRs) among notified cases for each species (hereafter referred to as notified CFRs, defined as number of PCR-confirmed *P. knowlesi*, *P. falciparum*, and *P. vivax* malaria-associated deaths per 1,000 microscopy-based *P. malariae*/*P. knowlesi*, *P. falciparum*, and *P. vivax* malaria notifications). PCR-confirmed *P. malariae* infection is rare in Sabah, accounting for <1% of clinical samples diagnosed by microscopy as *P. malariae* or *P. knowlesi* (1); thus, most notifications of *P. malariae*/*P. knowlesi* can be assumed to be *P. knowlesi*. During 2010–2014 in Sabah, only 1 fatal malaria case, a microscopy-diagnosed *P. malariae* infection, lacked PCR confirmation; an adjusted CFR was calculated following inclusion of this case.

Results

Sixteen malaria-associated deaths were reported in Sabah during 2012–2014, of which 15 were confirmed by PCR to be caused by *P. knowlesi* (7 cases; cases 1–7, online

Technical Appendix, <http://wwwnc.cdc.gov/EID/article/22/1/15-1305-Techapp1.pdf>); *P. falciparum* (7 cases); or *P. vivax* (1 case). Details of 1 *P. knowlesi* case (case 2) were previously reported (11). The remaining fatal case was microscopy diagnosed (without PCR confirmation) as *P. malariae* infection (online Technical Appendix).

Fatal PCR-Confirmed *P. knowlesi* Malaria

All 7 fatal *P. knowlesi* malaria cases occurred in adults (median age 61 [range 31–73] years); 4 were women. Six of these cases had been misdiagnosed by microscopy as *P. malariae* (4), *P. falciparum* (1), or *P. vivax* (1) infections. Severe malaria was recognized in 5 patients when they sought medical care; all received intravenous artesunate within 90 (median 30) minutes of diagnosis. Severity criteria at admission for these patients were jaundice (4 patients), acute kidney injury (3), metabolic acidosis (4), hyperparasitemia (2), respiratory distress (2), and coma (1) (online Technical Appendix Tables 1, 2). Shock and respiratory distress developed in all patients before death. All patients were intubated and ventilated; 2 received hemodialysis. Death occurred within 5–117 (median 41) hours of admission.

Two patients with fatal *P. knowlesi* malaria were not recognized to have severe malaria at admission; they received oral antimalarial treatment. One of these patients (case-patient 7, online Technical Appendix Table 1) had a blood film result reported as 22,666 *P. malariae* parasites/mL and was given oral artesunate/mefloquine for apparent uncomplicated malaria. Her creatinine level was 124 (reference 63–133) $\mu\text{mol/L}$; bilirubin, lactate, and bicarbonate results were not available. Within 12 hours, she became hypotensive and tachypneic; chest radiographs showed diffuse opacities in both lung fields. She was intubated and started on intravenous artesunate but died within 23 hours of admission. Subsequent reexamination of her initial blood slide showed 263,772 parasites/mL. The other patient (case 5, online Technical Appendix Table 1) was also thought to have uncomplicated malaria; her blood film result was reported as 9,866 *P. vivax* parasites/mL, her bilirubin level was 46 (reference <17) $\mu\text{mol/L}$, and her creatinine level was 143 $\mu\text{mol/L}$. She was treated with 1 dose of intravenous artesunate followed by chloroquine and primaquine. Blood film results the next day indicated *P. knowlesi* infection with 20,000 parasites/mL. Acute respiratory distress syndrome (ARDS) and metabolic acidosis developed, and the patient died on day 3, despite recommencement of intravenous artesunate. Postmortem reexamination of her initial blood slide showed 55,111 *P. knowlesi* parasites/mL.

One patient, a 56-year-old man, was comatose, a condition not previously reported in *P. knowlesi* malaria. He was unresponsive when brought into a health clinic by relatives, who reported a 1-day history of weakness and drowsiness and a 3-day history of fever, chills, arthralgia,

and myalgia. At hospital referral, his blood pressure was 85/60 mm Hg, pulse rate 150 beats/min, and oxygen saturation 81% on room air. He had a Glasgow Coma Scale score of 6/15. Pupils were reactive but asymmetric (right 2 mm, left 4 mm). Meningism was not present, and neurologic examination showed normal tone, symmetrically reduced reflexes, and downgoing plantar reflexes. Blood investigations showed 6,471 *P. knowlesi* parasites/mL and metabolic acidosis. Computed tomography brain scan and lumbar puncture were not performed. The patient was intubated and begun on intravenous artesunate and ceftriaxone but died 41 hours after admission. Blood cultures for bacterial infections were negative.

Fatal PCR-Confirmed *P. vivax* Malaria

One person, a 53-year-old man, died from *P. vivax* malaria. At admission, he had a 7-day history of fever, rigors, myalgia, nonproductive cough, and abdominal pain. Physical examination results were unremarkable. Thrombocytopenia was present, and his bilirubin level was 21.5 $\mu\text{mol/L}$; creatinine and hemoglobin levels were normal. A blood film result was reported as 2,090 *P. vivax* parasites/mL; oral chloroquine and primaquine treatment were begun. The next day, the parasite count was 890 parasites/mL of blood, but the patient became hypotensive, and ARDS developed; a postintubation chest radiograph showed bilateral opacities. Intravenous artesunate and antibiotic drugs were initiated, and hemodialysis was performed for acute kidney injury (creatinine level 316 $\mu\text{mol/L}$), but the patient died 4 days after admission. Blood cultures for bacterial infections were negative.

