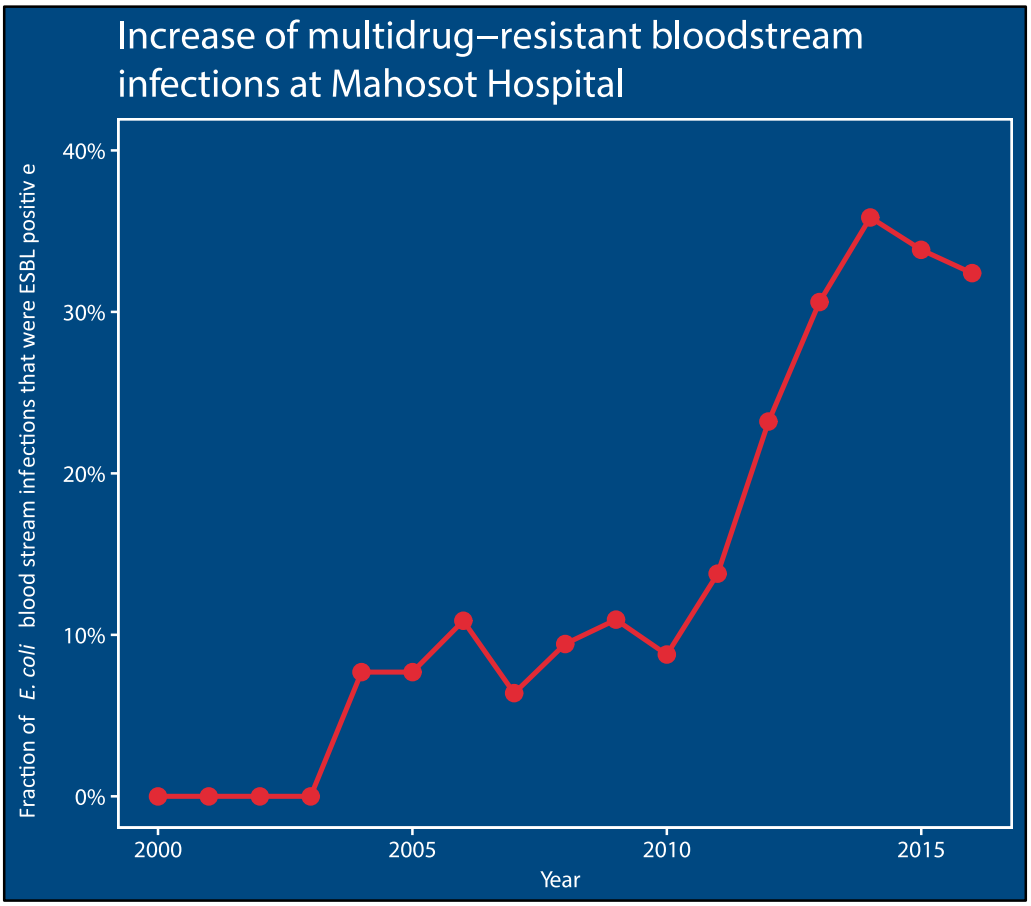
Lao - Oxford - Mahosot Hospital - Wellcome Trust Research Unit

SCIENTIFIC ANNUAL REPORT FOR 2016

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



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Ferry across the Mekong River, Champasak, 2002

IN MEMORIAM

Dr Rattanaphone Phetsouvanh MD, PhD

Director of the Microbiology Laboratory, Mahosot Hospital
Honorary Fellow, Nuffield Department of Medicine, University of Oxford

Dr Rattanaphone Phetsouvanh

It is with profound sadness and sorrow that we inform you of the death of Dr Rattanaphone Phetsouvanh on the evening of Wednesday 23rd November 2016. She had been ill for two years and bore her illness with enormous courage and completed her PhD on scrub typhus during her treatment. Dr Rattanaphone was a dear friend, colleague, leader and an inspiration to all in the Microbiology Laboratory at Mahosot Hospital and in Laos and elsewhere. She was an enormous inspiration to us all for restarting the microbiology services in Laos in 1993, for accepting the offer of collaboration with MORU that led to the founding of LOMWRU, for her wonderful leadership, encouragement and support for all in the Microbiology Laboratory, for fostering LOMWRU, for her research on bacteriology and scrub typhus and for her humour, courage and example in her illness. She will be greatly missed by many people, near and far. Our profound condolences to Dr Rattanaphone's family and all in the Microbiology Laboratory

ອາໄລຮັກເຖິງດວງວິນຍານຂອງ
ທ່ານ ປອ. ດຣ. ລັດຕະນະພອນ ເພັດສຸວັນ
ອາດີດຫົວໜ້າພະແນກວິເຄາະຈຸລິນຊີ, ໂຮງໝໍມະໂຫສົດ
ນັກຄົ້ນຄວ້າກິດຕິມະສັກ ປະຈຳພະແນກການແພດນາບຟີ, ມະຫາວິທະຍາໄລອ໋ອກຟອດ

ທ່ານ ປອ. ດຣ. ລັດຕະນະພອນ ເພັດສຸວັນ ພ້ອມປະກາສະນິຍະບັດປະລິນຍາເອກ

ພວກເຮົາຂໍສະແດງຄວາມໂສກເສົ້າ ແລະ ເສຍໃຈຢ່າງສຸດຊຶ້ງ ຕໍ່ການຈາກໄປໂດຍບໍ່ມີວັນກັບຄືນ ຂອງ ທ່ານ ປອ. ດຣ. ລັດຕະນະພອນ ເພັດສຸວັນ ໃນຄັ້ງວັນທີ 23 ພະຈິກ 2016 ທີ່ຜ່ານມາ ພາຍຫລັງທີ່ໄດ້ຕໍ່ສູ້ກັບພະຍາດຮ້າຍຂອງຜູ້ກ່ຽວເອງເປັນເວລາ 2 ປີ ດ້ວຍຄວາມເຂັ້ມແຂງ ແລະ ອົດທົນ ພ້ອມທັງເຮັດສຳເລັດການສຶກສາລະດັບປະລິນຍາເອກ ໃນຂະນະທີ່ທຳການປິ່ນປົວ. ທ່ານ ປອ. ດຣ. ລັດຕະນະພອນ ເປັນໝູ່, ເປັນເພື່ອນຮ່ວມງານທີ່ເຄົາລົບຮັກ, ເປັນຜູ້ນຳ ແລະ ເປັນແບບຢ່າງທີ່ດີເລີດ ໃຫ້ແກ່ພວກເຮົາທຸກຄົນພາຍໃນພະແນກວິເຄາະຈຸລິນຊີ ໂຮງໝໍມະໂຫສົດ, ໃນ ສປປ ລາວ ແລະ ໃນແຫ່ງອື່ນໆ. ທ່ານເປັນແບບຢ່າງທີ່ຍິ່ງໃຫ່ຍໃຫ້ແກ່ພວກເຮົາທຸກຄົນ ເລີ່ມຈາກການຟື້ນຟູຂະແໜງວິເຄາະຈຸລິນຊີ ໃນ ສປປ ລາວ ໃນປີ 1993, ຮ່ວມມືກັບໜ່ວຍງານຄົ້ນຄວ້າພະຍາດເຂດຮ້ອນມະຫາວິທະຍາໄລມະຫິດົນ-ອ໋ອກຟອດ ຈົນນຳໄປສູ່ການສ້າງຕັ້ງໂຄງການຄົ້ນຄວ້າພະຍາດເຂດຮ້ອນທີ່ໂຮງໝໍມະໂຫສົດ (LOMWRU). ທ່ານຍັງເປັນແບບຢ່າງໃນເລື່ອງພາວະຄວາມເປັນຜູ້ນຳ, ຄວາມເຂັ້ມແຂງ-ອົດທົນ ແລະ ນ້ຳໃຈເພື່ອແຜ່-ຊ່ວຍເຫລືອທຸກຄົນພາຍໃນພະແນກຈຸລິນຊີ ກໍຄືໂຄງການ LOMWRU ລວມທັງຜົນງານຄົ້ນຄວ້າຂອງທ່ານໃນຂະແໜງຈຸລິນຊີ ແລະ ພະຍາດ scrub typhus. ທ່ານຍັງເປັນແບບຢ່າງທີ່ດີເລີດໃນເລື່ອງຂອງອາລົມຂີບຂັນ, ຄວາມທ້າທາຍ ແລະ ຄວາມເດັດດ່ຽວເຂັ້ມແຂງ ເຊິ່ງເປັນແບບຢ່າງທີ່ດີທີ່ສຸດໃນຂະນະເຈັບເປັນ. ພວກເຮົາທຸກຄົນບ່ວ່າຢູ່ໄກ້ ແລະ ໄກ ລ້ວນແຕ່ຄິດຮອດທ່ານຢ່າງສຸດຊຶ້ງ. ພວກເຮົາສະແດງຄວາມເສຍໃຈ-ອາໄລອາວອນຢ່າງສຸດຊຶ້ງ ຕໍ່ຄອບຄົວ ແລະ ທຸກຄົນໃນພະແນກວິເຄາະຈຸລິນຊີ ຕໍ່ການຈາກໄປຂອງທ່ານ ປອ. ດຣ. ລັດຕະນະພອນ ເພັດສຸວັນ ແລະ ຂໍໃຫ້ດວງວິນຍານຂອງທ່ານ ຈົ່ງໄປສຸຂະຕິເຖີດ.

ບົດສັງລວມຫຍໍ້

ກ. ໂຄງການຄົ້ນຄວ້າພະຍາດເຂດຮ້ອນລະຫວ່າງໂຮງໝໍມະໂຫສົດ-ແວວຄຳຕັ໊ສ-ມະຫາວິທະຍາໄລອ໋ອກຟອດ ຫຼື The Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit (LOMWRU) ເປັນໜ່ວຍງານຄົ້ນຄວ້າທາງຄລີນິກ ເຊິ່ງນອນຢູ່ໃນພະແນກວິເຄາະຈຸລິນຊີ, ໂຮງໝໍມະໂຫສົດ. ໂຄງການນີ້ ຖືກສ້າງຕັ້ງຂຶ້ນໃນປີ 2000 ພາຍໄຕ້ເຄືອຂ່າຍຂອງ MORU Tropical Network ແລະ ຕິດພັນຢ່າງຊະນິດແໜ້ນກັບໜ່ວຍງານຄົ້ນຄວ້າພະຍາດເຂດຮ້ອນມະຫາວິທະຍາໄລມະຫິດົນ-ອ໋ອກຟອດ ປະຈຳບາງກອກ (MORU-Bangkok) ແລະ ປະມານ 41% ຂອງວຽກຄົ້ນຄວ້າຂອງພວກເຮົາ ແມ່ນໄດ້ຕິດພັນກັບໜ່ວຍງານດັ່ງກ່າວ.

ຂ. ໂຄງການ LOMWRU ໄດ້ຮັບທຶນຊ່ວຍເຫລືອຫລັກ ຈາກທາງແວວຄຳຕັ໊ສ ປະເທດ ອັງກິດ ແລະ ທຶນອີກສ່ວນໜຶ່ງແມ່ນໄດ້ຈາກ US Naval Medical Research Centre, the Bill & Melinda Gates Foundation, The European Union, Department for International Development-UK (DFID), US-CDC – Laos, Fondation Total/Institute Pasteur, Global Good, Foundation for Innovative New Diagnostics, DTRA, Global Antibiotic Research Partnership, INTERPOL, Joint Inter-Agency Task Force and the Asian Development Bank. ນອກນີ້ ທາງໂຄງການຍັງໄດ້ຮັບການຊ່ວຍເຫລືອ ເປັນເຄື່ອງອຸປະກອນ ຈາກສະຖາບັນຄົ້ນຄວ້າເພື່ອການພັດທະນາ/ມະຫາວິທະຍາໄລແອ໋ກຊ-ມາກໄຊ ປະເທດຝລັ່ງ ແລະ ໂຄງການຄົ້ນຄວ້າພະຍາດ ອິກເກັດເຊຍ ຂອງສູນຄົ້ນຄວ້າທາງການແພດກອງທັບເຮືອ ສະຫະລັດອາເມລິກາ.

