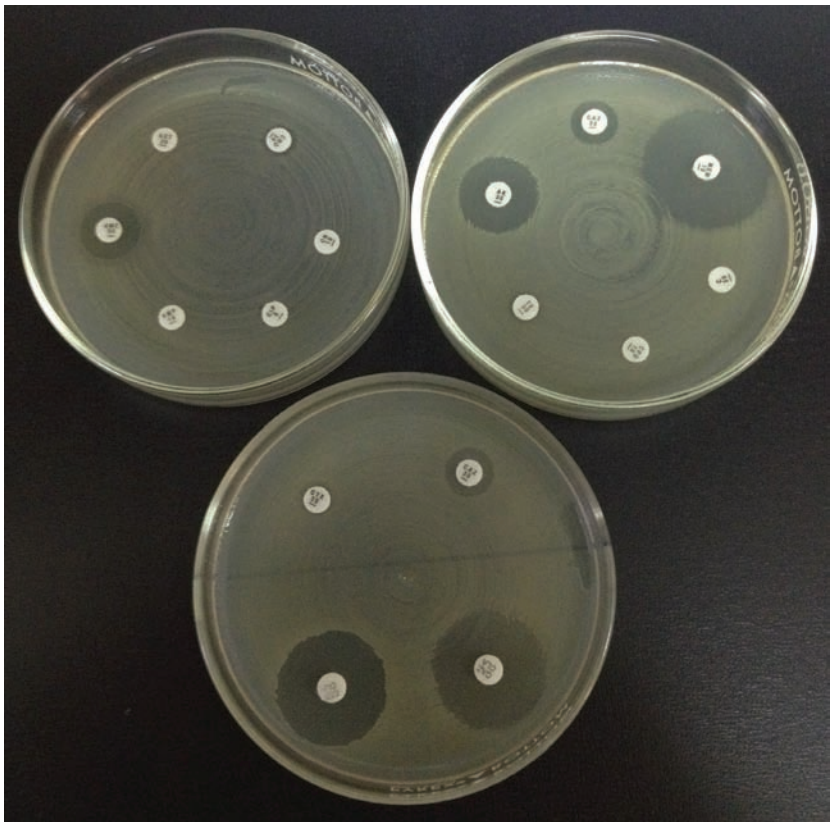
Lao - Oxford - Mahosot Hospital - Wellcome Trust Research Unit

SCIENTIFIC ANNUAL REPORT FOR 2015

LAO-OXFORD-MAHOSOT HOSPITAL-WELLCOME TRUST RESEARCH UNIT (LOMWRU)
MICROBIOLOGY LABORATORY
MAHOSOT HOSPITAL
VIENTIANE, LAO PDR

TO

MINISTRY OF HEALTH
GOVERNMENT OF THE LAO PDR



Antibiotic susceptibility test on *Escherichia coli* cultured from a foot infection in a 50 year old Lao woman with diabetes. The organism is resistant to all antibiotics tested apart from amikacin, a potentially toxic agent, and meropenem, which is not yet easily available in Laos. The bottom plate shows that this resistance is due in part to the production of an extended spectrum beta lactamase (ESBL) enzyme. Such infections are seen increasingly in Laos and reflect a growing global threat

CONTENTS

Meeting in Nong District, Savanakhet Province to discuss targeted malaria elimination with the community

ບົດສັງລວມຫຍໍ້

ກ. ໂຄງການຄົ້ນຄວ້າພະຍາດເຂດຮ້ອນລະຫວ່າງໂຮງໝໍມະໂຫສົດ-ແອວຄຳຕູ້ສ-ມະຫາວິທະຍາໄລອ໋ອກຟອດ ຫຼື The Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit (LOMWRU) ເປັນໜ່ວຍງານຄົ້ນຄວ້າທາງຄລີນິກ ເຊິ່ງນອນຢູ່ໃນພະແນກວິເຄາະຈຸລິນຊີ, ໂຮງໝໍມະໂຫສົດ ແລະ ເລີ່ມດຳເນີນໂຄງການມາແຕ່ປີ 2000 ເປັນຕົ້ນມາ. ໂຄງການນີ້ໄດ້ຮັບທຶນຊ່ວຍເຫລືອຫລັກ ຈາກທາງແອວຄຳຕູ້ສ ປະເທດ ອັງກິດ ແລະ ທຶນອີກສ່ວນໜຶ່ງແມ່ນໄດ້ຈາກ US Naval Medical Research Centre, US Centres for Disease Control (CDC), the Bill & Gates Foundation, The European Union, Fondation Total/Institute Pasteur, the Foundation for Innovative New Diagnostics (FIND), the World Health Organisation (WHO), the French Government Ministry of Foreign and International Affairs and the Joint Inter-Agency Task Force of the Global Fund, PMI, and UNDP. ນອກນີ້ ທາງໂຄງການຍັງໄດ້ຮັບການຊ່ວຍເຫລືອ ເປັນເຄື່ອງອຸປະກອນຈາກສະຖາບັນຄົ້ນຄວ້າເພື່ອການພັດທະນາ/ມະຫາວິທະຍາໄລແອ໋ກຊ-ມາກໄຊ ປະເທດຝລັ່ງ ແລະ ໂຄງການຄົ້ນຄວ້າພະຍາດອີກາເມັດເຊຍ ຂອງສູນຄົ້ນຄວ້າທາງການແພດກອງທັບເຮືອ ສະຫະລັດອາເມລິກາ.

ຂ. ພະແນກວິເຄາະຈຸລິນຊີ ມີພະນັກງານ (ພາກລັດ) ທັງໝົດ 31 ຄົນ, ສ່ວນ LOMWRU ມີພະນັກງານໂຄງການ 45 ຄົນ, ໃນນີ້ 93% ແມ່ນຄົນລາວ ແລະ 61% ເປັນເພດຍິງ. ພວກເຮົາມີຫ້ອງວິເຄາະຈຸລະຊີວະວິທະຍາທາງຄລີນິກ, ຫ້ອງວິເຄາະທາງພັນທຸກຳສາດ, ຫ້ອງວິເຄາະເຊໂຮໂລຊີ, ແລະ ຫ້ອງວິເຄາະລະດັບ 3 (BSL3). ການປະຕິບັດງານໃນຫ້ອງວິເຄາະດັ່ງກ່າວ ແມ່ນເປັນໄປຕາມແນວທາງ-ລະບຽບການຄວາມປອດໄພ ຂອງມະຫາວິທະຍາໄລອ໋ອກຟອດ.

ຄ. LOMWRU ຊ່ວຍບໍລິການຍິ່ງມະຕິພະຍາດຊຶມເຊື້ອພາຍໃນໂຮງມະໂຫສົດ ແລະ ໂຮງໝໍຕ່າງແຂວງ ເຊັ່ນ ໂຮງໝໍແຂວງ ຫລວງນ້ຳທາ, ສາລະວັນ ແລະ ຊຽງຂວາງ, ເຮັດການຄົ້ນຄວ້າທາງຄລີນິກ, ແລະ ສ້າງຄວາມເຂັ້ມແຂງໃຫ້ແກ່ພະນັກງານພາກລັດໃນດ້ານການຍິ່ງມະຕິພະຍາດ ແລະ ການເຮັດຄົ້ນຄວ້າ ໂດຍຜ່ານການຝຶກອົບຮົມ ແລະ ປະຕິບັດງານຕົວຈິງ. LOMWRU ຍັງມີການຮ່ວມມືກັບສູນໄຂ້ຍູງ, ແມ່ກາຝາ ແລະ ແມງໄມ້ ເຮັດໂຄງການຄົ້ນຄວ້າຢູ່ແຂວງເຊກອງ ແລະ ສະຫວັນນະເຂດ ແລະ ຍັງຮ່ວມມືກັບກົມອາຫານ ແລະ ຢາ ເຮັດໂຄງການຄົ້ນຄວ້າກ່ຽວກັບຄຸນນະພາບຂອງຢາ.

ງ. ຈຸດສຸມສຳລັບວຽກຄົ້ນຄວ້າຂອງພວກເຮົາໄດ້ແກ່ ສາເຫດ ແລະ ລະບາດວິທະຍາຂອງໄຂ້ ລວມທັງການຍິ່ງມະຕິ ແລະ ປິ່ວປົວທີ່ສົມເຫດສົມຜົນ ໃນ ສປປ ລາວ, ການຍິ່ງມະຕິ, ລະບາດວິທະຍາ ແລະ ການປ້ອງກັນພາວະຂາດວິຕະມິນເບ1 ໃນເດັກລຸ່ມ 1 ປີ, ແລະ ການສຶກສາຄົ້ນຄວ້າກ່ຽວກັບຄຸນນະພາບຂອງຢາ ໃນລະດັບສາກົນ.

ຈ. ໃນປີ 2015 ທາງໂຄງການໄດ້ສະໜັບສະໜູນພະນັກງານລາວຈຳນວນ 23 ເທື່ອຄົນ ເຂົ້າຮ່ວມກອງປະຊຸມຢູ່ຕ່າງປະເທດ 5 ຄັ້ງ ແລະ ໄດ້ໃຫ້ການສະໜັບສະໜູນພະນັກງານພາກລັດ 2 ຄົນ ສຶກສາຕໍ່ໃນລະດັບປະລິຍາເອກ ແລະ ປະລິນຍາໂທ.

ສ. ໃນປີ 2015 ພວກເຮົາໄດ້ຕີພິມ ຫລື ກຳລັງຖືກຮັບຕີພິມເຜີຍແຜ່ຜົນຂອງການຄົ້ນຄວ້າລົງໃນວາລະສານການແພດສາກົນ ຈຳນວນ 47 ບົດ, ລົງໃນປຶ້ມຕຳລາທາງການແພດຈຳນວນ 3 ພາກ ແລະ ລົງໃນບົດລາຍງານຂອງອົງການອະນາໄມໂລກ 1 ບົດ. ນັບແຕ່ປີ 2000 ເປັນຕົ້ນມາ, ນັກຄົ້ນຄວ້າຂອງໂຄງການ LOMWRU ມີຜົນງານຕີພິມເຜີຍແຜ່ຜົນການຄົ້ນຄວ້າຫລາຍຫວ່ານໝູ່ໃນວົງການສາທາລະນະສຸກຂອງລາວ ເຊິ່ງລວມມີ ທັງໝົດ 255 ບົດ ທີ່ຖືກຕີພິມ ຫລື ກຳລັງຖືກຮັບຕີພິມເຜີຍແຜ່ ລວມທັງປຶ້ມຕຳລາທາງການແພດອີກຈຳນວນ 16 ພາກ.

ຊ. ຜົນການຄົ້ນຄວ້າຂອງ LOMWRU ທີ່ຖືກນຳໄປເຜີຍແຜ່ຂະຫຍາຍ ເປັນແນວທາງນະໂຍບາຍ ດ້ານສາທາລະນະສຸກພາຍໃນປະເທດ ລວມມີ: ການຈັດຕັ້ງປະຕິບັດການສັກຢາກັນພະຍາດທີ່ເກີດຈາກເຊື້ອ Pneumococcus ແລະ ການສັກຢາກັນພະຍາດອັກເສບສະໝອງຍີ່ປຸ່ນ, ການປ່ຽນແປງແນວທາງການປິ່ນປົວພະຍາດໄຂ້ຍູງ ແລະ ພະຍາດໄຂ້ທໍລະພິດ (Typhoid). ນອກນີ້ ພວກເຮົາຍັງໄດ້ຄົ້ນພົບພະຍາດທີ່ສຳຄັນບາງຢ່າງ ເປັນຄັ້ງທຳອິດໃນປະເທດລາວ, ໄດ້ຊີ້ໃຫ້ເຫັນຄວາມສຳຄັນຂອງພະຍາດໄຂ້ແມງແດງ, ໄຂ້ຍ່ຽວໜູ, ໄຂ້ທໍລະພິດ, ເມລີອອຍໂດສຊີສ, ອັກເສບສະໝອງຍີ່ປຸ່ນ ເຊິ່ງເຮັດໃຫ້ພວກເຮົາເຂົ້າໃຈຢ່າງເລິກເຊິ່ງຕື່ມ ກ່ຽວກັບລັກສະນະດ້ານລະບາດວິທະຍາ ແລະ ແນວທາງການປ້ອງກັນພະຍາດດັ່ງກ່າວ.

ຍ. ສະຫລູບຜົນຂອງການຄົ້ນຄວ້າທີ່ມີຄວາມໝາຍສຳຄັນ ຕໍ່ປະເທດລາວ ທີ່ໄດ້ຕີພິມເຜີຍແຜ່ ຫລື ກຳລັງຈະຖືກຕີພິມເຜີຍແຜ່ ໃນປີ 2015 ມີດັ່ງຕໍ່ໄປນີ້ (ກະລຸນາເບິ່ງລາຍລະອຽດຕື່ມໃນບົດລາຍງານ):

- ລັກສະນະການຕ້ານຂອງເຊື້ອຈຸລິນຊີ ຕໍ່ຢາຕ້ານເຊື້ອ ກຳລັງເປັນບັນຫາສຳຄັນດ້ານສາທາລະນະສຸກ ແລະ ກໍ່ໃຫ້ເກີດຄວາມກັງວົນ ນັບມື້ຫລາຍຂື້ນໃນລາວ ກໍ່ຄືໃນທົ່ວໂລກ. ບັນຫາການຕ້ານ (ການດື້) ຂອງເຊື້ອ Enterobacteriaceae ຕໍ່ຢາຕ້ານເຊື້ອໃນກຸ່ມ Beta-lactamin (ESBL) ບໍ່ພຽງແຕ່ເປັນສາເຫດການຊຶມເຊື້ອທີ່ສຳຄັນ ແລະ ກຳລັງນັບມື້ເພີ່ມຫລາຍຂື້ນໃນໂຮງໝໍມະໂຫສົດເທົ່ານັ້ນ ແຕ່ມັນຍັງພົບເຫັນຢ່າງຫລວງຫລາຍໃນອາຈົມຂອງເດັກໂຮງຮຽນອະນຸບານທີ່ມີສຸຂະພາບແຂງແຮງ ໃນນະຄອນຫລວງວຽງຈັນ ແລະ ແຂວງວຽງຈັນ ລວມທັງພົບເຫັນໃນອາຈົມຂອງຄົນທີ່ມີສຸຂະພາບແຂງແຮງໃນເຂດຫ່າງໄກສອກຫລີກຂອງແຂວງຊຽງຂວາງ. ນອກນີ້ ພວກເຮົາຍັງໄດ້ຊ່ວຍເຫລືອ ກໍລະນີການລະບາດຄັ້ງໃຫ່ຍຂອງຊຶມເຊື້ອເລືອດ (ຍ້ອນເຊື້ອ ESBL – *Klebsiella pneumoniae*) ທີ່ເກີດຂື້ນໃນໂຮງໝໍເດັກ. ພ້ອມກັນນີ້ ພວກເຮົາຍັງພົບວ່າ ຄວາມຮູ້ກ່ຽວກັບລັກສະນະການຕ້ານຂອງເຊື້ອຈຸລິນຊີຕໍ່ຢາຕ້ານເຊື້ອ ແລະ ການສັ່ງຢາຕ້ານເຊື້ອທີ່ສົມເຫດສົມຜົນ ຂອງທ່ານໝໍ ແມ່ນຍັງບໍ່ທັນໄດ້ດີເທື່ອ ເຊິ່ງອັນນີ້ຊີ້ໃຫ້ເຫັນວ່າ ເຮົາຕ້ອງຫາແນວທາງແກ້ໄຂບັນຫາດັ່ງກ່າວຢ່າງຮີບດ່ວນທີ່ສຸດ. ການແຜ່ກະຈາຍຂອງເຊື້ອທີ່ຕ້ານຕໍ່ຢາຕ້ານເຊື້ອໃນລາວ ຈະມີຜົນກະທົບຢ່າງໃຫ່ຍຫລວງທີ່ສຸດຕໍ່ຄົນເຈັບ, ຊຸມຊົນ ແລະ ຕໍ່ເສດຖະກິດ ເພາະສະນັ້ນ ເຮົາຈະຕ້ອງເອົາໃຈໃສ່ຢ່າງຕັ້ງໜ້າຕໍ່ ວຽກງານຄວບຄູມການຊຶມເຊື້ອ, ລວມທັງການຄວບຄຸມ ແລະ ອອກລະບຽບການນຳໃຊ້ຢາຕ້ານເຊື້ອຢ່າງສົມເຫດສົມຜົນ ຢ່າງຮີບດ່ວນ.
- ພວກເຮົາພົບວ່າ ສ່ວນໃຫ່ຍຂອງນ້ຳໄຂສັນຫລັງທີ່ສົ່ງກວດ ແມ່ນມີຈຳນວນເມັດເລືອດຂາວຜິດປົກກະຕິ ເຊິ່ງຊີ້ໃຫ້ເຫັນວ່າ: ເຮົາຄວນເອົາໃຈໃສ່ໃຫ້ມີການເຈາະນ້ຳໄຂສັນຫລັງໃນຄົນເຈັບທີ່ສົງໃສວ່າ ມີການຊຶມເຊື້ອລະບົບປະສາດສູນກາງທຸກກໍລະນີ ເພື່ອຫລີກເວັ້ນການພາດໂອກາດປິ່ນປົວຄົນເຈັບດັ່ງກ່າວ.
- ພວກເຮົາສາມາດບົ່ງມະຕິພະຍາດໄຂ້ທໍລະພິດ ຢ່າງໄວວາ, ແມ່ນຢຳ ແລະ ມີລາຄາຖືກ ໂດຍນຳໃຊ້ຊຸດການກວດວິເຄາະແບບໄວວາເພື່ອຊອກຫາແອນຕິເຈນ ຂອງເຊື້ອໄຂ້ທໍລະພິດ ໃນແກ້ວປູກເລືອດ. ນອກຈາກນີ້ ການກວດທາງພັນທຸກຳ (PCR) ຈາກຊຸດການກວດແບບໄວວາທີ່ໃຫ້ຜົນບວກ ຍັງສາມາດບອກໄດ້ຢ່າງທັນການວ່າ ເຊື້ອດັ່ງກ່າວຕອບສະໜອງຕໍ່ຢາ Fluoroquinolones ຫລືບໍ່. ເຕັກນິກນີ້ ອາດຊ່ວຍໃຫ້ຫ້ອງວິເຄາະຂອງໂຮງໝໍແຂວງ ສາມາດບົ່ງມະຕິພະຍາດດັ່ງກ່າວໄດ້ ໃນອະນາຄົດ ແລະ ສາມາດສົ່ງແຜ່ນກວດໄວວາທີ່ມີຜົນບວກ ໄປກວດທາງພັນທຸກຳຢູ່ຂັ້ນສູນກາງ ເພື່ອເບິ່ງລັກສະນະການດື້ຂອງເຊື້ອຕໍ່ຢາ Fluoroquinolones.
- ພວກເຮົາພົບວ່າ ພະຍາດໄຂ້ແມງແດງ, ມູຣິນໄທຟັສ, ແລະ ໄຂ້ຍ່ຽວໜູ ເປັນສາເຫດທີ່ສຳຄັນຂອງການຊຶມເຊື້ອລະບົບປະສາດສູນກາງໃນວຽງຈັນ ແລະ ອາດພົບໃນແຫ່ງອື່ນໆຂອງຂົງເຂດອາຊີເຊັ່ນກັນ.

ຂໍ້ມູນນີ້ ຊີ້ໃຫ້ເຫັນວ່າ ເຮົາຄວນພິຈາລະນານໍາໃຊ້ຢາ Doxycycline ຕື່ມໃນຄົນເຈັບທີ່ເປັນອັກເສບເຍື່ອຫຸ້ມສະໝອງ/ສະໝອງອັກເສບ ຍ້ອນວ່າ ຢາກຸ່ມ Cephalosporins ລຸ້ນທີ 3 ທີ່ມັກໃຊ້ປິ່ນປົວພະຍາດຫຸ້ມສະໝອງ/ສະໝອງອັກເສບ ແບບເປັນປະເພນີນັ້ນ ບໍ່ສາມາດປິ່ນປົວພະຍາດໄທຟັສ.

- ຂໍ້ມູນຈາກການສຶກສາກ່ຽວກັບສາເຫດຂອງໄຂ້ ໃນລາວ, ກໍາປູເຈຍ ແລະ ຊາຍແດນໄທ-ພະມ້າ ຊີ້ໃຫ້ເຫັນວ່າ ການກວດ C-reactive protein ສາມາດນໍາໃຊ້ເປັນຕົວຊີ້ບອກວ່າ ຄົນເຈັບທີ່ເຂົ້າມາດ້ວຍອາການໄຂ້ ຈໍາເປັນຕ້ອງໄດ້ຮັບຢາຕ້ານເຊື້ອ ຫລືບໍ່. ນອກນີ້ ຫລັກຖານຈາກການຄົ້ນຄວ້າຢູ່ເຂດຊົນນະບົດຂອງລາວ ຍັງຊີ້ໃຫ້ເຫັນວ່າ ຊຸດການກວດ CRP ແບບໄວວາ ແມ່ນມີຄວາມແມ່ນຢໍາສູງ ແລະ ອາດໃຊ້ເປັນເຄື່ອງມືຕັດສິນ (ໃນບ່ອນທີ່ຂາດເຂີນຫ້ອງວິເຄາະ) ເບິ່ງວ່າ ຄົນເຈັບຈໍາເປັນຕ້ອງໄດ້ຮັບຢາຕ້ານເຊື້ອ ຫລືບໍ່.
- ຜົນການສຶກສາທົດລອງກ່ຽວກັບຢາສະເຕຣອຍ ໃນຄົນເຈັບ HIV ທີ່ເປັນເຍື່ອຫຸ້ມສະໝອງອັກເສບຈາກເຊື້ອເຫັດ (Cryptococcus) ຊີ້ໃຫ້ເຫັນວ່າ ຄົນເຈັບທີ່ໄດ້ຢາສະເຕຣອຍ ແມ່ນມີກໍາມະຜົນຂ້າງຄຽງໃນອັດຕາທີ່ສູງ ເພາະສະນັ້ນຈຶ່ງບໍ່ຄວນໃຊ້ປິ່ນປົວສົມທົບກັບຢາ Amphotericin.
- ເມລີອອຍໂດຊິສ ເປັນສາເຫດທີ່ສໍາຄັນຂອງການຊຶມເຊື້ອເລືອດທີ່ມັກຈະຖືກຫລົງລືມ ໃນລາວ ແລະ ໃນຂົງເຂດປະເທດເຂດຮ້ອນ. ນັບແຕ່ປີ 2009 ມາຮອດປະຈຸບັນ ພວກເຮົາໄດ້ບົ່ງມະຕິຄົນເຈັບເປັນພະຍາດເມລີອອຍຈໍານວນຫລາຍກວ່າ 940 ຄົນ.
- ມາຮອດປະຈຸບັນ ພວກເຮົາພົບຄົນເຈັບທີ່ຕິດເຊື້ອ Sennetsu (*Neorickettsia sennetsu*) ໃນ ສປປ ລາວ ຈໍານວນ 5 ຄົນ ເຊິ່ງການຄົ້ນພົບນີ້ ໄດ້ນໍາໄປສູ່ການພັດທະນາ ວິທີການບົ່ງມະຕິພະຍາດ *Neorickettsia sennetsu* ໂດຍກົງ. ການຄົ້ນພົບຈໍານວນຄົນເຈັບດັ່ງກ່າວ ແມ່ນມີໜ້ອຍກວ່າທີ່ພວກເຮົາຄາດການໄວ້ມາກ່ອນ.
- ພວກເຮົາຄົ້ນພົບເຊື້ອ *Tropheryma whipplei* ທີ່ກໍ່ໃຫ້ເກີດພະຍາດ Whipple ໃນອາຈົມຂອງເດັກທີ່ມີສຸຂະພາບແຂງແຮງໃນວຽງຈັນ. ເຊື້ອຈຸລິນຊີນີ້ ກໍ່ໃຫ້ເກີດພະຍາດຖອກທ້ອງ, ການດູດຊຶມອາຫານບໍ່ດີ, ບັນຫາທາງລະບົບປະສາດສູນກາງ ແລະ ບາງຄັ້ງພາໃຫ້ເກີດອັກເສບຫົວໃຈຊັ້ນໃນ (ແຕ່ບໍ່ສູ້ພົບ) - ແຕ່ວ່າ ຄວາມສໍາຄັນຂອງການກໍ່ໃຫ້ເກີດພະຍາດສໍາລັບເຊື້ອພະຍາດນີ້ ໃນລາວ ຍັງບໍ່ທັນຊັດເຈນ.
- ການຄົ້ນຄວ້າຂອງພວກເຮົາພົບວ່າ ແຜ່ນກວດພະຍາດໄຂ້ເລືອດອອກ (Standard Diagnostics, NS1/IgM/IgG) ທີ່ເກັບຮັກສາໄວ້ໃນອຸນຫະພູມຮ້ອນຂອງບ້ານເຮົາເປັນໄລຍະເວລາດົນນານ ຍັງສາມາດນໍາໃຊ້ໄດ້ເປັນຢ່າງດີ. ທີ່ຜ່ານມາ ເຄີຍພົບບັນຫາດັ່ງກ່າວກັບແຜ່ນຈຸ່ມກວດພະຍາດໄຂ້ຍູງແບບໄວວາ ທີ່ເກັບຮັກສາໄວ້ໃນສະພາບເງື່ອນໄຂອາກາດຮ້ອນ, ການຄົ້ນພົບນີ້ ເຮັດໃຫ້ເຮົາໝັ້ນໃຈ ໃນການນໍາໃຊ້ແຜ່ນກວດພະຍາດໄຂ້ເລືອດອອກໃນເຂດຊົນນະບົດຂອງປະເທດລາວ ແຕ່ພວກເຮົາໄດ້ເຮັດການຄົ້ນຄວ້າກັບແຜ່ນກວດພຽງຍີ່ຫໍ້ດຽວເທົ່ານັ້ນ.
- ພວກເຮົາພົບວ່າ ການບົ່ງມະຕິອັກເສບສະໝອງຍີ່ປຸ່ນດ້ວຍການກວດຫາ IgM ໃນນໍ້າໄຂສັນຫລັງໂດຍເຕັກນິກ ELISA ທີ່ພວກເຮົາເຄີຍໃຊ້ຜ່ານມາ ແມ່ນມີຄວາມຈໍາເພາະ (Specificity) ຕໍ່າກວ່າທີ່ຄາດການໄວ້ ແລະ ຈໍາເປັນຕ້ອງມີການພັດທະນາເຕັກນິກການບົ່ງມະຕິໃໝ່ ຢ່າງຮີບດ່ວນທີ່ສຸດ.
- ຜົນການຄົ້ນຄວ້າພົບວ່າ ພວກເຮົາສາມາດກວດວິເຄາະຫາຊະນິດ (Serotype) ຂອງໄວຣັສໄຂ້ເລືອດອອກ ດ້ວຍການເຮັດ PCR ຈາກແຜ່ນຈຸ່ມກວດໄຂ້ເລືອດອອກທີ່ໃຫ້ຜົນບວກ ເຊິ່ງການຄົ້ນພົບນີ້ ຈະຊ່ວຍໃຫ້ເຮົາສາມາດເຮັດການເຝົ້າລະວັງຊະນິດຂອງໄວຣັສໄຂ້ເລືອດອອກ ໃນຫລາຍໆພື້ນທີ່ຂອງທະວີບອາຊີ ທີ່ບໍ່ເຄີຍເຮັດມາກ່ອນ.

