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PHOTO QUIZ

Answer to Photo Quiz: *Plasmodium knowlesi* Infection

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Figure 1A and B show small *Plasmodium* trophozoite ring forms about one-third of the diameter of the infected, normal-sized, erythrocyte. Figure 1A demonstrates multiple infection, and Fig. 1B demonstrates a "bird's eye" appearance (not species specific). Figure 1C shows an immature schizont (mature *Plasmodium knowlesi* schizonts have elongated, segmented merozoites); in contrast, *Plasmodium falciparum* schizonts are rarely seen in peripheral blood except in severe cases. Figure 1D shows an oval-shaped gametocyte with scattered brown pigment similar to that of *Plasmodium malariae*; in contrast, *P. falciparum* gametocytes are sausage-shaped. (The observed acanthocytosis is artefactual and does not affect morphology of *Plasmodium*.)

Morphologic diagnosis of *P. knowlesi* is difficult due to overlapping features and geographical distribution with *P. falciparum* and *P. malariae* and because it is infrequently encountered outside regions where the disease is endemic. *P. knowlesi*-infected erythrocytes are normal sized, and early trophozoites resemble *P. falciparum* (double chromatin dots, multiple infection, appliqué forms), while older trophozoites may develop band forms resembling *P. malariae* (1). Due to the short 24-h asexual cycle of *P. knowlesi*, hyperparasitemia reminiscent of *P. falciparum* may occur, with levels as high as 27% reported (2). However, in *P. falciparum* (longer asexual cycle of 48 h), this instead occurs because all erythrocyte stages can be infected.

Aside from morphological similarities on microscopy, diagnosis of *P. knowlesi* infection may also be missed by rapid immunochromatographic tests due to suboptimal cross-reaction of "pan-malarial antibodies" against *P. knowlesi* antigens or low parasitemia counts (3). This underscores the importance of molecular diagnostic methods to allow definitive diagnosis of *P. knowlesi* malaria, including identifying coinfections. In our patient, definitive identification was obtained by both morphology and PCR (real-time PCR followed by nested PCR of positive samples for species identification, backed by confirmatory sequencing of the reverse transcriptase PCR [RT-PCR] amplicon) (4). He, fortunately, had low parasitemia (0.3% at diagnosis) and made a full recovery.

P. knowlesi is a simian zoonotic malaria endemic to Southeast Asia (5). The long-tailed, pig-tailed, and northern pig-tailed macaques and the banded leaf monkey are its natural hosts; the *Anopheles leucosphyrus* group is its vector. Once thought uncommon, it is now the preeminent malaria in Malaysia, even being reported in areas previously considered "malaria-free," such as neighboring Singapore (6) and Brunei Darussalam (7) and as far as southern China (Yunnan) (5) and the Andaman Islands (8). A 2014 study showed that *P. knowlesi* accounted for nearly 80% of malaria cases in Johor state (which adjoins Singapore) where this patient's infection was acquired, compared with 56.5% of malaria across Malaysia (9).

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