ຄ. ພະແນກວິເຄາະຈຸລິນຊີ ມີພະນັກງານ (ພາກລັດ) ທັງໝົດ 29 ຄົນ, ສ່ວນ LOMWRU ມີພະນັກງານໂຄງການ 42 ຄົນ, ໃນນີ້ 88% ແມ່ນຄົນລາວ ແລະ 56% ເປັນເພດຍິງ. ພວກເຮົາມີຫ້ອງວິເຄາະຈຸລະຊີວະວິທະຍາທາງຄລີນິກ, ຫ້ອງວິເຄາະທາງພັນທຸກຳສາດ, ຫ້ອງວິເຄາະເຊໂຣໂລຊີ, ແລະ ຫ້ອງວິເຄາະລະດັບ 3 (BSL3). ການປະຕິບັດງານໃນຫ້ອງວິເຄາະດັ່ງກ່າວ ແມ່ນເປັນໄປຕາມແນວທາງ-ລະບຽບການຄວາມປອດໄພ ຂອງມະຫາວິທະຍາໄລອ໋ອກຟອດ.

ງ. LOMWRU ຊ່ວຍບໍລິການບ່ຽມະຕິພະຍາດຊຶມເຊື້ອພາຍໃນໂຮງມະໂຫສົດ ແລະ ໂຮງໝໍຕ່າງແຂວງ ເຊັ່ນ ໂຮງໝໍແຂວງ ຫລວງນ້ຳທາ, ສາລະວັນ ແລະ ຊຽງຂວາງ, ເຮັດການຄົ້ນຄວ້າທາງຄລີນິກ, ແລະ ສ້າງຄວາມເຂັ້ມແຂງໃຫ້ແກ່ພະນັກງານພາກລັດໃນດ້ານການບ່ຽມະຕິພະຍາດ ແລະ ການເຮັດຄົ້ນຄວ້າ ໂດຍຜ່ານການຝຶກອົບຮົມ ແລະ ປະຕິບັດງານຕົວຈິງ. LOMWRU ຍັງມີການຮ່ວມມືກັບສູນໄຂ້ຍູງ, ແມ່ກາຝາ ແລະ ແມງໄມ້ ເຮັດໂຄງການຄົ້ນຄວ້າຢູ່ແຂວງເຊກອງ ແລະ ສະຫວັນນະເຂດ, ຮ່ວມມືກັບກົມອາຫານ ແລະ ຢາ ເຮັດໂຄງການຄົ້ນຄວ້າກ່ຽວກັບຄຸນນະພາບຂອງຢາ, ແລະ ມີໂຄງການຮ່ວມມືກ່ຽວກັບເຊື້ອຈຸລິນຊີຕ້ານຕ່ຢາຕ້ານເຊື້ອ ຮ່ວມກັບກົມຄວບຄຸມພະຍາດຕິດຕໍ່ ກະຊວງສາທາລະນະສຸກ.

ຈ. ຈຸດສຸມສຳລັບວຽກຄົ້ນຄວ້າຂອງພວກເຮົາໄດ້ແກ່ ສາເຫດ ແລະ ລະບາດວິທະຍາຂອງໄຂ້, ລັກສະນະການຕ້ານຂອງເຊື້ອພະຍາດຕ່ຢາປິ່ນປົວ ລວມທັງການບ່ຽມະຕິ ແລະ ປົ່ວປົວທີ່ສົມເຫດສົມຜົນ ເຊິ່ງທັງໝົດນີ້ແມ່ນແນໃສ່ເພື່ອສະໜອງຂໍ້ມູນສຳລັບສ້າງເປັນນະໂຍບາຍດ້ານສາທາລະນະສຸກໃນຂົງເຂດອາຊີ ແລະ ດ້ານຢາປິ່ນປົວໃນລະດັບສາກົນ.

ສ. ໃນປີ 2016 ທາງໂຄງການໄດ້ສະໜັບສະໜູນພະນັກງານລາວຈຳນວນ 19 ເທື່ອຄົນ ເຂົ້າຮ່ວມກອງປະຊຸມຢູ່ຕ່າງປະເທດຈຳນວນ 10 ຄັ້ງ ແລະ ໄດ້ໃຫ້ການສະໜັບສະໜູນພະນັກງານພາກລັດ 3 ຄົນ ສຶກສາຕໍ່ໃນລະດັບປະລິຍາເອກ ແລະ ປະລິນຍາໂທ ຢູ່ມະຫາວິທະຍາໄລມະຫິດົນ ແລະ ມະຫາວິທະຍາໄລອຳສະເຕີດຳ ປະເທດ ຮອນແລນ, ແລະ ອີກ 1 ຄົນ ສຶກສາໃນລະດັບປະລິນຍາຕີ ທີ່ມະຫາວິທະຍາໄລຂອນແກ່ນ.

ຊ. ໃນປີ 2016 ພວກເຮົາໄດ້ຕີພິມ ຫລື ກຳລັງຖືກຮັບຕີພິມເຜີຍແຜ່ຜົນຂອງການຄົ້ນຄວ້າລົງໃນວາລະສານການແພດສາກົນ ຈຳນວນ 47 ບົດ, ເຊິ່ງໃນນີ້ ມີ 39 ບົດທີ່ຖືກພິມເຜີຍແຜ່ໃນວາລະສານທີ່ມີການທົບທວນຄັກແນ່, ເປັນຈົດໝາຍເຫດ 3 ບົດ, ພິມລົງໃນປຶ້ມຕຳລາທາງການແພດຈຳນວນ 3 ພາກ, ເປັນບົດຄັດຫຍໍ້ 1 ບົດ ແລະ ລົງໃນບົດລາຍງານຂອງອົງການອະນາໄມໂລກ 1 ບົດ. ນັບຕັ້ງແຕ່ໂຄງການຖືກສ້າງຕັ້ງເປັນຕົ້ນມາ, ນັກຄົ້ນຄວ້າ ຂອງໂຄງການ LOMWRU ມີຜົນງານຕີພິມເຜີຍແຜ່ຜົນການຄົ້ນຄວ້າ ທັງໝົດ 295 ບົດ ລວມທັງປຶ້ມຕຳລາ ທາງການແພດອີກ ຈຳນວນ 18 ພາກ.

ຍ. ຜົນການຄົ້ນຄວ້າຂອງ LOMWRU ທີ່ຖືກນຳໄປຜັນຂະຫຍາຍ ເປັນແນວທາງນະໂຍບາຍ ດ້ານສາທາລະນະສຸກພາຍໃນປະເທດ ລວມມີ: ການຈັດຕັ້ງປະຕິບັດການສັກຢາກັນພະຍາດທີ່ເກີດຈາກເຊື້ອ Pneumococcus ແລະ ການສັກຢາກັນພະຍາດອັກເສບສະໝອງຍີ່ປຸ່ນ, ການປ່ຽນແປງແນວທາງການປິ່ນປົວພະຍາດໄຂ້ຍູງ ແລະ ພະຍາດໄຂ້ທໍລະພິດ (Typhoid). ນອກນີ້ ພວກເຮົາຍັງໄດ້ຄົ້ນພົບພະຍາດທີ່ສຳຄັນບາງຢ່າງ ເປັນຄັ້ງທຳອິດ ໃນ ປະເທດລາວ, ໄດ້ຊີ້ໃຫ້ເຫັນຄວາມສຳຄັນຂອງພະຍາດໄຂ້ແມງແດງ, ໄຂ້ຢ່ຽວໜູ, ໄຂ້ທໍລະພິດ, ເມລິອອຍໂດສ ຊິສ, ແລະ ອັກເສບສະໝອງຍີ່ປຸ່ນ ເຊິ່ງເຮັດໃຫ້ພວກເຮົາເຂົ້າໃຈຢ່າງເລິກເຊິ່ງຕື່ມ ກ່ຽວກັບລັກສະນະດ້ານລະ ບາດວິທະຍາ ແລະ ແນວທາງການປ້ອງກັນພະຍາດດັ່ງກ່າວ.

ດ. ສະຫລຸບຜົນຂອງການຄົ້ນຄວ້າທີ່ມີຄວາມໝາຍສຳຄັນ ຕໍ່ປະເທດລາວ ທີ່ໄດ້ຕີພິມເຜີຍແຜ່ ຫລື ກຳລັງຈະຖືກ ຕີພິມເຜີຍແຜ່ ໃນປີ 2016 ມີດັ່ງຕໍ່ໄປນີ້ (ກະລຸນາເບິ່ງລາຍລະອຽດຕື່ມໃນບົດລາຍງານ):

*** ລັກສະນະການຕ້ານຂອງເຊື້ອຈຸລິນຊີ ຕໍ່ຢາຕ້ານເຊື້ອ ກຳລັງເປັນບັນຫາສຳຄັນດ້ານສາທາລະ ນະສຸກ ແລະ ກໍ່ໃຫ້ເກີດຄວາມກັງວົນນັບມື້ຫລາຍຂຶ້ນໃນ ສປປ ລາວ ກໍ່ຄືໃນທົ່ວໂລກ.**

- ບັນຫາການຕ້ານ (ການດື້) ຂອງເຊື້ອ Enterobacteriaceae ຕໍ່ຢາຕ້ານເຊື້ອໃນກຸ່ມ Beta-lactamin (ESBL) ເປັນສາເຫດການຊຶມເຊື້ອສຳຄັນ ທີ່ພົບເຫັນໃນໂຮງໝໍມະໂຫສົດ. ອັນນີ້ຖືເປັນບັນຫາ ເຊື້ອຈຸ ລິນຊີ ຕ້ານຕໍ່ຢາຕ້ານເຊື້ອທີ່ພົບເຫັນເລື້ອຍທີ່ສຸດໃນຫ້ອງວິເຄາະຈຸລິນຊີວິທະຍາຂອງພວກເຮົາ ເມື່ອງ ຈາກອັດຕາການກວດພົບໃນໂຮງໝໍແມ່ນເພີ່ມຂຶ້ນເລື້ອຍໆ ແລະ ເຊື້ອດັ່ງກ່າວກໍຕ້ານຕໍ່ຢາຕ້ານເຊື້ອທີ່ມັກ ໃຊ້ເປັນປະຈຳ ເຊັ່ນ cephalosporins and penicillins. ພວກເຮົາພົບເຫັນເຊື້ອດັ່ງກ່າວໃນຄົນເຈັບ ບາງຄົນ ແລະ ເຊື້ອກໍຕ້ານຕໍ່ຢາຕ້ານເຊື້ອເກືອບທຸກຕົວ ເຮັດໃຫ້ພວກເຮົາກັງວົນວ່າ ພວກເຮົາຈະບໍ່ມີ ຢາທີ່ສາມາດປິ່ນປົວ-ຂ້າເຊື້ອໄດ້ໃນອະນາຄົດ ຫລື ຖ້າມີ ກໍຕ້ອງໃຊ້ຢາທີ່ມີລາຄາແພງທີ່ສຸດ ເຊິ່ງຄົນ ເຈັບອາດບໍ່ສາມາດຈ່າຍໄດ້.
- ເຊື້ອ ESBL bacteria ຍັງກວດພົບເຫັນໃນເປເຊັນທີ່ສູງ ໃນອາຈົມຂອງເດັກໂຮງຮຽນອະນຸບານທີ່ມີສຸຂະ ພາບແຂງແຮງ ໃນນະຄອນຫລວງວຽງຈັນ ແລະ ແຂວງວຽງຈັນ.
- ທີ່ໜ້າແປກໃຈກໍຄືວ່າ ESBL bacteria ຍັງພົບເຫັນໃນອາຈົມຂອງຄົນທີ່ມີສຸຂະພາບແຂງແຮງ ໃນບ້ານ ແຫ່ງໜຶ່ງ ຢູ່ເຂດຫ່າງໄກສອກຫລີກຂອງແຂວງຊຽງຂວາງ ເຊິ່ງຂໍ້ມູນນີ້ ຊີ້ໃຫ້ເຫັນວ່າ ESBL ເປັນ ບັນຫາຢູ່ຊຸມຊົນບົດຂອງລາວເຮົາແລ້ວ ແລະ ກໍຈະເປັນບັນຫາທີ່ໃຫ່ຍຫລວງໃນອະນາຄົດ ຖ້າເຮົາບໍ່ມີ ມາດຕະການຫຍັງ. ສິ່ງໜຶ່ງທີ່ໜ້າແປກໃຈກໍຄືວ່າ ປະຊາກອນພາຍໃນບ້ານດັ່ງກ່າວຈຳນວນຫລາຍສິມ ຄວນ (13.4%) ໄດ້ຮັບຢາຕ້ານເຊື້ອ ພາຍໃນ 2 ອາທິດ ກ່ອນໜ້າທີ່ມງານລົງໄປເຮັດການສຳຫລວດ ພາຍໃນບ້ານ.
- ນອກຈາກນີ້ ພວກເຮົາຍັງພົບວ່າ ຄົນຕ່າງປະເທດທີ່ມາຮ່ວມສຳມະນາທາງການແພດ ໃນນະຄອນ ຫລວງວຽງຈັນ (ໄດ້ເອົາຕົວຢ່າງອາຈົມໄປກວດທັນທີ ທີ່ມາເຖິງ ແລະ 3 ອາທິດຕໍ່ມາ) ແມ່ນພົບມີເຊື້ອ ຈຸລິນຊີທີ່ຕ້ານຕໍ່ຢາ cephalosporin ເຊິ່ງຂໍ້ມູນນີ້ຊີ້ໃຫ້ເຫັນວ່າ ເຊື້ອ ESBL ແມ່ນພົບໄດ້ຫລາຍໃນຄົນ