- ຜົນການສຶກສາຄົ້ນຄວ້າຂອງພວກເຮົາຊີ້ໃຫ້ເຫັນວ່າ ຍັງມີຄວາມກັງວົນຫລາຍ ສຳລັບການແຜ່ຫລາຍຂອງເຊື້ອໄຂ້ຍຸງຟານຊີປາຣອມທີ່ດື້ຕໍ່ຢາອາເທມີຊີນິນ ຢູ່ທາງພາກໃຕ້ຂອງລາວ, ເຊິ່ງບັນຫານີ້ຈຳເປັນຕ້ອງນຳໄປຫາລືກັນຢ່າງຮີບດ່ວນເພື່ອວາງແຜນວ່າ ຈະໃຊ້ຮູບແບບການປິ່ນປົວໃດໃນອະນາຄົດ ຫາກຢາປະສົມ 2 ຕົວ ຂອງ ACTs ທີ່ນຳລັງໃຊ້ໃນຂະນະນີ້ ບໍ່ໄດ້ຜົນອີກຕໍ່ໄປ.
- ພວກເຮົາພົບວ່າ ຄຸນນະພາບຂອງຢາປິ່ນປົວ ຍັງເປັນບັນຫາທີ່ໜັກໜ່ວງໃນທົ່ວໂລກ.
- ຫລັກຖານຈາກການສຶກສາ ໃນ ສປປ ລາວ ຊີ້ໃຫ້ເຫັນວ່າ ຄຸນນະພາບຂອງຢາປິ່ນປົວໄຂ້ຍຸງ ແມ່ນມີການປັບປຸງດີຂຶ້ນກວ່າເກົ່າ ເມື່ອທຽບກັບ 10 ປີ ທີ່ຜ່ານມາ, ແຕ່ຍັງມີຄວາມຈຳເປັນຕ້ອງໄດ້ສືບຕໍ່ເຝົ້າລະວັງກ່ຽວກັບຄຸນນະພາບຂອງຢາປິ່ນປົວໄຂ້ຍຸງ ອີກຕໍ່ໄປ.
- ຄວາມຮັບຮູ້ຂອງປະຊາຊົນໃນວົງຈັນໃນເລື່ອງຄວາມສ່ຽງຂອງຄຸນນະພາບຢາ ແມ່ນຍັງຢູ່ໃນລະດັບຕ່ຳ ເຊິ່ງຈຳເປັນຕ້ອງໃຫ້ສຸຂະສຶກສາແກ່ເຂົາເຈົ້າ ແນໃສ່ເພື່ອເພີ່ມທະວີການນຳໃຊ້ຢາໃຫ້ສົມເຫດສົມຜົນ.
- ພວກເຮົາພົບວ່າ ແນວທາງສຳລັບລາຍງານຜົນການທົດລອງຢາ (CONSORT guidelines) ຄວນມີພາກທີ່ຂຽນລາຍງານກ່ຽວກັບຄຸນນະພາບຂອງຢາທີ່ໃຊ້ໃນການສຶກສາທົດລອງ ເພື່ອເປັນມາດຕະການແກ້ໄຂບັນຫາຄຸນນະພາບຂອງຢາທີ່ໃຊ້ໃນການສຶກສາທົດລອງ (ທີ່ເຄີຍຖືກມອງຂ້າມໃນໄລຍະຜ່ານມາ).

Preparations for Womens' Day at Mahosot Hospital

SUMMARY

CryptoDex trial investigators meeting in Ho Chi Minh City

A. The Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit (LOMWRU) is a clinical research unit embedded within the Microbiology Laboratory of Mahosot Hospital. It was founded in 2000. It is funded predominantly by the Wellcome Trust of Great Britain, with significant additional support from the US Naval Medical Research Centre, US Centres for Disease Control (CDC), the Bill & Melinda Gates Foundation, The European Union, Fondation Total/Institute Pasteur, the World Health Organisation (WHO), the French Government Ministry of Foreign and International Affairs and the Joint Inter-Agency Task Force of the Global Fund. Considerable assistance in kind is given by the Institut de Recherche pour le Développement/Aix-Marseille University and the Rickettsial Diseases Research Program, Naval Medical Research Center, USA.

B. The Microbiology Laboratory is composed of 31 Lao Government staff and 45 project-funded staff; 91% are Lao and 61% are female. The Microbiology Laboratory has clinical microbiology, molecular, serology and BSL3 laboratories. It follows University of Oxford biosafety policies and guidelines.

C. LOMWRU supports the infectious disease diagnostic service of Mahosot Hospital, assists provincial hospitals in Luang Nam Tha, Salavan and Xieng Khouang Provinces, performs clinical research and builds diagnostic and research human capacity through training and active participation. LOMWRU also works with the Centre for Malariology, Parasitology and Entomology on malaria projects in Sekong and Savannakhet Provinces and with the Food and Drug Department (FDD) on the quality of medicines.

D. The main focus of the research work is on the causes of fever and their epidemiology, their optimal diagnosis and optimum treatment, the diagnosis, epidemiology and prevention of infantile beriberi, and the quality of medicines globally.

E. We supported 23 visits by Lao staff to five international meetings and supported two Lao staff to read for PhD/MSc degrees in 2015.

F. In 2015 we published or have in press 47 peer-reviewed papers, three book chapters, and one WHO report. LOMWRU authors are the most cited in the Lao public health literature since 2000, with 255 papers published or in press, including 16 book chapters.

G. Previous LOMWRU research translated into policy in Laos includes the implementation of vaccination against the pneumococcus and the Japanese encephalitis virus (JEV) and the change in national antimalarial and

typhoid treatment policies. It also demonstrated the presence of numerous important pathogens for the first time in Laos, and highlighted the global importance of scrub typhus, leptospirosis, typhoid, melioidosis and JEV, providing evidence on their epidemiology and prompting interventions.

H. The main findings, in brief, from work published, in press or in preparation in 2015 of immediate relevance to Laos (please see caveats in text!), are:

- Antimicrobial resistance (AMR) is, as elsewhere in the world, increasingly becoming a cause of significant concern for Lao public health. Extended spectrum beta-lactamase producing Enterobacteriaceae are not only increasingly important causes of infection in Mahosot Hospital but are also common in stools of healthy kindergarten children in Vientiane City and Province and in the stools of healthy people in a remote village of Xieng Khouang Province. We have also assisted with a large outbreak of hospital-acquired sepsis due to multidrug resistant ESBL producing *Klebsiella pneumoniae* at the Children's Hospital, Vientiane. Furthermore, knowledge about antibiotic resistance patterns and appropriate prescribing amongst doctors was relatively low – interventions targeting these gaps are urgently needed. The spread of drug resistance in Laos will have many deleterious consequences for patients, the community and the economy and greater emphasis on infection control, antibiotic stewardship and regulation is urgently needed.
- The majority of cerebrospinal fluid examinations have abnormal CSF cell counts, suggesting that lumbar punctures in inpatients with suspected central nervous system (CNS) infections should be facilitated to ensure that patients with these infections are not missed.
- Typhoid bacteria can be accurately, quickly and relatively inexpensively identified in blood culture fluid using typhoid antigen detecting rapid diagnostic tests (RDTs). Furthermore, PCR assays of the pad of positive RDTs can quickly determine whether the bacteria are likely to be susceptible to fluoroquinolones. These techniques may enable provincial hospital laboratories to diagnose this important disease with a centralised system of PCR testing for markers of fluoroquinolone resistance and facilitate investigation of typhoid outbreaks.
- Scrub typhus, murine typhus and leptospirosis are important causes of central nervous system infections in Vientiane and probably elsewhere in Asia. These data suggest a low threshold for inclusion of doxycycline in patients with meningitis/encephalitis along with third generation cephalosporins as the conventional empirical therapy as the later alone will not treat typhus.
- Data from fever studies in Laos, Cambodia and the Thai/Myanmar border suggest that C-reactive protein (CRP) assays are potentially useful markers of the need for antibiotic therapy amongst those presenting with fever. In addition, evidence from rural Laos suggests that CRP rapid diagnostic tests are accurate and may offer a mechanism for determining whether antibiotics are needed in settings without formal laboratory diagnostic microbiology tests.
- The results of a clinical trial of steroids in HIV-associated cryptococcal meningitis suggests that steroids are associated with a high incidence of adverse effects and should not be used in conjunction with amphotericin.
- Melioidosis is an important and under-recognised cause of sepsis in Laos, as well as other tropical regions. We have diagnosed over 940 culture-positive patients since 1999.
- We have now documented five patients with sennetsu (*Neorickettsia sennetsu*) infection in Laos and have established direct diagnostic methods for *Neorickettsia sennetsu*. We have found fewer patients than we were expecting.
- The agent of Whipple's disease, *Tropheryma whipplei,* occurs in the stools of healthy children in Vientiane. This bacterium can cause diarrhoea, malabsorption, CNS problems and, rarely, endocarditis – its importance in causing disease in Laos is uncertain.
- At least one brand of dengue RDT (Standard Diagnostics, NS1/IgM/IgG) retains diagnostic accuracy when exposed to Lao hot season temperatures long term. There are issues with malaria RDT stability in hot climates so these data are reassuring for the use of dengue RTDs in rural Laos, but we only evaluated one brand.
- There are concerns that the conventional CSF anti-JEV IgM ELISA for diagnosing Japanese virus encephalitis has lower than appreciated specificity and that new diagnostic tests are urgently needed.
- Dengue 'serotype' can be determined by PCR assays of the pad in NS1 dengue positive rapid diagnostic tests, potentially facilitating surveillance of dengue serotypes in the large areas of Asia without such surveillance.
- There remain serious concerns about the spread of artemisinin resistance *P. falciparum* parasites in southern Laos and discussions are needed as to what therapy will be recommended if double-therapy ACTs fail.
- There remain severe, at least focal, problems with the quality of diverse medicines globally.
- In Laos, there is evidence that the quality of antimalarials has improved over the last decade but that it is vital that surveillance of antimalarial quality is continued.
- There is limited awareness in Vientiane residents of the risks of medicines and more engagement with the public would make a valuable contribution to the appropriate use of medicines
- We argue that the CONSORT guidelines on clinical trial reports should include a requirement to determine

and describe the quality of medicines used, as a measure to correct this under-recognized and neglected critical weak link in clinical trials.

Dr Stuart Blacksell teaching IFA microscope use

PneuCAPTIVE study team en route for patient follow up

Beetles for sale in Phalanxay

INTRODUCTION

Team from the Microbiology Laboratory participating in tak bhat at Wat Sisaket for Khao Pansa

The Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit (LOMWRU) is a clinical research unit embedded within the Microbiology Laboratory of Mahosot Hospital, a Lao Government primary-tertiary hospital in Vientiane. The majority of the funding is from the Wellcome Trust of the United Kingdom (UK), a charity, through the University of Oxford. LOMWRU was founded in 2000 and is guided by a Memorandum of Understanding between Mahosot Hospital of the Lao Ministry of Health, the Wellcome Trust and the University of Oxford (2012-2022). It is housed in two buildings. The old Microbiology Laboratory (from the 1920s), houses the clinical microbiology laboratory, offices, administration and the medicine quality project, and was extended to create a modern microbiology laboratory in 2011 with funding from the University of Oxford. The upper floor of the Infectious Disease Centre (construction was funded by the Wellcome Trust and opened in 2008) contains the Molecular, Serology and BSL3 Laboratories and offices.

Oxford University headquarters are at the Centre for Tropical Medicine & Global Health, in the Nuffield Department of Medicine on the Churchill Hospital site. We are a part of the Mahidol-Oxford Research Unit (MORU) Network based in the Faculty of Tropical Medicine, Mahidol University, Bangkok, and are greatly assisted by the supplies, logistic and accounting staff of MORU and have many scientific liaisons with MORU - ~ 40% of our research projects are jointly with MORU-Bangkok.

MORU, the Shoklo Malaria Research Unit (SMRU), in Mae Sot, Thailand, the Cambodia-Oxford Medical Research Unit (COMRU) and LOMWRU are integrated into the Thailand Major Overseas Programme of the Wellcome Trust and Oxford University. We are also linked to the Oxford University Clinical Research Unit (OUCRU), based in Ho Chi Minh City, Vietnam, and have important collaborations with them.

The Microbiology Laboratory is composed of 31 Lao Government staff and 45 project-funded staff; 91% are Lao and 61% are female. In addition, we have goats, resident in the Laboratory garden, which assist with the preparation of blood agar. LOMWRU has received significant recent support, in addition to that from the Wellcome Trust, from the US Naval Medical Research Centre, the US Centres for Disease Control (CDC), the Foundation for Innovative New Diagnostics (FIND), the Institut de Recherche sur l'Asie du Sud-Est Contemporaine (IRASEC), the B&M Gates Foundation, DFID - UK and the Joint Inter-Agency Task Force of the Global Fund. Considerable assistance in kind is given by the Institut de Recherche pour le Développement (IRD)/Aix-Marseille University and the University Rickettsial Diseases Research Program, Naval Medical Research Center, USA

LOMWRU supports the infectious disease diagnostic service of Mahosot Hospital and assists provincial hospitals in the far northwest (Luang Nam Tha), the northeast (Xieng

Khouang), the far south (Salavan) and other hospitals and institutions on request, performs clinical research and builds diagnostic and research human capacity. In 2015 the Laboratory processed blood cultures from 6,067 patients, cerebrospinal fluid from 222, urine from 1,291, stool from 465, pus from 842, genital swabs from 3,383 and throat swabs from 1,436 patients. Dengue IgG, IgM and NS1 ELISAs were performed for 2,619 patients and scrub typhus and murine typhus rapid diagnostic tests for 504 patients. We assisted responding to disease outbreaks in Laos, such as helping the Mahosot Cardiac Centre and new Mothers and Neonates' Hospital in the identification and investigation of nosocomial infections. LOMWRU also works with the Centre for Malariology, Parasitology and Entomology on malaria projects in Sekong and Savannakhet Provinces and with the Food and Drug Department on the quality of medicines.

Since 2000 we have published or have in press 255 papers, including 16 book chapters. LOMWRU authors are the most cited in the Lao public health literature since 2000 and are the most cited authors on medicine quality & public health. In 2015 we published or have in press 47 peer-reviewed papers, 3 book chapters, and one World Health Organization report. We supported 23 visits by Lao staff to five international meetings and supported two Lao staff to read for PhD/Msc. Here we describe this work and briefly summarize diverse activities over the past year.

Microbiology Laboratory dance troupe

STAFF AND HUMAN CAPACITY BUILDING

Numerous students and doctors in diverse health disciplines studied in the Microbiology Laboratory in 2015 and residents wrote their theses related to the work of the Laboratory. The Laboratory staff assisted with the post-graduate internal medicine and paediatric training programme teaching. Five Lao postgraduate fellows in infectious disease are conducting their research theses with us - Dr Bandith Soumphonphakdy, Dr Phouvieng Douangdala, Dr Ko Chang, Dr Othila Rasphone and Dr Savandalat Phouangsouvanh.

We are delighted that Dr Rattanaphone Phetsouvanh has completed her PhD from Mahidol University, Bangkok, on scrub typhus, and that Dr Manivanh Vongsouvath has completed her MSc in Clinical Tropical Medicine also at Mahidol University. Ms Kristin Mullins of the Uniformed Services University, USA, is completing her PhD with us on scrub typhus disease severity and *O. tsutsugamushi* genotypes. We hosted two MSc students, Audrey Rachlin and Tehmina Bharucha, from the London School of Hygiene and Tropical Medicine in 2015, working, respectively, on MLST analysis of *B. pseudomallei* from patients with repeated melioidosis and testing whether anti-JEV IgM could be accurately detected in cerebrospinal fluid dried onto filter paper. An MSc student from the University of Oslo, Scott Tschida, worked with us on a global review of the quality of antibiotics. Dr Nguyen Văn Hoàn, from Hai Phong Medical University and a PhD student at University of Aix-Marseille, spent two months with us working on respiratory infections.

Dr Sabine Dittrich left LOMWRU after nearly five years productive work to join the Foundation for Innovative New Diagnostics in Geneva and her successor, Dr Mathew Robinson, will start in May 2016.

Ms Bountoy Sibounheuang spent a further two months in the laboratory of Professor Xavier Nicolas de Lamballerie at Marseille working with Dr Audrey Dubot-Pérès, funded by the Institut de Recherche pour le Développement. Dr Audrey Dubot-Pérès, who leads the LOMWRU virology, is based in Marseille but returned for three months of intensive virology work in LOMWRU in 2015.

We are also fortunate to have strong links with Public Health England (PHE) who support a microbiology/infectious disease registrar to spend a year of training with us. Dr Kate Woods conducted much laboratory, clinical and research work during 2015. This has been a very useful synergistic programme that we hope will resume soon. In addition, Dr Andrew Taylor from the UK worked in LOMWRU for a year, contributing much to the clinical and research work. Drs Ruth Lim and Jana Lai from Australia have been working with us on the PneuCAPTIVE study (below).

Regular classes have been held in English. We are planning Good Clinical Practice, mapping and statistics training in 2016 and MORU-Bangkok is planning a mathematical modelling for policy makers workshop in Vientiane.

In 2015, we supported 23 visits by Lao staff to five international meetings and supported two Lao staff to read for their PhD/MSc degrees.

We have continued to build capacity within the Unit with hands-on training in microbiology, ELISA, molecular diagnostic and BSL3 Laboratory work. In addition we have daily ad hoc teaching during board rounds and weekly teaching sessions for the doctors working within the Unit (both at Mahosot and those visiting from the Provinces) covering clinical and laboratory aspects of infectious diseases and microbiology directly relevant to both their clinical and research activities. We have a Lao Deputy Safety Officer, a Lao Head of Field Research, a Lao deputy head of Virology, a Lao deputy WWARN Antimalarial Quality Coordinator, a Lao Laboratory Manager and a Lao BSL3 Manager. A Laboratory Management Adviser is co-ordinating a programme of work towards ISO15189 accreditation for the Microbiology Laboratory, and we are working closely with other laboratories in Laos, working towards such accreditation.

LOMWRU staff teach at the University of Health Sciences and Institut de la Francophonie pour la Médecine Tropicale, Vientiane, the DTM&H of the London School of Hygiene and Tropical Medicine and the International Health MSc at the University of Oxford.

Professor Mayfong presenting a leaving gift to Ms Viphaphone Vongsavanh

RESEARCH RESULTS AND THEIR PUBLIC HEALTH IMPLICATIONS

Dr Tiengkham Pongvongsa and Professor Mayfong Mayxay discussed targeted malaria elimination in Nong District, southern Laos

Infectious Disease Epidemiology and Treatment

A. Fever in rural Laos. The data published in Mayxay *et al.* (2013) in Lancet Global Health (see 2014 Annual Report) demonstrated the importance of a wide spectrum of neglected infectious diseases, especially dengue, scrub typhus, leptospirosis and the Japanese encephalitis virus, as the causes of non-malarial fever in patients in rural Laos. We expanded this work in 2015, with the support of the US Naval Medical Research Centre, to include Xieng Khouang Provincial Hospital, along with those in Salavan and Luang Nam Tha and a physician and technician were posted to run the study in each of the three hospitals. The National Centre for Laboratory & Epidemiology (NCLE) has analyzed nasopharyngeal swabs from these patients, contributing to national influenza surveillance. This study is on hold after completing one year but we hope to restart in May 2016.

Further analysis of the non-malarial fever data from Laos suggests that serum C-Reactive Protein (CRP) is a potentially accurate predictor for the need for antibiotics amongst patients with fever (Lubell *et al.* 2015). Serum procalcitonin and CRP levels were measured in stored serum samples from febrile patients enrolled in three prospective studies conducted in Cambodia, Laos and, Thailand. Of the 1,372 patients with a microbiologically confirmed diagnosis, 1,105 had a single viral, bacterial or malarial infection. Serum concentrations of both biomarkers were significantly higher in bacterial infections and malaria than in viral infections. The AUROC for CRP in discriminating between bacterial and viral infections was 0.83 (0.81–0.86) compared with 0.74 (0.71–0.77) for procalcitonin ($p < 0.0001$). For CRP at a threshold of 10 mg/L, the sensitivity of detecting bacterial infections was 95% with a specificity of 49%. Use of a CRP rapid test in peripheral health settings could potentially be a simple and affordable measure to better identify patients in need of antibacterial treatment and combat the emergence of antibiotic resistance through avoiding unnecessary antibiotic use.

