Institution: London School of Hygiene & Tropical Medicine (LSHTM)

Unit of Assessment: UoA1 – Clinical Medicine

Title of case study: Improving methodologies for the detection and identification of malaria parasites in human blood

1. Summary of the impact

Work by LSHTM researchers has led to a greater understanding of *Plasmodium* malaria parasite species and contributed new methodologies for diagnosis. As a result, patients with the uncommon species *P. knowlesi* and many hundreds with *P. ovale* spp. have been correctly diagnosed by polymerase chain reaction (PCR), and the rapid detection of parasite DNA is revolutionising clinical trial design. The work has led to the successful commercialisation of a low-cost, easy-to-use malaria testing kit for use in developing countries. Through media outputs and further research, the work has taken awareness of the issues surrounding malaria diagnostics to an international audience.

2. Underpinning research

The life cycle of *Plasmodium* malaria parasites was revealed by nineteenth-century scientists using microscopic examination of blood samples. Distinguishing parasite species accurately, however, has become an important part of diagnosis since different drugs are used to treat different species, and requires more sensitive methods.

LSHTM researchers have developed DNA-based detection and species discrimination methods. Led by Colin Sutherland (Reader in Parasitology, LSHTM since 1998, then Research Fellow), key contributors include John Williams (Principal Scientific Officer, LSHTM since 1975), David Conway (Professor of Biology, LSHTM since 1993, then Research Fellow) and Khalid Beshir (Research Assistant, since 2009).

In 2004 the team, with Malaysian partners, published key evidence that the simian parasite *Plasmodium knowlesi* was infecting residents on the island of Borneo. Around a fifth of 1999 malaria cases had been identified as *Plasmodium malariae*, but the infections appeared atypical and a nested polymerase chain reaction (PCR) assay failed to identify *P malariae* DNA. Between 2000–2002 the researchers took blood samples from 208 people with malaria in the Kapit division of Malaysia. By PCR assay, 58% of the samples tested positive for *P knowlesi*, a malaria parasite of long-tailed macaque monkeys.[3.1] A 2006 study, evidenced five further cases of human infection by *P knowlesi* in the Philippines.[3.2]

Difficulties in identifying *Plasmodium ovale* infection in the UK Malaria Reference Laboratory at LSHTM led to the surprising finding, published in 2010, that *ovale* malaria is caused by two related but distinct species, indistinguishable by microscopy. Wellcome Trust funding enabled the researchers to demonstrate that both species occur across Africa, much of Asia and the South-West Pacific.[3.3]

From 2007 a project carried out with the Hospital for Tropical Diseases trialled the use of a loop-mediated isothermal amplification (LAMP) kit as an alternative method of parasite detection. A trial of 705 blood samples from returned travellers, between January–July 2011, showed that diagnostic accuracy of the kits was comparable to PCR in sensitivity, and required minimal training.[3.4]

Recent studies of antimalarial drug efficacy on the Thai-Cambodian border have shown that data from the first 72 hours following treatment provide a crucial indicator of drug failure. These findings came from six-hourly blood sampling and exhaustive quantitative microscopy, impractical for African studies where most patients are children. Beshir and Sutherland have developed a simple quantitative PCR method for detecting parasite clearance in the first 72 hours of treatment. This has been used very effectively in a 2009 clinical trial of Kenyan children,[3.5] and will now be deployed in large trials in West Africa.

Since parasite detection is key to ensuring the safety of blood products, researchers worked with the NHS and National Blood Service to improve methodologies for sensitive detection. Currently all mothers donating umbilical cord blood who may have been exposed to malaria undergo serological screening, with a positive result excluding the use of the blood for transplant therapy. By significantly increasing the sensitivity of the nucleic acid amplification technique used to detect malaria parasites, researchers developed new protocols, meaning fewer units will be needlessly discarded.[3.6]

3. References to the research

3.1 Singh, B, Sung, LK, Matusop, A, Radhakrishnan, A, Shamsul, SSG, Cox-Singh, J, Thomas, A and Conway, DJ (2004) A large focus of naturally acquired *Plasmodium knowlesi* infections in human beings, *Lancet*, 363(9414): 1017–1024, doi: 10.1016/S0140-6736(04)15836-4. Citation count: 264.

3.2 Luchavez, J, Espino, F, Curameng, P, Espina, R, Bell, D, Chiodini, P, Nolder, D, Sutherland, C, Lee, KS and Singh B (2008) Human infections with *Plasmodium knowlesi*, the Philippines, *Emerging Infectious Diseases*,14(5): 811–813, http://wwwnc.cdc.gov/eid/article/14/5/pdfs/07-1407.pdf (accessed 14 November 2013). Citation count: 70.