Fatal PCR-Confirmed *P. falciparum* Malaria

Of the 7 *P. falciparum* malaria-associated deaths, 2 (29%) occurred in children (a boy and a girl 2–3 years of age, both Filipino) and 5 (71%) occurred in adults (3 men and 2 women 31–80 years of age; 2 Filipino, 2 Malaysian, and 1 Indonesian). At the initial examination, all patients met WHO criteria for severe malaria: jaundice (5 patients), cerebral malaria (4 patients), renal failure (3 patients), respiratory distress (3 patients), and anemia (2 patients). Within 2 hours of malaria diagnosis, 1 patient was given oral artesunate/mefloquine and all others were given intravenous artesunate. All patients were intubated and ventilated, 6 received inotropes, and dialysis was performed on 3. All patients died within 2–9 days of admission. Blood cultures for 1 child and 1 adult were positive for *Klebsiella pneumoniae* and coagulase-negative staphylococci, respectively; the latter was thought to represent contamination.

Review of 2010–2014 Malaria Notification Data

The overall notified CFR for *P. knowlesi* malaria was 3.08 deaths/1,000 cases, compared with 4.83 and 0.87

deaths/1,000 cases of *P. falciparum* and *P. vivax* malaria, respectively (Table). Among adults (persons ≥ 15 years of age), notified CFRs were 3.37, 4.17, and 1.02 deaths/1,000 cases for *P. knowlesi*, *P. falciparum*, and *P. vivax* malaria, respectively. Despite 373 notifications of *P. knowlesi* malaria and 611 notifications of *P. vivax* malaria among children (persons < 15 years of age) during 2010–2014, no deaths from either species were reported. However, children with *P. falciparum* malaria had a notified CFR of 6.7 deaths/1,000 cases. Notified CFRs among adults with *P. knowlesi* malaria declined from 9.2 to 1.6 deaths/1,000 notifications in 2010 and 2014, respectively (χ^2 test for trend, $p = 0.11$).

During 2010–2014, female patients accounted for only 783 (19%) of the 4,217 *P. malariae/P. knowlesi* notifications, but they accounted for 6 (46%) of the 13 fatal *P. knowlesi* malaria cases. Thus, the notified CFR for *P. knowlesi* malaria was 7.66 deaths/1,000 cases for female patients, compared with 2.04 deaths/1,000 cases for male patients (Fisher exact test, $p = 0.021$). However, this difference was not significant in a multivariate logistic regression model adjusting for age (odds ratio 2.60, $p = 0.095$). For *P. falciparum* and *P. vivax* malaria, no difference was seen in the number of notified CFRs for male and female patients.

Discussion

Despite ongoing microscopy-based misdiagnoses of *P. knowlesi* infections, management of severe malaria in Sabah appears to have improved; all patients recognized to have severe *P. knowlesi* malaria on admission received intravenous artesunate as initial therapy. Although our findings clearly demonstrate the ability of *P. knowlesi* to cause

fatal malaria despite optimal therapy, *P. knowlesi* notified CFRs in Sabah have fallen over the past 5 years in association with the increased early use of artesunate documented in this and other reports (6). Death from *P. knowlesi* malaria remains unreported in children, and all but 1 of the *P. knowlesi*-associated deaths in this series occurred in adults > 50 years of age.

The absence of *P. knowlesi*-associated deaths among children, despite 373 *P. malariae/P. knowlesi* notified cases in this age group during 2010–2014, contrasts with the well-recognized risk for childhood deaths from *P. falciparum* malaria (17) and extends the lack of previous reports of either severe or fatal outcomes in children with *P. knowlesi* malaria (5,8,9). Furthermore, the large number of notified cases in children in this series suggests that the lack of *P. knowlesi*-associated deaths among children may not be due solely to the relative underrepresentation of children in previous series of *P. knowlesi* malaria (6,7,18,19). A lower risk for severe and fatal *P. knowlesi* malaria in children may be due to the previously documented strong association between age and parasitemia (6); the level of parasitemia in children is generally insufficient to cause severe and fatal disease (20). In addition, younger age may be associated with physiologic protection from severe and fatal *P. knowlesi* malaria, as suggested by previous findings of a lower risk of severe malaria after primary exposure to *P. falciparum* in nonimmune children compared with nonimmune adults (21).