With evidence that CRP is a promising test we evaluated the diagnostic accuracy of three different CRP rapid diagnostic tests (RDT) in provincial hospitals, against the reference NycoCard Reader II. All three tests showed high sensitivity, specificity and kappa values suggesting that CRP RDTs could offer an inexpensive and effective

approach to improve the targeting of antibiotics in remote settings where health facilities are basic and laboratories are absent (Phommasone *et al.* 2016). Further programmatic and cost-effectiveness analysis of different algorithms is being planned.

We are also looking in the stored blood samples of patients without an aetiological diagnosis for diverse other pathogens such as *Bartonella* spp., *Neorickettsia sennetsu*, *Anaplasma* and *Ehrlichia* species at the three provincial sites and at Mahosot Hospital (see below).

Data collected on the causes of fever amongst patients with suspected malaria presenting at Phalanxay District Hospital, Savannakhet Province, suggested that dengue, leptospirosis, Japanese encephalitis virus infection and scrub typhus were the predominant causes (Mayxay *et al.* 2015).

B. Causes of fever at Mahosot Hospital. We are working on amalgamating all the data on common causes of fever (conventional bacteraemia, rickettsia, leptospira, dengue and JEV) over four recent years so that we can estimate the frequency of hospital admission of diverse aetiologies for a large series of patients and describe their comparative clinical features. For the 'conventional' bacteria we are also analyzing how antimicrobial resistance patterns have changed since 2000 and are working with mathematical modelers in MORU-Bangkok to try to understand this better. We work with Stanford University on messenger RNA signatures in infections, especially investigating whether the mRNA signature of scrub typhus is especially characteristic of this disease.

C. Scrub typhus, murine typhus, leptospirosis and central nervous system disease. We investigated the relative importance of *Orientia tsutsugamushi*, *R. typhi*/*Rickettsia* spp. and *Leptospira* spp. among meningitis and encephalitis patients at Mahosot Hospital (Dittrich *et al.* 2015a). Among 1,051 patients, 24% had a central nervous system (CNS) infection attributable to a bacterial or fungal pathogen and 35% of those were caused by *O. tsutsugamushi*, *R. typhi*/*Rickettsia* spp. or *Leptospira* spp. These pathogens were found significantly more frequently than 'conventional' bacterial infections, such as *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae* and *S. suis*. CNS infections had a high mortality (27%), with 18% for *R. typhi*/*Rickettsia* spp., *O. tsutsugamushi* and *Leptospira* spp. combined, and 33% for 'conventional' bacterial infections. These data suggest that *R. typhi*/*Rickettsia* spp., *O. tsutsugamushi* and *Leptospira* spp. infections are important causes of CNS infections in Laos and probably across rural Asia. Antibiotics, such as tetracyclines, required for the treatment of murine typhus and scrub typhus, are not routinely advised for empirical treatment of CNS infections.

These findings suggest that tetracyclines should be used for CNS infections when rickettsial infections are suspected and that typhus needs to be considered as important but treatable causes of CNS disease in Asia and elsewhere. We caution that, given the variable specificity of antibody based antibody tests for diagnosing scrub typhus and murine typhus, that doxycycline should not be used alone in severe disease, such as CNS syndromes, and that serious 'conventional' pathogens should also be covered by, for example, additional ceftriaxone.

D. Central nervous system infections. We have nearly completed analyzing the data from the first ~1,000 patients to have a lumbar puncture at Mahosot Hospital since 2003 to describe the aetiologies for a large series of patients with meningitis/encephalitis and describe their comparative clinical features and impact. Both 'conventional' bacterial, fungal and viral infections are important but rickettsial and leptospiral infections are also key – see above. This is a collaborative project with multiple partners, especially with Institut de Recherche pour le Développement (IRD)/Aix-Marseille University.

This work has been expanded since 2014 as the SEAe project in collaboration with the Institut Pasteur, Paris, funded by the Total Foundation to investigate the aetiology and impact of encephalitis and meningoencephalitis in Vietnam (at National Institute of Hygiene and Epidemiology & National Children's Hospital, Hanoi), Cambodia (at Kantha Bopha Hospital, Phnom Penh) and Laos (at Mahosot Hospital and the Children's Hospital) using common study protocols. This allows first line PCR diagnosis of 21 pathogens in the first 24 hours after LP.

The majority of CSF samples collected give abnormal results, suggesting that a higher frequency of LP is needed to ensure that patients, especially at the extremes of age, do not have serious CNS infections missed.

We work with the Centre d'Infectiologie Christophe Mérieux du Laos on detection of molecular markers of *M. tuberculosis* drug resistance from patients with TB meningitis.

We also examined the function of the blood-brain barrier in patients with diverse CNS infections finding that the albumin index (AI) and glial fibrillary acidic protein (GFAP) levels were significantly higher in bacterial and tuberculous meningitis patients than other diseases but were also raised in individual rickettsial patients (Dittrich *et al.* 2015b). Interestingly, total tau protein was significantly raised in the CSF of JEV patients.

The diagnosis of encephalitis and meningitis due to JEV infection is based on detection of anti-JEV IgM in CSF by ELISA. PCR is seldom useful as a diagnostic tool as

Dr Céline Caillet and Dr Chanvilay Sichanh, working on the WWARN medicine quality project

JEV is usually undetectable in blood or CSF by the time patients present at hospitals. We presented data from Laos suggesting that the positive predictive value of anti-JEV IgM detection in serum and CSF is low for diagnosing JEV encephalitis (Dubot Pérès *et al.* 2015). This raises concern that the incidence of acute neurological infections due to JEV might be overestimated, and that detection of anti-JEV IgM in patients with acute infections of the CNS should not rule out other treatable diseases, particularly bacterial/tuberculous infections. Innovative techniques such as CSF proteomics might give information allowing more sensitive and specific diagnostic tests to be developed.

There are few data globally on whether the disability associated with JEV encephalitis improves in the longer term and we are investigating this using the Liverpool Outcome Score for patients with long-term follow up.

E. Aetiology and impact of fever in pregnancy. The large pilot cohort study of the causes and impact of fevers in pregnancy in Pak Gnum District, Vientiane, is completed. Maternal mortality in Laos is reported as the highest in SE Asia and data from Mahosot Hospital (Chansamouth *et al.* in press) suggests that common infectious diseases, such as dengue and scrub typhus may be important contributors. This study is linked to the National Centre of Laboratory and Epidemiology for surveillance of respiratory infections in pregnant women, supported by US CDC in Laos. One thousand pregnant women were recruited, 92% of all pregnant women known in the district, of whom 110 developed intra-or post-partum fevers. The aetiologies of fever are being examined in relation to outcome measures such as low birth weight and stillbirth. Among the 1,000 women there were 18 miscarriages, 6 perinatal deaths, 3 neonatal deaths, 1 maternal death (ectopic pregnancy) and 11 congenital abnormalities.

F. Do steroids reduce mortality in cryptococcal meningitis? The Cryptodex clinical trial of dexamethasone *versus* placebo in HIV-positive patients receiving amphotericin B for cryptococcal meningitis, coordinated by OUCRU, was stopped after 451 patients were recruited on the recommendation of the Trial Steering Committee and the Data Monitoring and Ethics Committee. This was because of a significantly higher incidence of adverse events in the dexamethasone arm. As the study was stopped early it was underpowered to determine whether dexamethasone reduced mortality from cryptococcal meningitis but it seems unlikely that it does (Beardsley *et al.* 2016).

G. Mapping. We are working with the London School of Hygiene & Tropical Medicine (LSHTM), the WorldWide Antimalarial Resistance Network (WWARN) and FIND on the mapping of the aetiology of fevers globally, building on our earlier collaboration - Acestor *et al.* (2012; *PLoS One 7,* e44269).

Clinical Bacteriology

A. Typhoid diagnosis & genomes. Typhoid (*Salmonella enterica* serovar Typhi) remains an important pathogen in Laos but there are very few health facilities with accessible blood culture and antimicrobial susceptibility testing facilities. We demonstrated that brief blood culture bacterial incubation followed by testing of blood culture fluid for *S.* Typhi antigen using rapid diagnostic tests (RDT) is an accurate and inexpensive tool for the accelerated diagnosis of patients with acute typhoid in Laos (Castonguay-Vanier *et al.* 2013, See 2014 Annual Report).

We expanded this work, demonstrating that molecular markers of *S.* Typhi fluoroquinolone resistance could be detected by PCR assays on extracts from the RDTs (Nic Fhogartaigh *et al.* 2015). This work suggests that *S.* Typhi antigen detecting RDTs could be used in rural Asia for diagnosing typhoid after brief blood culture incubation. Positive RDTs could be sent to a central facility for the rapid determination of fluoroquinolone resistance by PCR. Such a novel system could help with individual patient management, outbreak investigations and typhoid antibiotic resistance surveillance.

Although azithromycin is an effective treatment for uncomplicated infections with *Salmonella enterica* serovar Typhi and serovar Paratyphi A, there are no clinically validated MIC and disk zone size interpretative guidelines. We participated in a consortium of investigators to help inform this. Data from three randomized controlled trials of antimicrobial treatment in enteric fever in Vietnam, with azithromycin used in one treatment arm, were used to determine the relationship between azithromycin treatment response and the azithromycin MIC of the infecting isolate. The azithromycin MIC and the disk susceptibility zone

Inspection by our goat, Himakhao, of visitors from the US Naval Medicine Research Centre, Dr Rebecca Pavlicek, Dr Brian Pike and Dr Ian Sutherland with Paul Newton and Mayfong Mayxay

sizes of 1,640 *S.* Typhi and *S.* Paratyphi A clinical isolates from seven Asian countries, including Laos, were then compared; 99.5% of Asian *S.* Typhi isolates had an MIC of ≤16 μg/ml. An azithromycin MIC of ≤ 16 μg/ml or disk inhibition zone size of ≥ 13 mm enabled the detection of susceptible *S.* Typhi isolates that respond to azithromycin treatment (Parry *et al.* 2015).

B. Extended spectrum beta-lactamase (ESBL) carriage in kindergartens. We completed a pilot study with the Institut de la Francophonie pour la Médecine Tropicale (IFMT) on childhood faecal carriage of Extended spectrum beta-lactamase producing Enterobacteriaceae (ESBLE) in Vientiane kindergartens, finding that 23% of children were colonised with ESBLE, mainly *Escherichia coli* carrying $bla_{\text{CTX-M}}$ and *Klebsiella pneumoniae* carrying bla_{SHV} or $bla_{\text{CTX-M}}$, which were frequently resistant to multiple unrelated antibiotics (Stoesser *et al.* 2015). Faecal carriage of ESBLE was more common in Vientiane Capital (30%) than the more rural Vientiane Province (16%). Only antibiotic use in the last three months was found to be an independent risk factor for ESBLE carriage on multivariable analysis. The high prevalence of paediatric colonization with ESBLE in Laos, one of the highest reported in Asia, is probably the result of inappropriate antibiotic use and needs urgent attention.

C. Extended spectrum beta-lactamase (ESBL) carriage in a remote Lao village. We extended the above work by estimating the prevalence of colonisation with ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* in a remote village, Yod Teui, in Xieng Khouang Province. Rectal swabs were taken from 268 human inhabitants and 252 domestic animals. Overall 14 humans and 21 animals (including chickens, dogs and pigs) were found to be colonised with ESBL-positive organisms, despite the remoteness of the village and the fact that no commercial animal feedstuffs were being used. However, a surprising proportion of the human population (13.4%) had taken antibiotics in the preceding 2 weeks. Further data analysis is underway and whole genome sequencing of the isolates is planned in order to investigate the epidemiology of antimicrobial resistance within this interesting and relatively isolated community.

D. Extended spectrum beta-lactamase (ESBL) acquisition amongst visitors. The acquisition of ESBL-producing *E. coli* and *K. pneumoniae* was studied amongst 21 European doctors participating in a tropical medicine course in Vientiane during September and October 2015, by collecting daily rectal swabs. Ten of the participants were already excreting organisms that grew on the selective screening medium by the time they submitted their first sample, and all of the participants had had one or more probable ESBL positive cultures by the end of the course. Further characterisation of these isolates and data analysis is underway.

E. *Burkholderia pseudomallei* and the environment. We optimised molecular methods for the detection of *B. pseudomallei* in soil and developed methodologies to be used with water samples (Knappik *et al.* 2015). Molecular detection following an enrichment step was a sensitive and reliable approach for *B. pseudomallei* detection in Lao environmental samples and is recommended as the preferred method for future surveys.

We investigated the environmental factors that influence the presence (and absence) of *B. pseudomallei* in a tropical watershed in Salavan (Ribolzi *et al.* 2016). Soil type in the surrounding catchment and turbidity had a strong positive influence on the presence (acrisols and luvisols) or absence (ferralsols) of *B. pseudomallei*.

Tauran *et al.* (2015) reported patients with melioidosis in Sulawesi, Indonesia. This has rarely been reported in Indonesia but may be under-recognized. Nasner-Posso *et al.* (2015) reviewed reports of human melioidosis reported by ProMED. Most of the cases were reported from Australia, Thailand, Singapore, Vietnam, and Malaysia, with sporadic reports from other countries. It was concluded that Internet-based reporting systems such as ProMED are useful to gather information and synthesize knowledge on emerging infections. Reports of infectious disease to ProMed should be encouraged.

Mapping the distribution of humans and animals with *B. pseudomallei* and the presence of environmental *B. pseudomallei* were combined in a formal modelling framework to estimate the global burden of melioidosis (Limmathurotsakul *et al.* 2016). This estimated that there may be ~165,000 human melioidosis cases per year worldwide, from which ~89,000 people die. The estimates suggest that melioidosis may be severely underreported in the 45 countries in which it is known to be endemic (including Laos) and that melioidosis may be endemic in a further 34 countries that have never reported the disease.

F. *Burkholderia pseudomallei* and clinical microbiology. We participated in a workshop to discuss the current state of melioidosis diagnostics, diagnostic needs, and future directions (Hoffmaster *et al.* 2015). We are evaluating a new RDT for *B. pseudomallei* antigen detection in blood cultures and other body fluids using the same principle as the work on the detection of typhoid (see above).

Ms Audrey Rachlin, an MSc student from the LSHTM, conducted her thesis research with us, using a multilocus sequence typing (MLST) scheme specific for *B. pseudomallei* to investigate nine cases of culture-positive recurrence occurring in 514 patients with melioidosis between 2010-2015: four were suspected to be relapses whilst the other five represented re-infections.

We collaborate with Dr Ivo Steinmetz, University of Greifswald, Germany, evaluating a highly sensitive PCR method for the detection of *B. pseudomallei* DNA in EDTA blood and with Professor K Thong Wong, University of Malaya, on the intracellular localisation of *B. pseuodomallei* in human cells.

G. Bacterial genomics and epidemiology. We are working with OUCRU and the Wellcome Trust Sanger Institute on the comparative genomics of *Salmonella* species in Asia, both typhoid and non-typhoidal, as well as *Shigella* species. We are working with the Murdoch Children's Research Institute, Melbourne, Australia on the genomics of *Streptococcus pyogenes*, with the Oxford University Clinical Research Unit in Hanoi on the genomics of *Klebsiella pneumoniae* and the epidemiology of *Streptococcus suis*, with Public Health England, the Sanger Institute in Cambridge, MORU in Bangkok and the Wellcome Trust Centre for Human Genetics, Oxford, on the epidemiology and genomics of *Burkholderia pseudomallei*, and with Public Health England on the genomics of *Staphylococcus aureus*.

We participated in a large multi-country study of the whole-genome sequence analysis of 1,832 *Salmonella enterica* serovar Typhi (*S.* Typhi) isolates (Wong *et al.* 2015). This identified a single dominant multi-drug resistance lineage, H58, that has emerged and spread throughout Asia and Africa over the last 30 years. H58 was described from Laos. The data suggest that H58 *S.* Typhi are displacing antibiotic-sensitive isolates, transforming the global population structure of this pathogen.

We characterized the emm types, emm clusters and the antibiotic resistance profile of 124 Group A streptococcal isolates cultured in Laos during 2004–2013 (Rattanavong *et al.* 2015). Most strains were recovered from skin and invasive infections. Thirty-four emm types were identified as belonging to 12 emm clusters and no novel emm types were identified. There was moderate strain diversity in Laos but considerable differences in emm-type distribution between Laos, Thailand and Cambodia. Expected vaccine coverage was high for the J8 vaccine candidate but the theoretical coverage for the 30-valent vaccine candidate needs further investigation. Antibiotic resistance was moderate to erythromycin and chloramphenicol (8% and 7%, respectively) and low to ofloxacin (<1%)

H. *Tropheryma whipplei* stool carriage in Vientiane. *Tropheryma whipplei* is the bacterium causing Whipple's Disease, manifested as diarrhoea, malabsorption, CNS problems and, rarely, endocarditis. The organism has been shown to be commonly carried in the faeces of young children in central and west Africa but there are no published data from Asia. Working with the University of Aix-Marseille, France, PCR detected *T. whipplei* in the stools of 48% of 106 well children in Vientiane (Keita *et al.* 2015). Positive samples were genotyped. Eight genotypes were detected including 7 specific to Laos. Further research is needed to identify the public health significance of this finding.

I. Respiratory infections. We are continuing a prospective description of the clinical features and aetiologies of respiratory illness in children (ARIVI). This has given the first evidence that *Mycoplasma pneumoniae* does occur in Laos.

Within this study and working with the Murdoch Children's Research Institute, Melbourne, we are also estimating the hospital incidence of *S. pneumoniae* invasive disease, pneumococcal carriage and its serotypes to examine how their frequencies change with the introduction of 13 valent *S. pneumoniae* vaccination in Laos – the PneuCAPTIVE study funded by the B&M Gates Foundation. We also work with the Centre d'Infectiologie Christophe Mérieux on the LaCoRIS study, funded by the US Naval Medical Research Centre, that is a large cohort study examining the aetiology of respiratory illness in Vientiane.

J. *Nocardia aobensis* mycetoma. Together with colleagues from the Bernhard Nocht Institute for Tropical Medicine in Hamburg, we described a 30 year old farmer with actinomycetoma caused by *Nocardia aobensis* in Savannakhet Province (Vongphoumy *et al.* 2015). This is the first record of this pathogen in actinomycetoma. A treatment course of only 14 days with amikacin and trimethoprim-sulfamethoxazole was apparently sufficient to cure the infection, although long-term treatment up to one year is currently recommended. Treatment trials or prospective descriptions of outcome for actinomycetoma should investigate treatment efficacy for the different members of Actinomycetales, particularly *Nocardia* spp., with short term and long-term treatment courses.

K. *Clostridium difficile.* We have looked for *Clostridium difficile* in the stools of patients at Mahosot Hospital and have found it – we hope to publish this soon. The occurrence of this organism is not unexpected with the high cephalosporin use in Vientiane hospitals and argues for enhanced antibiotic stewardship.

3. Leptospirosis

A. Leptospiral DNA detection in blood cultures. We are investigated whether we can detect *Leptospira* species by PCR in blood culture fluid. Despite initial promising results, a subsequent large prospective evaluation showed very low sensitivity compared with PCR of venous blood samples (Dittrich *et al.* in press).

B. Leptospirosis rapid diagnostic tests. We have been determining the optimal fraction of blood for the molecular diagnosis of leptospirosis and conducted a large prospective evaluation of different RDTs and PCR assays for diagnosing leptospirosis, with the aim that this will inform the optimal diagnostic techniques for leptospiral infections in rural Asia and will be reported soon.

C. Untreated mortality of leptospirosis. We conducted a review of the literature to estimate the untreated mortality of leptospirosis – there are no previous evidence-based estimates (Taylor *et al.* 2015a). Thirty-five studies, comprising 41 patient series and 3,390 patients, were included. A high degree of bias within studies was shown due to limitations in study design, diagnostic tests and missing data. Median series mortality was 2.2%, but mortality was high in jaundiced patients (19.1%), those with renal failure 12.1% and in patients aged over 60 years (60%), but low in anicteric patients (0%).

4. Rickettsiology and related pathogens

A. Rapid diagnostic tests for scrub typhus. We are conducting a prospective study in 2015/2016 of the diagnostic accuracy of 5 different scrub typhus RDTs to determine which one(s) are the optimal for diagnosis this disease in rural Asia. We plan to complete this in 2016.

B. Scrub typhus genotypes. We have been working on the genetic diversity of *Orientia tsutsugamushi* across Laos using a multilocus sequence typing (MLST) scheme to characterize 74 clinical isolates from three geographic locations in Laos, and compare them with isolates described from Udon Thani, northeast Thailand (Phetsouvanh *et al.* 2015). The data confirm high levels of diversity and recombination within the natural *O. tsutsugamushi* population, and a rate of mixed infection of ~8%. These analyses point towards low levels of population differentiation between isolates from Vientiane and Udon Thani, cities. However, a very distinct population was found in Salavan, southern Laos.

The collaboration with Rickettsial Diseases Research Program, Naval Medical Research Center, USA, is progressing with the whole genome sequencing (WGS) of multiple Lao *Orientia tsutsugamushi* genotypes to examine whether different genotypes are associated with disease severity. Twenty isolates have had successful WGS performed and more genotypes are expected soon – these data will help us understand if severe scrub typhus is associated with particular *Orientia tsutsugamushi* genotypes.

C. Pathophysiology or rickettsial diseases. Cell-mediated immunity is essential in protection against rickettsial illnesses, but the role of neutrophils in these intracellular vasculotropic infections remains unclear. We analyzed the plasma levels of nucleosomes, FSAP-activation (nucleosome-releasing factor), and neutrophil activation, as evidenced by neutrophil-elastase (ELA) complexes, in sympatric Lao patients with scrub typhus and murine typhus (Paris *et al.* 2015). Elevated nucleosome and ELA complex levels were associated with a 4.8-fold and 4-fold increased risk of developing severe scrub typhus, respectively. In murine typhus, nucleosome levels associated with pro-inflammatory cytokines and the duration of illness, while ELA complexes correlated strongly with inflammation markers, jaundice and increased respiratory rates. The data suggest that increased neutrophil activation relates to disease progression and severe complications, and increased plasma levels of nucleosomes and ELA complexes represent independent risk factors for developing severe scrub typhus.

D. Antibiotic susceptibility of rickettsial species. The project examining the antibiotic susceptibility of diverse isolates of *O. tsutsugamushi* is progressing well and we expect to have more information later in 2016.

E. Ticks and potential human pathogens. We are working with the Institut Pasteur-Laos and the US Naval Medical Research Centre on the detection of *Rickettsia, Bartonella, Orientia, Anaplasma* and *Ehrlichia* species in a large collection of ticks from Khammouane Province. We found that a significant proportion contain *Rickettsia* spp. DNA including three probable new species. In addition, *Ehrlichia chaffeensis*, *Coxiella burnetii, Anaplasma phagocytophilum* and *Borellia* spp. were identified. This should help us to narrow down what tick-borne bacterial pathogens we may find in patients in Laos.

F. Leeches and rickettsial diseases. We described a Lao patient from Luang Namtha with PCR evidence for *Rickettsia felis* infection in an eschar at the site of a leech bite (Slesak *et al.* 2015). The potential importance of leeches as vectors for rickettsial infections is discussed. We plan to investigate this possibility further.

G. Mapping of scrub typhus. We are working with VectorMap (http://www.vectormap.org/), the Spatial Ecology and Epidemiology Group of Oxford University, Liverpool University and many partners on the global mapping of chigger vectors/reservoirs and infected rodents and humans. We hope that this work will lead to a greater understanding of the relationships between humans rodents and chiggers in the ecology of scrub typhus.

H. Untreated mortality of scrub typhus. We conducted a review of the literature to estimate the untreated mortality of scrub typhus – the first evidence-based estimate (Taylor *et al.* 2015b). A total of 76 studies containing 89 patient series and 19,644 patients were included in the final analysis. The median mortality of all patient series was 6.0% with a wide range (min-max) of 0–70%. Many studies used clinical diagnosis alone and had incomplete data on secondary outcomes. Mortality varied by location and increased with age and in patients with myocarditis, delirium, pneumonitis, or signs of hemorrhage, but not according to sex or the presence of an eschar or meningitis. The untreated mortality from scrub typhus appears lower than previously reported estimates. More data are required to clarify mortality according to location and host factors, clinical syndromes including myocarditis and central nervous system disease, and in vulnerable mother-child populations.