ແລະ ສິ່ງແວດລ້ອມຢູ່ວຽງຈັນ, ແລະ ກໍຊີ້ໃຫ້ເຫັນວ່າ ແຂກທີ່ມາຢ້ຽມຢາມ ກໍມີການຕິດເຊື້ອເຂົ້າໃນຕົວ ຢ່າງໄວວາ ເຊິ່ງເຮັດໃຫ້ມີຄວາມສ່ຽງຕໍ່ສຸຂະພາບຂອງເຂົາເຈົ້າ ແລະ ສ່ຽງຕໍ່ການແຜ່ກະຈາຍເຊື້ອໄປຫາ ບ່ອນອື່ນໆ.

- ພວກເຮົາພົບເຊື້ອ Enterobacteriaceae ທີ່ຕ້ານຕໍ່ຢາ carbapenem ເປັນຄັ້ງທຳອິດໃນ ສປປ ລາວ ແລະ ເຊື້ອ *E. coli* ດັ່ງກ່າວແມ່ນພົບທັງຢູ່ໃນຕົວຢ່າງໜອງ ແລະ ຕົວຢ່າງຈາກລະບົບຖ່າຍເທ. ເນື່ອງ ຈາກວ່າ ກຳລັງມີການລິເລີ່ມໃຊ້ຢາ carbapenems ໃນລາວ, ສະນັ້ນ ການຄົ້ນພົບດັ່ງກ່າວຈຶ່ງເຮັດໃຫ້ ພວກເຮົາມີຄວາມກັງວົນຫລາຍທີ່ສຸດ ແລະ ມັນຍ້ງກ້ອງໃຫ້ຕ້ອງມີການຕິດຕາມສຳລັບການນຳໃຊ້ຢາ ດັ່ງກ່າວຢ່າງເຂັ້ມງວດ.
- ສະພາບການຕ້ານຂອງເຊື້ອ *Streptococcus pyogenes* ຕໍ່ຢາຕ້ານເຊື້ອທີ່ພົບເຫັນໃນລາວມີຄື: ມີການ ຕ້ານຕໍ່ຢາ erythromycin and chloramphenicol ໃນລະດັບປານກາງ, ແຕ່ຍັງບໍ່ພົບ ການຕ້ານຕໍ່ຢາ penicillin ເຊິ່ງຂໍ້ມູນດັ່ງກ່າວບ່ົ່ງບອກວ່າ: ເຮົາບໍ່ຄວນໃຊ້ຢາ erythromycin and chloramphenicol ເພື່ອປິ່ນປົວຄົນເຈັບທີ່ຕິດເຊື້ອ *Streptococcus pyogenes* ແຕ່ໃຫ້ໃຊ້ຢາ penicillin.
- ໃນຂະນະທີ່ຂໍ້ມູນຫລັກຖານຫລາຍຢ່າງຊີ້ບອກວ່າ ມີຄວາມຈຳເປັນຕ້ອງເພີ່ມທະວີເອົາໃຈໃສ່ ເລື່ອງການ ນຳໃຊ້ຢາຕ້ານເຊື້ອ ແລະ ການຄວບຄຸມການຕິດເຊື້ອຢ່າງເຂັ້ມງວດນັ້ນ, ພວກເຮົາພົບຄົນພົບເຊື້ອ *Clostridium difficile* ໃນອາຈົມຂອງຄົນເຈັບຢູ່ໂຮງໝໍມະໂຫສົດ. ການເກີດມີເຊື້ອດັ່ງກ່າວ (ທີ່ເປັນສາ ເຫດສຳຄັນຂອງຖອກທ້ອງໃນໂຮງໝໍ) ແມ່ນບໍ່ແປກເລີຍ ເນື່ອງຈາກມີການນຳໃຊ້ຢາ cephalosporin ຢ່າງແຜ່ຫລາຍໃນໂຮງໝໍແຫ່ງຕ່າງໆຂອງນະຄອນຫລວງວຽງຈັນ.
- ຜົນການທົດສອບເຊື້ອ *N. gonorrhoeae* ໃສ່ຢາຕ້ານເຊື້ອຈຳນວນ 158 ເຊື້ອ ພົບວ່າ: 100% ແມ່ນ ຖືກກັບຢາ ceftriaxone and spectinomycin ແຕ່ພົບມີການຕ້ານໃນລະດັບສູງ ຕໍ່ຢາ ciprofloxacin, penicillin and tetracycline. ຂໍ້ມູນນີ້ ຊີ້ໃຫ້ເຫັນວ່າ ຢາ ceftriaxone and spectinomycin ໜ້າຈະ ມີປະສິດທິພາບສູງຕໍ່ເຊື້ອ *N. gonorrhoeae* ໃນ ສປປ ລາວ. ການຕິດຕໍ່ຫາຄູ່ນອນຂອງຄົນເຈັບທີ່ຕິດ ເຊື້ອ ເພື່ອມາປິ່ນປົວຮ່ວມກັນ ຖືເປັນແນວທາງສຳຄັນສຳລັບການຫລຸດຜ່ອນພະຍາດ ພຕພ.
- ພວກເຮົາພົບວ່າ ຄວາມຮູ້ກ່ຽວກັບລັກສະນະການຕ້ານຂອງເຊື້ອຕໍ່ຢາຕ້ານເຊື້ອ ແລະ ການສັ່ງຢາຕ້ານ ເຊື້ອທີ່ສົມເຫດສົມຜົນຂອງທ່ານໝໍ ແມ່ນຍັງບໍ່ທັນສູງ. ສະນັ້ນ ຈຶ່ງມີຄວາມຈຳເປັນຢ່າງຮີບດ່ວນ ທີ່ຈະ ຕ້ອງແກ້ໄຂບັນຫາດັ່ງກ່າວ.
- ການແຜ່ຂະຫຍາຍຂອງເຊື້ອຈຸລິນຊີທີ່ຕ້ານຕໍ່ຢາຕ້ານເຊື້ອໃນ ສປປ ລາວ ຈະສິ່ງຜົນສະທ້ອນຢ່າງໃຫ່ຍ ຫລວງຕໍ່ຄົນເຈັບ, ຊຸມຊົນ ແລະ ເສດຖະກິດ. ສະນັ້ນ ຈຶ່ງມີຄວາມຈຳເປັນຢ່າງຮີບດ່ວນ ທີ່ຈະຕ້ອງ ເອົາໃຈໃສ່ວຽກງານຄວບຄຸມການຕິດເຊື້ອ, ການນຳໃຊ້ຢາຕ້ານເຊື້ອຢ່າງສົມເຫດສົມຜົນ, ແລະ ລະບຽບ ຫລັກການຕ່າງໆທີ່ກ່ຽວຂ້ອງ.
- ເພື່ອຊ່ວຍໃຫ້ຂໍ້ມູນ-ຫລັກຖານ ແລະ ແນວທາງປະຕິບັດທີ່ເໝາະສົມ, ພວກເຮົາໄດ້ຮ່ວມມືກັບ ກະຊວງ ສາທາລະນະສຸກ ລວມທັງຄູ່ຮ່ວມງານອື່ນໆ ຈັດຕັ້ງຄະນະກຳມະການຄວບຄຸມເຊື້ອຈຸລິນຊີທີ່ຕ້ານຕໍ່ຢາ ຕ້ານເຊື້ອ ເຊິ່ງຄະນະກຳມະການນີ້ ຈະເຮັດວຽກສົມທົບກັບຄະນະກຳມະການຂອງ WHO/ FAO/OIE.

*** ສາເຫດຂອງໄຂ້ໃນເຂດຊົນນະບົດຂອງ ສປປ ລາວ.** ພາຍໄຕ້ການສະໜັບສະໜູນຂອງ US Naval Medical Research Centre ພວກເຮົາໄດ້ຂະຫຍາຍໂຄງການຄົ້ນຄວ້າຫາສາເຫດຂອງໄຂ້ ໃນຄົນ ເຈັບເຂດນອກ ຂອງໂຮງໝໍແຂວງຊຽງຂວາງ, ສາລະວັນ ແລະ ຫລວງນ້ຳທາ. ສາເຫດຕົ້ນຕໍຂອງໄຂ້ທີ່ພົບ ໄດ້ແກ່: ພະຍາດໄຂ້ຫວັດ (60%), ໄຂ້ຍ່ຽວໜູ (15%), ໄຂ້ຍູງລາຍ (10%), Scrub typhus (5%), ຊຶມເຊື້ອເລືອດ (5%), murine typhus (3%), ອັກເສບສະໝອງຍີ່ປຸ່ນ (1%) ແລະ *Rickettsia* spp.

(1%). ພວກເຮົາມີແຜນດຳເນີນການສຶກສາໃນລັກສະນະນີ້ອີກ ແຕ່ຈະເຮັດໃນຄົນເຈັບນອນ ພາຍຫລັງປີໃໝ່ ລາວປີນີ້ ແລະຈະນຳໃຊ້ຜົນຈາກການຄົ້ນຄວ້າມາສົນທະນາເພື່ອວາງເປັນແນວທາງການປິ່ນປົວໃນອະນາຄົດ.

*** ໃນ ສປປ ລາວ ມີເຊື້ອພະຍາດຈັກຊະນິດ?** ໃນ 15 ປີຜ່ານມາ ປະກົດວ່າໄດ້ມີຈຳນວນເຊື້ອພະຍາດທີ່ຖືກກວດພົບຫລາຍຂຶ້ນຢ່າງໄວວາ. ແລ້ວຍັງຈະຖືກຄົ້ນພົບອີກບໍ່? ຜົນການຊອກຄົ້ນຫາໃນປົດຕີພົມເຜີຍແຜ່ ແຕ່ປີ 1874 ຫາ ປີ 2016 ພົບວ່າ: ມີເຊື້ອພະຍາດໃນຄົນຈຳນວນ 148 ຊະນິດ ທີ່ຖືກບັນທຶກໃນ ສປປ ລາວ ບໍ່ວ່າຈະດ້ວຍເຕັກນິກການປູກເຊື້ອ ຫລື ເຕັກນິກທາງພັນທຸກຳ. ຜົນຈາກການໃຊ້ mathematical model ແບບໃໝ່ ໄດ້ຄາດຄະເນໄວ້ວ່າ: ໃນ ສປປ ລາວ ມີເຊື້ອພະຍາດຂອງຄົນປະມານ 157 ຊະນິດ - ສະແດງວ່າ ເຊື້ອພະຍາດຈຳນວນຫລວງລາຍທີ່ມີໃນລາວ ແມ່ນໄດ້ຖືກຄົ້ນພົບແລ້ວ.