Clinical and demographic characteristics for adult *P. knowlesi* malaria patients in this study were consistent with those in previous reports (1,6,7,18). Patients had a median age of 61 years. Because of the strong correlation between

Table. CFRs among persons with notified cases of *Plasmodium* spp. malaria, Sabah, Malaysia, 2010–2014*

| Age group, year | <i>P. knowlesi</i> † | | | <i>P. falciparum</i> | | | <i>P. vivax</i> | | |
|----------------------------|----------------------|------------|---------------|----------------------|------------|--------------|-------------------|------------|--------------|
| | No. notifications | No. deaths | Notified CFR‡ | No. notifications | No. deaths | Notified CFR | No. notifications | No. deaths | Notified CFR |
| Persons ≥ 15 y of age | | | | | | | | | |
| 2010 | 327 | 3 | 9.17 | 736 | 2 | 2.72 | 720 | 0 | 0.00 |
| 2011 | 608 | 3 | 4.93 | 467 | 2 | 4.28 | 479 | 1 | 2.09 |
| 2012 | 744 | 4§ | 5.38 | 543 | 2 | 3.68 | 390 | 0 | – |
| 2013 | 927 | 1 | 1.08 | 239 | 1 | 4.18 | 211 | 1 | 4.74 |
| 2014 | 1,246 | 2 | 1.61 | 173 | 2 | 11.56 | 165 | 0 | – |
| Total | 3,852 | 13 | 3.37 | 2,158 | 9 | 4.17 | 1,965 | 2 | 1.02 |
| Persons < 15 y of age | | | | | | | | | |
| 2010 | 57 | 0 | – | 355 | 2 | 5.63 | 262 | 0 | – |
| 2011 | 95 | 0 | – | 138 | 1 | 7.25 | 149 | 0 | – |
| 2012 | 73 | 0 | – | 171 | 2 | 11.70 | 88 | 0 | – |
| 2013 | 69 | 0 | – | 58 | 0 | – | 52 | 0 | – |
| 2014 | 79 | 0 | – | 21 | 0 | – | 60 | 0 | – |
| Total | 373 | 0 | – | 743 | 5 | 6.73 | 611 | 0 | – |

*Notified CFR, case-fatality rate determined on the basis of the no. of PCR-confirmed malaria-associated deaths/1,000 notifications of microscopy-based malaria cases.

†Includes all cases notified as *P. knowlesi* or *P. malariae*. PCR-confirmed *P. malariae* infection is rare in Sabah, accounting for $< 1\%$ of clinical samples diagnosed by microscopy as *P. malariae* or *P. knowlesi* (1); thus, most notifications of *P. malariae/P. knowlesi* can be assumed to be *P. knowlesi*.

‡ $p = 0.110$ (χ^2 test for trend) for reduction in *P. knowlesi* fatality rate among adults during 2010–2014.

§Excludes 1 fatal case of microscopy-diagnosed *P. malariae*. The notified CFR in 2012 with this case included as a *P. knowlesi*-associated death was 6.72 deaths/1,000 notifications, and the overall notified CFR for *P. malariae/P. knowlesi* in adults was 3.63 deaths/1,000 notifications. The p value for the test for trend is unchanged at 0.11

age and parasitemia, older patients are known to be at increased risk for severe *P. knowlesi* malaria (6). Of the 7 patients who died, 4 were female; thus the notified CFR was significantly higher among female than male patients. Although this discrepancy appears to be primarily due to the older age of female patients with *P. knowlesi* malaria (1), a trend toward increased notified CFRs for female *P. knowlesi* patients remained even after adjusting for age. This finding is consistent with the increased risk for severe *P. knowlesi* malaria found for female patients in some (7,18), but not all (6), previous studies. Larger studies are needed to clarify the association between sex and risk for severe *P. knowlesi* malaria.

The complications experienced by the *P. knowlesi* malaria patients in this study were generally consistent with those in other reports; hyperparasitemia, respiratory distress, shock, jaundice, and acute kidney injury were common in this and previous reports (6,7,18,22–24). Metabolic acidosis occurred in 5 patients in this series. This complication of severe *P. knowlesi* malaria was uncommon in a previous tertiary referral hospital study that involved early, including prereferral, use of artesunate and in which no deaths occurred (6). However, metabolic acidosis has been reported in most fatal *P. knowlesi* malaria cases (7,10,11,18,25), and, as with *P. falciparum* malaria, is likely a late complication signifying poor outcome. Acute lung injury was present at admission in 2 patients and developed after treatment initiation in all remaining *P. knowlesi* patients; this finding is consistent with a posttreatment inflammatory response, as previously postulated (6).

Coma has not previously been reported in *P. knowlesi* malaria. Although decreased conscious state occurred in 1 patient in this study, blood cultures, lumbar puncture, and computed tomography brain scan were not performed, and pupillary asymmetry, reported in this patient, is unusual in coma due to *P. falciparum* malaria. Therefore, while decreased consciousness directly associated with *P. knowlesi* remains possible, alternative causes are plausible.

Microscopy-based misdiagnosis of *P. knowlesi* infection occurred in 6 of 7 cases. Four of the 6 case-patients had misdiagnoses of *P. malariae* infection; all had high parasitemia (2 had >100,000 parasites/ μ L of blood), which is inconsistent with a diagnosis of *P. malariae* infection but highly suggestive of *P. knowlesi* infection. Malaysia's malaria guidelines recommend that blood films with parasites resembling *P. malariae* be reported as *P. knowlesi*/*P. malariae* (12); however, high parasitemia, particularly in the context of a very low statewide prevalence of *P. malariae* (1), makes *P. malariae* infection unlikely. The frequent microscopy-based misdiagnosis of *P. knowlesi* malaria in this and other reports (26) in Sabah highlights the need for alternative rapid diagnostic methods.