I. Revisiting the natural history of scrub typhus. Dr Ivo Elliott has been awarded a Wellcome Trust Fellowship to work in LOMWRU from 2015, revisiting the research on the natural history of scrub typhus in the 1930s/1950s using modern techniques such as whole genome sequencing and geographical information systems. There are many uncertainties about the ecology of scrub typhus and this work will increase our understanding and inform interventions to reduce transmission.

J. *Neorickettsia sennetsu.* We have identified a further four patients with PCR evidence for sennetsu (Dittrich *et al.* 2015c). This is fewer than we expected, giving the high apparent seroprevalence, but the transmission of this pathogen, thought to be from trematodes in fish, remains unclear. It is possible that the high Lao seroprevalence reflects exposure to other non-pathogenic *Neorickettsia* species. We continue to work on the epidemiology of *Neorickettsia sennetsu*, with the University of North Dakota, looking for this pathogen in a variety of invertebrate and vertebrate taxa to try to elucidate the natural history of this intriguing pathogen.

Plus, We are working on determining the optimal fraction of blood for the molecular diagnosis of rickettsial diseases, disease severity scores for scrub typhus, and analyzing the scrub typhus and murine typhus clinical trials of doxycycline and azithromycin and the first PK-PD work on typhus and doxycycline and azithromycin therapy with the Pharmacology Department of MORU-Bangkok.

5. Virology

The virology work of LOMWRU is strongly supported by the Institut de Recherche pour le Développement (IRD)/ Aix-Marseille University. Virological aspects of CNS infections are discussed above.

A. Dengue epidemiology. Thankfully 2015 had a low incidence of dengue in Laos, unlike 2013. We are analyzing the distribution of dengue serotypes in Vientiane 2006-2010 and in Luang Nam Tha and Salavan 2008-2010.

B. Dengue RDTs. We are evaluating the diagnostic accuracy of dengue rapid diagnostic tests (RDTs) and determining whether dengue PCR can be performed, for determination of dengue serotype, on extracts of the pad of dry NS1 positive RDTs. Dr Manivanh Vongsouvath performed this research for her Mahidol University MSc thesis. This technique could facilitate the Lao national monitoring of dengue serotypes by the shipping of RDTs from the provinces to Vientiane for dengue PCR of NS1 positive RDTs.

There are problems with the thermal stability of malaria RDTs and we therefore examined the long term thermal stability of the diagnostic accuracy of dengue RDTs in the laboratory and in the field. The data suggest that at least one brand of dengue NS1/IgM/IgG RDT maintains diagnostic accuracy long term at hot Lao temperatures. The RDTs had

100% consistency over the two-year study, despite high temperatures, including in a hut in which temperatures exceeded the manufacturer's recommendations for 29% of time points (Phommasone *et al.* 2015).

C. Filter paper CSF and JEV diagnosis. Dr Tehmina Bharucha, a LSHTM Msc student spent two months in LOMWRU investigating whether CSF dried onto filter papers could be used for anti-JEV IgM ELISAs. She developed a novel method of pre-cut filter paper saturated with CSF that could provide a useful tool for JEV diagnostics in settings with limited laboratory access (Bharucha *et al.* in press).

C. Hand, Foot and Mouth disease. We continue to support enteroviral PCR for surveillance of Hand, Foot and Mouth disease (HFMD) as it is likely that there will be a large outbreak in Laos in the future, as has happened in adjoining countries in the last decade. We are liaising with OUCRU in Ho Chi Minh City over a multicentre placebo controlled trial of the efficacy of immunoglobulin in severe EV71 disease for when the expected epidemic occurs.

D. Hepatitis C. The full-length genomes of 22 hepatitis C virus genotype 6 (HCV-6) isolates were determined: 10 from Vietnam, one from China and 11 from the Lao PDR (representing a new subtype 6xe plus eight novel variants (Li *et al.* 2016). Lao HCV isolates are genetically very diverse and are phylogenetically distributed in multiple lineages within genotype 6.

E. Zika virus infection. With current global interest in this pathogen and possible association of infection with microcephaly we are working with partners to build diagnostic capacity at Mahosot Hospital.

6. Malaria

A. Artemisinin resistance – clinical aspects. With the spread of artemisinin resistance in Asia there is an urgent need to explore alternative antimalarial treatments, including triple combination artemisinin combination therapies (ACTs). We are participating, with the Centre for Malariology, Parasitology and Entomology, in the multicentre TRAC-2 study, coordinated by MORU-Bangkok, at Sekong Provincial Hospital. This is a randomised clinical trial comparing uncomplicated falciparum malaria parasite clearance times between artemether-lumefantrine and artemether-lumefantrine plus amodiaquine. We hope that these data will be useful for informing optimal ACT use in Laos.

We participated in a WorldWide Antimalarial Resistance Network (WWARN) study to collate data from clinical trials of artemisinin derivatives in falciparum malaria with frequent parasite counts to provide reference parasite clearance estimates stratified by location, treatment and time, to examine host factors affecting parasite clearance, and to assess the relationships between parasite clearance and risk of recrudescence during follow-up (WWARN 2015a). As substantial heterogeneity in parasite clearance exists between locations, early detection of artemisinin resistance requires reference PC1/2 data. Studies with frequent parasite count measurements to characterize PC1/2 should be encouraged.

B. Molecular markers of antimalarial resistance. The description of a molecular marker of artemisinin resistance ('K-13 propeller') (see http://www.wwarn.org/molecular/surveyor/k13/index.html?t=201505211240#0) has facilitated the mapping the extent of these parasites. We are working with MORU-Bangkok to examine the distribution of different K-13 mutations across southern Laos. With independent emergence of mutations in different geographic areas such monitoring will be very important (Takala-Harrison *et al.* 2015). We participated in the analysis of the in vivo transcriptomes of 1,043 *P. falciparum* isolates from patients with acute malaria, including from Laos, and found that artemisinin resistance is associated with increased expression of unfolded protein response (UPR) pathways involving the major PROSC and TRiC chaperone complexes (Mok *et al.* 2015). Artemisinin-resistant parasites also exhibit decelerated progression through the first part of the asexual intraerythrocytic development cycle. These findings suggest that artemisinin-resistant parasites remain in a state of decelerated development at the young ring stage, whereas their up-regulated UPR pathways mitigate protein damage caused by artemisinin. Miotto *et al.* (2015) examined the genomes of *P. falciparum* parasites, including from Laos, and found evidence that the risk of new resistance-causing mutations emerging is determined by specific predisposing genetic factors in the underlying parasite population. However, Brown *et al.* (2015) tested, using evidence from Laos and elsewhere, the hypothesis that hypermutator *P. falciparum* parasites exist in Southeast Asia and that an increased rate of acquisition of new mutations in these parasites may explain the repeated emergence of drug resistance there. However, the evidence obtained did not support this hypothesis.

We have been collecting filter paper blood spots from malaria patients all over Laos for the last ten years, with the Centre for Malariology, Parasitology & Entomology, to examine how the frequency of molecular markers of anti-malarial resistance have changed with the reduction in chloroquine and sulphadoxine-pyrimethamine (SP) use in collaboration with the Southwest Foundation for Biomedical Research in Texas and MORU-Bangkok.

C. Antimalarial dosing. We participated in a large pooled analysis of individual pharmacokinetic-pharmacodynamic data from patients treated with artemether-lumefantrine for uncomplicated *Plasmodium falciparum* malaria, to

The taking of goat blood

define therapeutic day 7 lumefantrine concentrations and identify patient factors that substantially alter these concentrations (WWARN 2015b,c). This concluded that current artemether-lumefantrine dosing recommendations achieve day 7 lumefantrine concentrations ≥200 ng/ml and high cure rates in most uncomplicated malaria patients. Three groups are at increased risk of treatment failure: very young children (particularly those underweight-for-age); patients with high parasitaemias; and patients in very low transmission intensity areas with emerging parasite resistance. In these groups, adherence and treatment response should be monitored closely. Higher, more frequent, or prolonged dosage regimens should now be evaluated in very young children, particularly if malnourished, and in patients with hyperparasitaemia.

D. Vivax malaria treatment. The clinical trial of the efficacy of chloroquine in *P. vivax* malaria is continuing.

E. Glucose-6-phosphate deficiency and malaria. Glucose-6-phosphate deficiency is thought to be common in Laos but the lack of information on the prevalence of different types of deficiency impairs decision making on the use of primaquine in vivax malaria. We have therefore conducted surveys of the prevalence of phenotypic and genotypic markers of G6PD deficiency in Sekong and Salavan Provinces in collaboration with CMPE, IRD and SMRU. A G6PD deficiency survey conducted in six randomly selected villages of two districts of Sekong province demonstrated that, using Trinity fluorescence spot test, the frequency of people with phenotypic G6PD deficiency was ~ 4% (70/1,897). Molecular G6PD analysis is continuing.

F. Targeted malaria elimination. A large study of the potential implementation of Targeted Malaria Elimination (TME) in Savannakhet Province, funded by the Bill and Melinda Gates Foundation, has started with CMPE and MORU-Bangkok.

Recently it has been realised that a significant proportion of apparently well people in rural Asia have *Plasmodium falciparum* infections that are not detected by RDTs or microscopy but are evident by high blood volume ultra-sensitive quantitative PCR (uPCR). To understand the situation of asymptomatic falciparum malaria in rural Laos and to prepare for TME evaluations, we conducted a cross-sectional survey in Thapangthong and Nong Districts, Savannakhet Province. Of 888 blood samples collected from afebrile consenting, well villagers aged ≥15 years old in 18 villages during March and July 2015, uPCR detected *Plasmodium* infections in 175/888 samples (20%) of people. The species detected were *P. falciparum* (32/888; 3.6%), *P. vivax* (99/888; 11.1%), mixed infections with *P. falciparum* and *P. vivax* (14/888; 1.6%) and *Plasmodium* undetermined species (30/888; 3.4%).

Of additional concern, the mutation C580Y in the K13 kelch propeller domain, which is associated with reduced susceptibility to artemisinin derivatives, was found in 75% (12/18) *P. falciparum* strains from Thapangthong and in 7% (2/28) strains from Nong ($p<0.001$). The prevalence of *P. falciparum* mono-infections was higher in Nong 5% (24/455) compared to Thapangthong 2% (8/433; $p=0.1$).

With these high frequencies of apparently asymptomatic *P. falciparum,* trials of mass drug administration with DHA-piperaquine in Nong District with key public engagement actions are planned for 2016.

7. Medicine quality & pharmacy

A. The Worldwide Antimalarial Resistance Network (WWARN). The WWARN Antimalarial Quality Scientific Group is based at Mahosot Hospital and continues to tabulate and map reports of the quality of antimalarials (see http://www.wwarn.org/resistance/surveyors/antimalarial-quality). We are tabulating the accessible data on the quality of maternal health medicines, antibiotics, anti-retrovirals and veterinary medicines and hope to be able to map these in 2016.

We contributed five papers to the special supplement of the American Journal of Tropical Medicine & Hygiene on medicine quality.

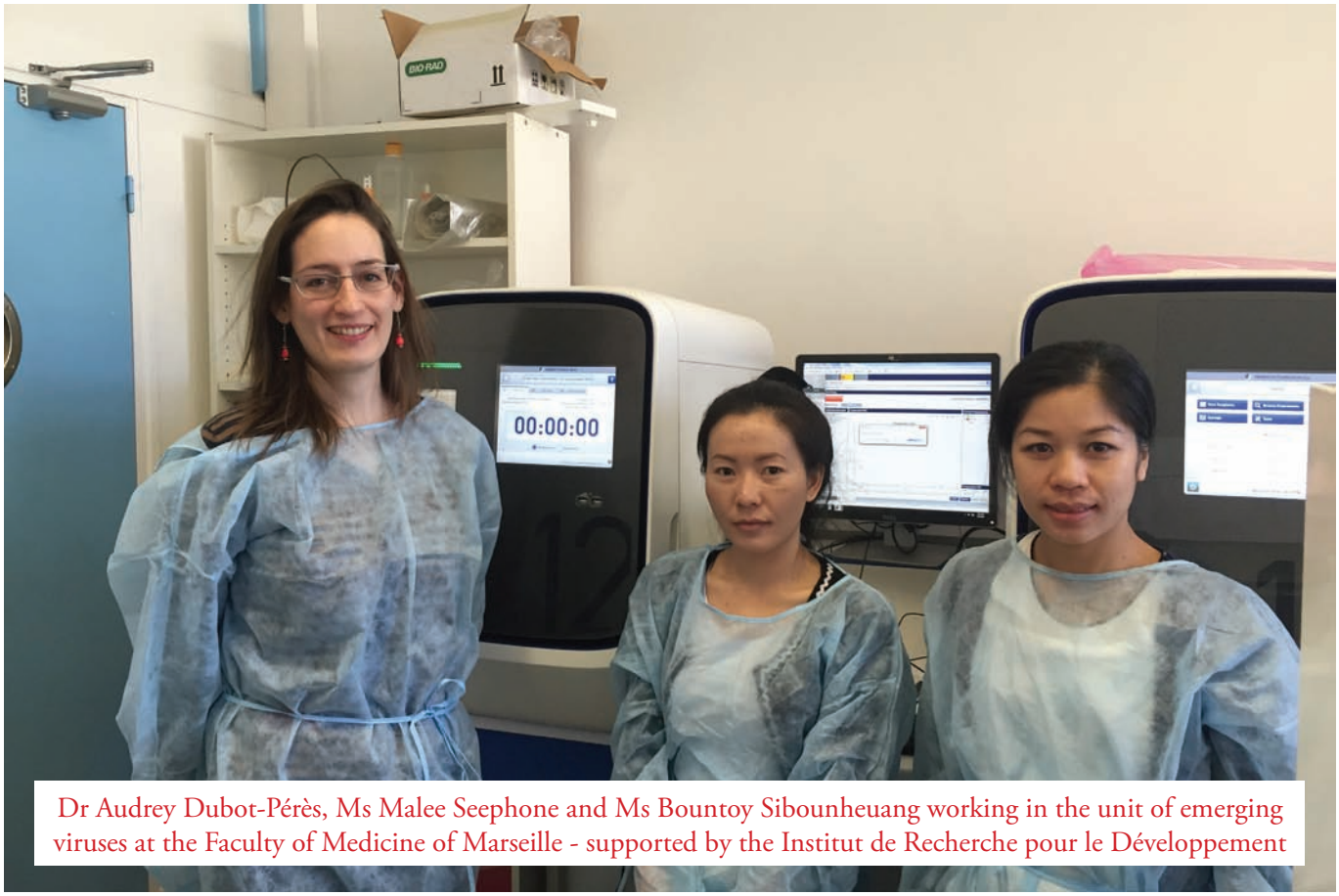
A. Repeat random sampling of antimalarial quality in southern Laos. In 2003 we performed a stratified random sampling survey in Laos to study the availability and quality of antimalarials in the private sector. In 2012 we repeated this survey, with the Food and Drug Department and CMPE, using a similar random sampling design, to allow an objective and statistically valid analysis of change through time (Tabernero *et al.* 2015). Results obtained from the 2012 survey demonstrate that the availability of oral artesunate monotherapies had decreased from 22.9% of 96 outlets in southern Laos in 2003 to 4.8% of 144 outlets in 2012. All the samples collected in the 2012 survey contained the correct Active Pharmaceutical Ingredient (API) in contrast to the 21 (84%) falsified artesunate samples found in the 2003 survey. Yaa chut, small plastic bags of multiple individual tablets/capsules, were found. The new portable CD3 tool allowed checking of packaging quality in the field. Although none of the medicines found in the 2012 survey had evidence for falsification, there was great variation of the quantity of active ingredient within the samples; 25.4% (37) of the samples were outside the 90-110% pharmacopeial limits of the label claim and 6.85% (10) were outside the 85-115% cut off, suggesting that they were substandard. Although these data suggest that the quality of antimalarials has improved, the quality of ACTs used in Laos should be monitored; especially as substitutions of falsified ACTs in the distribution chain is possible and would have devastating consequences. In addition, there is an urgent need of reducing the availability and use of medicines with potential fatal side effects and the indiscriminate use of antibiotics as found in yaa chud. We also sampled antibiotics and the chemical analysis of these is nearly completed and will be reported in 2016.

B. Operation Storm. We described the findings of Operation Storms I & II conducted in 2008/2009 to combat falsified medicines through partnership between INTERPOL and national customs, Drug Regulatory Agencies (DRAs) and police in Cambodia, Indonesia, Laos, Myanmar, Singapore, Thailand and Vietnam (Yong *et al.* 2015). Ninety-three antibiotic and 95 antimalarial suspect samples were collected. Of the antibiotics, 31% had % active pharmaceutical ingredient content (%API) <85% or >115% (including one falsified). Of the 95 antimalarials, 32% had %API <85 >115% API (including one falsified). A significant minority of samples, antimalarials (13%) and antibiotics (15%), were collected in plastic bags with minimal or no labeling. Of 20 ampicillin samples, 13 (65%) contained <85%API (with one counterfeit containing additional unstated amoxicillin). Of 34 oral artesunate samples, 7 (21%) contained %API out of the 85-115% range. Coordinated and synergistic partnership adopted by the participating countries, INTERPOL, WHO and laboratories facilitated a platform for discussions and intelligence sharing, helping to improve each participating country's capacity to combat poor quality medicines.

C. Estimation of the number of excess deaths due to poor quality antimalarials. We estimated the number of deaths of children under five years old associated with consumption of poor-quality antimalarials in 39 sub-Saharan countries (Renschler *et al.* 2015). This suggested that ~122,350 under-five malaria deaths were associated with consumption of poor-quality antimalarials, representing ~3.75% of all under-five deaths in our sample of 39 countries. There is considerable uncertainty surrounding these results because of gaps in data on case fatality rates and prevalence of poor-quality antimalarials. However, this is the first objective estimate based on field data and will be improved as better quality data are acquired.

D. Novel Low-cost Colorimetric, Laser Photometric and Visual Fluorescent Techniques for Rapid Identification of Falsified Medicines. Scientists at CDC-USA developed a series of low-cost approaches to evaluating medicine quality - a novel colorimetric assay for the simultaneous assessment of both lumefantrine and artemether in co-formulated Coartem tablets, was integrated with two novel, low-cost, fluorescence and laser photometric devices (Green *et al.* 2015). The CoDI laser device laser fires near-UV laser light through a tablet producing different colour intensities with and without a red filter, dependent on the actual tablet composition. The results suggest that these low cost techniques could be valuable tools and more field investigations of their accuracy are warranted.

E. Responding to the Pandemic of Falsified Medicines. We discussed the obstacles and potential solutions in Nayyar *et al.* (2015). Obstacles in combating falsified pharmaceuticals include 1) lack of consensus on definitions, 2) paucity



Dr Audrey Dubot-Pérès, Ms Malee Seephone and Ms Bountoy Sibounheuang working in the unit of emerging viruses at the Faculty of Medicine of Marseille - supported by the Institut de Recherche pour le Développement

of reliable and scalable technology to detect fakes before they reach patients, 3) poor global and national leadership and accountability systems for combating this scourge, and 4) deficient manufacturing and regulatory challenges, especially in China and India where fake products often originate. The major needs to improve the quality of the world's medicines fall into three main areas: 1) research to develop and compare accurate and affordable tools to identify high-quality drugs at all levels of distribution; 2) an international convention and national legislation to facilitate production and utilization of high-quality drugs and protect all countries from the criminal and the negligent who make, distribute, and sell life-threatening products; and 3) a highly qualified, well-supported international science and public health organization that will establish standards, drug-quality surveillance, and training programs.

F. Falsified phenobarbital and outbreaks of seizures. Two outbreaks of seizures in community epilepsy services in Guinea-Bissau and Nigeria were described, associated with change in the brands of phenobarbital used. The phenobarbital concentrations in tablets from the two suspect brands were either not detectable or extremely low. It is advised that if a change of the frequency of seizures occurs in such community programs, a key first step must be to investigate the quality of the anti-epileptic drugs administered (Otte *et al.* 2015).

G. Proposal to adapt the CONSORT guidelines**.** The CONSORT guidelines have been widely adopted to guide the design, conduct and reporting of clinical trials. We give examples of medicines used in clinical research that were not of good quality, risking such work giving wrong results and wrong policy recommendations (Newton *et al.* 2015). We proposed that the CONSORT guidelines on trial reports should include a requirement to determine and state the quality of medicines, as a measure to correct this under-recognized and neglected critical weak link in trials (Newton *et al.* 2015). This proposed change will require development of infrastructure and accessible analytical capacity but enormous investment in trials will be wasted and their interpretation into public policy incorrect if the quality of medicines and medical devices used is not assured.

H. Guidelines for medicine quality surveys. We have revised the MEDQUARD guidelines (Newton *et al.* 2009; *PLoS Medicine* 6, e1000052) on conducting and reporting surveys for the quality of medicines for the WHO. These have been reviewed by a WHO committee and are being revised as a WHO report to be published in May 2016. See:

http://www.who.int/medicines/areas/quality_safety/quality_assurance/WHO_SamplingProcedures_GeneralDocument_QAS14_590_10062014.pdf?ua=1

I. Population awareness of the risks of medicines. We investigated the awareness of Lao residents regarding medicine risks amongst 144 residents in 12 randomly selected villages out of the 146 villages with at least one health structure in Vientiane Capital (Caillet *et al.* 2015). This found that more than half of the respondents had never heard of poor quality medicines and 1/3rd thought that traditional medicines could not cause harm. Communication on medicinal product risks to patients through well-trained healthcare providers would make a valuable contribution towards the appropriate use of medicines, reducing the risk of antibiotic resistance.

J. Reports for the Joint Inter-Agency Task Force (JIATF). We write reports every two months on medicine quality problems, from a public health perspective, for the Joint Inter-Agency Task Force (JIATF) of The Global Fund's Office of Inspector General, USAID's Office of the Inspector General, and UNDPs Office of Audit and Investigations.

K. Forensics. We are working on innovative techniques to look for DNA in falsified medicines and using stable isotope ratios in starch excipients to try to determine the geographical origin of such 'medicines' in comparison to the genuine products.

L. Packaging. Working with the Institut de la Francophonie pour la Médecine Tropicale (IFMT), we have been surveying the information and language of antimalarial packaging – much of which is in the wrong language or too small a font to read!

M. Legal and definitions mapping. We have completed a pilot WWARN project to map national laws related to medicine quality and the definitions of different types of poor quality medicines used, funded by INTERPOL.

N. Access to Medicines Index. The reporting of poor quality medicines between stakeholders globally is woeful. We proposed to the Access to Medicines Index (AMI) (http://www.accesstomedicineindex.org/), based in The Netherlands, that they include evaluation of the policies that the pharmaceutical industry have, and their adherence to these, for the rapid reporting of poor quality medicines to national medicine regulatory authorities and the WHO RapidAlert system. This has been included and company rankings will be published in the 2016 AMI report.

O. The quality of antibiotics. An MSc student from the University of Oslo, Mr Scott Tschida, spent 6 months in LOMWRU, with the WWARN project, to review the global accessible literature on the quality of antibiotics. We hope to be able to map these data, as we have done for antimalarials, and use the results to inform models of the consequences of poor quality antibiotics on patient outcome and drug resistance.

P. The quality of medicines for maternal health. We are working on a review of the quality of medicines for maternal and sexual health globally and discussing their public health impact.

Q. The quality of medicines for HIV infections. We are working on a review of the quality of medicines for treating HIV infection and discussing their public health impact.

R. The ethics of medicine quality sampling. Medicine quality sampling is an increasingly important field but there are no discussion papers of the ethical issues and pitfalls with such work. We coordinated a group of scientists and ethicists involved in such work and developed a discussion document on these issues.

Dr Tiengkham Pongvongsa engaging with the Nong community for targeted malaria elimination

PneuCAPTIVE team travelling to a patient's home

ENGAGEMENT

Microbiology Laboratory staff in traditional Lao dress

Community perceptions and engagement

Now that there are more data on infectious disease epidemiology in Laos we are planning both public engagement research and implementation. This is a key component of the TME project (above) with intensive work to understand how to optimally engage with people in Laos so that the benefits/risks of different interventions, such as mass drug administration, can be explored and communities can make informed decisions.