*** ສາເຫດຂອງການຊຶມເຊື້ອລະບົບປະສາດສູນກາງ ໃນຄົນເຈັບຈຳນວນ 1,065 ຄົນ ທີ່ໄດ້ຮັບການແທງນ້ຳໄຂສັນຫລັງໄປກວດ ທີ່ໂຮງໝໍມະໂຫສົດ ຊ່ວງປີ 2003-2011** ກຳລັງຖືກວິເຄາະຂໍ້ມູນ. ປະມານ 42% ຂອງຄົນເຈັບ ແມ່ນຮູ້ສາເຫດຈາກການກວດນ້ຳໄຂສັນຫລັງ ແລະ ຈາກການກວດເລືອດ. ໃນຈຳນວນຄົນເຈັບທີ່ຕິດເຊື້ອພຽງຊະນິດດຽວ, ສາເຫດທີ່ພົບຫລາຍກວ່າໝູ່ແມ່ນ ອັກເສບສະໝອງຍີ່ປຸ່ນ (8.8%), *Cryptococcus* spp. (6.6%), *Orientia tsutsugamushi* (2.9%), Dengue virus (2.5%), *Leptospira* spp. (2.3%), *Rickettsia* spp. (2.3%), *Streptococcus pneumoniae* (2.1%), *Mycobacterium tuberculosis* (1.9%), *Herpes simplex virus* (HSV) (1.4%), *Cytomegalovirus* (CMV) 12 (1.1%), *Enterovirus* (0.9%), *Varicellazoster virus* (VZV) (0.6%), *Mumps virus* (0.5%) and *P. falciparum* (0.4%). ອັດຕາການຕາຍແມ່ນສູງເຖິງ 26.3%. ປັດໄຈທີ່ພົວພັນກັບສາເຫດການຕາຍຢ່າງມີຄວາມສຳຄັນດ້ານສະຖິຕິໄດ້ແກ່: ການທີ່ມີລະດັບ Lactate ສູງໃນນ້ຳໄຂສັນຫລັງ (p=0.001) ແລະ ຄະແນນ GCS ຕ່ຳ (p<0.001). ຂໍ້ມູນນີ້ ຊີ້ໃຫ້ເຫັນວ່າ: ເຮົາຕ້ອງເອົາໃຈໃສ່ເປັນພິເສດຕໍ່ຄົນເຈັບທີ່ມີຄະແນນ GCS ຕ່ຳ ເຊິ່ງຄົນເຈັບກຸ່ມນີ້ ມີແນວໂນ້ມທີ່ຈະເສຍຊີວິດສູງ - ສະນັ້ນ ການປິ່ນປົວປະຄັບປະຄອງຄົນເຈັບທີ່ສະຕິບໍ່ດີ ເຊັ່ນ ການຕິດຕາມໃນຫ້ອງມໍລະສູມ ສົມທົບກັບການໃຫ້ຢາຕ້ານເຊື້ອທີ່ເໝາະສົມ ໃນທັນທີອາດເປັນແນວທາງຫລັກໃນການຫລຸດຜ່ອນອັດຕາການຕາຍລົງ. ຂໍ້ມູນນີ້ ຈະຖືກນຳໃຊ້ເພື່ອພິຈາລະນາເປັນແນວທາງປິ່ນປົວການຊຶມເຊື້ອລະບົບປະສາດສູນກາງ.

* ພວກເຮົາຍັງພົບວ່າ ສ່ວນໃຫ່ຍຂອງນ້ຳໄຂສັນຫລັງທີ່ສົ່ງກວດ (94%) ແມ່ນຜິດປົກກະຕິ ເຊິ່ງຊີ້ໃຫ້ເຫັນວ່າ: ເຮົາຄວນເອົາໃຈໃສ່ໃຫ້ມີການເຈາະນ້ຳໄຂສັນຫລັງໃນຄົນເຈັບທີ່ສົງໃສວ່າ ມີການຊຶມເຊື້ອລະບົບປະສາດສູນກາງທຸກກໍລະນີ ເພື່ອຫລີກເວັ້ນການພາດໂອກາດປິ່ນປົວຄົນເຈັບດັ່ງກ່າວ.

* ການສຶກສາຄົ້ນຄວ້າຂະໜາດໃຫ່ຍ ເພື່ອຫາສາເຫດ ແລະ ຜົນກະທົບຂອງໄຂ້ໃນແມ່ຍິງຖືພາ ທີ່ເມືອງປາກງື່ມ, ນະຄອນຫລວງວຽງຈັນ ໄດ້ສຳເລັດແລ້ວ. ອັດຕາການຕາຍຂອງແມ່ທີ່ສູງ ໃນ ສປປ ລາວ (ສູງກວ່າໝູ່ໃນອາຊີຕາເວັນອອກຊ່ຽງໄຕ້) ເຮັດໃຫ້ພວກເຮົາເຮັດການສຶກສາຄັ້ງນີ້. ໃນຈຳນວນແມ່ມານ 1,000 ຄົນ ທີ່ເຮັດການສຶກສາ, ມີ 110 ຄົນ ທີ່ມີອາການໄຂ້ໃນລະຫວ່າງຖືພາ ຫລື ຫລັງເກີດລູກ. ໃນຈຳນວນນີ້ 18 ຄົນ ລຸລູກ, ເດັກຕາຍຮອບເກີດມີ 6 ຄົນ, 3 ຄົນ ຕາຍຫລັງເກີດມາໃໝ່ໆ, ແມ່ 1 ຄົນເສຍຊີວິດ (ຍ້ອນຖືພາລູກນອກທ້ອງ) ແລະ ມີເດັກຈຳນວນ 11 ຄົນ ທີ່ເກີດມາຜິດປົກກະຕິ. ປະຈຸບັນ ພວກເຮົາກຳລັງວິເຄາະຫາສາເຫດຂອງໄຂ້ ແລະ ຫາຄວາມສຳພັນລະຫວ່າງອາການໄຂ້ ກັບພາວະອື່ນໆ ເຊັ່ນ ນ້ຳໜັກເກີດຕ່ຳ ແລະ ເດັກເກີດມາເສຍຊີວິດ.

* ພວກເຮົາພົບວ່າ: ການກວດຫາ 'serotype' ຂອງເຊື້ອໄຂ້ຍຸງລາຍ ສາມາດເຮັດໄດ້ດ້ວຍການກວດ PCR ຈາກສິ້ນຂອງແຜ່ນຈຸ່ມກວດແບບໄວທີ່ມີຜົນກວດ NS1 ບວກ. ການຄົ້ນພົບນີ້ ຈະເປັນປະໂຫຍດສຳລັບການເຝົ້າລະວັງຊະນິດຂອງເຊື້ອໄວຣັສໄຂ້ເລືອດອອກໃນຫລາຍໆພື້ນທີ່ຂອງຂົງເຂດອາຊີ ທີ່ຍັງບໍ່ມີການເຝົ້າລະວັງພະຍາດເທື່ອ.

* ການບິ່ງມະຕິອັກເສບສະໝອງຍີ່ປຸ່ນ. ພວກເຮົາພົບວ່າ ການເກັບຕົວຢ່າງນ້ຳໄຂສັນຫລັງໃສ່ເຈ້ຍຊັບ ສາ ມາດນຳໄປບິ່ງມະຕິອັກເສບສະໝອງຍີ່ປຸ່ນໄດ້ ໂດຍສະເພາະໃນບ່ອນທີ່ບໍ່ມີເຄື່ອນໄຂ ສຳລັບບິ່ງມະຕິໃນຫ້ອງ ວິເຄາະ. ນອກນີ້ ມັນຍັງຈະຊ່ວຍປັບປຸງການເຝົ້າລະວັງລະດັບຊາດສຳລັບພະຍາດດັ່ງກ່າວໃຫ້ດີຂຶ້ນກວ່າເກົ່າ ແນໃສ່ເພື່ອສະໜອງຂໍ້ມູນສຳລັບນະໂຍບາຍການສັກຢາວັກແຊງກັນພະຍາດດັ່ງກ່າວ.

* ການບິ່ງມະຕິໄຂ້ຍຸງແບບໄວວາ. ພວກເຮົາໄດ້ປະເມີນຄວາມສາມາດຂອງພະນັກງານແພດ ໃນການນຳໃຊ້ ຊຸດກວດສອບຄຸນນະພາບຂອງແຜ່ນຈຸ່ມກວດໄຂ້ຍຸງ - ເຊິ່ງຖືກນຳໃຊ້ຢ່າງແຜ່ຫລາຍ ເພື່ອບິ່ງມະຕິໄຂ້ຍຸງໃນ ສປປ ລາວ (ທີ່ອາດເຊື່ອມຄຸນນະພາບ ເວລາຢູ່ໃນສະພາບອາກາດຮ້ອນ). ພາຍຫລັງການອົບຮົມວິທີນຳໃຊ້ ຊຸດກວດສອບຄຸນນະພາບດັ່ງກ່າວ, ຜູ້ເຂົ້າຮ່ວມການອົບຮົມສ່ວນໃຫ່ຍສາມາດເຮັດການກວດສອບ 6 ຂັ້ນ ຕອນຫລັກໄດ້ຢ່າງຖືກຕ້ອງ ແລະ ປະມານ 97% ຂອງຜູ້ເຂົ້າຮ່ວມ ສາມາດບອກແນວທາງທີ່ຈະຕ້ອງປະຕິ ບັດສຳລັບການນຳໃຊ້ຊຸດກວດສອບຄຸນນະພາບໃນພາກສະໜາມໄດ້ຢ່າງຖືກຕ້ອງ. ຊຸດກວດສອບຄຸນນະພາບ ດັ່ງກ່າວ ຈະເຮັດໃຫ້ພະນັກງານແພດມີຄວາມໝັ້ນໃຈໃນຜົນກວດໄຂ້ຍຸງຫລາຍຂຶ້ນກວ່າເກົ່າ ເຊິ່ງຈະສົ່ງຜົນດີ ໃຫ້ແກ່ໂຄງການຄວບຄຸມພະຍາດໄຂ້ຍຸງ. ຂໍ້ມູນຈາກການສຶກສາຄັ້ງນີ້ ຊີ້ໃຫ້ເຫັນວ່າ ເຮົາສາມາດເອົາຊຸດ ກວດສອບຄຸນນະພາບ ມານຳໃຊ້ ເພື່ອກວດສອບຄຸນນະພາບຂອງແຜ່ນຈຸ່ມໄຂ້ຍຸງໄດ້.

* ຂໍ້ມູນຈາກການສຶກສາກ່ຽວກັບສາເຫດຂອງໄຂ້ ໃນລາວ, ກຳປູເຈຍ ແລະ ຊາຍແດນໄທ-ພະມ້າ ຊີ້ໃຫ້ ເຫັນວ່າ ການກວດ C-reactive protein ສາມາດນຳໃຊ້ເປັນຕົວຊີ້ບອກວ່າ ຄົນເຈັບທີ່ເຂົ້າມາດ້ວຍ ອາການໄຂ້ ຈຳເປັນຕ້ອງໄດ້ຮັບຢາຕ້ານເຊື້ອ ຫລືບໍ່. ນອກນີ້ ຫລັກຖານຈາກການຄົ້ນຄວ້າຢູ່ເຂດຊົນນະ ບົດຂອງລາວ ຍັງຊີ້ໃຫ້ເຫັນວ່າ ຊຸດການກວດ CRP ແບບໄວວາ ແມ່ນມີຄວາມແມ່ນຍຳສູງ ແລະ ອາດໃຊ້ ເປັນເຄື່ອງມືຕັດສິນ (ໃນບ່ອນທີ່ຂາດເຂີນຫ້ອງວິເຄາະ) ເບິ່ງວ່າ ຄົນເຈັບຈຳເປັນຕ້ອງໄດ້ຮັບຢາຕ້ານເຊື້ອ ຫລືບໍ່. ການວິເຄາະເບິ່ງຄວາມຄຸ້ມຄ່າ ຊີ້ໃຫ້ເຫັນວ່າ ການນຳໃຊ້ຊຸດການກວດ CRP ແບບໄວວາ ໃນເຂດ ຊົນນະບົດຂອງລາວ ອາດມີຄວາມຄຸ້ມຄ່າ ແລະ ອາດຊ່ວຍຫລຸດຜ່ອນການນຳໃຊ້ຢາຕ້ານເຊື້ອລົງໄດ້ຫລາຍ ທີ່ສຸດສຳລັບການຊຶມເຊື້ອຈາກໄວຣັສ.