Despite the frequent misdiagnoses in this series, all patients with fatal *P. knowlesi* infection who were recognized as having severe malaria at admission were appropriately treated with early intravenous artesunate. In contrast, in our 2010–2011 review of malaria deaths (10), only 2 of 5 patients with severe *P. knowlesi* malaria received parenteral treatment; the other 3, who had misdiagnoses of *P. malariae* or *P. vivax* malaria, received oral chloroquine or sulfadoxine/pyrimethamine. In the current study, 2 patients were thought to have uncomplicated malaria and received oral therapy (case-patient 5 was given chloroquine after 1 dose of intravenous artesunate; case-patient 7 was given artesunate/mefloquine). Postmortem reexamination of these patients' initial blood films showed a parasite count substantially higher than initially reported (55,111 parasites/ μ L vs. 9,866 parasites/ μ L for case-patient 5; 263,772 parasites/ μ L vs. 22,666 parasites/ μ L for case-patient 7). Parasitemia has been shown to be a major risk factor for severe *P. knowlesi* malaria: in a recent prospective study, severity criteria were present in >50% of patients with >20,000 parasites/ μ L of blood and >80% of patients with >100,000 parasites/ μ L of blood (6). WHO guidelines now recommend that intravenous artesunate be used for all patients with *P. knowlesi* malaria and >100,000 parasites/ μ L of blood or, if testing for laboratory criteria for severe malaria is not available, >20,000 parasites/ μ L blood (14,15). The failure of oral therapy in case-patient 7 (initial blood slide reported as 22,666 parasites/ μ L; bilirubin not available) highlights the value of this recommendation. Moreover, these 2 cases demonstrate that parasitemia must be accurately quantified in patients with *P. knowlesi* malaria.

The use of chloroquine in case-patient 5 may have contributed to the poor outcome. Compared with artesunate/mefloquine, chloroquine has been associated with reduced parasite clearance time in *P. knowlesi* malaria (27,28) and is no longer recommended as first-line treatment for *P. knowlesi* malaria in Malaysia (12). In Sabah, parasite clearance time for *P. vivax* malaria treated with chloroquine is reduced compared with that for cases treated with artesunate/mefloquine, and treatment failures are common (29). Moreover, *P. knowlesi* and *P. vivax* are frequently confused in microscopy examination (26); hence, a unified treatment approach should be considered in Sabah, using artemisinin for all malaria cases.

Although this case series highlights the ability of *P. knowlesi* malaria to cause fatal disease in adults even after prompt administration of intravenous artesunate, it must be noted that the number of deaths has not increased over recent years, despite a rise in *P. knowlesi* malaria notifications from 384 in 2010 to 1,325 in 2014. Thus, the adult notified CFR has declined from 9.2 deaths/1,000 notifications in 2010 to 1.6 deaths/1,000 notifications in 2014. This improvement likely resulted from increased

recognition of the ability of *P. knowlesi* to cause severe disease and to increased use of intravenous artesunate. Although intravenous artesunate has been recommended in Sabah since December 2008, intravenous quinine was still in use until at least 2010 (10). In a retrospective study of severe *P. knowlesi* malaria at a tertiary referral hospital in Sabah during 2007–2009, a total of 5 (31%) of 16 patients treated with intravenous quinine died. In contrast, at the same hospital during 2010–2011, none of the severe *P. knowlesi* malaria patients treated with early intravenous artesunate died (6). In addition, in Sabah, the increasing use of artemisinin combination treatment instead of chloroquine for uncomplicated *P. knowlesi* malaria (6) may also have contributed to the decline in notified CFRs, particularly for cases in which severe disease is unrecognized.

We also reported a case of fatal *P. vivax* malaria with ARDS, an increasingly well-recognized complication of *P. vivax* malaria that has resulted in fatalities (30–34). The pathophysiologic mechanism likely involves soluble mediators and endothelial damage, exacerbated by shock and leading to diffuse damage to alveolar membranes (30). As with the case in our study, most ARDS cases occur after treatment initiation (32,35), possibly resulting from an exacerbated inflammatory response to parasite killing. Most of the initial cases of *P. vivax*-associated ARDS were in returned travelers with single organ dysfunction and nonfatal outcome; however, more recent series from countries where *P. vivax* is endemic have reported ARDS cases with multiorgan dysfunction and considerable mortality (35,36).

This study had several limitations. First, the retrospective design of the case series resulted in unavoidably incomplete laboratory and clinical data. In particular, alternative diagnoses cannot be excluded in the case of possible *P. knowlesi*-associated coma. Second, our calculation of the microscopy-based notified CFR represents only an estimate of the true *P. knowlesi*-associated CFR. The accuracy of this estimate will depend on the accuracy of microscopy-based identification of all *Plasmodium* species, the notification rate of malaria cases, and the proportion of persons with malaria who seek care at a health clinic. We do not have data on the proportion of malaria cases in Sabah that are notified; it is probable, however, that some are not notified, so the notified CFR likely overestimates the true CFR. In addition, we cannot exclude the possibility that the reduction in the *P. knowlesi*-associated notified CFRs during 2010–2014 is due to an increase in the proportion of malaria cases that are notified. However, notification of malaria cases in Sabah has been mandatory since 1992, and there is no reason to suspect that the notification rate would have changed substantially since 2010. It is similarly unlikely that the proportion of *P. knowlesi* malaria cases diagnosed as *P. falciparum* malaria, and vice versa, changed sufficiently

over the 5-year period to account for the observed decline in notified CFRs (1). Nonetheless, larger prospective studies involving molecular diagnostic methods are needed to obtain a more accurate assessment of the true *P. knowlesi* malaria CFR, including changes over time. Although we report notified CFRs for *P. knowlesi*, *P. falciparum*, and *P. vivax* malaria, these data may not reflect the relative virulence of each species. In this series, non-Malaysian citizens accounted for a higher proportion (5/7) of patients with fatal *P. falciparum* malaria than fatal *P. knowlesi* malaria, and it is possible that a delay in seeking care at a healthcare facility may be a confounding factor in comparing CFRs for malaria caused by these *Plasmodium* spp.