3.3 Sutherland, CJ, Tanomsing, N, Nolder, D, Oguike, M, Jennison, C, Pukrittayakamee, S, Dolecek, C, Tran, TH, do Rosário, VE, Arez, AP, Pinto, J, Michon, P, Escalante, AA, Nosten, F, Burke, M, Lee, R, Blaze, M, Otto, TD, Barnwell, JW, Pain, A, Williams, J, White, NJ, Day, NPJ, Snounou, G, Lockhart, PJ, Chiodini, PL, Imwong, M and Polley, SD (2010) Two nonrecombining sympatric forms of the human malaria parasite *Plasmodium ovale* occur globally, *Journal of Infectious Diseases*, 201(10): 1544–1550, doi: 10.1086/652240. Citation count: 45.

3.4 Polley, SD, González, IJ, Mohamed, D, Daly, R, Bowers, K, Watson, J, Mewse, E, Armstrong, M, Gray, C, Perkins, MD, Bell, D, Kanda, H, Tomita, N, Kubota, Y, Mori, Y, Chiodini, PL and Sutherland, CJ (2013) Clinical evaluation of a loop-mediated amplification kit for diagnosis for imported malaria, *Journal of Infectious Diseases*, 208(4): 637–644, doi: 10.1093/infdis/jit183. Citation count: 0.

3.5 Beshir, KB, Sutherland, CJ, Sawa, P, Drakeley, CJ, Okell, L, Mweresa, CK, Omar, SA, Shekalaghe, SA, Kaur, H, Ndaro, A, Chilongola, J, Schallig, HDFH, Sauerwein, RW, Hallett, RL and Bousema, T (2013) Residual *Plasmodium falciparum* parasitemia in Kenyan children after artemisinin-combination therapy is associated with increased transmission to mosquitoes and parasite recurrence, *Journal of Infectious Diseases*, 208(12): 2017-2024, doi: 10.1093/infdis/jit431.

3.6 Polley, SD, Sutherland, CJ, Regan, F, Hassan and M, Chiodini, PL (2012) Increased sensitivity for detecting malaria parasites in human umbilical cord blood using scaled-up DNA preparation, *Malaria Journal*, 11(62), doi: 10.1186/1475-2875-11-62. Citation count: 3.

Key funding

Sutherland co-PI (with Chiodini, Hospital for Tropical Diseases [HTD]), UK Malaria Reference Laboratory, Service Contract, UK HPA/PHE, 2004–present, £287,000 pa, ongoing.
Sutherland co-PI (with Chiodini, HTD), Developing a Simple Molecular Test for Malaria, FIND Geneva, 2007–2012, £310,000.
Sutherland PI, Population Biology and Epidemiology of Two Newly Recognized Human Malaria Parasite Species, Wellcome Trust 9/2010–12/2013, £250,000.
Sutherland Consortium Partner EDCTP, An Integrated Approach to Clinical Trials, Capacity Building and Networking in West Africa, WANECAM Consortium, 2010–2014, LSHTM €475,000.

4. Details of the impact

LSHTM has made notable contributions to malaria treatment through the development of improved protocols and methodologies, leading to the commercialisation of a low-cost, easy-to-use malaria testing kit suitable for developing countries. Through media outputs and further research, the work

has raised awareness of issues surrounding malaria diagnostics among an international audience.

From a patient-care perspective, the team's examination of the risks posed by *Plasmodium knowlesi* means this species is now widely considered a possible cause of malaria throughout Southeast Asia. Evidence published by LSHTM is now used throughout Malaysian Borneo (where at least 1,731 cases of *knowlesi* malaria have been correctly diagnosed and treated between 2004–2012) and the region, such that 'human cases have been described in virtually all Southeast Asian countries, and *P. knowlesi* is now considered the fifth species of *Plasmodium* causing malaria in humans'.[5.1]

The description of ovale malaria as two distinct species has generated a new (small) literature focusing on *Plasmodium ovale curtisi* and *P. ovale wallikeri*. This is now influencing understanding of malaria epidemiology in countries including Angola, Ethiopia and Bangladesh, and the practice of malaria diagnosis in non-endemic laboratories. For example, parasite DNA from a recent fatal case of ovale malaria was used as evidence that *P. ovale curtisi* was the infective agent.[5.2] A US soldier returned from Liberia with malaria symptoms, microscopic evidence of malaria lacking species-discriminating features, and a negative antigen detection test, was diagnosed as infected with *P. ovale wallikeri* by comparison with published sequence data from the LSHTM studies.[5.3]

Research into the use of a LAMP kit as an alternative method of parasite detection has brought both commercial impact and patient benefits. The testing kits pioneered at LSHTM received CE marking in 2011 and became commercially available via the Tokyo-based Eiken Chemical Corporation in July 2012.[5.4] The Foundation for Innovative New Diagnostics (FIND) is currently coordinating orders from the manufacturers. Advantages of the kits relative to existing tests include: they can be performed by non-specialist health workers, do not need refrigeration, and take less than an hour. Rapid detection is key to rapid treatment, before complications and risks escalate, so could save money for health services.