* ໂຕເຫັບ ແລະ ເຊື້ອພະຍາດໃນຄົນ. ພວກເຮົາໄດ້ຮ່ວມມືກັບສະຖາບັນປາສເຕີ ແລະ US Navy ໃນ ການຄົ້ນຫາເຊື້ອພະຍາດຂອງຄົນໃນໂຕເຫັບທີ່ເກັບມາຈາກແຂວງຄຳມ່ວນ ແລະ ພົບວ່າ ມີໂຕເຫັບຈຳນວນ ຫລາຍສົມຄວນ ທີ່ພົບເຫັນ ດີເອັນເອ ຂອງເຊື້ອພະຍາດ *Rickettsia* spp ລວມທັງ 3 ເຊື້ອຊະນິດໃໝ່ ຄື: *Candidatus* Rickettsia laoensis, *Candidatus* Rickettsia mahosotii and *Candidatus* Rickettsia khammouanensis. ນອກນີ້ ພວກເຮົາຍັງພົບ ເຊື້ອ *Ehrlichia* spp., *Coxiella burnetii*, *Anaplasma* spp. and *Borrelia* spp. ການຄົ້ນພົບນີ້ ຊ່ວຍໃຫ້ພວກເຮົາຕີກອບການຄົ້ນພົບເຊື້ອພະຍາດທີ່ເກີດຈາກເຫັບ ໃນວົງ ແຄບຫລາຍຂຶ້ນກວ່າເກົ່າ.

* ເຊື້ອ *Orientia tsutsugamushi* ໃນປະເທດຊີລີ. ພວກເຮົາໄດ້ຊ່ວຍນັກຄົ້ນຄວ້າປະເທດເຢຍລະມັນ ແລະ ຊີລີ ໃນການບິ່ງມະຕິທາງພັນທຸກຳສຳລັບເຊື້ອ *Orientia tsutsugamushi* ໃນຕົວຢ່າງຂອງຄົນເຈັບທີ່ເດີນທາງ ມາຈາກພາກໃຕ້ຂອງປະເທດຊີລີ. ການຄົ້ນພົບນີ້ ໄດ້ເຮັດໃຫ້ເຮົາຮູ້ ແລະ ເຂົ້າໃຈກ່ຽວກັບການກະຈາຍ ຂອງພະຍາດ scrub typhus ແລະ ນຳໄປສູ່ການຕັ້ງຫລາຍໆຄຳຖາມວ່າ: ເຊື້ອ *Orientia tsutsugamushi* ພົບໃນອາເມລິກາໃຕ້ຫລາຍປານໃດ ແລະ ມັນມີການແຜ່ເຊື້ອຄືແນວໃດ?

* ເມລິອອຍໂດຊິສ ເປັນສາເຫດທີ່ສຳຄັນຂອງການຊຶມເຊື້ອເລືອດທີ່ມັກຈະຖືກຫລົງລືມ ໃນລາວ ແລະ ໃນ ຂົງເຂດປະເທດເຂດຮ້ອນ. ນັບແຕ່ປີ 1999 ມາຮອດປະຈຸບັນ ພວກເຮົາໄດ້ບິ່ງມະຕິຄົນເຈັບເປັນພະຍາດ ເມລິອອຍ ຈຳນວນຫລາຍກວ່າ 1,100 ຄົນ (ສະເພາະໃນປີ 2016 ພົບ 158 ຄົນ) ແລະ ພວກເຮົາກັ່ງວິນ

ວ່າ ຍັງມີຄົນເຈັບພະຍາດດັ່ງກ່າວອີກຫລາຍໆຄົນ ທີ່ບິ່ງມະຕິບໍ່ໄດ້ ແລະ ອາດເສຍຊີວິດຈາກການຕິດເຊື້ອ ພະຍາດດັ່ງກ່າວ ໂດຍສະເພາະໃນເຂດພາກໄຕ້ຂອງລາວ.

* ຄວາມສ່ຽງຂອງຜູ້ຄ້າຂາຍສັດປ່າ. ຕະຫລາດໃນ ສປປ ລາວ ເປັນສູນລວມຂອງຄົນໃນສັງຄົມ ແຕ່ພວກ ເຮົາຍັງບໍ່ທັນມີຂໍ້ມູນກ່ຽວກັບຄວາມຮັບຮູ້ຂອງຜູ້ຄ້າຂາຍ ຕໍ່ກັບຄວາມສ່ຽງດ້ານສຸຂະພາບທີ່ອາດເກີດຈາກສິ່ງທີ່ ເຂົາເຈົ້າຂາຍ ເຊັ່ນດຽວກັນກັບສັດປ່າ ແລະ ສັດບ້ານ ທີ່ສາມາດເປັນຕົວການນຳເຊື້ອພະຍາດມາສູ່ຄົນ. ສະນັ້ນ ພວກເຮົາຈຶ່ງໄດ້ເຮັດການຄົ້ນຄວ້າໃນຕະຫລາດກັບຊາວຄ້າຂາຍສັດປ່າ ແລະ ພົບວ່າ ເກືອບທັງໝົດ ຂອງຜູ້ຄ້າຂາຍດັ່ງກ່າວ ແມ່ນມີຄວາມຮັບຮູ້ທີ່ຕ່ຳຕໍ່ກັບຄວາມສ່ຽງຕໍ່ສຸຂະພາບຂອງເຈົ້າເຈົ້າໃນສິ່ງທີ່ເຂົາເຈົ້າ ຂາຍ. ຂໍ້ມູນນີ້ ຊີ້ໃຫ້ເຫັນວ່າ ມີຄວາມຈຳເປັນຕ້ອງເຮັດການຄົ້ນຄວ້າ ດ້ານສຸຂະພາບໜຶ່ງດຽວຕື່ມອີກ ເພື່ອ ໃຫ້ເຂົ້າໃຈກ່ຽວກັບຄວາມສ່ຽງ ແລະ ໃຫ້ໂຄສະນາສຸຂະສຶກສາແກ່ຊາວຄ້າຂາຍ ແລະ ຜູ້ທີ່ມາຊື້ສິນຄ້າ.

* ເຊື້ອພະຍາດໃນຊີ້ນສັດປ່າ. ການວິໄຈຕົວຢ່າງຊີ້ນສັດປ່າ ທາງພັນທຸກຳ ຈຳນວນ 400 ຕົວຢ່າງ ຈາກສັດ ປ່າທີ່ມີກະດູກສັນຫລັງ (ສ່ວນໃຫ່ຍແມ່ກະຮອກ-ກະແຕ) ທີ່ໄດ້ມາຈາກຕະຫລາດ ພົບວ່າ ມີສັດຈຳນວນ 44 ໂຕ ທີ່ມີເຊື້ອ *Leptospira* spp., 6 ໂຕ ມີເຊື້ອ *Rickettsia* spp. (ລວມທັງເຊື້ອ *R. felis* 1 ເຊື້ອ) ກັບເຊື້ອ *L. garvieae, Kurthia* spp., *Ehrlichia* spp. TC251-2, and *A. marginale*. ການກວດພົບເຊື້ອ *Leptospira* spp. ໃນອັດຕາທີ່ສູງ ຈາກຕົວຢ່າງທີ່ຕ້ອຍມາຈາກທ່ຍ່ງວ-ອະໄວຍະວະເພດຂອງສັດ ຊີ້ໃຫ້ ເຫັນເຖິງຄວາມເປັນໄປໄດ້ສຳລັບການສົ່ງຕໍ່ພະຍາດໄຂ້ຍ່ງວໜູ ຈາກໂຕກະຮອກ-ກະແຕ ໄປສູ່ຄົນໃນຕະ ຫລາດ. ການຄົ້ນພົບເຊື້ອ *R. felis* ເຊິ່ງເປັນເຊື້ອຕະກຸນຣິກເກັດເຊຍທີ່ພົບໃໝ່ ຖືເປັນລາຍງານຄັ້ງທຳອິດ ໃນສັດຕະກຸນກະຮອກ-ກະແຕ.

* ພະຍາດໄຂ້ຍູງໃນແມ່ຍິງຖືພາ. ທີ່ຜ່ານມາ ໃນ ສປປ ລາວ ຍັງບໍ່ທັນມີຂໍ້ມູນກ່ຽວກັບພະຍາດໄຂ້ຍູງໃນແມ່ ຍິງຖືພາເທື່ອ. ພວກເຮົາໄດ້ເຮັດການສຳຫລວດ 2 ຄັ້ງ ໃນປີ 2014 ເພື່ອຊອກຫາຄວາມຊຸກຊຸມຂອງໄຂ້ຍູງ ໃນແມ່ຍິງຖືພາ ທີ່ເມືອງວາປີ ແຂວງສາລະວັນ. ຜົນການສຳຫລວດພົບວ່າ ໃນຈຳນວນແມ່ຍິງຖືພາ 204 ຄົນ ນັ້ນ, 12 ຄົນ (5.9%) ແມ່ນກວດພົບເຊື້ອໄຂ້ຍູງດ້ວຍເຕັກນິກ RTqPCR ເຊິ່ງ 11 ຄົນ ແມ່ນເຊື້ອວິ ວັກ ແລະ ອີກ 1 ຄົນ ແມ່ນເຊື້ອປະສົມວິວັກ-ຟານຊີປາຣອມ. ໃນຈຳນວນດັ່ງກ່າວ 9 ຄົນ ບໍ່ສາມາດກວດ ພົບຈາກການສ່ອງກ້ອງຈຸລະທັດ. ການຕິດເຊື້ອສ່ວນໃຫ່ຍບໍ່ມີອາການໄຂ້ ແລະ ເປັນເຊື້ອວິວັກ ທີ່ບໍ່ສາມາດ ກວດພົບດ້ວຍກ້ອງຈຸລະທັດ. ຂໍ້ມູນນີ້ເປັນຫລັກຖານທີ່ສຳຄັນສຳລັບການວາງຍຸດທະສາດລະດັບຊາດໃນ ການກວດກັ່ນຕອງ ແລະ ການປ້ອງກັນພະຍາດໄຂ້ຍູງໃນແມ່ຍິງຖືພາ ພາຍໃນ ສປປ ລາວ.

* ໄຂ້ຍູງທີ່ບໍ່ມີອາການໄຂ້. ການພັດທະນາຮູບແບບການບິ່ງມະຕິພະຍາດໄຂ້ຍູງແບບໃໝ່ທີ່ກ້າວໜ້າ-ທັນສະ ໄໝ ໄດ້ເຮັດໃຫ້ເຮົາສາມາດກວດພົບຄົນທີ່ຕິດເຊື້ອພະຍາດໄຂ້ຍູງໃນອາຊີ ແຕ່ບໍ່ມີອາການໄຂ້ - ເຂົາເຈົ້າ ເຫລ່ານີ້ ຖືເຊື້ອເປັນເວລາດົນນານໃນກະແສເລືອດໃນລະດັບທີ່ບໍ່ສາມາດກວດພົບດ້ວຍກ້ອງຈຸລະທັດ ແລະ ແຜ່ນຈຸ່ມໄຂ້ຍູງ. ການຕິດເຊື້ອຮູບແບບນີ້ອາດເປັນແຫລ່ງເກັບເຊື້ອທີ່ສຳຄັນ ແລ້ວແຜ່ກະຈາຍຕໍ່ໄປໃຫ້ຜູ້ອື່ນ. ການສຶກສາຄົ້ນຄວ້າຂອງພວກເຮົາທີ່ແຂວງສະຫວັນນະເຂດພົບວ່າ: ປະມານ 20% ຂອງຊາວບ້ານ ແມ່ນມີ ເຊື້ອໄຂ້ຍູງໃນເລືອດ ຈາກການກວດດ້ວຍເຕັກນິກ ultra-sensitive quantitative PCR. ນອກນີ້ ຍັງພົບວ່າ ເຊື້ອໄຂ້ຍູງຟານຊີປາຣອມ ມີລັກສະນະການຕ້ານຕໍ່ຢາປິ່ນປົວໄຂ້ຍູງອາກເຕມີຊີນິນ ຢູ່ລະຫວ່າງ 7-75%. ຂໍ້ ມູນນີ້ ເຮັດໃຫ້ເຮົາມີຄວາມກັງວົນ ແຕ່ກໍມີຄວາມສຳຄັນສຳລັບວຽກງານຄວບຄຸມພະຍາດໄຂ້ຍູງໃນ ສປປ ລາວ ລວມທັງບັນດາປະເທດລຸ່ມແມ່ນ້ຳຂອງ. ສະນັ້ນ ການຄົ້ນຄວ້າ-ພິຈາລະນາເພື່ອກຳຈັດພະຍາດໄຂ້ຍູງ ຊະນິດຟານຊີປາຣອມ ຈຶ່ງຖືເປັນບູລິມະສິດອັນດັບໜຶ່ງໃນ ສປປ ລາວ ແລະ ໃນປະຈຸບັນພວກເຮົາກຳລັງ ດຳເນີນໂຄງການກຳຈັດພະຍາດໄຂ້ຍູງຕົວແບບ ຢູ່ເມືອງນອງ, ແຂວງສະຫວັນນະເຂດ.