In conclusion, our findings show that despite increasing notifications of *P. knowlesi* malaria cases in Sabah, the number of fatal cases has not increased. The reduction in notified CFRs may be associated with the increased recognition of the ability of *P. knowlesi* to cause severe and fatal malaria and improved use of intravenous artesunate for severe malaria caused by any *Plasmodium* spp, as per recent policy changes (6,12). Nonetheless, this study demonstrates the ability of *P. knowlesi* to cause fatal malarial disease in adults, despite optimal therapy, and that *P. knowlesi* remains the most common cause of fatal malaria in adults in Sabah. In contrast, the study shows a notable absence of deaths among children with *P. knowlesi* malaria.

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Falling *Plasmodium knowlesi* Malaria Death Rates among Adults despite Rising Incidence, Sabah, Malaysia, 2010–2014

Technical Appendix

Fatal Cases of PCR-Confirmed *P. knowlesi* Malaria, 2012–2014

Case 1

A 31 year old man with no known comorbidities presented for the second time in 3 days to a tertiary-referral hospital with seven days of fever, rigors and abdominal pain. On examination he was jaundiced and tachycardic but other vital signs were normal. Respiratory examination was normal. He had tenderness over the epigastric region and right hypochondrium. A provisional diagnosis of ascending cholangitis was made and he was admitted under the surgical team. Blood investigations revealed jaundice (bilirubin 254 $\mu\text{mol/L}$), acute kidney injury (creatinine 915 $\mu\text{mol/L}$, urea 37.9 $\mu\text{mol/L}$) and metabolic acidosis (pH=7.21, bicarbonate 11.5 mmol/L). Blood film was reported as *P. falciparum* with 92,500 parasites/ μL . He was commenced on intravenous artesunate and antibiotics (intravenous ceftriaxone and metronidazole and oral doxycycline), however deteriorated rapidly with progressive shock and respiratory distress. He was intubated and ventilated and transferred to the intensive care unit (ICU) where he commenced haemodialysis and inotropic support. Chest radiograph post-intubation was reported as normal. Parasite clearance occurred on day 3 however the patient required ongoing ventilatory and haemodialysis support, with his ICU admission also complicated by *Pseudomonas aeruginosa* ventilator-associated pneumonia for which he was commenced on intravenous ceftazidime. He was extubated and discharged from ICU on day 9, however developed acute respiratory distress the following day while being commenced on haemodialysis. Resuscitation was unsuccessful, and cause of death was reported as acute

pulmonary oedema, although no chest radiograph was performed. Blood cultures taken prior to antibiotics on admission were negative, and PCR confirmed *P. knowlesi* monoinfection.

Case 2

Details of this case have been previously reported (Rajahram et al. Med J Malaysia 2013;68). In brief, a 71 year old woman with a history of hypertension and peptic ulcer disease presented with 5 days of fever associated with chills, rigors, abdominal pain, myalgia and arthralgia. She was jaundiced, tachycardic and tachypnoeic. Bilateral lower zone crepitations were noted on chest auscultation, although her chest radiograph was reported as normal, and arterial blood gas revealed metabolic acidosis. Blood film was reported as *P. malariae* 120,000 parasites/ μ L and the patient was commenced on intravenous artesunate, oral doxycycline and intravenous ceftriaxone, however her condition deteriorated rapidly requiring intubation and inotropic support. CXR showed bilateral heterogeneous opacities. She died 12 hours later, with a diagnosis of severe malaria complicated by acute kidney injury, hyperbilirubinemia, hypoglycaemia, acute respiratory distress syndrome and metabolic acidosis. Blood cultures done on admission were negative.

Case 3

A 61 year old woman with history of hypertension presented to a district hospital with 7 days of epigastric pain, fever, reduced urine output and dyspnoea. She was tachycardic and tachypnoeic, with an oxygen saturation of 86% on 5 litres of oxygen via facemask. Chest auscultation was unremarkable, and abdominal examination noted hepatomegaly. Blood film was reported as *P. malariae* 320,000 parasites/ μ L and acute kidney injury was present (creatinine 453 μ mol/L, urea 33.8 μ mol/L). The patient was commenced on intravenous artesunate and ceftriaxone and transferred to a tertiary referral hospital where she was intubated and ventilated and commenced on inotropic support. Repeat chest auscultation noted bilateral crepitations. Chest radiograph was reported to be normal, and arterial blood gas revealed metabolic acidosis (pH=7.24, bicarbonate 10.3 mmol/L). The patient died 7 hours later, with cause of death reported as acute respiratory distress syndrome. Blood cultures taken on admission prior to antibiotics were negative.