A. E-Library. We have been working with the University of Health Sciences (UHS) to build a page on their website as an e-library – as a repository of published and grey literature information about Lao public health. This is now completed – see: (http://www.uhs.edu.la/elibrary/Elibrary.php). If you have any open access papers relevant to public health in Laos please submit by sending the pdfs to mayfong@tropmedres.ac. We hope very much that this will become a Lao national resource for health workers and policy makers.

B. Lao Medical Journal. We assist with the publication of the Lao Medical Journal (LMJ), the first Lao language medical journal. Assoc. Professor Mayfong Mayxay is an editor. We hope that the LMJ will be fully bilingual soon. It is freely downloadable on the e-library at UHS. See: http://www.uhs.edu.la/elibrary/Elibrary.php?&parentID=0&CatID=10

C. LOMWRU website. The LOMWRU aspects of the www.tropmedres.ac website were updated in 2015. See http://www.tropmedres.ac/lomwru-laos

D. Science café. Science café's are now popular in other Asian cities, such as Ho Chi Minh City and Bangkok, and we are hoping to start a science café devoted to medical topics in Vientiane in the near future.

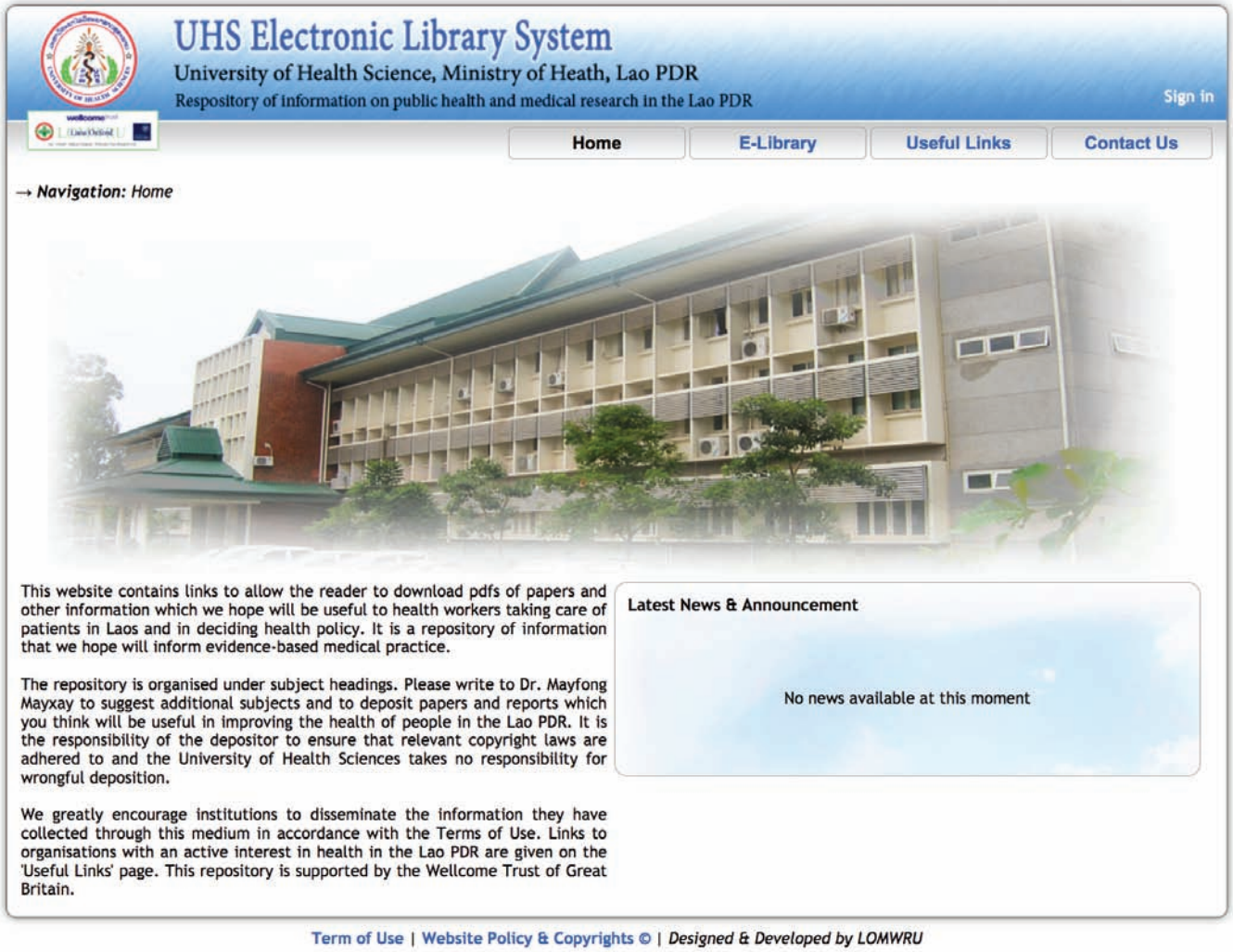
F. London School of Hygiene and Tropical Medicine Short Course on Medicine Product Quality & Public Health. We organised the first course on medicine quality and public health at the LSHTM in July 2015, with support from the Wellcome Trust, Medicine for Malaria Venture and the ACT Consortium at the LSHTM. This course lasted for a week with 18 students and 17 lecturers from all over the world. We hope that this will become a yearly event to build capacity and interest in this neglected subject.

G. Antibiotic knowledge and prescribing. We also participated in an assessment of antibiotic prescribing of doctors in Laos through a knowledge, attitude and practice survey (Quet *et al.* 2015). Sixty percent of participants declared not to have enough information about antibiotics. Knowledge about antibiotic prescribing was poor and only 14% recognized cephalosporin cross-resistance in methicillin-resistant-*Staphylococcus aureus*. Most participants had no information about local antibiotic resistance patterns for *Salmonella* Typhi and hospital-acquired pneumonia. Unnecessary antibiotic prescriptions were considered as harmless by 30% of doctors and 38% considered locally available antibiotics to be of poor quality. Generic antibiotics were perceived by 34% of participants as substandard. Most participants (373/386) welcomed educational programmes on antibiotic prescribing and 65.0% (249/383) preferred local over international antibiotic guidelines.

These data, in conjunction with the severe issues with AMR, especially ESBL, (see above) suggest an urgent need to engage with health workers and the public in Laos to encourage appropriate use of antibiotics and try to prevent the current situation becoming worse.

Goats and blood donors, Mr Huakuk and Mr Phonephang-1, in the garden of the Microbiology Laboratory

The University of Health Sciences E-library - a repository of information about health in Laos, supported by LOMWRU. See: http://www.uhs.edu.la/elibrary/index.php



OTHER ACTIVITIES

Basci and presentation to Dr Ruth Lim, Mr Scott Tschida and Dr Phonevilay Thongsith on leaving LOMWRU

A. External quality assurance. We participate in the UK National External Quality Assessement Service (NEQAS) scheme for general bacteriology, antimicrobial susceptibility testing, AAFB microscopy and mycobacterial culture and the WPRO scheme for JEV IgM ELISA QA.

B MOPSOP and Safety liaison. We have multiple links for liaison across the Major Overseas Programme for building consensus on Standard Operating Procedures for laboratory assays and for laboratory safety.

C. Talks etc. The Laboratory runs monthly lunchtime journal clubs, monthly scientific seminars, has frequent talks by academic visitors and contributes to the monthly scientific talks of Mahosot Hospital.

D. Pathogen Asset Control System (PACS). With the kind support of DTRA of the USA, LOMWRU, along with other medical organisations in Vientiane, have a new Pathogen Asset Control System (PACS) for the barcoding and cataloguing of samples so that they can accurately stored and located.

E. Wellcome Trust Review. In March we were visited by the Wellcome Trust Review Panel to evaluate the work of the MORU-Network and to advise the Wellcome Trust on the Network's application for a further five years of core funding. The additional funding was granted so we now have core funding support until 2020.

First Aid training

KEY COLLABORATIONS

Our yearly fire training by the Vientiane Fire Brigade

Within Lao PDR

Centre for Malariology, Parasitology & Entomology
National Centre for Laboratory & Epidemiology
Food and Drug Department, Ministry of Health
University of Health Sciences
Provincial Hospitals of Luang Nam Tha, Xieng Khouang, Salavan and Sekong
Mittaphab, Sethathirat, Childrens, Police and Army Hospitals, Vientiane
National Animal Health Laboratory

World Health Organisation Lao Country Office, Vientiane
Institut de la Francophonie pour la Médecine Tropicale
Institut de Recherche pour le Développement
Centre d'Infectiologie Christophe Mérieux du Laos
Institut Pasteur – Laos
Health Frontiers, Vientiane
US CDC, US Embassy

International (in addition to collaborations with MORU, SMRU, COMRU, MOCRU and OUCRU), in alphabetical order of institution

Wim Leereveld, Access to Medicine Foundation, Haarlem, The Netherlands

Dr Robert Gibbons, Department of Virology, Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand

Dr Joerg Blessmann, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany

Professor Sharon Peacock, University of Cambridge, UK

Dr Mike Green, CDC, Atlanta, Georgia, USA

Dr Christopher Gregory and Dr Sean Griffing, Thailand MOPH-US CDC Collaboration, Bangkok, Thailand

Professor Muhammad Zaman, Department of Biomedical Engineering, Boston University, Boston, USA

Professor Adrian Linacre, Flinders University, Australia

Nicola Ranieri, Forensic Chemistry Center, Food & Drug Administration, Cincinnati, Ohio, USA

Professor Facundo Fernandez, Georgia Institute of Technology, Atlanta, Georgia, USA

Dr Dallas Mildenhall, GNS Science, New Zealand

Dr Mariana Mirabel, Paris Cardiovascular Research Centre, Inserm U970, European Georges Pompidou Hospital, Paris Descartes University, Cardiology Department, European Georges Pompidou Hospital, Paris, France

Prof Anne Roussin, Faculté de Pharmacie, UMR1027 Inserm-Universite Toulouse III, France

Dr Raffaella Ravinetto, QUAMED, Institute of Tropical Medicine, Antwerp, Belgium

Drs Paul Horwood and Didier Menard, Institut Pasteur, Phnom Penh, Cambodia

Professor Marc Lecuit and colleagues, Institut Pasteur, Paris, France

Dr Mathieu Picardeau, Unite de Biologie des Spirochetes, Institut Pasteur, Paris

Dr Alain Pierret and Dr Olivier Ribolzi, Institut de recherche pour le développement, Laos

Dr Guillaume Lacombe, International Water Management Institute, Laos

Mrs Aline Plançon, INTERPOL, Lyon, France

Drs Lesley Chesson and Jim Ehleringer, IsoForensics Inc., and Thure Cerling, University of Utah, USA

Drs Lee Smythe & Scott Craig, Leptospiral Reference Laboratory, Coopers Plains, Australia

Professor David Mabey, Professor David Schellenberg, Dr Shunmay Yeung, Dr Heidi Hopkins and Dr Harparkash Kaur, London School of Hygiene and Tropical Medicine, London, UK

Dr Martin Cinnamond, Joint Inter-Agency Task Force, Geneva, Switzerland

Prof Bart Currie, Menzies School of Health Research, Australia

Prof. Paul Keim and Dr David Wagner, Northern Arizona University, USA

Ms Lorna Cox, Nutritional Biomarker Analysis Laboratory, MRC Nutrition, Cambridge, UK

Dr David Litt, Respiratory and Vaccine Preventable Bacteria Reference Unit, Public Health England, London, UK

Dr SJ Gray, Meningococcal Reference Unit, Public Health England, Manchester, UK

Professor Angela Kearns, Staphylococcus Reference Service, Public Health England, Colindale, UK

Professor Al Richards, Rickettsial Diseases Research Program, Naval Medical Research Center, USA

Professor Ramanan Laxminarayan, Public Health Foundation of India, New Delhi, India

Dr Damien Chaussabel, Sidra Medical and Research Center, Qatar

Professor David Relman and Dr Stephen Popper, Department of Microbiology and Immunology, Stanford University, California, USA

Dr Esther Kuenzli and colleagues, Swiss Tropical and Public Health Institute, Basel, Switzerland

Professor Tim Anderson, Texas Biomedical Research Institute, San Antonio, Texas, USA

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Professor Albert Ko, Yale School of Public Health, USA

Dr Julie Logan, Molecular Identification Services Unit, Public Health England

Professor Guy Thwaites, Director of OUCRU, giving a talk on TB meningitis at Mahosot Hospital

TITLES AND ABSTRACTS OF PAPERS PUBLISHED OR IN PRESS 2015

In alphabetical order by first author. If the paper does not include an abstract a brief summary is given in [ ].

1. Anspacher M, Phetsouvanh R, Newton PN (2015) Meliodiosis. IN: Atlas of Pediatrics in the tropics. Ed: Spector JM & Gibson TE, American Academy of Pediatrics.

[A brief review of meliodiosis in children, clinical features and management]

2. Beardsley J, Wolbers M, Kibengo FM, Ggayi AM, Ruzagira E, Kamali A, Cuc NTK, Binh TQ, Chau TTH, Chau NVV, Farrar J, Hien TT, Loc TT, Merson L, Phuong L, Thwaites G, Kinh NV, Thuy PT, Wertheim HFL, Chierakul W, Konpan P, Onsanit S, Siriboon S, Thiansukhon E, Chan AK, Heyderman R, Mwinjiwa E, van Oosterhout JJ, Imran D, Basri H, Ganiem AR, Tjahjani N, Wahyuningsih R, Mayxay M, Dance D, Phimmasone P, Rattanavong S, Lalloo DG, Day JN (2016) Adjunctive steroids in HIV-associated cryptococcal meningitis: a randomized controlled trial in African and Southeast Asian countries. *NEJM* 374(6):542-554.

Background HIV-associated cryptococcal meningitis causes >600,000 deaths yearly. Treatment has changed little in 20 years, and there are no imminent novel agents. Adjuvant corticosteroids reduce mortality in other forms of meningitis in some populations, but are untested in cryptococcal meningitis. We performed a double-blind randomised controlled trial to determine whether adjunctive treatment with dexamethasone reduces mortality in HIV-associated cryptococcal meningitis.
Methods We recruited adult patients in Vietnam, Thailand, Indonesia, Laos, Uganda and Malawi. All patients received dexamethasone or placebo for 6 weeks, and combination antifungal therapy with amphotericin B and fluconazole.
Results The trial was stopped for safety concerns following enrolment of 451 patients. Mortality by 10 weeks was 47% for dexamethasone vs 41% for placebo (hazard ratio (HR) of time to death 1.11 (95%CI 0.84 to 1.47); P=0.45) and 57% vs 49% by 6 months (HR 1.18 (95%CI 0.91 to 1.53); P=0.20). Disability at 10 weeks was more frequent in patients receiving dexamethasone ('good' outcome 13% versus 25% with placebo, odds ratio 0.42 (95%CI 0.25 to 0.69) P<0.001). Clinical adverse events were more common in the dexamethasone group (total number of events = 667 vs 494, P= 0.01), including grade 3 or 4 infectious (48 vs 25 patients, P=0.003), renal (22 vs 7, P= 0.004) and cardiac events (8 vs 0, P=0.004). Cerebrospinal fluid fungal clearance was slower in the dexamethasone group. Results were consistent across Asian and African sites.
Conclusion Dexamethasone does not reduce mortality in HIV-associated cryptococcal meningitis and is associated with more adverse events and disability.

Dr Ivo Elliott test driving a tok-tok during scrub typhus fieldwork

3. Brown TS, Jacob CG, Silva JC, Takala-Harrison S, Djimdé A, Dondorp AM, Fukuda M, Noedl H, Nyunt MM, Kyaw MP, Mayxay M, Hien TT, Plowe CV, Cummings MP (2015) *Plasmodium falciparum* field isolates from areas of repeated emergence of drug resistant malaria show no evidence of hypermutator phenotype. *Infect Genet Evol* 30:318-22.

Multiple transcontinental waves of drug resistance in *Plasmodium falciparum* have originated in Southeast Asia before spreading westward, first into the rest of Asia and then to sub-Saharan Africa. In vitro studies have suggested that hypermutator *P. falciparum* parasites may exist in Southeast Asia and that an increased rate of acquisition of new mutations in these parasites may explain the repeated emergence of drug resistance in Southeast Asia. This study is the first to test the hypermutator hypothesis using field isolates. Using genome-wide SNP data from human *P. falciparum* infections in Southeast Asia and West Africa and a test for relative rate differences we found no evidence of increased relative substitution rates in *P. falciparum* isolates from Southeast Asia. Instead, we found significantly increased substitution rates in Mali and Bangladesh populations relative to those in populations from Southeast Asia. Additionally we found no association between increased relative substitution rates and parasite clearance following treatment with artemisinin derivatives.

4. Buisson Y, Rattanavong S, Keoluangkhot V, Vongphayloth K, Manivanh L, Phetsouvanh R, Pierret A, Maeght J-L, Wuthiekanun V, Newton PN, Dance D (2015) Melioidosis in Laos. In: *Socio-Ecological Dimensions*

LOMWRU Steering Committee Meeting - Assoc Prof Bounthaphany Bounxouai, Professor Nick Day, Assoc Prof Ounkham Phanthaly, Dr Rattanaphone Phetsouvanh, Assoc Prof Mayfong Mayxay and Prof Paul Newton

of Infectious Diseases in Southeast Asia. Edited by Morand S, Dujardin J-P, Lefait-Robin R, Apiwathnasorn C. Springer.

[A review of research on *Burkholderia pseudomallei* in soil and water in Laos and how this may be related to melioidosis in humans]

5. Caillet C, Sichanh C, Syhakhang L, Delpierre C, Manithip C, Mayxay M, Lapeyre-Mestre M, Newton PN, Roussin A (2015) Population awareness of risks related to medicinal product use in Vientiane Capital, Lao PDR: a cross-sectional study for public health improvement in low and middle income countries. *BMC Public Health* 15, 590. Background: While essential medicines have been made more available in all but the most remote areas in low and middle income countries (L/MICs) over the past years, inappropriate and incorrect use of good quality medicines remains a key impediment for public health. In addition, as medicines have a potential to cause harm (medicine risks), adequate awareness by medicine users of the risks of adverse reactions is essential, especially as self-medication is common in L/MICs. This study aimed to investigate the awareness of Lao residents regarding medicine risks in Vientiane Capital, Lao People's Democratic Republic. Methods: Face-to-face interviews using structured questionnaires of 144 residents older than 16 years were carried out in 12 randomly selected villages out of the 146 villages of Vientiane Capital with at least one health facility. Results: The respondents were mainly (85.0 %) the heads of households or their husband/spouse . The majority of the respondents were unaware (61.8 %) of medicine risks. Compared to residents living in the urban district of Xaysetha, living in peri-urban and even more in rural areas were identified as factors associated with being unaware of medicine risks [adjusted odds ratio (aOR) =3.3, 95 % Confidence Interval (CI) = 1.1–9.4]) and aOR =7.5 (95 % CI = 2.3–24.2), respectively]. In addition, more than half of the respondents had never heard of poor quality medicines, with a higher rate in rural/peri-urban compared to urban districts (55.6 % vs 38.9 %, respectively, p = 0.02). Finally, approximately one third of all respondents thought that traditional medicines could not cause harm. Conclusions: Overall, these results suggest a lack of awareness about medicinal product risks. Differences according to the place of residence are apparent and could be partly explained by a lower level of training of healthcare providers in contact with the population in the rural districts in particular. Communication on medicinal product risks to patients through well-trained healthcare providers could probably make a valuable contribution towards the appropriate use of medicines in L/MICs.

6. Cheeseman IH, Miller B, Tan JC, Tan A, Nair S, Nkhoma SC, De Donato M, Rodulfo H, Dondorp A, Branch OH, Mesia LR, Newton P, Mayxay M, Amambua-Ngwa A, Conway DJ, Nosten F, Ferdig MT, Anderson TJ (2015) Population Structure Shapes Copy Number Variation in Malaria Parasites. *Mol Biol Evol* pii: msv282.

Abstract. If copy number variants (CNVs) are predominantly deleterious, we would expect them to be more efficiently purged from populations with a large effective population size (N_e) than from populations with a small N_e. Malaria parasites (*Plasmodium falciparum*) provide an excellent organism to examine this prediction, because this protozoan shows a broad spectrum of population structures within a single species, with large, stable, outbred populations in Africa, small unstable inbred populations in South America and with intermediate population characteristics in South East Asia. We characterized 122 single-clone parasites, without prior laboratory culture, from malaria-infected patients in seven countries in Africa, South East Asia and South America using a high-density single-nucleotide polymorphism/CNV microarray. We scored 134 high-confidence CNVs across the parasite exome, including 33 deletions and 102 amplifications, which ranged in size from <500 bp to 59 kb, as well as 10,107 flanking, biallelic single-nucleotide polymorphisms. Overall, CNVs were rare, small, and skewed toward low frequency variants, consistent with the deleterious model. Relative to African and South East Asian populations, CNVs were significantly more common in South America, showed significantly less skew in allele frequencies, and were significantly larger. On this background of low frequency CNV, we also identified several high-frequency CNVs under putative positive selection using an F_{ST} outlier analysis. These included known adaptive CNVs containing rh2b and pfmdr1, and several other CNVs (e.g., DNA helicase and three conserved proteins) that require further investigation. Our data are consistent with a significant impact of genetic structure on CNV burden in an important human pathogen.

7. Dance DA. Melioidosis in Puerto Rico: The Iceberg Slowly Emerges. *Clin Infect Dis* 60(2), 251-3.
[A Comment on 'Contact investigation of melioidosis cases reveals regional endemicity in Puerto Rico.' Physicians should consider the diagnosis of melioidosis in anyone who has spent time in the Caribbean who presents with sepsis, severe pneumonia, or abscesses, particularly if they have predisposing factors such as diabetes.]

8. Day N, Newton PN (in press) Scrub typhus and other tropical rickettsioses. IN: *Infectious Diseases*. Eds: Cohen J and Powderly WG. Mosby. Fourth Ed.

[A textbook chapter on scrub typhus and rickettsial pathogens]

Vientiane from the air

9. Dittrich S, Rattanavong S, Lee SJ, Panyanivong P, Craig SB, Tulsiani SM, Blacksell SD, Dance DAB, Dubot-Pérès A, Sengduangphachanh A, Phoumin P, Paris DH, Newton PN (2015a) Rickettsia and leptospira as neglected but treatable causes of central nervous system infection. *Lancet Global Health* 3, e104-e111.

Background. Scrub typhus (caused by *Orientia tsutsugamushi*), murine typhus (caused by *Rickettsia typhi*), and leptospirosis are common causes of febrile illness in Asia; meningitis and meningoencephalitis are severe complications. However, scarce data exist for the burden of these pathogens in patients with CNS disease in endemic countries. Laos is representative of vast economically poor rural areas in Asia with little medical information to guide public health policy. We assessed whether these pathogens are important causes of CNS infections in Laos.

Methods. Between Jan 10, 2003, and Nov 25, 2011, we enrolled 1112 consecutive patients of all ages admitted with CNS symptoms or signs requiring a lumbar puncture at Mahosot Hospital, Vientiane, Laos. Microbiological examinations (culture, PCR, and serology) targeted so-called conventional bacterial infections (*Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae, S suis*) and O *tsutsugamushi, Rickettsia typhi/Rickettsia* spp, and *Leptospira* spp infections in blood or cerebrospinal fl uid (CSF). We analysed and compared causes and clinical and CSF characteristics between patient groups. Findings. 1051

(95%) of 1112 patients who presented had CSF available for analysis, of whom 254 (24%) had a CNS infection attributable to a bacterial or fungal pathogen. 90 (35%) of these 254 infections were caused by *O tsutsugamushi, R typhi/Rickettsia* spp, or *Leptospira* spp. These pathogens were significantly more frequent than conventional bacterial infections (90/1051 [9%] vs 42/1051 [4%]; p<0·0001) by use of conservative diagnostic definitions. CNS infections had a high mortality (236/876 [27%]), with 18% (13/71) for *R typhi/Rickettsia* spp, *O tsutsugamushi*, and *Leptospira* spp combined, and 33% (13/39) for conventional bacterial infections (p=0·076).