Sutherland shared the benefits of the methodology with a wider audience when he demonstrated the kits on a 30-minute BBC World Service programme, broadcast in August 2013 on the BBC Arabic programme *4Tech*. Interest has been high, with FIND and LSHTM receiving an average of one query a week from hospitals and universities worldwide. Media coverage (for example in the *Mail Online*, May 2013)[5.5] has also generated interest, with enquiries coming from countries including Malaysia, Sierra Leone, India, Tanzania and, from Kenya, the US Army Medical Research Unit. One typical email sent to Sutherland in May 2013 reads: 'Great to read about the test for malaria … I am now working in East Malaysia … The diagnosis here is still based on microscopic examination. If your test is available (and hopefully not too expensive) then it would reduce the likelihood of missing malaria cases, especially in areas where we lack expert technicians.'[5.6]

Further impact has been achieved through involvement in the West African Network for Clinical Trials of Antimalarial Drugs (WANECAM) project, a consortium of West African and European researchers focusing on trials for developing countries. They contacted Sutherland and the LSHTM team in 2009, asking them to become a consortium partner. LSHTM's quantitative PCR method for detecting parasite clearance within the first 72 hours of treatment was built into large-scale phase III/IV monitoring of antimalarial drug efficacy in children.[5.7] Khalid Beshir received funding from the European and Developing Countries Clinical Trial Partnership (EDCTP) to work on the project, which began in early 2013 with the collection of thousands of samples at five sites within three West African countries: Mali, Burkina Faso and Guinea Conakry. The samples will be analysed over the coming year to examine the efficacy of four different combination drugs, with findings feeding into policy recommendations.

LSHTM's scaled-up method permitting parasite DNA detection down to a level of 0.05 parasites per microlitre of blood was used to screen a series of umbilical cord blood donations from seropositive mothers, and found no evidence that any of the infants were exposed to viable malaria parasites (N=54). The paper with these findings was received for consideration by the Parasite Advisory Group (Sutherland is a member) of the Standing Advisory Committee for Transfusion-

Transmitted Infections (SACTII).[5.8] The findings support current policy at NHS Blood and Transplant, which seeks to minimise loss of donated units.

5. Sources to corroborate the impact

5.1 Singh, B and Daneshvar, C (2013) Human infections and detection of *Plasmodium knowlesi*, *Clinical Microbiology Reviews*, 26(2): 165–184, doi: 10.1128/CMR.00079-12.

5.2 Lau, YL, Lee, WC, Tan, LH, Kamarulzaman, A, Syed Omar, SF, Fong, MY, Cheong, FW and Mahmud, R (2013) Acute respiratory distress syndrome and acute renal failure from *Plasmodium ovale* infection with fatal outcome, *Malaria Journal*, 12(1): 389–397 doi: 10.1186/1475-2875-12-389.

5.3 Cohen, R, Feghali, K, Alameyhu, S, Komisar, J, Hang, J, Weina, PJ, Coggeshall, P, Kamau, E and Zapor, M (2013) Use of qPCR and genomic sequencing to diagnose *Plasmodium ovale wallikeri* malaria in a returned soldier in the setting of a negative rapid diagnostic assay, *The American Journal of Tropical Medicine and Hygiene*, 89(3): 501–506, doi: 10.4269/ajtmh.12-0724.

5.4 FIND: Foundation for Innovative New Diagnostics (2013) Loop mediated isothermal amplification (LAMP) for malaria, http://www.finddiagnostics.org/programs/malaria-afs/malaria/product_development/lamp-for-malaria.html (accessed 14 November 2013).

5.5 *Mail Online* (2013) Yellow fever booster no longer necessary, World Health Organization tells tourists, 20 May, http://www.dailymail.co.uk/travel/article-2327425/Tourists-longer-need-year-Yellow-fever-booster-says-World-Heath-Organisation.html (accessed 14 November 2013) (LAMP test covered in second part of article as 'potential breakthrough with treatment of malaria').

5.6 Email from Malaysian medical microbiologist and LSHTM alumnus (available on request).

5.7 WANECAM: West African Network for Clinical Trials of Antimalarial Drugs, http://www.wanecam.org (accessed 14 November 2013).

5.8 NHS Blood and Transplant (2013) Blood supply: focus on safety', in *Annual review 2012-13: saving and improving lives*, NHS Blood and Transplant, Watford, pp. 22-24. Available online: http://www.nhsbt.nhs.uk/annualreview/blood-supply/focus-on-safety/ (accessed 15 November 2013).