Case 4

A 56 year old man with no known comorbidities was brought unresponsive to a primary health clinic by relatives who reported a one day history of progressive weakness and drowsiness on a background of 3 days of fever, chills, arthralgia and myalgia. On referral to a district hospital he was afebrile, hypotensive (blood pressure 85/60 mmHg) and tachycardic (pulse rate 150 beats per minute). Oxygen saturation was 81% on room air, and 97% on 15 litres oxygen via high flow mask with a respiratory rate of 25 breaths per minute. Glasgow Coma Scale (GCS) was 6/15. Pupils were reactive and measured as 2mm on the right and 4mm on the left. There was no meningism, and neurological examination noted normal tone, symmetrically reduced reflexes and downgoing plantar reflexes. Chest auscultation and abdominal examination were unremarkable. Arterial blood gas revealed severe metabolic acidosis (pH= 7.28, bicarbonate 11.6 mmol/L), and blood film was reported as *P. knowlesi* with 6471 parasites/ μ L. Chest radiograph was reported as normal. No CT brain or lumbar puncture was performed. The patient was intubated and ventilated, and commenced on inotropic support in addition to intravenous ceftriaxone, artesunate and oral doxycycline. The patient died 41 hours following admission, with blood film prior to death reported as *P. knowlesi* 1134 parasites/ μ L. Blood cultures taken on admission were negative.

Case 5

A 57 year old woman with a history of type 2 diabetes and hypertension presented to a district hospital with 7 days of fever, lethargy and myalgia. She was febrile and tachycardic, but clinical examination was otherwise unremarkable. Initial blood film was reported as *P. vivax* 9866 parasites/ μ L and she was commenced on oral chloroquine and primaquine following a single dose of intravenous artesunate. Blood film the following day was reported as *P. knowlesi* 20,000 parasites/ μ L , and the patient was referred to a tertiary referral hospital. Shortly after arrival she was noted to be tachypnoeic (respiratory rate of 34 breaths per minute), and hypoxic (oxygen saturation 70% on 15 liter oxygen via high flow mask), with bilateral crepitations on chest auscultation, and metabolic acidosis on arterial blood gas (pH 7.35, bicarbonate 14.3 mmol/L). She was intubated, ventilated and commenced on intravenous artesunate, ceftriaxone and furosemide, however died shortly after intubation. Cause of death was reported as acute pulmonary oedema, although chest radiograph had not been performed. Blood cultures

performed on admission were negative. Post- mortem re-examination of her initial blood film was reported as *P. knowlesi* 55,111 parasites/ μ L.

Case 6

A 73 year old man with no known comorbidities presented to a district hospital with a 7-day history of fever, chills and diarrhoea. On examination he was jaundiced and hypotensive, with hepatomegaly. Blood film was reported as *P. malariae* “4+”. He was commenced on intravenous artesunate and ceftriaxone and oral doxycycline, however developed worsening respiratory distress, acute kidney injury (creatinine 356 μ mol/L) and metabolic acidosis (pH 7.1, bicarbonate 11.8 mmol/L), and was intubated and transferred to the ICU. Chest radiograph showed bilateral lung infiltrates. Tracheal aspirate was positive for acid fast bacilli “2+” and antituberculosis therapy was commenced. Despite haemodialysis and inotropic support, the patient died 3 days later. Cause of death was stated as severe malaria and active tuberculosis with multiorgan failure. Blood cultures performed on admission were negative.

Case 7

A 62 year old woman with a history of hypertension presented with a 6-day history of fever, chills, rigors and dry cough. On examination she was febrile and tachycardic, but examination was otherwise unremarkable and initial chest radiography was normal. Blood film was reported as *P. malariae* with 22,666 parasites/ μ L, and she was commenced on oral artesunate+mefloquine for uncomplicated malaria. Within 12 hours she became hypotensive (blood pressure 77/49 mmHg) and tachypnoeic (respiratory rate 32 breaths per minute). Oxygen saturation was 98% on room air. Chest auscultation revealed crackles in both lungs and a repeat chest radiograph noted diffuse heterogenous opacities in both lung fields. Arterial blood gas was not available. The patient was intubated and commenced on intravenous artesunate and inotropes, however died within 23 hours of admission. No blood cultures were done. PCR confirmed *P. knowlesi* mono-infection. Later cross-check of initial parasite count showed her true initial *P. knowlesi* parasitemia was 263,772 parasites/ μ L.

Fatality due to Microscopy-Diagnosed “*P. malariae*”

A previously well 27 year old man presented to a district hospital with a 3-day history of fever and dyspnoea, and reduced conscious state on the day of admission. His Glasgow Coma

Score (GCS) was recorded as 3/15, and pupils were fixed and dilated. Blood pressure was 149/96 mmHg and oxygen saturation was 81% on room air, with bilateral crackles on chest auscultation. Blood film was reported as *P. malariae* “3+”, and kidney injury was present (creatinine 210 µmol/L, urea 9.4 µmol/L). Chest radiograph was reported as normal, and arterial blood gas was not performed. The patient was intubated and ventilated and commenced on intravenous artesunate and antibiotics, however died 21 hours later. No CT brain or lumbar puncture was performed. Cause of death was reported as severe malaria with acute kidney injury and ARDS. Neither blood cultures nor PCR were performed.