* ປະຈຸບັນ ຍັງມີຄວາມກັງວົນກ່ຽວກັບການແຜ່ກະຈາຍຂອງເຊື້ອໄຂ້ຍຸງຟານຊີປາຣອມທີ່ຕ້ານຕ່ຍາ ອາກເຕມີຊີນິນ ຢູ່ທາງພາກໄຕ້ຂອງລາວ ແລະ ມີຄວາມຈຳເປັນຈະຕ້ອງໄດ້ຫາລືກັນວ່າ ເຮົາຈະໃຊ້ຍາປິ່ນປົວຊະນິດໃດ ຖ້າຍາປະສົມ ACTs ສອງຕົວຫາກໃຊ້ບໍ່ໄດ້ຜົນແລ້ວ. ສະນັ້ນ ພວກເຮົາກຳລັງຮ່ວມມືກັບສູນໄຂ້ຍຸງ, ແມ່ກາຝາກ ແລະ ແມງໄມ້ ເຮັດການຄົ້ນຄວ້າແບບຫລາຍສູນ (TRAC-2) ເພື່ອສຶກສາການຕ້ານຂອງເຊື້ອໄຂ້ຍຸງຟານຊີປາຣອມຕ່ຍາອາກເຕມີຊີນິນ ຢູ່ທີ່ໂຮງໝໍແຂວງເຊກອງ ໂດຍແມ່ນໜ່ວຍງານຄົ້ນຄວ້າ MORU ຢູ່ບາງກອກເປັນຜູ້ປະສານງານ. ການສຶກສານີ້ ເປັນໃນຮູບແບບການທົດລອງທາງຄລີນິກແບບຊຸ່ມ ເພື່ອປຽບທຽບໄລຍະເວລາທີ່ເຊື້ອໝົດໄປຈາກກະແສເລືອດ ລະຫວ່າງຢາ artemether-lumefantrine ແລະ artemether-lumefantrine plus amodiaquine. ພວກເຮົາຄາດວ່າ ຂໍ້ມູນທີ່ໄດ້ຈາກການສຶກສານີ້ ຈະເປັນປະໂຫຍດສຳລັບການວາງແນວທາງນະໂຍບາຍປິ່ນປົວໄຂ້ຍຸງ ຂອງ ສປປ ລາວ ໃນຕໍ່ໜ້າ.

* ຜົນການສຶກສາທົດລອງກ່ຽວກັບຢາສະເຕຣອຍ ໃນຄົນເຈັບ HIV ທີ່ເປັນເຍື່ອຫຸ້ມສະໝອງອັກເສບຈາກເຊື້ອເຫັດ (Cryptococcus) ຊີ້ໃຫ້ເຫັນວ່າ ຄົນເຈັບທີ່ໄດ້ຢາສະເຕຣອຍ ແມ່ນມີການຜົນຂ້າງຄຽງໃນ ອັດຕາທີ່ສູງ ເພາະສະນັ້ນຈຶ່ງບໍ່ຄວນໃຊ້ປິ່ນປົວສົມທົບກັບຢາ Amphotericin.

* ບັນຫາຄຸນນະພາບຂອງຢາປິ່ນປົວຫລາຍຊະນິດ ຍັງເປັນເລື່ອງທີ່ໜ້າກັງວົນໃນທົ່ວໂລກ. ການສຶກສາຂອງພວກເຮົາ ພົບວ່າ ມີຢາ sofosbuvir ປອມ ທີ່ປະເທດພະມ້າ ເຊິ່ງອັນນີ້ເຮັດໃຫ້ເກີດມີຄວາມສ່ຽງຕ່ຳການປິ່ນປົວພະຍາດຕັບອັກເສບຊີ ເພາະຢາດັ່ງກ່າວກໍມີຂາຍໃນ ສປປ ລາວ ເຊັ່ນກັນ.

* ການຍົກລະດັບຄວາມສາມາດຂອງນັກກວດສອບຢາປິ່ນປົວ. ການສຶກສາປະເມີນຮ່ວມກັບ Global Good ໃນກຸ່ມນັກການຢາທີ່ໂຮງໝໍມະໂຫສົດ, ຄະນະເພສັຊສາດ - ມວສ, ແລະ ໜ່ວຍງານກວດສອບອາຫານ ແລະ ຢາ ຂອງກົມອາຫານ ແລະ ຢາ ຊີ້ໃຫ້ເຫັນວ່າ ເຄື່ອງມືກວດສອບທີ່ເອີ້ນວ່າ Near-Infrared device ແມ່ນມີຄວາມແມ່ນຍຳສູງໃນການກວດຫາຢາທີ່ບໍ່ໄດ້ຄຸນນະພາບ. ປະຈຸບັນ ພວກເຮົາກຳລັງຫາລື ເພື່ອເຮັດການສຶກສາໃນລະດັບທີ່ໃຫ່ຍກວ່າເກົ່າ ສຳລັບການນຳໃຊ້ເຄື່ອງດັ່ງກ່າວ ເຂົ້າໃນການກວດສອບຄຸນນະພາບຂອງຢາທີ່ສົງໃສວ່າ ມີບັນຫາເລື່ອງຄຸນນະພາບ.

* ການສຶກສາຂອງພວກເຮົາພົບວ່າ ປະຊາຊົນໃນນະຄອນຫລວງວຽງຈັນ ຍັງມີຄວາມຮັບຮູ້ທີ່ຈຳກັດ ໃນເລື່ອງຄວາມສ່ຽງຂອງຢາປິ່ນປົວ. ຂໍ້ມູນນີ້ ຊີ້ໃຫ້ເຫັນວ່າ ການປຸກລະດົມ ແລະ ໃຫ້ສຸຂະສຶກສາແກ່ປະຊາຊົນ ຈະເຮັດໃຫ້ການນຳໃຊ້ຢາ ມີລັກສະນະສົມເຫດສົມຜົນຫລາຍຂຶ້ນກວ່າເກົ່າ.

* Science Café. ປະຈຸບັນ ສປປ ລາວ ມີຂໍ້ມູນດ້ານສຸຂະພາບຫລາຍຂຶ້ນກວ່າເກົ່າ, ສະນັ້ນ ການເຜີຍແຜ່ຂໍ້ມູນດັ່ງກ່າວໃຫ້ແກ່ຜູ້ສ້າງນະໂຍບາຍ, ພະນັກງານສາທາລະນະສຸກ, ແລະ ປະຊາຊົນທົ່ວໄປ ຈຶ່ງມີຄວາມສຳຄັນເປັນຢ່າງຍິ່ງ. ພວກເຮົາໄດ້ຮ່ວມມືກັບ ມະຫາວິທະຍາໄລວິທະຍາສາດສຸຂະພາບ ຈັດການສົນທະນາທີ່ເອີ້ນວ່າ Science Café ເປັນຄັ້ງທຳອິດໃນ ສປປ ລາວ ແລະ ພວກເຮົາຄາດວ່າ ຈຸດເລີ່ມຕົ້ນນີ້ຈະກາຍເປັນສິ່ງທີ່ຄວນເຮັດຢ່າງເປັນປະຈຳໃນອະນາຄົດ ໃນຮູບແບບຂອງການສົນທະນາໃນຫົວຂໍ້ວິທະຍາສາດ.

SUMMARY

Ivo Elliott, Sungsit Sungvornyothin, Chanthala Vilaihong, Serge Morand, Rawadee Kumlert setting off for fieldwork in Feuang District

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 as a part of the MORU Tropical Network and is strongly linked to MORU-Bangkok with whom 41% of our studies are shared projects.

B. LOMWRU is funded predominantly by the Wellcome Trust of Great Britain, with significant additional support from the US Naval Medical Research Centre, the Bill & Melinda Gates Foundation, the European Union, Department for International Development-UK (DFID), US-CDC – Laos, Fondation Total/Institute Pasteur, Global Good, Foundation for Innovative New Diagnostics, DTRA, Global Antibiotic Research Partnership, INTERPOL, Joint Inter-Agency Task Force and the Asian Development Bank. Considerable assistance in kind is given by the Institut de Recherche pour le Développement/Aix-Marseille University, France, and the Rickettsial Diseases Research Program, Naval Medical Research Center, USA.

C. The Microbiology Laboratory is composed of 29 Lao Government staff and 42 project-funded staff; 88% are Lao and 56% are female. The Microbiology Laboratory has clinical microbiology, molecular, serology and BSL3 laboratories. It follows University of Oxford safety policies and guidelines.

D. 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, with the Food and Drug Department (FDD) on the quality of medicines and with the MoH CDC on antibiotic resistance.

E. The main current focus of the research work is on the causes of fever and their epidemiology, their antimicrobial resistance patterns, their optimal diagnosis and optimum treatment, to inform policy in rural Asia and the quality of medicines globally.

F. We supported 19 Lao staff to attend 10 international meetings and three Lao staff to read for PhD/MSc degrees at Mahidol University, one to read for a PhD at the University of Amsterdam and one to read for a BSc degree at Khon Kaen University in 2016.

G. In 2016 we published or have in press 47 publications, including 39 peer-reviewed papers, three letters, three book chapters, one abstract and one WHO report. Since LOMWRU was founded, its staff has published 295 papers, including 18 book chapters.

H. Previous LOMWRU research translated into policy in Laos includes the implementation of vaccination against the pneumococcus and the *Japanese encephalitis*

Dr Rattanaphone Phetsouvanh teaching in 2002

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.

I. The main findings, in brief, from work published or in press or in preparation in 2016 of immediate relevance to Laos (please see caveats in main 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 (ESBL) are increasingly important causes of infection in Mahosot Hospital (see front cover). These are the commonest AMR problem the Microbiology Laboratory encounters, their hospital incidence is increasing and they are resistant to commonly used antibiotics such as cephalosporins and penicillins. We see susceptibility results for bacteria from patients with extremely limited options for therapy and we are greatly concerned that soon we will commonly see untreatable infections and infections that will require unaffordable antibiotics.
- ❖ ESBL bacteria are also common in the stools of healthy kindergarten children in Vientiane City and Province.
- ❖ ESBL bacteria are also surprisingly common in the stools of healthy people in a remote village of Xieng Khouang Province, suggesting that ESBL are a problem in rural Laos and will become a greater problem unless action is taken. A surprisingly high proportion of this rural human population (13.4%) had taken antibiotics in the preceding 2 weeks.
- ❖ In addition, we found that overseas visitors attending a medical course in Vientiane, who provided their stools for analysis on arrival, rapidly acquired cephalosporin resistant bacteria in their stools during three weeks in Vientiane. These data suggest that ESBL bacteria are abundant in people and the environment in Vientiane and that those visiting rapidly become colonised,

Don Kho in the Mekong River

risking their health and the dissemination of these bacteria elsewhere.

- We have found the first isolates of carbapenem resistant Enterobacteriaceae in Laos, both *E. coli*, one from pus and one from the urinary tract. With the recent beginning of use of carbapenems in Laos, this is extremely worrying and calls for increasing oversight of their use.
- *Streptococcus pyogenes* antibiotic resistance amongst Lao isolates was moderate to erythromycin and chloramphenicol. No isolate was resistant to penicillin and these data suggest that erythromycin and chloramphenicol should not be used for *Streptococcus pyogenes*, but that penicillin should be used.
- Reinforcing the evidence that enhancing of antibiotic stewardship and infection control practices is urgently needed, we have found *Clostridium difficile* in the stools of patients at Mahosot Hospital. The occurrence of this organism, an important cause of hospital-associated diarrhoea, is not unexpected with the frequent cephalosporin use in Vientiane hospitals.
- Of 158 *N. gonorrhoeae* isolates with antibiotic susceptibility data, all were susceptible to ceftriaxone and spectinomycin, but with very high levels of resistance to ciprofloxacin, penicillin and tetracycline. These data suggest that ceftriaxone and spectinomycin are likely to be efficacious against *N. gonorrhoeae* in Laos. Contact tracing and treatment of partners will be a key intervention to reduce the burden of STIs.
- Knowledge about antibiotic resistance patterns and appropriate prescribing amongst doctors was relatively low – interventions targeting these gaps are urgently needed.
- The spread of bacteria resistant to common antibiotics 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.
- To assist with an evidence base and appropriate action/intervention we have joined with the Ministry of Health and key stakeholders to form an AMR Working Group/Committee that will be synergistic with the WHO/FAO/OIE AMR committee.