Interpretation. Our data suggest that *R typhi/Rickettsia* spp, *O tsutsugamushi*, and *Leptospira* spp infections are important causes of CNS infections in Laos. Antibiotics, such as tetracyclines, needed for the treatment of murine typhus and scrub typhus, are not routinely advised for empirical treatment of CNS infections. These severely neglected infections represent a potentially large proportion of treatable CNS disease burden across vast endemic areas and need more attention.

10. Dittrich S, Sunyakumthorn P, Rattanavong S, Phetsouvanh R, Panyanivong P, Sendouangphachanh A, Phouminh P, Anantatat T, Chanthongthip A, Lee S, Dubot-Pérès A, Day N, Paris D, Newton PN, Turner G (2015b) Blood Brain Barrier Function and Biomarkers of Central Nervous System Injury in Rickettsial versus other Neurological Infections in Laos. *American Journal of Tropical Medicine & Hygiene*, 93, 232–237.

Blood–brain barrier (BBB) function and cerebrospinal fluid (CSF) biomarkers were measured in patients admitted to hospital with severe neurological infections in the Lao People's Democratic Republic (N = 66), including bacterial meningitis (BM; N = 9) or tuberculosis meningitis (TBM; N = 11), Japanese encephalitis virus (JEV; N = 25), and rickettsial infections (N = 21) including murine and scrub typhus patients. The albumin index (AI) and glial fibrillary acidic protein (GFAP) levels were significantly higher in BM and TBM than other diseases but were also raised in individual rickettsial patients. Total tau protein was significantly raised in the CSF of JEV patients. No differences were found between clinical or neurological symptoms, AI, or biomarker levels that allowed distinction between severe neurological involvement by *Orientia* tsutsugamushi compared with *Rickettsia* species.

11. Dittrich S, Phuklia W, Turner GDH, Rattanavong S, Chansamouth V, Dumler SJ, Ferguson DJ, Paris PN, Newton PN (2015c) *Neorickettsia sennetsu* as a neglected cause of fever in South-East Asia. *PLoS Negl Trop Dis* 9, e0003908.

Neorickettsia sennetsu infection is rarely recognized, with less than 100 globally reported patients over the last 50 years. The disease is thought to be contracted by eating raw fish, a staple of many South-East Asian cuisines. In 2009, the first patient with sennetsu was identified in the Lao PDR (Laos), raising the question as to how common this organism and related species are in patients presenting with fever. We investigated the frequency of *N. sennetsu* infection at hospitals in diverse areas of Laos. Consenting febrile hospital inpatients from central (Vientiane: n = 1,013), northern (Luang Namtha: n = 453) and southern (Salavan: n = 171) Laos were screened by PCR for N. sennetsu, if no previous positive direct diagnostic test was available. A PCR-restriction fragment length polymorphism assay was developed to differentiate between *N. sennetsu, Ehrlichia chaffeensis* and *Anaplasma phagocytophilum*. To allow more detailed studies of *N. sennetsu*, culture was successfully established using a reference strain (ATCC VR-367), identifying a canine-macrophage cell line (DH82) to be most suitable to visually identify infection. After screening, *N. sennetsu* was identified and sequence confirmed in four (4/1,637; 0.2%) Lao patients. Despite the previously identified high seroprevalence of *N. sennetsu* antibodies in the Lao population (~17%), acute *N. sennetsu* infection with sufficient clinical signs to prompt hospitalization appears to be rare. The reservoir, zoonotic cycle and pathogenicity of *N. sennetsu* remain unclear and require further investigations.

12. Dittrich S, Rudgard W, Woods K, Sirisouk J, Phuklia W, Davone V, Vongsouvath M, Phommasone K, Rattanavong S, Knappik M, Craig S, Weier S, Tulsiani S, Dance D, Newton PN (in press) The utility of blood culture fluid for the molecular diagnosis of *Leptospira*: a prospective evaluation. *American Journal of Tropical Medicine & Hygiene*

Abstract. Leptospirosis is an important zoonosis worldwide, with infections occurring after exposure to contaminated water. Despite being a global problem, laboratory diagnosis remains difficult with culture results taking up to 3 months, serology being retrospective by nature, and polymerase chain reaction showing limited sensitivity. Leptospira have been shown to survive and multiply in blood culture media, and we hypothesized that extracting DNA from incubated blood culture fluid (BCF), followed by quantitative real-time polymerase chain reaction (qPCR) could improve the accuracy and speed of leptospira diagnosis. We assessed this retrospectively, using preincubated BCF of *Leptospira* spp. positive (N = 109) and negative (N = 63) febrile patients in Vientiane, Lao PDR. The final method showed promising sensitivities of 66% (95% confidence interval [CI]: 55–76) and 59% (95% CI: 49–68) compared with direct or direct and indirect testing combined, as the respective reference standards (specificities > 95%). Despite these promising diagnostic parameters, a subsequent prospective evaluation in a Lao hospital population (N = 352) showed that the

Microbiology Laboratory dance troupe in the hospital competition at Lao New Year

sensitivity was very low (30%) compared with qPCR on venous blood samples. The disappointingly low sensitivity does suggest that venous blood samples are preferable for the clinical microbiology laboratory, although BCF might be an alternative if leptospirosis is only suspected postadmission after antibiotics have been used.

13. Dubot-Pérès A, Sengvilaipaseuth O, Chanthongthip A, Newton PN, de Lamballerie X (2015) How many patients with anti-JEV IgM in cerebrospinal fluid really have Japanese encephalitis? *Lancet Infectious Diseases* 15, 1377-1378.

[Data are presented from Laos suggesting that the positive predictive value of anti-JEV IgM detection in serum and CSF is low for diagnosing JEV encephalitis. Hence the incidence of acute neurological infections due to JEV might be overestimated, and that detection of anti-JEV IgM in patients with acute infections of the CNS should not rule out other treatable diseases, particularly bacterial infections.]

14. Green M, Hostetler D, Nettey H, Swamidoss I, Ranieri N, Fernandez FM, Newton PN, Bremen JG, Herrington J (2015) Integration of Novel Low-cost Colorimetric, Laser Photometric and Visual Fluorescent Techniques for Rapid Identification of Falsified Medicines in Resource-poor Areas: Application to Artemether-lumefantrine. *American Journal of Tropical Medicine & Hygiene* 92(6 Suppl), 8-16.
Abstract. The availability of falsified antimalarial drugs can be reduced with effective drug regulatory agencies and proper enforcement. Fundamental to these agencies taking action, rapid identification must be made as soon as they appear in the market place. Since falsified antimalarials occur mostly in developing countries, performing drug analysis presents itself with unique challenges. A fundamental factor in choosing a useful technique is affordability and simplicity. Therefore, we suggest a three-tiered drug evaluation strategy for identifying a falsified drug in resource-poor areas. Tier I is a simple comparison of a tablet's weight and dimensions with official specifications. Tier II uses inexpensive photometric devices (laser and fluorescence) to evaluate a tablet. Suspicious samples from Tier I and II assessments are then subjected to a colorimetric assay for active ingredients identification and quantification. In this article, we evaluate a novel colorimetric assay for the simultaneous assessment of both lumefantrine and artemether in co-formulated Coartem™ tablets, and integrate the method with two novel, low-cost, fluorescence and laser photometric devices. Image analysis software is used for the assessments. Although artemether–lumefantrine is used as an example, the strategy may be adapted to other medicines.

Professor Bounthapany Bounxouei, Director of Mahosot Hospital, with visitors for the 5 -Year Wellcome Trust review and Microbiology Laboratory staff in March 2015

15. Hoffmaster A, AuCoin D, Baccam S, Baggett H, Baird R, Bhengsi S, Blaney D, Brett P, Brooks T, Brown K, Chantratita N, Cheng A, Dance D, Decuypere S, Defenbaugh D, Dixon D, Gee J, Houghton R, Jorakate P, Lertmemongkolchai G, Limmathurotsakul D, Merlin T, Mukhopadhyay C, Mukhopadhay S, Norton R, Peacock S, Pickett T, Rolim D, Simpson A, Steinmetz I, Stoddard R, Stokes M, Sue D, Tuanyok A, Whistler T, Wuthiekanun W, Walke H (2015) Melioidosis Diagnostic Workshop, 2013: Diagnostic gaps in endemic and non-endemic areas. *Emerging Infectious Diseases* 21(2). doi: 10.3201/eid2102.141045.

Melioidosis is a severe disease that can be difficult to diagnose because of its diverse clinical manifestations and a lack of adequate diagnostic capabilities for suspected cases. There is broad interest in improving detection and diagnosis of this disease not only in melioidosis-endemic regions but also outside these regions because melioidosis may be underreported and poses a potential bioterrorism challenge for public health authorities. Therefore, a workshop of academic, government, and private sector personnel from around the world was convened to discuss the current state of melioidosis diagnostics, diagnostic needs, and future directions.

16. Keita AK, Dubot-Pérès A, Phommasone K, Sibounheuang B, Vongsouvath M, Mayxay M, Raoult D, Newton PN, Fenollar F (2015) High Prevalence of *Tropheryma whipplei* in Lao Kindergarten Children. *PLoS Negl Trop Dis* 9, e0003538.

Abstract. Background. *Tropheryma whipplei* is a bacterium commonly found in feces of young children in Africa, but with no data from Asia. We estimated the prevalence of *T. whipplei* carriage in feces of children in Lao PDR (Laos). Methods/Principal Findings. Using specific quantitative real-time PCR, followed by genotyping for each positive specimen, we estimated the prevalence of *T. whipplei* in 113 feces from 106 children in Vientiane, the Lao PDR (Laos). *T. whipplei* was detected in 48% (51/106) of children. Those aged 4 years were significantly less frequently positive (17/52, 33%) than older children (34/54, 63%; p< 0.001). Positive samples were genotyped. Eight genotypes were detected including 7 specific to Laos. Genotype 2, previously detected in Europe, was circulating (21% of positive children) in 2 kindergartens (Chompet and Akad). Genotypes 136 and 138 were specific to Chompet (21% and 15.8%, respectively) whereas genotype 139 was specific to Akad (10.55%). Conclusions/Significance. *T. whipplei* is a widely distributed bacterium, highly prevalent in feces of healthy children in Laos. Further research is needed to identify the public health significance of this finding.

17. Knappik M, Dance D, Rattanavong S, Pierret A, Ribolzi O, Davong V, Silisouk J, Vongsouvath M, Newton PN, Dittrich S (2015) Evaluation of molecular methods to improve the detection of *Burkholderia pseudomallei* in soil and water samples from Laos. *Applied and Environmental Microbiology* 81(11), 3722-7.

Burkholderia pseudomallei is the cause of melioidosis, a severe and potentially fatal disease of humans and animals. It is endemic in northern Australia and Southeast Asia and is found in soil and surface water. The environmental distribution of *B. pseudomallei* worldwide and within countries where it is endemic, such as the Lao People's Democratic Republic (Laos), remains unclear. However, this knowledge is important to our understanding of the ecology and epidemiology of *B. pseudomallei* and to facilitate public health interventions. Sensitive and specific methods to detect *B. pseudomallei* in environmental samples are therefore needed. The aim of this study was to compare molecular and culture-based methods for the detection of B. pseudomallei in soil and surface water in order to identify the optimal approach for future environmental studies in Laos. Molecular detection by quantitative real-time PCR (qPCR) was attempted after DNA extraction directly from soil or water samples or after an overnight enrichment step. The positivity rates obtained by qPCR were compared to those obtained by different culture techniques. The rate of detection from soil samples by qPCR following culture enrichment was significantly higher (84/100) than that by individual culture methods and all culture methods combined (44/100; P<0.001). Similarly, qPCR following enrichment was the most sensitive method for filtered river water compared with the sensitivity of the individual methods and all individual methods combined. In conclusion, molecular detection following an enrichment step has proven to be a sensitive and reliable approach for *B. pseudomallei* detection in Lao environmental samples and is recommended as the preferred method for future surveys.

18. Li C, Barnes E, Newton PN, Fu Y, Vongsouvath M, Klenerman P, Okamoto H, Abe K, Pybus OG, Lu L (2015) An expanded taxonomy of hepatitis C virus genotype 6: Characterization of 22 new full-length viral genomes. *Virology* 476C, 355-363.

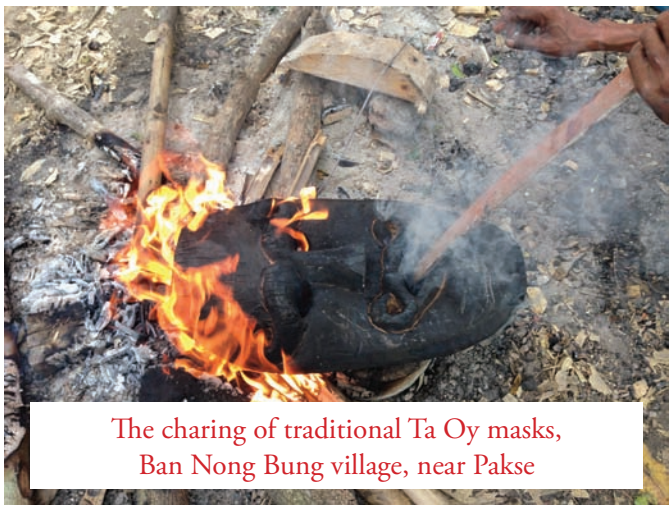
We characterized the full-length genomes of 22 hepatitis C virus genotype 6 (HCV-6) isolates: 10 from Vietnam (classified into subtypes 6e, 6h, 6p, 6r, 6s, and 6u), one from China (confirmed as a new subtype 6xd), and 11 from the Lao PDR (representing a new subtype 6xe plus eight novel variants). With these 22 new genomes, HCV-6 now has a diverse and extended taxonomic structure, comprised of 28 assigned subtypes (denoted 6a-6xe) and 27 unassigned lineages, all of which have been represented by full-length genomes. Our phylogenetic analyses also included many partially-sequenced novel variants of HCV-6 from Lao PDR. This revealed that Lao HCV isolates are genetically very diverse and are phylogenetically distributed in multiple lineages within genotype 6. Our results suggest that HCV-6 has been maintained in Laos, a landlocked country, since the common ancestor of genotype 6 and indicates historical dispersal of HCV-6 across Southeast Asia.

19. Limmathurotsakul D, Golding N, Dance D, Messina J, Pigott D, Moyes C, Rolim D, Bertherat E, Day N, Peacock S, Hay S (2016) Predicted global distribution of *Burkholderia pseudomallei* and burden of melioidosis. *Nature Microbiology Reviews* 150008.

Burkholderia pseudomallei, a highly pathogenic bacterium that causes melioidosis, is commonly found in soil in Southeast Asia and Northern Australia. Melioidosis can be difficult to diagnose due to its diverse clinical manifestations and the inadequacy of conventional bacterial identification methods. The bacterium is intrinsically resistant to a wide range of antimicrobials, and treatment with ineffective antimicrobials may result in case fatality rates (CFRs) exceeding 70%. The importation of infected animals has, in the past, spread melioidosis to non-endemic areas. The global distribution of *B. pseudomallei* and the burden of melioidosis, however, remain poorly understood. Here, we map documented human and animal cases and the presence of environmental *B. pseudomallei* and combine this in a formal modelling framework to estimate the global burden of melioidosis. We estimate there to be 165,000 (95% credible interval 68,000–412,000) human melioidosis cases per year worldwide, from which 89,000 (36,000–227,000) people die. Our estimates suggest that melioidosis is severely underreported in the 45 countries in which it is known to be endemic and that melioidosis is probably endemic in a further 34 countries that have never reported the disease. The large numbers of estimated cases and fatalities emphasize that the disease warrants renewed attention from public health officials and policy makers. Melioidosis is a disease of public health importance.

20. Lubell Y, Blacksell SD, Dunachie S, Tanganuchitcharnchai A, Althaus T, Watthanaworawit W, Paris DH, Mayxay M, Peto TJ, Dondorp AM, White NJ, Day NP, Nosten F, Newton PN, Turner P (2015) Performance of C-reactive protein and procalcitonin to distinguish viral from bacterial and malarial causes of fever in Southeast Asia. *BMC Infect Dis* 15(1), 511

Abstract. Background: Poor targeting of antimicrobial drugs contributes to the millions of deaths each year from malaria, pneumonia, and other tropical infectious diseases. While malaria rapid diagnostic tests have improved use of antimalarial drugs, there are no similar tests to guide the use of antibiotics in undifferentiated fevers. In this study we estimate the diagnostic accuracy of two well established biomarkers of bacterial infection, procalcitonin and C reactive



The charing of traditional Ta Oy masks, Ban Nong Bung village, near Pakse

protein (CRP) in discriminating between common viral and bacterial infections in malaria endemic settings of Southeast Asia. Methods: Serum procalcitonin and CRP levels were measured in stored serum samples from febrile patients enrolled in three prospective studies conducted in Cambodia, Laos and, Thailand. Of the 1372 patients with a microbiologically confirmed diagnosis, 1105 had a single viral, bacterial or malarial infection. Procalcitonin and CRP levels were compared amongst these aetiological groups and their sensitivity and specificity in distinguishing bacterial infections and bacteraemias from viral infections were estimated using standard thresholds. Results: Serum concentrations of both biomarkers were significantly higher in bacterial infections and malaria than in viral infections. The AUROC for CRP in discriminating between bacterial and viral infections was 0.83 (0.81–0.86) compared with 0.74 (0.71–0.77) for procalcitonin ($p < 0.0001$). This relative advantage was evident in all sites and when stratifying patients by age and admission status. For CRP at a threshold of 10 mg/L, the sensitivity of detecting bacterial infections was 95 % with a specificity of 49 %. At a threshold of 20 mg/L sensitivity was 86 % with a specificity of 67 %. For procalcitonin at a low threshold of 0.1 ng/mL the sensitivity was 90 % with a specificity of 39 %. At a higher threshold of 0.5 ng/ul sensitivity was 60 % with a specificity of 76 %. Conclusion: In samples from febrile patients with mono-infections from rural settings in Southeast Asia, CRP was a highly sensitive and moderately specific biomarker for discriminating between viral and bacterial infections. Use of a CRP rapid test in peripheral health settings could potentially be a simple and affordable measure to better identify patients in need of antibacterial treatment and part of a global strategy to combat the emergence of antibiotic resistance.

21. Mayxay M, Sengvilaipaseuth O, Chanthongthip A, Dubot-Pérès A, Rolain JM, Parola P, Craig S, Tulsiani S, Burns M-A, Khanthavong M, Keola S, Pongvongsa T, Raoult D, Dittrich S, Newton PN (2015) Causes of Fever in Rural Southern Laos. *Am J Trop Med Hyg* 93(3), 517-20.

Abstract. The etiology of fever in rural Lao People's Democratic Republic (Laos) has remained obscure until recently owing to the lack of laboratory facilities. We conducted a study to determine the causes of fever among 229 patients without malaria in Savannakhet Province, southern Laos; 52% had evidence of at least one diagnosis (45% with single and 7% with apparent multiple infections). Among patients with only one diagnosis, dengue (30.1%) was the most common, followed by leptospirosis (7.0%), Japanese encephalitis virus infection (3.5%), scrub typhus (2.6%), spotted fever group infection (0.9%), unspecified flavivirus infection (0.9%), and murine typhus (0.4%). We discuss the empirical treatment of fever in relation to these findings.

22. Miotto O, Amato R, Ashley EA, MacInnis B, Almagro-Garcia J, Amaratunga C, Lim P, Mead D, Oyola SO, Dhorda M, Imwong M, Woodrow C, Manske M, Stalker J, Drury E, Campino S, Amenga-Etego L, Thanh TN, Tran HT, Ringwald P, Bethell D, Nosten F, Phyo AP, Pukrittayakamee S, Chotivanich K, Chuor CM, Nguon C, Suon S, Sreng S, Newton PN, Mayxay M, Khanthavong M, Hongvanthong B, Htut Y, Han KT, Kyaw MP, Faiz MA, Fanello CI, Onyamboko M, Mokuolu OA, Jacob CG, Takala-Harrison S, Plowe CV, Day NP, Dondorp AM, Spencer CC, McVean G, Fairhurst RM, White NJ, Kwiatkowski DP (2015) Genetic architecture of artemisinin-resistant *Plasmodium falciparum*. *Nat Genet* 47(3): 226-34.

Abstract. We report a large multicenter genome-wide association study of *Plasmodium falciparum* resistance to artemisinin, the frontline antimalarial drug. Across 15 locations in Southeast Asia, we identified at least 20 mutations in kelch13 (PF3D7_1343700) affecting the encoded propeller and BTB/POZ domains, which were associated with a slow parasite clearance rate after treatment with artemisinin derivatives. Nonsynonymous polymorphisms in fd (ferredoxin), arps10 (apicoplast ribosomal protein S10), mdr2 (multidrug resistance protein 2) and crt (chloroquine resistance transporter) also showed strong associations with artemisinin resistance. Analysis of the fine structure of the parasite population showed that the fd, arps10, mdr2 and crt polymorphisms are markers of a genetic background on which kelch13 mutations are particularly likely to arise and that they correlate with the contemporary geographical boundaries and population frequencies of artemisinin resistance. These findings indicate that the risk of new resistance-causing mutations emerging is determined by specific predisposing genetic factors in the underlying parasite population.

23. Mok S, Ashley EA, Ferreira PE, Zhu L, Lin Z, Yeo T, Chotivanich K, Imwong M, Pukrittayakamee S, Dhorda M, Nguon C, Lim P, Amaratunga C, Suon S, Hien TT, Htut Y, Faiz MA, Onyamboko MA, Mayxay M, Newton PN, Tripura R, Woodrow CJ, Miotto O, Kwiatkowski

DP, Nosten F, Day NP, Preiser PR, White NJ, Dondorp AM, Fairhurst RM, Bozdech Z. (2015) Population transcriptomics of human malaria parasites reveals the mechanism of artemisinin resistance. *Science* 347, 431-5.

Artemisinin resistance in *Plasmodium falciparum* threatens global efforts to control and eliminate malaria. Polymorphisms in the kelch domain–carrying protein K13 are associated with artemisinin resistance, but the underlying molecular mechanisms are unknown. We analyzed the in vivo transcriptomes of 1043 *P. falciparum* isolates from patients with acute malaria and found that artemisinin resistance is associated with increased expression of unfolded protein response (UPR) pathways involving the major PROSC and TRiC chaperone complexes. Artemisinin-resistant parasites also exhibit decelerated progression through the first part of the asexual intraerythrocytic development cycle. These findings suggest that artemisinin-resistant parasites remain in a state of decelerated development at the young ring stage, whereas their up-regulated UPR pathways mitigate protein damage caused by artemisinin. The expression profiles of UPR-related genes also associate with the geographical origin of parasite isolates, further suggesting their role in emerging artemisinin resistance in the Greater Mekong Subregion.

24. Nasner-Posso KM, Cruz-Calderón S, Montúfar-Andrade FE, Dance DA, Rodriguez-Morales AJ (2015) Human melioidosis reported by ProMED. *Int J Infect Dis* 35, 103-106.

Objective: There are limited sources describing the global burden of emerging diseases. A review of human melioidosis reported by ProMED was performed and the reliability of the data retrieved assessed in comparison to published reports. The effectiveness of ProMED was evaluated as a source of epidemiological data by focusing on melioidosis. Methods: Using the keyword 'melioidosis' in the ProMED search engine, all of the information from the reports and collected data was reviewed using a structured form, including the year, country, gender, occupation, number of infected individuals, and number of fatal cases. Results: One hundred and twenty-four entries reported between January 1995 and October 2014 were identified. A total of 4630 cases were reported, with death reported in 505 cases, suggesting a misleadingly low overall case fatality rate (CFR) of 11%. Of 20 cases for which the gender was reported, 12 (60%) were male. Most of the cases were reported from Australia, Thailand, Singapore, Vietnam, and Malaysia, with sporadic reports from other countries. Conclusions: Internet-based reporting systems such as ProMED are useful to gather information and synthesize knowledge on emerging infections. Although certain areas need to be improved, ProMED provided good information about melioidosis.