Fatal *P. vivax* Malaria

A 53-year-old- man with a history of hypertension presented with 7-day history of fever with chills, rigors, myalgia, non-productive cough and abdominal pain. Heart rate was 125 beats per minute and blood pressure was 159/34 mmHg, with examination otherwise normal. Blood investigations noted thrombocytopenia (platelets 74,000 cells/µL) and mildly elevated bilirubin (21.5 µmol/L), but other blood parameters on admission were normal, including haemoglobin (17 g/dL), creatinine (108 µmol/L) and urea (6 µmol/L). Blood film was reported as *P. vivax* with 2090 parasites/µL, and the patient was commenced on oral chloroquine and primaquine. The following day the parasite count was 890 parasites/µL, however the patient became hypotensive (blood pressure 95/51 mmg Hg), tachypnoeic (respiratory rate 34 breaths/minute) and hypoxic (oxygen saturation 70% on room air) with generalized crackles on chest auscultation, and he was intubated and transferred to ICU. Chest radiograph showed bilateral heterogeneous opacities, and FiO₂:PaO₂ ratio was 132. The patient was commenced on intravenous artesunate and intravenous antibiotics in addition to inotropic support, and subsequently required haemodialysis for acute kidney injury (creatinine 316 µmol/L, urea 19.6 µmol/L). Despite supportive care the patient died 4 days after admission. Cause of death was reported as severe malaria with ARDS and acute kidney injury. PCR identified *P. vivax* monoinfection. Blood cultures done on admission were negative.

Technical Appendix Table 1. Demographic, clinical and laboratory features of fatal cases of PCR-confirmed *P. knowlesi*, and one microscopy-diagnosed "*P. malariae*"

| No. | Sex, age, y | Nationality | Initial microscopic diagnosis, parasites/ μ L | PCR | Severity criteria on presentation | | | | | | Time to death, h | Initial antimalarial treatment | Complications developing following commencement of antimalarial treatment |
|-----|-------------|-------------|---|-----|--|---|----------------------------------|--|---|------|------------------|---|---|
| | | | | | Acute Kidney Injury (Cr $>265 \mu\text{mol/L}$) | Jaundice (Bil $>43 \mu\text{mol/L}$ with Cr $>132 \mu\text{mol/L}$ or parasite count $>20,000$ parasites/ μL) | Hypotension (BP ≤ 80 mm Hg) | Metabolic acidosis ($\text{HCO}_3^- < 15$ mmol/L) | Respiratory distress ($\text{SaO}_2 < 94\%$)* | Coma | | | |
| 1 | M, 31 | Malaysian | Pf 92,500 | Pk | Yes (Cr 915 $\mu\text{mol/L}$) | Yes (Bil 254 $\mu\text{mol/L}$) | No | Yes (HCO_3^- 11.5 mmol/L) | No | No | 117 | iv artesunate, oral doxycycline | Shock, respiratory distress |
| 2 | F, 71 | Malaysian | Pm 120,000 | Pk | Yes (Cr 662 $\mu\text{mol/L}$) | Yes (Bil 108 $\mu\text{mol/L}$) | No | Yes (HCO_3^- 4.2 mmol/L) | No | No | 12 | iv artesunate, oral doxycycline | Shock, respiratory distress (ARDS) |
| 3 | F, 61 | Malaysian | Pm 320,000 | Pk | Yes (Cr 453 $\mu\text{mol/L}$) | Yes (Bil 315 $\mu\text{mol/L}$) | No | Yes (HCO_3^- 10.3 mmol/L) | Yes (SaO_2 88%) | No | 5 | iv artesunate | Shock |
| 4 | M, 56 | Filipino | Pk 6471 | Pk | No | No | No | Yes (HCO_3^- 11.6 mmol/L) | Yes (SaO_2 81%) | Yes | 41 | iv artesunate, oral doxycycline | Shock |
| 5 | F, 57 | Filipino | Pv 9866 ¹ | Pk | No | Yes (Bil 46 $\mu\text{mol/L}$, Cr 143 $\mu\text{mol/L}$) | No | N/A | No | No | 63 | iv artesunate (single dose) followed by CQ and PQ | Shock, respiratory distress (ARDS), metabolic acidosis |
| 6 | M, 73 | Malaysian | Pm "4+" ² | Pk | No | Yes (Bil 234 $\mu\text{mol/L}$) ⁴ | Yes (BP 80/49 mm Hg) | No | No | No | 71 | iv artesunate, oral doxycycline | Respiratory distress, AKI, metabolic acidosis |
| 7 | F, 62 | Malaysian | Pm 22,666 ³ | Pk | No | N/A | No | N/A | No | No | 23 | oral artesunate/mefloquine | Shock, respiratory distress |
| X | M, 27 | Filipino | Pm "3+" ² | N/A | No | N/A (Cr 210 $\mu\text{mol/L}$) | No | N/A | Yes (SaO_2 81%) | Yes | 21 | iv artesunate | shock |

Pk = *P. knowlesi*; Pv = *P. vivax*, Pf = *P. falciparum*; Pm = *P. malariae*; Cr = creatinine; Bil = bilirubin, iv = intravenous; BP = blood pressure; HCO_3^- = bicarbonate; SaO_2 = oxygen saturation; CQ = chloroquine; PQ = primaquine; ARDS = acute respiratory distress syndrome; AKI = acute kidney injury; N/A = not available.