Map of Mahosot Hospital 1930. The Microbiology Laboratory is the second building to the left of the bottom right corner. The Mekong River is at the base of the map

- **The causes of fevers in rural Laos.** The Expanded Fever Surveillance (EFS) project, with the support of the US Naval Medical Research Centre, investigated the aetiology of fever in outpatients attending Xieng Khouang, Salavan and Luang Nam Tha Provincial Hospitals. The main diagnoses were influenza (60%), followed by leptospirosis (15%), dengue (10%), scrub typhus and bacteraemia (both 5%), murine typhus (3%), JEV and *Rickettsia* spp. (both 1%). We plan to restart this study, including inpatients, after Pii Mai Lao and are using these data to inform treatment algorithm discussions.
- **How many human pathogens are there in Laos?** There has been a rapid increase in the number of pathogens described in Laos over the last ~ 15 years. Are there more to find? Searches of publications, 1874 – 2016, yielded evidence that 148 human pathogens have been recorded in Laos by culture and molecular assays. A novel mathematical model, incorporating a time-varying discovery rate, estimated that a total of 157 species of human pathogens are in Laos, suggesting that most pathogens currently in Laos have now been described.
- **The causes of central nervous system infections for the first 1,065 lumbar punctures** at Mahosot Hospital, 2003-2011, are being analysed. Aetiology, based on assays from blood and CSF, was confirmed in 42% patients. Among single infections, the most frequent aetiologies were the *Japanese encephalitis virus* (JEV) (8.8%), *Cryptococcus* spp. (6.6%), *Orientia tsutsugamushi* (2.9%), *Dengue virus* (2.5%), *Leptospira* spp. (2.3%), *Rickettsia* spp. (2.3%), *Streptococcus*

pneumoniae (2.1%), *Mycobacterium tuberculosis* (1.9%), *Herpes simplex virus* (HSV) (1.4%), *Cytomegalovirus* (CMV) 12 (1.1%), *Enterovirus* (0.9%), *Varicella-zoster virus* (VZV) (0.6%), *Mumps virus* (0.5%) and *P. falciparum* (0.4%). The mortality was high at 26.3%. Factors that showed strong association with death were higher CSF lactate (p=0.001) and lower GCS (p<0.001). The Lao data suggest that particular attention should be paid to patients presenting with decreased GCS, who are more likely to have a poor outcome, and supportive care for unconscious patients, such as high-dependency units (HDU) along with appropriate urgent antimicrobial therapy may be a key factor in improving outcome. These data will be used to inform evidence-based treatment guidelines for central nervous system infections.

- **The majority of laboratory cerebrospinal fluid examinations (94%) are abnormal**, suggesting that more lumbar punctures in inpatients with suspected central nervous system (CNS) infections should be facilitated to ensure that patients with these infections are not missed.
- **A large pilot cohort study of the causes and impact of fevers in pregnancy in Pak Gnum District, Vientiane was completed.** That Lao has the highest estimated maternal mortality in SE Asia prompted this large pilot study. Of 1,000 pregnant patients recruited, 110 developed intra-or post-partum fevers. There were 18 miscarriages, 6 perinatal deaths, 3 neonatal deaths, 1 maternal death (ectopic pregnancy) and 11 congenital abnormalities. The final diagnostic assays are being conducted before final analysis in relation to outcome measures such as low birth weight and stillbirth.
- **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 currently without such surveillance.
- **Japanese encephalitis virus diagnostics.** We demonstrated that pre-cut filter paper saturated with CSF could provide a useful tool for JEV ELISA diagnostics in settings with limited laboratory access. It has the potential to improve national JEV surveillance and inform vaccination policies.
- **Malaria Rapid Diagnostic Tests (RDTs).** We assessed health workers' (HW) ability to use a potential improvement on malaria RDTs, the mainstay of malaria diagnosis in Laos, that may become degraded

Nong District Targeted Malaria Elimination Engagement

Sengkham Symanivong, Phonelavanh Phouminh, Amphaivanh Seubsanith & Kingthong Sisouphanthaklar taking goat blood

in hot climates. The improvement includes positive control wells (PCW) to detect degraded RDTs. After training, most participants correctly performed the six key individual PCW steps and 97% reported a correct action based on PCW use at routine work sites. PCW availability can improve HWs' confidence in RDT results, and benefit malaria diagnostic programs. These data support the implementation of PCWs in RDTs.

- **Data from fever studies in Laos, Cambodia and the Thai/Myanmar border suggest that C-reactive protein 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. Cost-effectiveness analysis suggests that CRP testing, in rural Laos, is likely to be consistently cost-effective and could offer substantial reductions in overuse of antimicrobials in viral infections.
- **Ticks & human pathogens.** With the Institut Pasteur du Laos and the US Navy we looked for potential human pathogens in a large collection of ticks from Khammouane Province. A significant proportion contained *Rickettsia* spp. DNA, including three novel genotypes that might be new species, *Candidatus* Rickettsia laoensis, *Candidatus* Rickettsia mahosotii and *Candidatus* Rickettsia khammouanensis. In addition, evidence for *Ehrlichia* spp., *Coxiella burnetii, Anaplasma* spp. and *Borrelia* spp. were identified. This should help to narrow down what tick-borne bacterial pathogens we may find in patients in Laos.
- ***Orientia tsutsugamushi* in Chile!** We supported German & Chilean colleagues in the molecular diagnosis of *Orientia tsutsugamushi* in patient samples from southern Chile. This dramatically extends the known distribution of scrub typhus and leads to many questions as to how common *Orientia tsutsugamushi* is in South America and how it is transmitted.
- **Melioidosis is an important and under-recognised cause of sepsis in Laos**, as well as other tropical regions. We have diagnosed over 1,100 culture-positive patients since 1999 (158 in 2016 alone) and are concerned that there is substantial unrecognised but potentially treatable mortality due to *B. pseudomallei* elsewhere, especially in southern Laos.
- **Wildlife vendors' risks.** Lao markets are fulcrums of society but there is no information on vendors' perception of health risk due to the food they sell.

As wild and domestic animals are potential carriers of diverse diseases, we conducted a descriptive cross-sectional study in markets with traders selling wildlife. Nearly all vendors had a very low perception of risk for health due to the food sold. Further One Health research is needed to understand risks and engage with the market communities, both buyers and sellers.

- **Pathogens in bushmeat.** Molecular assays on 400 specimens from 248 wild vertebrates (mostly squirrels) collected at markets demonstrated that 44 animals contained *Leptospira* spp., six animals contained *Rickettsia* spp. (including a confirmed *R. felis*) plus *L. garvieae*, *Kurthia* spp., *Ehrlichia* spp. TC251-2, and *A. marginale*. The high frequency of *Leptospira* spp. detected in urogenital swabs suggests the possibility of squirrel-human transmission of leptospirosis in markets. The discovery of *R. felis*, an emerging rickettsial pathogen, is the first reported case in a squirrel species.
- **Malaria in pregnancy.** There are no data on the burden of malaria in pregnancy (MiP) in Laos. Two cross-sectional surveys conducted in 2014 assessed the prevalence of MiP in Vapi District, Salavan Province; 12/204 pregnant women (5.9 %) were infected with malaria as determined by RTqPCR: 11 were *Plasmodium vivax* infections and 1 was mixed *Plasmodium vivax*/*Plasmodium falciparum* infection. Nine infections were sub-microscopic. Most infections were asymptomatic, submicroscopic vivax malaria - important evidence for national strategies for the screening and prevention of MiP in Laos.
- **Asymptomatic malaria.** A fascinating recent development has been the realization that there are foci in rural Asia of populations with high frequencies of *Plasmodium falciparum* asymptomatic malaria carriage that persist long term at densities in blood that are not detectable by microscopy or rapid diagnostic tests. These infections may be critical as a transmission reservoir in countries such as Laos. A cross-sectional survey in Savannakhet Province, found that of villagers without symptoms, 20% had *Plasmodium* infections detected by high volume, ultra-sensitive quantitative PCR. The mutation associated with reduced susceptibility to artemisinin derivatives, was found in 7-75 % of *P. falciparum* isolates. These worrying findings have wider implications for Laos and could reverse the gains achieved by the successful control of malaria in Laos and the Greater Mekong Sub-region. Consideration of rapid elimination of *P. falciparum* has to be a top priority in Laos and a Targeted Malaria Elimination pilot study has started in Nong District, Savannakhet.
- **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-**

Fieldwork improvisation

Targeted Malaria Elimination public meeting Nong District

therapy ACTs fails. We are participating, with CMPE, in the multicentre TRAC-2 study, coordinated by MORU-Bangkok, at Sekong Provincial Hospital. This is a randomised clinical trial comparing parasite clearance times between artemether-lumefantrine and artemether-lumefantrine plus amodiaquine. We hope that these data will be useful for informing optimal future national ACT policy.

- **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.**
- **There remain severe, at least focal, problems with the quality of diverse medicines globally.** Falsified sofosbuvir has been found in Myanmar and this is a risk for Lao Hepatitis C care as sofosbuvir is now available in Laos
- **Empowering drug inspectors.** Evaluation, with Global Good, by pharmacists at Mahosot Hospital, the Faculty of Pharmacy of the UHS and the Bureau of Food and Drug Inspection of Laos, suggests good diagnostic accuracy of a small Near-Infrared device in screening for poor quality medicines. We are discussing a large scale trial of this device to empower drug inspectors to detect medicines of suspicious quality.
- **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.
- **Science Café.** Now that there is more Lao public health information available, engagement with policy makers, health workers and the public is vital. With the University of Health Sciences we organised the first Science Café in Laos, that we hope will become a regular feature of the Vientiane scientific 'scene'.

INTRODUCTION

The new Mahosot Hospital Microbiology Laboratory Directors team are Dr Manivanh Vongsouvath Director (centre), Dr Sayapeth Rattanavong (left) and Mrs Viegmon Davong (right) Deputy Directors.

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 Great Britain, a charity now named 'Wellcome', 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.

The roof of the old Microbiology Laboratory has deteriorated over the last few years and with funding from the Wellcome Trust the roof is being replaced and the building renovated.

Oxford University headquarters are at the Centre for Tropical Medicine & Global Health, in the Nuffield Department of Medicine on the Old Road Campus, in Oxford, next to the Churchill Hospital. We are linked within the Mahidol-Oxford Research Unit (MORU) Network 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; 41% of our research is jointly with MORU-Bangkok.

MORU, the Shoklo Malaria Research Unit (SMRU), in Mae Sot, Thailand, the Cambodia-Oxford Medical Research Unit (COMRU), Myanmar Oxford Clinical Research Unit (MOCRU), Kinshasa Mahidol Oxford Research Unit (KIMORU) in the Democratic Republic of the Congo, 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. We are very grateful for all the help of MORU-Bangkok for vital logistical and auditing support for LOMWRU.

We have been part of the WorldWide Antimalarial Resistance Network (WWARN) for 5 years. WWARN maps evidence on antimalarial resistance and treatment and includes published data from Laos, and at Mahosot we run the WWARN Antimalarial Quality group and mapping system (http://www.wwarn.org/aqsurveyor/). This system is being extended to other infectious diseases under the Infectious Diseases Data Observatory (https://www.iddo.org/medicine-quality) umbrella and we are expanding our work to map the quality of a diversity of essential medicines.

These data will graded, mapped and released progressively during 2017.