The Mekong near Pakse from the air at sunset

25. Nayyar G, Attaran A, Clark J, Culzoni J, Fernandez F, Kendall M, Newton PN, Herrington J, Breman JG (2015) Responding to the Pandemic of Falsified Medicines. *American Journal of Tropical Medicine & Hygiene* 92(6 Suppl), 113-8.

Abstract. Over the past decade, the number of countries reporting falsified (fake, spurious/falsely labeled/counterfeit) medicines and the types and quantities of fraudulent drugs being distributed have increased greatly. The obstacles in combating falsified pharmaceuticals include 1) lack of consensus on definitions, 2) paucity of reliable and scalable technology to detect fakes before they reach patients, 3) poor global and national leadership and accountability systems for combating this scourge, and 4) deficient manufacturing and regulatory challenges, especially in China and India where fake products often originate. The major needs to improve the quality of the world's medicines fall into three main areas: 1) research to develop and compare accurate and affordable tools to identify high-quality drugs at all levels of distribution; 2) an international convention and national legislation to facilitate production and utilization of high-quality drugs and protect all countries from the criminal and the negligent who make, distribute, and sell life-threatening products; and 3) a highly qualified, well-supported international science and public health organization that will establish standards, drug-quality surveillance, and training programs like the U.S. Food and Drug Administration. Such leadership would give authoritative guidance for countries in cooperation with national medical regulatory agencies, pharmaceutical companies, and international agencies, all of which have an urgent interest and investment in ensuring that patients throughout the world have access to good quality medicines. The organization would also advocate strongly for including targets for achieving good quality medicines in the United Nations Millennium Development Goals and Sustainable Development Goals

Dr Sabine Dittrich and Professor Bounthaphany Bounxouai at Sabine's leaving party

26. Newton PN, Schellenberg D, Ashley EA, Ravinetto R, Green MD, ter Kuile FO, Tabernero P, White NJ, Guerin PJ (2015) Quality assurance of medicines used in clinical trials: Proposal for adapting guidelines. *BMJ* 350, h602.

[The paper discussed the CONSORT guidelines – that have been extremely important in improving practice and ensuring that trial results appropriately guide public policy. However, examples are given of trials and clinical research where poor quality medicines were used or nearly used in such research. The authors propose that the CONSORT guidelines on trial reports should include a requirement to state the quality of medicines used. Enormous investment in trials will be wasted and their interpretation into public policy incorrect if the quality of medicines and medical devices used is not assured]

27. Nic Fhogartaigh C, Dance DAB, Davong V, Tann P, Phetsouvanh P, Turner P, Dittrich S, Newton PN (2015) A novel technique for detecting antibiotic resistant typhoid from rapid diagnostic tests. *J Clin Micro* 53, 1758-60.

Fluoroquinolone-resistant typhoid is increasing. An antigen-detecting rapid diagnotic test (RDT) can rapidly diagnose typhoid from blood cultures. A simple, inexpensive molecular technique performed with DNA from positive RDTs accurately identified gyrA mutations consistent with phenotypic susceptibility testing results. Field diagnosis combined with centralized molecular resistance testing could improve typhoid management and surveillance in low-resource settings.

28. Otte WM, van Diessen E, van Eijsden P, van der Maas F, Patsalos PN, Newton PN, Alvarenga IC, Braun KP, Sander JW (2015) Counterfeit antiepileptic drugs threaten community services in Guinea-Bissau and Nigeria. *Lancet Neurol* 14, 1075-6.

[The paper describes two outbreaks of seizures in community epilepsy services in Guinea-Bissau and Nigeria when the brands of phenobarbital used were changed. The phenobarbital concentrations in tablets from the two suspect brands were either not detectable or extremely low (0.8–1.5%). It is advised that if a change of the frequency of seizures occurs in such community programs, a key first step must be to investigate the quality of the anti-epileptic drugs administered.]

29. Paris DH, Stephan F, Bulder I, Wouters D, van der Poll T, Newton PN, Day NPJ, Zeerleder S (2015) Increased Nucleosomes and Neutrophil Activation Link to Disease Progression in Patients with Scrub Typhus but Not Murine Typhus in Laos. *PLOS Negl Trop Dis* 9(8), e0003990.

Abstract. Cell-mediated immunity is essential in protection against rickettsial illnesses, but the role of neutrophils in these intracellular vasculotropic infections remains unclear. This study analyzed the plasma levels of nucleosomes, FSAP-activation (nucleosome-releasing factor), and neutrophil activation, as evidenced by neutrophil-elastase (ELA) complexes, in sympatric Lao patients with scrub typhus and murine typhus. In acute scrub typhus elevated nucleosome levels correlated with lower GCS scores, raised respiratory rate, jaundice and impaired liver function, whereas neutrophil activation correlated with fibrinolysis and high IL- 8 plasma levels, a recently identified predictor of severe disease and mortality. Nucleosome and ELA complex levels were associated with a 4.8-fold and 4-fold increased risk of developing severe scrub typhus, beyond cut off values of 1,040 U/ml for nucleosomes and 275 U/ml for ELA complexes respectively. In murine typhus, nucleosome levels associated with pro-inflammatory cytokines and the duration of illness, while ELA complexes correlated strongly with inflammation markers, jaundice and increased respiratory rates. This study found strong correlations between circulating nucleosomes and neutrophil activation in patients with scrub typhus, but not murine typhus, providing indirect evidence that nucleosomes could originate from neutrophil extracellular trap (NET) degradation. High circulating plasma nucleosomes and ELA complexes represent independent risk factors for developing severe complications in scrub typhus. As nucleosomes and histones exposed on NETs are highly cytotoxic to endothelial cells and are strongly pro-coagulant, neutrophil-derived nucleosomes could contribute to vascular damage, the pro-coagulant state and exacerbation of disease in scrub typhus, thus indicating a detrimental role of neutrophil activation.

The data suggest that increased neutrophil activation relates to disease progression and severe complications, and increased plasma levels of nucleosomes and ELA complexes represent independent risk factors for developing severe scrub typhus.

30. Parry CM, Tran VT, Dolecek C, Karkey A, Gupta R, Turner P, Dance D, Maude RR, Ha V, Nguyen TC, Le TP, Pham VB, Tran TP, Nguyen NR, Ghose A, Dongol S, Campbell JI, Duy PT, Tuyen HT, Moore CE, Sona S, Gaind R, Deb M, Vo AH, Nguyen VS, Tran TH, Day NP, Dondorp A, Thwaites G, Faiz MA, Phetsouvanh R, Newton P, Basnyat B, Farrar JJ, Baker S (2015) Clinically and microbiologically derived azithromycin susceptibility breakpoints for *Salmonella enterica* serovars Typhi and Paratyphi A. *Antimicrob Agents Chemother* 59, 2756-64.

Azithromycin is an effective treatment for uncomplicated infections with *Salmonella enterica* serovar Typhi and serovar Paratyphi A (enteric fever), but there are no clinically validated MIC and disk zone size interpretative guidelines. We studied individual patient data from three randomized controlled trials (RCTs) of antimicrobial treatment in enteric fever in Vietnam, with azithromycin used in one treatment arm, to determine the relationship between azithromycin treatment response and the azithromycin MIC of the infecting isolate. We additionally compared the azithromycin MIC and the disk susceptibility zone sizes of 1,640 *S.* Typhi and *S.* Paratyphi A clinical isolates collected from seven Asian countries. In the RCTs, 214 patients who were treated with azithromycin at a dose of 10 to 20 mg/ml for 5 to 7 days were analyzed. Treatment was successful in 195 of 214 (91%) patients, with no significant difference in response (cure rate, fever clearance time) with MICs ranging from 4 to 16 µg/ml. The proportion of Asian enteric fever isolates with an MIC of ≤ 16 µg/ml was 1,452/1,460 (99.5%; 95% confidence interval [CI], 98.9 to 99.7) for *S.* Typhi and 207/240 (86.3%; 95% CI, 81.2 to 90.3) ($P < 0.001$) for *S.* Paratyphi A. A zone size of ≥ 13 mm to a 5-µg azithromycin disk identified *S.* Typhi isolates with an MIC of ≤ 16 µg/ml with a sensitivity of 99.7%. An azithromycin MIC of ≤ 16 µg/ml or disk inhibition zone size of ≥ 13 mm enabled the detection of susceptible *S.* Typhi isolates that respond to azithromycin treatment. Further work is needed to define the response to treatment in *S.* Typhi isolates with an azithromycin MIC of >16 µg/ml and to determine MIC and disk breakpoints for *S.* Paratyphi A.

[Note – a correction appeared in *Antimicrob Agents Chemother* (2015) 59(7), 4364]

31. Phetsouvanh R, Sonthayanon P, Pukrittayakamee S, Paris DH, Newton PN, Feil EJ, Day NPJ (2015) The Diversity and Geographical Structure of *Orientia tsutsugamushi* Strains from Scrub Typhus Patients in Laos. *PLOS Negl Trop Dis* 9(8), e0004024.

Abstract. *Orientia tsutsugamushi* is the causative agent of scrub typhus, a disease transmitted by *Leptotrombidium* mites which is responsible for a severe and under-reported public health burden throughout Southeast Asia. Here we use multilocus sequence typing (MLST) to characterize 74 clinical isolates from three geographic locations in the Lao PDR (Laos), and compare them with isolates described from Udon Thani, northeast Thailand. The data confirm high levels of diversity and recombination within the natural *O. tsutsugamushi* population, and a rate of mixed infection of ~8%. We compared the relationships and geographical structuring of the strains and populations using allele based approaches (eBURST), phylogenetic approaches, and by calculating F-statistics (FST). These analyses all point towards low levels of population differentiation between isolates from Vientiane and Udon Thani, cities which straddle the Mekong River which defines the Lao/Thai border, but with a very distinct population in Salavan, southern Laos. These data highlight how land use, as well as the movement of hosts and vectors, may impact on the epidemiology of zoonotic infections.

32. Phommasone K, Sengvilaipaseuth O, de Lamballerie X, Vongsouvath M, Phonemixay O, Blacksell SD, Newton, PN, Dubot-Pérès A (2015) Temperature and the field stability of a dengue rapid diagnostic test in the tropics. *American Journal of Tropical Medicine & Hygiene* 93(1), 33-9.

Abstract. The global incidence of dengue has increased significantly in recent decades, resulting in a large public health burden in tropical and subtropical countries. Dengue rapid diagnostic tests (RDTs) can provide accurate, rapid accessible diagnosis for patient management and may be easily used by health workers in rural areas. However, in dengue-endemic areas, ambient temperatures are often higher than manufacturer's recommendation. We therefore evaluated the effect of high temperature over time on the performance of one commonly used dengue RDT, the Standard Diagnostics Bioline Dengue Duo. RDTs were kept in five different conditions (at 4°C, 35°C, 45°C, 60°C, and at fluctuant ambient temperatures in a free-standing hut) for between 2 days and 2 years in the Lao People's Democratic Republic (PDR). RDTs were tested with four control sera (negative, dengue nonstructural protein 1 [NS1], anti-dengue immunoglobulin [Ig] M, and antidengue IgG positive). The RDTs had 100% consistency over the 2-year study, despite high temperatures, including in the hut in which temperatures exceeded the manufacturer's recommendations for 29% of time points. These data suggest that the diagnostic accuracy of the SD Bioline Dengue Duo RDT remains stable even after long-term storage at high temperatures. Therefore, use at such ambient temperatures in tropical areas should not jeopardize the dengue diagnostic outcome.

Presentation to Microbiology Laboratory staff of certificates of training on the new Pathogen Asset Control System (PACS) by Mr Azamat Zhilokov and staff of Black & Veatch

33. Phommasone K, Althaus T, Souvanthong P, Phakhounthong K, Soyvienvong L, Malapheth P, Mayxay M, Pavlicek RL, Paris DH, Dance D, Newton PN, Lubell Y (2016) Accuracy of commercially available C-reactive protein rapid tests in the context of undifferentiated fevers in rural Laos. *BMC Infect Dis* 16:61.

Background: C-Reactive Protein (CRP) has been shown to be an accurate biomarker for discriminating bacterial from viral infections in febrile patients in Southeast Asia. Here we investigate the accuracy of existing rapid qualitative and semi-quantitative tests as compared with a quantitative reference test to assess their potential for use in remote tropical settings.
Methods: Blood samples were obtained from consecutive patients recruited to a prospective fever study at three sites in rural Laos. At each site, one of three rapid qualitative or semi-quantitative tests was performed, as well as a corresponding quantitative NycoCard Reader II as a reference test. We estimate the sensitivity and specificity of the three tests against a threshold of 10 mg/L and kappa values for the agreement of the two semi-quantitative tests with the results of the reference test. Results: All three tests showed high sensitivity, specificity and kappa values as compared with the NycoCard Reader II. With a threshold of 10 mg/L the sensitivity of the tests ranged from 87–98 % and the specificity from 91–98 %. The weighted kappa values for the semi-quantitative tests were 0.7 and 0.8. Conclusion: The use of CRP rapid tests could offer an inexpensive and effective approach to improve the targeting of antibiotics in remote settings where health facilities are basic and laboratories are absent. This study demonstrates that accurate CRP rapid tests

34. Quet F, Vlieghe E, Leyer C, Buisson Y, Newton PN, Philaysak N, Keoluangkhot V, Chomarat M, Longuet C, Steenkeste N, Jacobs (2015) Antibiotic prescribing behavior among doctors from hospitals in Lao PDR: a knowledge, attitude and practice survey in four selected provinces. *Bull WHO* 93, 219-227.

Objective To assess the antibiotic prescribing practices of doctors working in the Lao People's Democratic Republic and their knowledge of local antibiotic resistance patterns.

Methods Doctors attending morning meetings in 25 public hospitals in four provinces were asked to complete a knowledge, attitude and practice survey. The questionnaire contained 43 multiple choice questions that the doctor answered at the time of the meeting.

Findings The response rate was 83.4% (386/463). Two hundred and seventy doctors (59.8%) declared that they had insufficient information about antibiotics. Only 14.0% (54/386) recognized the possibility of cephalosporin cross-resistance in methicillin-resistant *Staphylococcus aureus*. Most participants had no information about local antibiotic resistance for *Salmonella* Typhi (211/385, 54.8%) and hospital-acquired pneumonia (253/384, 65.9%). Unnecessary antibiotic prescriptions were considered as harmless by 115 participants and 148 considered locally-available generic antibiotics to be of poor quality. Nearly three-quarters (280/386) of participants agreed that it was difficult to select the correct antibiotics. Most participants (373/386) welcomed educational programmes on antibiotic prescribing and 65.0% (249/383) preferred local over international antibiotic guidelines.

Conclusion Doctors in the Lao People's Democratic Republic seem to favour antibiotic prescribing interventions. Health authorities should consider a capacity building programme that incorporates antibiotic prescribing and hospital infection control.

35. Rattanavong S, Dance DA, Davong V, Baker C, Frost H, Phetsouvanh R, Vongsouvath M, Newton PN, Steer AC, Smeesters PR (2015) Group A streptococcal strains isolated in Lao People's Democratic Republic from 2004 to 2013. *Epidemiol Infect,* 1-4.

SUMMARY. Epidemiological data regarding group A streptococcal (GAS) infections in South East Asia are scarce with no information from Laos. We characterized emm types, emm clusters and the antibiotic resistance profile of 124 GAS isolates recovered in Laos during 2004–2013. Most strains were recovered from skin and invasive infections (76% and 19%, respectively). Thirty four emm types were identified as belonging to 12 emm clusters and no novel emm types were identified. No significant differences were observed in the distribution of emm types or emm clusters according to age or site of recovery (skin or invasive infections). There was moderate strain diversity in this country but considerable differences in emm-type distribution between Laos, Thailand and Cambodia. Vaccine coverage was high for the J8 vaccine candidate. The theoretical coverage for the 30-valent vaccine candidate needs further investigation. Antibiotic resistance was moderate to erythromycin and chloramphenicol (8% and 7%, respectively) and low to ofloxacin (<1%).

36. Renschler JP, Walters K, Newton PN, Laxminarayan R (2015) Estimated under-five deaths associated with poor-quality antimalarials in sub-Saharan Africa. *American Journal of Tropical Medicine & Hygiene* 92(6 Suppl), 119-26.

Abstract. Many antimalarials sold in sub-Saharan Africa are poor-quality (falsified, substandard, or degraded), and the burden of disease caused by this problem is inadequately quantified. In this article, we estimate the number of under five deaths caused by ineffective treatment of malaria associated with consumption of poor-quality antimalarials in 39 sub-Saharan countries. Using Latin hypercube sampling our estimates were calculated as the product of the number of private sector antimalarials consumed by malaria-positive children in 2013; the proportion of private sector antimalarials consumed that were of poor-quality; and the case fatality rate (CFR) of under-five malaria-positive children who did not receive appropriate treatment. An estimated 122,350 (interquartile range [IQR]: 91,577–154,736) under-five malaria deaths were associated with consumption of poor-quality antimalarials, representing 3.75% (IQR: 2.81–4.75%) of all under-five deaths in our sample of 39 countries. There is considerable uncertainty surrounding our results because of gaps in data on case fatality rates and prevalence of poor-quality antimalarials. Our analysis highlights the need for further investigation into the distribution of poor-quality antimalarials and the need for stronger surveillance and regulatory efforts to prevent the sale of poor-quality antimalarials.

37. Ribolzi O, Rochelle-Newall E, Dittrich S, Auda Y, Newton PN, Rattanavong S Knappik M, Soulileuth B, Sengtaheuanghoung O, Dance DAB, Pierret A (2016) Land use and soiltype determine the presence of the pathogen *Burkholderia pseudomallei* in tropical rivers. *Environmental Science and Pollution Research Jan 13*

Burkholderia pseudomallei is the bacterium that causes melioidosis in humans. While *B. pseudomallei* is known to be endemic in South East Asia (SEA), the occurrence of the disease in other parts of the tropics points towards a potentially large global distribution. We investigated the environmental factors that influence the presence (and absence) of *B. pseudomallei* in a tropical watershed in SEA. Our main objective was to determine whether there is a link between the presence of the organism in the hydrographic network and the upstream soil and land-use type. The presence of *B. pseudomallei* was determined using a specific quantitative real-time PCR assay following enrichment culture. Land use, soil, geomorphology and environmental data were then analyzed using Partial Least Squares Discriminant Analysis (PLSDA) to compare the *B. pseudomallei* positive and negative sites. Soil type in the surrounding catchment and turbidity had a strong positive influence on the presence (acrisols and luvisols) or absence (ferralsols) of *B. pseudomallei*. Given the strong apparent links between soil characteristics, water turbidity and the presence/absence of *B. pseudomallei*, actions to raise public awareness about factors increasing the risk of exposure should be undertaken in order to reduce the incidence of melioidosis in regions of endemicity.

38. Slesak G, Inthalth S, Dittrich S, Paris DH, Newton PN (2015) Leeches as further potential vectors for rickettsial infections. *PNAS* www.pnas.org/cgi/doi/10.1073/pnas.1515229112

[Describes a Lao patient with PCR evidence for *Rickettsia felis* infection in an eschar at the site of a leech bite. The potential importance of leeches as vectors for rickettsial infections is discussed]

39. Stoesser N, Xayaheuang S, Vongsouvath M, Phommasone K, Elliott I, Del Ojo Elias C, Crook D, Newton PN, Buisson Y, Lee S, Dance DAB (2015) Colonisation with Enterobacteriaceae producing extended spectrum beta-lactamases in children attending pre-school childcare facilities in the Lao People's Democratic Republic. *J Antimicrob Chemo* 70, 1893-7.

Objectives: Intestinal carriage constitutes an important reservoir of antimicrobial-resistant bacteria, with some of the highest rates reported from Asia. Antibiotic resistance has been little studied in Laos, where some antibiotics are available without restriction, but others such as carbapenems are not available. Patients and methods: We collected stools from 397 healthy children in 12 randomly selected pre-school childcare facilities in and around Vientiane. Colonization with ESBL-producing Enterobacteriaceae (ESBLE) and carbapenemase-producing Enterobacteriaceae (CPE) was detected using a disc diffusion screening test and ESBLE were characterized using WGS. Risk factor data were collected by questionnaire.

Results: Ninety-two children (23%) were colonized with ESBLE, mainly Escherichia coli carrying blaCTX-M and *Klebsiella pneumoniae* carrying blaSHV or blaCTX-M, which were frequently resistant to multiple antibiotic classes. Although residence in Vientiane Capital, foreign travel, higher maternal level of education, antibiotic use in the preceding 3 months and attending a childcare facility with a 'good' level of hygiene were all associated with ESBLE colonization on univariable analysis, a significant association remained only for antibiotic use when a stepwise approach was used with a multivariate random-effects model. WGS analysis suggested transmission in both childcare facilities and community settings.

Conclusions: The high prevalence of paediatric colonization with ESBLE in Laos, one of the highest reported in Asia, is probably the result of inappropriate antibiotic use. Paediatric colonization with CPE was not identified in this study,

40. Tabernero P, Mayxay M, Culzoni J, Dwivedi P, Swamidoss I, Allan L, Khanthavong, M, Phonlavong C, Vilayhong C, Sichanh C, Sengaloundeth S, Kaur H, Fernandez F, Green MD, Newton PN (2015) A repeat random survey of the prevalence of falsified and substandard antimalarials in the Lao PDR - a change for the better. *American Journal of Tropical Medicine & Hygiene* 92(6 Suppl), 95-104.

Professor Sasithon Pukrittayakamee, congratulating Dr Rattanaphone Phetsouvanh on passing her PhD viva

Abstract. In 2003, a stratified random sample survey was conducted in the Lao People's Democratic Republic (Laos) to study the availability and quality of antimalarials in the private sector. In 2012, this survey was repeated to allow a statistically valid analysis of change through time. The counterfeit detection device 3 (CD-3) was used to assess packaging quality in the field and HPLC and mass spectroscopy analysis chemical analysis performed. The availability of oral artesunate monotherapies had significantly decreased from 22.9% (22) of 96 outlets in southern Laos in 2003 to 4.8% (7) of 144 outlets in 2012 ($P < 0.0001$). All the samples collected in the 2012 survey contained the correct active pharmaceutical ingredients (APIs) in contrast to the 21 (84%) falsified artesunate samples found in the 2003 survey. Although none of the medicines found in 2012 survey had evidence for falsification, 25.4% (37) of the samples were outside the 90–110% pharmacopeial limits of the label claim, suggesting that they were substandard or degraded. Results obtained from this survey show that patients are still exposed to poorly manufactured drugs or to ineffective medicines such as chloroquine. The quality of artemisinin-based combination therapies (ACTs) used in Laos needs to be monitored, since falsified ACTs would have devastating consequences in public health.

Fun at the Oxford Tropical Network Meeting in Siam Reap

41. Tabernero P, Lee SJ, Stepniewska K, Newton PN (2014) Recommendations on the content of a survey protocol for surveys of the quality of medicines. (July 2014) World Health Organisation Working document QAS/14.590. http://www.who.int/medicines/areas/quality_safety/quality_assurance/WHO_SamplingProcedures_GeneralDocument_QAS14_590_10062014.pdf?ua=1

[A draft report for WHO on methods for designing, conducting and reporting surveys of medicine quality, building on the MEDQUARD guidelines published in 2009. The WHO report will be published in May 2016]

42. Takala-Harrison S, Jacob CG, Arze C, Cummings MP, Silva JC, Dondorp AM, Fukuda MM, Hien TT, Mayxay M, Noedl H, Nosten F, Kyaw MP, Nhien NTT, Imwong M, Bethell D, Se Y, Lon C, Tyner SD, Saunders DL, Ariey F, Mercereau-Puijalon O, Menard D, Newton PN, Khanthavong M, Hongvanthong B, Starzengruber P, Fuehrer H-P, Swoboda P, Khan WF, Phyo AP, Nyunt MM, Nyunt MH, Brown TS, Adams M, Pepin CS, Bailey J, Tan JC, Ferdig MT, Clark TG, Miotto O, MacInnis B, Kwiatkowski DP, White NJ, Ringwald P, Plowe CV (2015) Independent emergence of *Plasmodium falciparum* artemisinin resistance mutations in Southeast Asia. *J Infect Dis* 211(5), 670-9.