¹Post-mortem re-examination of blood film reported as *P. knowlesi* 55,111 parasites/ μL .

²"3+" refers to 1 - 10 parasites per thick field, and "4+" refers to >10 parasites per thick field.

³Post-mortem re-examination of blood film reported as *P. knowlesi* 262,772 parasites/ μL .

⁴Creatinine 132 $\mu\text{mol/L}$ and exact parasite count recorded only as "4+."

Note: Blood culture taken before antibiotics were negative in all patients except case 7, where they were not performed. Detailed case descriptions are listed in the text above.

Technical Appendix Table 2. Demographic, clinical and laboratory features of reported PCR-confirmed *P. knowlesi* deaths

| Details | Case 1 | Case 2 | Case 3 | Case 4 | Case 5 | Case 6 | Case 7 |
|--|-----------|-----------|-----------|----------|-----------------|-----------|-----------|
| Age | 31 | 71 | 61 | 56 | 57 | 73 | 62 |
| Sex | Male | Female | Female | Male | Female | Male | Female |
| Nationality | Malaysian | Malaysian | Malaysian | Filipino | Filipino | Malaysian | Malaysian |
| Time to death, hours | 117 | 12 | 5 | 41 | 63 | 71 | 23 |
| Blood pressure, mmHg | 129/72 | 127/87 | 112/79 | 85/60 | 193/96 | 80/49 | 116/71 |
| Heart rate per minute | 108 | 111 | 124 | 150 | 129 | 72 | 111 |
| Respiratory Rate per minute | 24 | 38 | 26 | 25 | 22 | 20 | 22 |
| Oxygen saturation on room air | 97 | 96 | 88 | 81 | 98 | 100 | 96 |
| PaO ₂ :FiO ₂ ratio | 297 | 86 | 159 | 164 | 79 ¹ | 288 | NA |
| Axillary temperature, °C | 37.4 | 37.0 | 36.8 | 36.2 | 39.0 | 37.7 | 39.9 |

| Details | Case 1 | Case 2 | Case 3 | Case 4 | Case 5 | Case 6 | Case 7 |
|--|-----------------------------------|-----------------------------------|----------------------------|-----------------------------------|---|-----------------------------------|--|
| Hemoglobin, g/dL (females 12.0-16.0, males 13.5-17.5) | 12.9 | 14.1 | 12.6 | 13.8 | 11.1 | 11.0 | 12.0 |
| WBC count, x 10 ³ cells/mL (4.5-11) | 6.9 | 13.3 | 20.5 | 12.1 | 5.3 | 8.2 | 11.2 |
| Platelet count, x 10 ³ cells/ μ L (150-450) | 25 | 53 | 8 | 73 | 26 | 67 | 60 |
| Serum creatinine, μ mol/L (63-133) | 915 | 662 | 453 | 171 | 143 | 132 | 124 |
| Serum urea, μ mol/L (1.0-8.3) | 37.9 | 36.3 | 33.8 | 11.8 | 12.1 | 13.8 | 6.1 |
| Total serum bilirubin, μ mol/L (<17) | 254 | 108 | 315 | 43 | 46 | 234 | NA |
| Serum aspartate aminotransferase, U/L (<37) | 67 | 322 | 732 | 373 | NA | 61 | NA |
| Serum alanine aminotransferase concentration, U/L (<40) | NA | 145 | 258 | 143 | NA | 36 | NA |
| Serum albumin, g/L (35-60) | 28 | 24 | 20 | 24 | 27 | 20 | NA |
| Serum bicarbonate, mmol/L (18-23) | 11.5 | 4.2 | 10.3 | 11.6 | 14.3 | 16 | NA |
| Serum lactate mmol/L (0.5-2.2) | 3.6 | NA | NA | NA | NA | NA | NA |
| Blood cultures | Negative | Negative | Negative | Negative | Negative | Negative | Not done |
| Initial microscopic diagnosis, parasites/ μ L | <i>P. falciparum</i> 92,500 | <i>P. malariae</i> 120,000 | <i>P. malariae</i> 320,000 | <i>P. knowlesi</i> 6471 | <i>P. vivax</i> 9866 ² | <i>P. malariae</i> "4+" | <i>P. malariae</i> 22,666 ³ |
| PCR result | <i>P. knowlesi</i> | <i>P. knowlesi</i> | <i>P. knowlesi</i> | <i>P. knowlesi</i> | <i>P. knowlesi</i> | <i>P. knowlesi</i> | <i>P. knowlesi</i> |
| Initial anti-malarial therapy received | IV artesunate/oral doxycycline | IV artesunate/oral doxycycline | IV artesunate | IV artesunate/oral doxycycline | IV artesunate (single dose), followed by oral chloroquine and primaquine | IV artesunate/oral doxycycline | oral artesunate + melfoquine |

NOTE. Laboratory reference ranges are given in parentheses. NA, not available; IV, intravenous
Unless otherwise stated, clinical and laboratory parameters are those obtained on admission.