Luchavez, J; Espino, F; Curameng, P; Espina, R; Bell, D; Chiodini, P; Nolder, D; Sutherland, C; Lee, KS; Singh, B (2008) Human Infections with Plasmodium knowlesi, the Philippines. Emerging infectious diseases, 14 (5). pp. 811-813. ISSN 1080-6040

Downloaded from: http://researchonline.lshtm.ac.uk/7696/

DOI:

Usage Guidelines

Please refer to usage guidelines at http://researchonline.lshtm.ac.uk/policies.html or alternatively contact researchonline@lshtm.ac.uk.

Available under license: http://creativecommons.org/licenses/by-nc-nd/2.5/
Human Infections with *Plasmodium knowlesi*, the Philippines

Jennifer Luchavez,* Fe Espino,* Peter Curameng,† Ronald Espina,† David Bell‡ Peter Chiodini,§ Debbie Nolder,§ Colin Sutherland,§ Kim-Sung Lee,¶ and Balbir Singh¶

Five human cases of infection with the simian malaria parasite *Plasmodium knowlesi* from Palawan, the Philippines, were confirmed by nested PCR. This study suggests that this zoonotic infection is found across a relatively wide area in Palawan and documents autochthonous cases in the country.

Human malaria is commonly caused by *Plasmodium falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*. However, a large focus of human infections with the simian malaria parasite, *P. knowlesi* (1), has recently been reported in Malaysian Borneo (2), and single case reports of infections acquired in Thailand (3) and Myanmar (4) have been documented. The diagnosis of *P. knowlesi* in humans may be missed by microscopy since the early blood stages of *P. knowlesi* morphologically resemble *P. falciparum*; the mature blood stages and gametocytes are similar to those of *P. malariae* (2).

The Study

Palawan is an island province lying southwest of the main islands of the Philippines. One of its smaller islands, Balabac, located off the southern tip, is separated from Borneo by the Balabac Strait (Figure). Malaria transmission occurs in all 19 municipalities of the province throughout the year. The *Anopheles fluviatilis* mosquito is the reported primary vector in the area (5). Based on national control program data in 2005, a total of 16,339 malaria cases were reported from Palawan, accounting for 35% of the country’s total. Of these, 11,580 (≈71%) were *P. falciparum*, 4,194 (26%) were *P. vivax*, 430 (3%) were *P. malariae*, and the remainder (135, <1%) were mixed-species infections.

Blood films of 2 patients (A and B), whose condition was diagnosed by microscopy as *P. malariae* at a local laboratory in Palawan, were sent to the malaria national reference laboratory of the Research Institute for Tropical Medicine (RITM) in Manila. Microscopy showed mature trophozoites indistinguishable from *P. malariae* and young ring forms of *P. falciparum*. This observation, and the fact that macaques in the Philippines can harbor *P. knowlesi* (6, 7), raised the possibility that these 2 patients were infected with *P. knowlesi*. Therefore, replicate slides were sent to the Malaria Research Centre, University of Malaysia Sarawak (UNIMAS), for confirmation of the identity of the *Plasmodium* species by molecular methods. DNA was extracted from the 2 slides as detailed previously (8) and examined by nested PCR assays for *P. falciparum*, *P. vivax*, *P. malariae*, *P. knowlesi*, and *P. ovale* as described by Singh and co-workers (2). One sample was positive for *P. knowlesi* mono-infection; the other was a mixed infection of *P. falciparum*, *P. malariae*, and *P. knowlesi*. PCR results were confirmed on subsequent testing at the malaria reference laboratory, London School of Hygiene & Tropical Medicine, United Kingdom.

The blood films had been collected in January 2006 from 2 men (>40 years of age) who lived in the villages of Tagbarungis and Bacungan near Puerto Princesa City, Pal-
ing sites ideal for their illness in forested foothills that contained many breed-
4). The farmers reportedly stayed overnight before onset of day 3; 3 tablets of primaquine (15 mg/tablet) on day 4. The farmers reportedly stayed overnight before onset of their illness in forested foothills that contained many breeding sites ideal for A. flavirostris mosquitoes. Long-tailed macaques (Macaca fascicularis), the natural hosts for P. knowlesi, were observed to be roaming freely in the area. An additional 9 samples (D, E, G, H, I, J, K, O, and P), consisting of 5 blood films and 4 blood spots on filter paper, were obtained from patients at Bataraza and Roxas municipalities (also in Palawan) and P. malariae infection was diagnosed by the local microscopists. These samples were subsequently examined by nested PCR assays at UNIMAS after DNA extraction. Three were identified as P. knowlesi, 4 as P. malariae, and the remaining 2 as mixed species infections (Table). The findings of autochthonous P. malariae infections further compounded the problem of accurate diagnosis of P. knowlesi by microscopy. The P. knowlesi patients came from 3 different villages in Roxas, 80–100 km north of where the original 2 P. knowlesi case-patients resided, near Puerto Princesa (Figure). This suggests that human P. knowlesi infections are found across a relatively wide area in Palawan. PCR examination of more blood samples in other areas where P. malariae infections have been diagnosed by microscopy are necessary to determine the geographic distribution and public health importance of human knowlesi infections in the Philippines.