Background. The emergence of artemisinin-resistant *Plasmodium falciparum* in Southeast Asia threatens malaria treatment efficacy. Mutations in a kelch protein encoded on *P. falciparum* chromosome 13 (K13) have been associated with resistance in vitro and in field samples from Cambodia. Methods. *P. falciparum* infections from artesunate efficacy trials in Bangladesh, Cambodia, Laos, Myanmar, and Vietnam were genotyped at 33 716 genome-wide single-nucleotide polymorphisms (SNPs). Linear mixed models were used to test associations between parasite genotypes and parasite clearance half-lives following artesunate treatment. K13 mutations were tested for association with artemisinin resistance, and extended haplotypes on chromosome 13 were examined to determine whether mutations arose focally and spread or whether they emerged independently. Results. The presence of nonreference K13 alleles was associated with prolonged parasite clearance half-life (P = 1.97 Å~ 10–12). Parasites with a mutation in any of the K13 kelch domains displayed longer parasite clearance half-lives than parasites with wild-type alleles. Haplotype analysis revealed both population-specific emergence of mutations and independent emergence of the same mutation in different geographic areas.

Conclusions. K13 appears to be a major determinant of artemisinin resistance throughout Southeast Asia. While we found some evidence of spreading resistance, there was no evidence of resistance moving westward from Cambodia into Myanmar.

43. Tauran PM, Sennang N, Rusli B, Wiersinga WJ, Dance D, Arif M, Limmathurotsakul D (2015) Emergence of Melioidosis in Indonesia. *Am J Trop Med Hyg* 93(6), 1160-3.

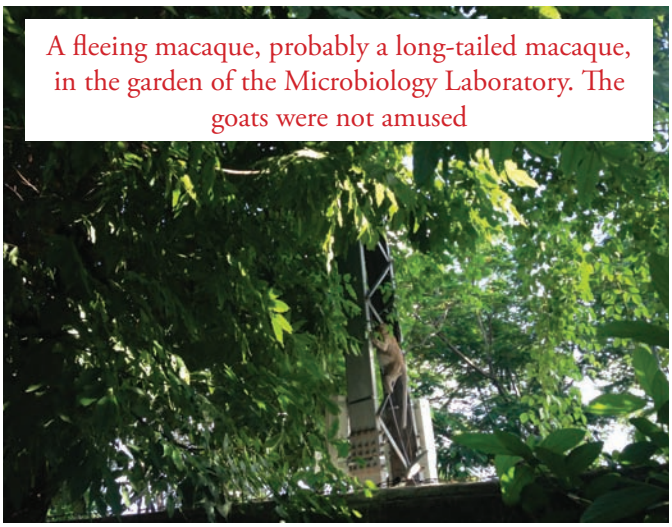
Melioidosis is known to be highly endemic in parts of southeast Asia and northern Australia; however, cases are rarely reported in Indonesia. Here we report three cases of melioidosis in Makassar, South Sulawesi, Indonesia occurring between 2013 and 2014. Two patients died and the other was lost to follow-up. *Burkholderia pseudomallei* isolates from all three cases were identified by the VITEK2 Compact installed in the hospital in 2012. None of the three patients reported received antimicrobials recommended for melioidosis because of the delayed recognition of the organism. We reviewed the literature and found only seven reports of melioidosis in Indonesia. Five were reported before 1960. We suggest that melioidosis is endemic throughout Indonesia but currently under-recognized. Training on how to identify *B. pseudomallei* accurately and safely in all available microbiological facilities should be provided, and consideration should be given to making melioidosis a notifiable disease in Indonesia.

44. Taylor AJ, Paris D, Newton PN (2015a) A systematic review of the mortality from untreated leptospirosis, *PLoS Negl Trop Dis* 9(6), e0003866.

Abstract. Background. Leptospirosis occurs worldwide, but the global incidence of human disease and its mortality are not well understood. Many patients are undiagnosed and untreated due to its nonspecific symptoms and a lack of access to diagnostics. This study systematically reviews the literature to clarify the mortality from untreated leptospirosis. Results will help quantify the global burden of disease and guide health policies. Methodology/ Principal Findings. A comprehensive literature search was performed to identify untreated patient series. Included patients were symptomatic, but asymptomatic patients and those who had received antibiotics, dialysis or who were treated on Intensive Care Units were excluded. Included patients had a confirmed laboratory diagnosis by culture, PCR, or serological tests. Data was extracted and individual patient series were assessed for bias. Thirty-five studies, comprising 41 patient series and 3,390 patients, were included in the study. A high degree of bias within studies was shown due to limitations in study design, diagnostic tests and missing data. Median series mortality was 2.2% (Range 0.0 – 39.7%), but mortality was high in jaundiced patients (19.1%) (Range 0.0 – 39.7%), those with renal failure 12.1% (Range 0-25.0%) and in patients aged over 60 (60%) (Range 33.3-60%), but low in anicteric patients (0%) (Range 0-1.7%). Conclusions. This systematic review contributes to our understanding of the mortality of untreated leptospirosis and provides data for the estimation of DALYs attributable to this disease. We show that mortality is significantly higher in older patients with icteric disease or renal failure but is lower in younger, anicteric patients. Increased surveillance and accurate point-of-care diagnostics are required to better understand the incidence and improve diagnosis of disease. Empirical treatment strategies should prioritize early treatment to improve outcomes from leptospirosis.

45. Taylor AJ, Paris DH, Newton PN (2015b) A systematic review of mortality from untreated scrub typhus (*Orientia tsutsugamushi*). *PLoS Negl Trop Dis* 9(8), e0003971.

Background. Scrub typhus, a bacterial infection caused by *Orientia tsutsugamushi*, is increasingly recognized as an important cause of fever in Asia, with an estimated one million infections occurring each year. Limited access to health care and the disease's non-specific symptoms mean that many patients are undiagnosed and untreated, but the mortality from untreated scrub typhus is unknown. This review systematically summarizes the literature on the untreated mortality from scrub typhus and disease outcomes. Methodology/Principal Findings A literature search was performed to identify patient series containing untreated patients. Patients were included if they were symptomatic and had a clinical or laboratory diagnosis of scrub typhus and excluded if they were treated with antibiotics. The primary outcome was mortality from untreated scrub typhus and secondary outcomes were total days of fever, clinical symptoms, and laboratory results. A total of 76 studies containing 89 patient series and 19,644 patients were included in the final analysis. The median mortality of all patient series was 6.0% with a wide range (min-max) of 0–70%. Many studies used clinical diagnosis alone and had incomplete data on secondary outcomes. Mortality varied by location and increased with age and in patients with myocarditis, delirium, pneumonitis, or signs of hemorrhage, but not according to sex or the presence of an eschar or meningitis. Duration of fever was shown to be long (median 14.4 days Range (9–19)). Conclusions Results show that the untreated mortality from scrub typhus appears lower than previously reported estimates. More data are required to clarify mortality according to location and host factors, clinical syndromes including myocarditis and central nervous system disease, and in vulnerable mother-child populations. Increased surveillance and improved access to diagnostic tests are required to accurately estimate the untreated mortality of scrub typhus. This information would facilitate reliable quantification of DALYs and guide empirical treatment strategies.



A fleeing macaque, probably a long-tailed macaque, in the garden of the Microbiology Laboratory. The goats were not amused

46. Vongphoumy I, Dance DA, Dittrich S, Logan J, Davong V, Rattanavong S, Blessmann J (2015) Case Report: Actinomycetoma Caused by *Nocardia aobensis* from Lao PDR with Favourable Outcome after Short-Term Antibiotic Treatment. *PLoS Negl Trop Dis* 9(4), e0003729.

Abstract. Background. Mycetoma is a neglected, chronic, localized, progressively destructive, granulomatous infection caused either by fungi (eumycetoma) or by aerobic actinomycetes (actinomycetoma). It is characterized by a triad of painless subcutaneous mass, multiple sinuses and discharge containing grains. Mycetoma commonly affects young men aged between 20 and 40 years with low socioeconomic status, particularly farmers and herdsmen. Methodology / Principal Findings A 30 year-old male farmer from an ethnic minority in Phin District, Savannakhet Province, Lao PDR (Laos) developed a painless swelling with multiple draining sinuses of his right foot over a period of approximately 3 years. X-ray of the right foot showed osteolysis of tarsals and metatarsals. Aerobic culture of sinus discharge yielded large numbers of *Staphylococcus aureus* and a slow growing Gram-positive rod. The organism was subsequently identified as *Nocardia aobensis* by 16S ribosomal RNA gene sequencing. The patient received antimicrobial treatment with amikacin and trimethoprim-sulfamethoxazole according to consensus treatment guidelines. Although slight improvement was noted the patient left the hospital after 14 days and did not take any more antibiotics. Over the following 22 weeks the swelling of his foot subsequently diminished together with healing of discharging sinuses. Conclusion. This is the first published case of Actinomycetoma caused by *Nocardia aobensis* and the second case of Actinomycetoma from Laos. A treatment course of only 14 days with amikacin and trimethoprim-sulfamethoxazole was apparently sufficient to cure the infection, although long-term treatment up to one year is currently recommended. Treatment trials or prospective descriptions of outcome for actinomycetoma should investigate treatment efficacy for the different members of Actinomycetales, particularly *Nocardia* spp., with short term and long-term treatment courses.

47. WWARN Parasite Clearance Study Group (2015a) Baseline data of parasite clearance in patients with falciparum malaria treated with an artemisinin derivative: an individual patient data meta-analysis. *Malar J* 14(1), 359.

Abstract. Background: Artemisinin resistance in *Plasmodium falciparum* manifests as slow parasite clearance but this measure is also influenced by host immunity, initial parasite biomass and partner drug efficacy. This study collated data from clinical trials of artemisinin derivatives in falciparum malaria with frequent parasite counts to provide reference parasite clearance estimates stratified by location, treatment and time, to examine host factors affecting parasite clearance, and to assess the relationships between parasite clearance and risk of recrudescence during follow-up. Methods: Data from 24 studies, conducted from 1996 to 2013, with frequent parasite counts were pooled. Parasite clearance half-life (PC1/2) was estimated using the WWARN Parasite Clearance Estimator. Random effects regression models accounting for study and site heterogeneity were used to explore factors affecting PC1/2 and risk of recrudescence within areas with reported delayed parasite clearance (western Cambodia, western Thailand after 2000, southern Vietnam, southern Myanmar) and in all other areas where parasite populations are artemisinin sensitive. Results: PC1/2 was estimated in 6975 patients, 3288 of whom also had treatment outcomes evaluated during 28–63 days follow-up, with 93 (2.8 %) PCR-confirmed recrudescences. In areas with artemisinin-sensitive parasites, the median PC1/2 following three-day artesunate treatment (4 mg/kg/day) ranged from 1.8 to 3.0 h and the proportion of patients with PC1/2 >5 h from 0 to 10 %. Artesunate doses of 4 mg/kg/day decreased PC1/2 by 8.1 % (95 % CI 3.2–12.6) compared to 2 mg/kg/day, except in populations with delayed parasite clearance. PC1/2 was longer in children and in patients with fever or anaemia at enrolment. Long PC1/2 (HR = 2.91, 95 % CI 1.95–4.34 for twofold increase, $p < 0.001$) and high initial parasitaemia (HR = 2.23, 95 % CI 1.44–3.45 for tenfold increase, $p < 0.001$) were associated independently with an increased risk of recrudescence. In western Cambodia, the region with the highest prevalence of artemisinin resistance, there was no evidence for increasing PC1/2 since 2007. Conclusions: Several factors affect PC1/2. As substantial heterogeneity in parasite clearance exists between locations, early detection of artemisinin resistance requires reference PC1/2 data. Studies with frequent parasite count measurements to characterize PC1/2 should be encouraged. In western Cambodia, where PC1/2 values are longest, there is no evidence for recent emergence of higher levels of artemisinin resistance.

Mr Davanh and Ms Amphyvanh performing at the hospital singing competition at Lao New Year

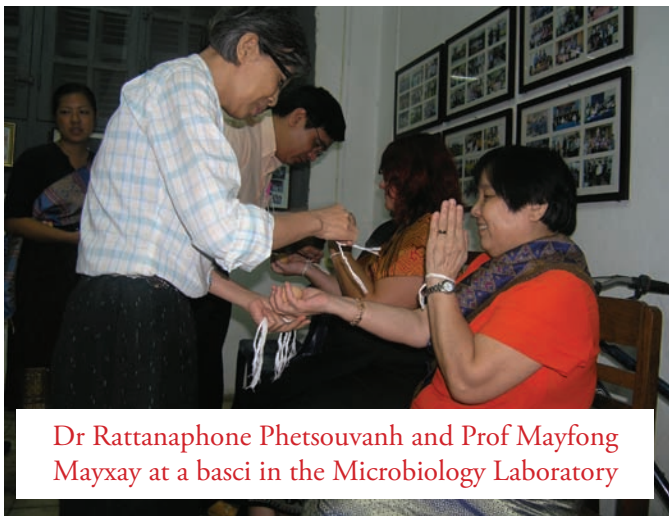
48. WorldWide Antimalarial Resistance Network (WWARN) Lumefantrine PK/PD Study Group (2015b) Artemether-lumefantrine treatment of uncomplicated *Plasmodium falciparum* malaria: a systematic review and meta-analysis of day 7 lumefantrine concentrations and therapeutic response using individual patient data. *BMC Med* 13, 227.

Abstract/ Background: Achieving adequate antimalarial drug exposure is essential for curing malaria. Day 7 blood or plasma lumefantrine concentrations provide a simple measure of drug exposure that correlates well with artemether-lumefantrine efficacy. However, the 'therapeutic' day 7 lumefantrine concentration threshold needs to be defined better, particularly for important patient and parasite sub-populations. Methods: The WorldWide Antimalarial Resistance Network (WWARN) conducted a large pooled analysis of individual pharmacokinetic-pharmacodynamic data from patients treated with artemether-lumefantrine for uncomplicated *Plasmodium falciparum* malaria, to define therapeutic day 7 lumefantrine concentrations and identify patient factors that substantially alter these concentrations. A systematic review of PubMed, Embase, Google Scholar, ClinicalTrials.gov and conference proceedings identified all relevant studies. Risk of bias in individual studies was evaluated based on study design, methodology and missing data. Results: Of 31 studies identified through a systematic review, 26 studies were shared with WWARN and 21 studies with 2,787 patients were included. Recrudescence was associated with low day 7 lumefantrine concentrations (HR 1.59 (95 % CI 1.36 to 1.85) per halving of day 7 concentrations) and high baseline parasitemia (HR 1.87 (95 % CI 1.22 to 2.87) per 10-fold increase). Adjusted for mg/kg dose, day 7 concentrations were lowest in very young children (<3 years), among whom underweight-for-age children had 23 % (95 % CI −1 to 41 %) lower concentrations than adequately nourished children of the same age and 53 % (95 % CI 37 to 65 %) lower concentrations than adults. Day 7 lumefantrine concentrations were 44 % (95 % CI 38 to 49 %) lower following unsupervised treatment. The highest risk of recrudescence was observed in areas of emerging artemisinin resistance and very low transmission intensity. For all other populations studied, day 7 concentrations ≥ 200 ng/ml were associated with >98 % cure rates (if parasitemia <135,000/μ L). Conclusions: Current artemether-lumefantrine dosing recommendations achieve day 7 lumefantrine concentrations ≥200 ng/ml and high cure rates in most uncomplicated malaria patients. Three groups are at increased risk of treatment failure: very young children (particularly those underweight-for-

age); patients with high parasitemias; and patients in very low transmission intensity areas with emerging parasite resistance. In these groups, adherence and treatment response should be monitored closely. Higher, more frequent, or prolonged dosage regimens should now be evaluated in very young children, particularly if malnourished, and in patients with hyperparasitemia.

49. Worldwide Antimalarial Resistance Network (WWARN) AL Dose Impact Study Group (2015c) The effect of dose on the antimalarial efficacy of artemether-lumefantrine: a systematic review and pooled analysis of individual patient data. *Lancet Infect Dis* 15(6):692-702.

Abstract. BACKGROUND: Artemether-lumefantrine is the most widely used artemisinin-based combination therapy for malaria, although treatment failures occur in some regions. We investigated the effect of dosing strategy on efficacy in a pooled analysis from trials done in a wide range of malaria-endemic settings. METHODS: We searched PubMed for clinical trials that enrolled and treated patients with artemether-lumefantrine and were published from 1960 to December, 2012. We merged individual patient data from these trials by use of standardised methods. The primary endpoint was the PCR-adjusted risk of Plasmodium falciparum recrudescence by day 28. Secondary endpoints consisted of the PCR-adjusted risk of P falciparum recurrence by day 42, PCR-unadjusted risk of P falciparum recurrence by day 42, early parasite clearance, and gametocyte carriage. Risk factors for PCR-adjusted recrudescence were identified using Cox's regression model with frailty shared across the study sites. FINDINGS: We included 61 studies done between January, 1998, and December, 2012, and included 14,327 patients in our analyses. The PCR-adjusted therapeutic efficacy was 97·6% (95% CI 97·4-97·9) at day 28 and 96·0% (95·6-96·5) at day 42. After controlling for age and parasitaemia, patients prescribed a higher dose of artemether had a lower risk of having parasitaemia on day 1 (adjusted odds ratio [OR] 0·92, 95% CI 0·86-0·99 for every 1 mg/kg increase in daily artemether dose; p=0·024), but not on day 2 (p=0·69) or day 3 (0·087). In Asia, children weighing 10-15 kg who received a total lumefantrine dose less than 60 mg/kg had the lowest PCR-adjusted efficacy (91·7%, 95% CI 86·5-96·9). In Africa, the risk of treatment failure was greatest in malnourished children aged 1-3 years (PCR-adjusted efficacy 94·3%, 95% CI 92·3-96·3). A higher artemether dose was associated with a lower gametocyte presence within 14 days of treatment (adjusted OR 0·92, 95% CI 0·85-0·99; p=0·037 for every 1 mg/kg increase in total artemether dose). INTERPRETATION: The recommended dose of artemether-lumefantrine provides reliable efficacy in most patients with uncomplicated malaria. However, therapeutic efficacy was lowest in young children from Asia and young underweight children from Africa; a higher dose regimen should be assessed in these groups.



Dr Rattanaphone Phetsouvanh and Prof Mayfong Mayxay at a basci in the Microbiology Laboratory

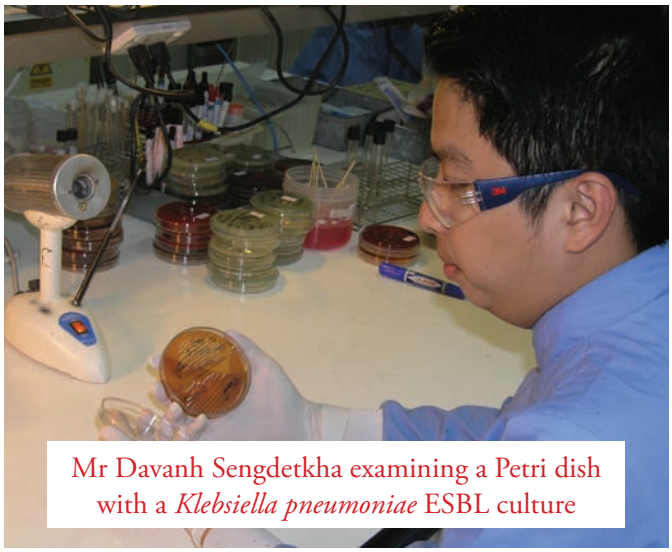
50. Wong V, Baker S, Pickard D, Parkhill J, Page AJ, Feasey N, Kingsley RA, Thomson NR, Keane JA, Weill F-X, Edwards DJ, Harris S, Mather A, Cain AK, Hadfield J, Hart P, Thieu NTV, Klemm EJ, Breiman R, Watson C, Kariuki S, Gordon M, Heyderman R, Okoro C, Jacobs J, Lunguya O, Edmunds WJ, Msefula C, Chabalgoity JA, Kama M, Jenkins K, Dutta S, Marks F, Campos J, Thompson C, Obaro S, MacLennan C, Dolecek C, Keddy KH, Smith AM, Parry C, Karkey A, Mulholland K, Campbell JI, Dongol S, Basnyat B, Dufour M, Bandaranayake D, Toleafoa TN, Singh SP, Hatta M, Newton PN, Onsare RS, Isaia L, Dance D, Thwaites G, Wijedoru L, Crump JA, Pinna ED, Nair S, Nilles E, Thanh DP, Turner P, Soeng S, Hogg G, Farrar J, Holt KE, Dougan G (2015) Phylogeographic analysis of the dominant multidrug-resistant H58 clade of *Salmonella* Typhi identifies inter-and intra-continental transmission events. *Nature Genetics* 47(6), 632-9.

The emergence of multidrug-resistant (MDR) typhoid is a major global health threat affecting many countries where the disease is endemic. Here whole-genome sequence analysis of 1,832 *Salmonella enterica* serovar Typhi (*S.* Typhi) identifies a single dominant MDR lineage, H58, that has emerged and spread throughout Asia and Africa over the last 30 years. Our analysis identifies numerous transmissions of H58, including multiple transfers from Asia to Africa and an ongoing, unrecognized MDR epidemic within Africa itself. Notably, our analysis indicates that H58 lineages are displacing antibiotic-sensitive isolates, transforming the global population structure of this pathogen. H58 isolates can harbor a complex MDR element residing either on transmissible IncHI plasmids or within multiple chromosomal integration sites. We also identify new mutations that define the H58 lineage. This phylogeographical analysis provides a framework to facilitate global management of MDR typhoid and is applicable to similar MDR lineages emerging in other bacterial species.

51. Yong YL, Plançon A, Lau YH, Hostetler DM, Fernández FM, Green MD, Sounvoravong S, Nara S, Boravann M, Dumrong T, Bangsawan N, Low MY, Lim CC, Ai RL, Newton PN (2015) Collaborative Health and Enforcement Operations on the Quality of Antimalarials and Antibiotics in Southeast Asia. *American Journal of Tropical Medicine & Hygiene* 92(6 Suppl), 105-12.

Abstract. Counterfeit (or falsified) and substandard medicines pose a major public health risk. We describe the findings of Operation Storm I and II conducted in 2008–2009 to combat counterfeit medicines through partnership between national customs, Drug Regulatory Agencies (DRAs), and police in Cambodia, Indonesia, Laos, Myanmar, Singapore, Thailand, and Vietnam. Samples were obtained from seizures and market surveillance by national DRAs. Laboratory analysis using spectroscopic and chromatographic techniques and examination of packaging were performed. Ninety-three suspect antibiotics and 95 antimalarial samples were collected. Of the 93 antibiotics, 29 (31%) had % active pharmaceutical ingredient content (%API) < 85%or > 115% (including one counterfeit). Of the 95 antimalarials, 30 (32%) had %API < 85 > 115% API (including one counterfeit). A significant minority of samples, antimalarials (13%) and antibiotics (15%), were collected in plastic bags with minimal or no labeling. Of 20 ampicillin samples, 13 (65%) contained < 85% API (with one counterfeit containing additional amoxicillin). Of 34 oral artesunate samples, 7 (21%) contained %API out of the 85–115% range. Coordinated and synergistic partnership adopted by the participating countries, International Criminal Police Organization (INTERPOL), World Health Organization (WHO), and laboratories facilitated a platform for discussions.

Dr Koukeo Phommasone arriving in the Nong Village community



Mr Davanh Sengdetkha examining a Petri dish with a *Klebsiella pneumoniae* ESBL culture

Meeting of MORU Network Science & Strategy Meeting in Vientiane