Conclusions

This report extends the geographic range of human P. knowlesi infections from Thailand (3), Myanmar (4), pen-
insular Malaysia (8), and Malaysian Borneo (2) to Palawan Island in the Philippines. Although the parasite has been isolated from local macaques in the Philippines in 1961 (6) and 1978 (7), this report documents autochthonous human cases in the country. Major progress in malaria control has been achieved in many malarious areas in the Philippines (9). However, P. knowlesi forms a previously unrecognized pool of infections that may be maintained in forested areas through its presence in a simian reservoir, despite control efforts in the human population. Current data suggest that human knowlesi malaria is strictly a zoonotic disease. To confirm this theory, further knowledge of the dynamics of human infection is needed.

Acknowledgments

We thank the staff of the Department of Health, Center for Health Development IV-B Extension Office, and Kilusan Ligtas Malaria (Movement Against Malaria), Palawan, Philippines, especially Ray Angluben, for their support during the field investigation; and Felisa Guballa and Philip Bugayong for microscopy validation and the map, respectively.

The malaria reference laboratory is supported by the Health Protection Agency, UK.

Ms Luchavez works in the Parasitology Department of the Research Institute for Tropical Medicine in the Philippines. Since 2003, her major research interests have included the development of quality assurance processes for malaria rapid diagnostic tests. She is one of the leading investigators in the WHO/Tropical Disease Research–coordinated network to develop a global quality assurance and product testing scheme for malaria diagnostics.

Table. Microscopy and PCR results of blood samples from Palawan, the Philippines

| Patient | Age, y/sex | Location | Microscopy | PCR |
|---------|------------|----------|------------|-----|
| A       | 50/M       | Bacungan, Puerto Princesa | P. falciparum (gametocytes), P. malariae | P. falciparum, P. malariae, P. knowlesi |
| B       | 49/M       | Inagawan, Tagbarungis, Puerto Princesa | P. falciparum, P. malariae | P. knowlesi |
| D       | 55/F       | Caibulo, Iraan, Roxas | P. malariae | P. knowlesi |
| E       | 3/M        | Balogo, San Miguel, Roxas | P. malariae | P. knowlesi |
| G       | 6/M        | Maninguin, Iraan, Roxas | P. malariae | P. malariae |
| H       | 25/M       | Minara, Roxas | P. malariae | P. malariae |
| I       | 10/F       | Taradungan, Roxas | P. malariae | P. knowlesi |
| J       | 5/M        | Bono-Bono, Bataraza | P. vivax, P. malariae | P. falciparum, P. vivax, P. malariae |
| K       | 14/F       | Bono-Bono, Bataraza | P. malariae | P. malariae |
| O       | 9/M        | Inogbong, Bataraza | P. malariae | P. malariae |
| P       | 5/F        | Inogbong, Bataraza | P. falciparum, P. malariae | P. falciparum, P. vivax |
Human Infections with *Plasmodium knowlesi*

References

1. Garnham PCC. Malaria parasites and other haemosporidia. Oxford: Blackwell Scientific Publications; 1966. p. 317–32.
2. Singh B, Kim Sung L, Matusop A, Radhakrishnan A, Shamsul SS, Cox-Singh J, et al. A large focus of naturally acquired *Plasmodium knowlesi* infections in human beings. Lancet. 2004;363:1017–24.
3. Jongwutiwes S, Putaporntip C, Iwasaki T, Sata T, Kanbara H. Naturally acquired *Plasmodium knowlesi* malaria in humans, Thailand. Emerg Infect Dis. 2004;10:2211–3.
4. Zhu HM, Li J, Zheng H. Human natural infection of *Plasmodium knowlesi*. Chinese Journal of Parasitology and Parasitic Diseases. 2006;24:70–1.
5. Oberst R, Schultz G, Laughlin L, Sy N, Santos M, Casimiro C. Epidemiological study of malaria in Palawan. The Philippine Journal of Microbiology and Infectious Diseases. 1988;17:41–8 [cited 2008 Mar 8]. Available from http://www.psmid.org.ph/index.php?fid=journals/V olate17Number2.
6. Lambrecht FL, Dunn FL, Eyles DE. Isolation of *Plasmodium knowlesi* from Philippine macaques. Nature. 1961;191:1117–8.
7. Tsukamoto M, Miyata A, Miyagi I. Surveys on simian malaria parasites and their vectors in Palawan Island, the Philippines. Institute of Tropical Medicine, Nagasaki University, Japan. 1978;20:29–50 [cited 2008 Mar 8]. Available from http://naosite.lab.nagasaki-u.ac.jp/dspace/handle/10069/4234
8. Cox-Singh J, Davis TME, Lee KS, Shamsul SSG, Divis PCS, Matusop A, et al. *Plasmodium knowlesi* malaria in humans is widely distributed and potentially life threatening. Clin Infect Dis. 2008;46:165–71
9. National Objective for Health. Philippines, 2005–2010 [cited 2008 Jan 7]. Available from www2.doh.gov.ph/noh2007/NOHWeb32/ NOHperSubj/Chap4/Malaria.pdf

Address for correspondence: Jennifer Luchavez, Research Institute for Tropical Medicine – Parasitology, Fillinvest Compound, Alabang, Muntinlupa City, Muntinlupa 1781, the Philippines; email: jluchavez@yahoo.com