The Microbiology Laboratory and LOMWRU together are staffed by 29 Lao Government staff and 42 project-funded staff; 88% are Lao and 56% 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 the core funding from the Wellcome Trust, from the US Naval Medical Research Centre, the Bill & Melinda Gates Foundation, the European Union, Department for International Development-UK (DFID), US-CDC – Laos, Fondation Total/Institut Pasteur, Global Good, Foundation for Innovative New Diagnostics, US-DTRA, Global Antibiotic Research Partnership, INTERPOL, Joint Inter-Agency Task Force and the Asian Development Bank. Considerable assistance in kind is given by the Institut de Recherche pour le Développement (IRD)/Aix-Marseille University, France, 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 2016 the Laboratory processed blood cultures from 2,538 patients, cerebrospinal fluid from 253, urine from 1,709, stool from 544, pus from 1060, genital swabs from 3,865, other body fluids from 388, cryptococcal antigen tests from 279 and throat swabs from 1,575 patients. Dengue IgG, IgM and NS1 ELISAs were performed for 447 patients, JEV IgM ELISAs for 386 patients, scrub typhus and murine typhus rapid diagnostic tests (RDTs) for 1,201 patients and dengue RDTs for 470 patients. LOMWRU also works with the Centre for Malariology, Parasitology and Entomology (CMPE) on malaria projects in Sekong and Savannakhet Provinces, with the Food and Drug Department on the quality of medicines and with the MoH CDC on antibiotic resistance. We have been collaborating with the Lao Red Cross in collection of sera and other blood samples as 'normal' controls.

Now that there is more information on public health in Laos we are increasing our public and health worker engagement, from the Lao Medical Journal, the University of Health Sciences e-library, to the Targeted Malaria Elimination engagement and the first Science Café in Laos.

Since 2000 we have published or have in press 295 papers, including 18 book chapters. In 2016 we published or have in press 47 publications, including 39 peer-reviewed papers, three letters, three book chapters, one abstract and one WHO report. We supported 19 Lao staff to attend 10 international meetings and supported three Lao staff to read for PhD/MSc degrees at Mahidol University, one at the University of Amsterdam and one to read the BSc degree in Medical Technology at Khon Kaen University. Here we describe this work and briefly summarize diverse activities over the past year.

LACANET Molecular Microbiology Course in Vientiane attendees

STAFF AND HUMAN CAPACITY BUILDING

To our profound sadness and sorrow Dr Rattanaphone Phetsouvanh died, after a long illness, on 23rd November 2016. She successfully completed her PhD from Mahidol University, Bangkok, on scrub typhus whilst undergoing treatment.

Dr Manivanh Vongsouvath, Dr Rattanaphone's successor as Director of the Microbiology Laboratory, successfully completed her Mahidol University research on dengue diagnostics (see below) for her MSc in Clinical Tropical Medicine.

New staff who joined in 2016 include Mrs Athirat Black – Operations Manager, Mr Phonepasith Boupha – Research Assistant-Medicine Quality, Mr Kem Boutsamay – Research Assistant-Medicine Quality, Ms Touny Chindavong – Data Entry Officer, Dr Pruksa Nawtaisong – Molecular Biologist, Dr Rene Neihus – Mathematical Modeller, Dr Matthew Robinson – Molecular Microbiologist, Mr Soubon Saysana – Research Assistant/Data Entry – MCRI, Ms Neeranuch Thangnimitchok - Research Assistant, Dr Serena Vickers - Research Physician.

Five Lao postgraduate fellows in infectious disease have been conducting their University of Health Sciences research theses with us - Dr Phouvieng Douangdala (on JEV), Dr Ko Chang (on ESBL), and Dr Savandalat Phouangsouvanh (on gonorrhoea) have successfully defended their theses and Dr Bandith Soumphonphakdy (on respiratory infections) will complete in 2017. Dr Chanfong Philavong successfully completed her IFMT MSc thesis on the perceptions of market traders of health risks of wildlife and other foods.

Dr Céline Caillet completed her University of Toulouse PhD thesis on pharmacovigilance in Laos and started as Coordinator of the Medicine Quality project in LOMWRU/IDDO/WWARN (see below). We hosted three overseas MSc students in 2016. Madeleine Clarkson, from the London School of Hygiene and Tropical Medicine, conducting a mathematical modelling study to estimate the number of human pathogen species in Laos. Dr Kartika Saraswati, from the University of Oxford, reviewed the evidence on the global quality of diabetes medicines and Scott Tschida, from the University of Oslo, conducted his MSc thesis on a global review of the quality of antibiotics.

Dr Nguyễn Văn Hoàn, from Hai Phong Medical University and a PhD student at University of Aix-Marseille, spent four months with us working on respiratory infections. Dr Koukeo Phommasone is conducting his University of Amsterdam PhD fieldwork, Dr Tiengkham Pongvongsa his Mahidol University PhD fieldwork and Dr Bipin Adhikari his Oxford University DPhil as part of the TME project in Savannakhet Province. Mr Weerawat Phuklia is conducting his PhD on the antibiotic susceptibility of diverse isolates of *O. tsutsugamushi*. Dr Ivo Elliott is conducting his fieldwork on scrub typhus for his Oxford DPhil thesis and Dr Cat Wootton is planning dermatology projects.

Ms Bountoy Sibounheuang and Ms Malee Seephone spent two and three months each, respectively, in the Unit of Emerging Viruses of Professor Xavier Nicolas de Lamballerie, Faculty of Medicine in Marseille working with Dr Audrey Dubot-Pérès, funded by the Institut de Recherche pour le Développement (IRD). 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 2016. Ms Bountoy Sibounheuang is now studying for the BSc in Medical Technology at Khon Kaen University.

We are fortunate to have strong links with Public Health England (PHE) who will support a further microbiology/infectious disease registrar to spend a year of training with us starting in 2017. Dr Serena Vickers is visiting us for a year to work especially on medicine quality and a diabetes pilot study. Dr Ruth Lim and Jana Lai from Australia have been working with us on the PneuCAPTIVE study (below) and Dr Tamalee Roberts was with us for six months to help with the cleaning of the blood culture database, with Naomi Waithira of MORU-Bangkok, and has returned in 2017. Dr Rene Niehus, from MORU-Bangkok, is based in LOMWRU for his mathematical modelling work on antimicrobial resistance. Dr Olivier Celhay and Dr Phetsavanh Chanthavilay, also modellers from MORU-Bangkok, are based in CMPE and affiliated with LOMWRU.

Dr Rosalie Zimmerman of the Swiss Tropical and Public Health Institute worked on the Adult Infectious Disease (IDA) Ward with Professor Valy Kelouangkhot and colleagues and conducted extensive work across Laos with LOMWRU and IRD, on the distribution of *B. pseudomallei*

Dr Rattanaphone Phetsouvanh & Assoc Prof May-fong Mayxay at the MOU signing 2007

Assoc. Professor Phaikyeong Cheah discussing ethics at the Science Cafe, University of Health Sciences

in rivers, funded by DTRA. Rosalie was followed by Dr Kerstin Kling, also of the Swiss Tropical Institute & Public Health Institute, who worked on the IDA Ward and with LOMWRU on the portable ultrasound (POCUS) project. Dr Luci Stimmler worked with us part time on leptospirosis projects. Dr Jennifer Yan, whilst working on the University of Melbourne project at Mahosot Hospital, also worked with us on rickettsial infections in children.

Prof Daniel Paris, of MORU-Bangkok, has moved to the Swiss Tropical Institute & Public Health Institute and Dr Matthew Robinson has taken over as the MORU Network Rickettsial Coordinator.

Numerous students and doctors in diverse health disciplines studied in the Microbiology Laboratory in 2016. The Laboratory staff assisted with the post-graduate internal medicine and paediatric training programme teaching. As part of the LACANET project, Dr Matthew Robinson, Dr Pruksa Nawtaisong & Mr Weerawat Phuklia ran a molecular biology training course with colleagues from Institut Pasteur-Cambodia and the National Animal Health Laboratory of Laos. Regular classes have been held in English language. Dr Daniel Parker of SMRU ran a week long QGIS training course and Drs Mavuto Mukaka & Atthanee Jeeyapant of MORU-Bangkok ran a statistics training course. MORU-Bangkok is also planning a mathematical modelling for policymakers' workshop in Vientiane. We plan to hold further medical ethics and Good Clinical Practice courses in 2017. We supported 19 Lao staff to attend 10 international meetings.

We run a monthly journal club, have regular talks and participate in the Mahosot Hospital scientific monthly talks. We also regularly join MORU colleagues via Webex for scientific seminars. LOMWRU staff teach at the University of Health Sciences (UHS) and Institut de la Francophonie pour la Médecine Tropicale (IFMT), Vientiane, the DTM&H of the London School of Hygiene and Tropical Medicine and the International Health MSc at the University of Oxford.

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 Clinical Safety Officer, two Lao Deputy Safety Officers, a Lao Head of Field Research, a Lao Deputy Head of Virology, a Lao Administrator, a Lao Deputy WWARN Antimalarial Quality Coordinator, and a Lao Laboratory 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. Our administrative procedures are evolving, led by our Network COO, Ms Karen Valentine, with initial help by Dr Abby Taylor, seconded by the Wellcome Trust, working with our Administrator Ms Sengmany Symanivong and then also with our new Operations Manager, Mrs Athirat Black.

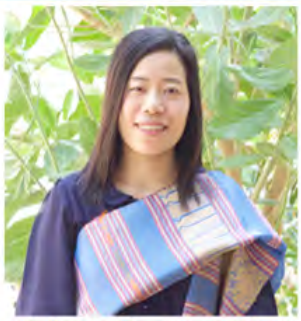
New Staff 2016

Mrs Athirat Black
Operations Manager

Mr Phonepasith Boupha
RA (Pharmacist)

Mr Kem Boutsamay
RA (Pharmacist)

Ms Touny Chindavong
Data Entry Clerk



Dr Pruksa Nawtaisong (Jaa)
Molecular Microbiologist

Dr Rene Niehus
Mathematical Modeller

Dr Tamalee Roberts
Scientific Microbiologist & Data Manager

Dr Matthew Robinson
Molecular Microbiologist

Ms Neeranuch Thangnimitchok
RA (Lab Technician)

Dr Serena Vickers
Research Physician

Mr Souban Xaysana
Research Assistant

RESEARCH RESULTS AND THEIR PUBLIC HEALTH IMPLICATIONS

Somsavanh Syhalath & Latsaniphone Boudthasavong working in the Microbiology Laboratory

1. Infectious Disease Diagnosis, 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, as the Expanded Fever Surveillance (EFS) project with the support of the US Naval Medical Research Centre, to include Xieng Khouang Provincial Hospital (XK), along with those in Salavan (SV) and Luang Nam Tha (LNT), investigating the aetiology of fever in outpatients. The National Centre for Laboratory & Epidemiology (NCLE) has analyzed nasopharyngeal swabs from these patients, contributing to national influenza surveillance.

Outpatients of any age giving informed written consent were recruited to EFS, if they presented with history of fever for ≤8 days and/or admission temperature ≥38°C (measured as tympanic but corrected to oral), during the study blocks. Each week was divided into ten slots in which outpatients were seen and study blocks were chosen using random numbers. This gave a total recruitment time of 51% of the time outpatients were seen.

It commenced on the 1st December 2014 and was completed on 30th November 2015. A total of 2,070 patients were recruited (900 from Luang-Namtha (LNT), 576 from Xieng Khouang (XK) and 594 from Salavan (SV)). 21 patients (1%) were admitted from outpatients to the hospitals. The majority of the recruited patients were children less than 15 years old (60%), with a median (range) age of 9 (2 days to 85 years) years old, and just over half of them (52%) were male.

Influenza PCR could not be completed for all recruited patients and from 1st of September 2015 until the end of this study a random selection of one day a week was chosen. Of the 2,070 patients, 347 (17%) were given a conservative laboratory diagnosis, 175 (20%) at LNT, 65 (11%) at SV and 107 (19%) at XK. The main overall diagnoses were influenza (60%), followed by leptospirosis (15%), dengue (10%), scrub typhus and bacteraemia (both 5%), murine typhus (3%), JEV and undetermined *Rickettsia* spp. (both 1%).

The main diagnosis at LNT was influenza (72%), leptospirosis was the second most frequently found (11%), followed by dengue (8%), scrub typhus (4%), JEV and *Rickettsia* spp. (both 2%), bacteraemia (1%) and murine typhus (0.5%). The main diagnoses at SV were also influenza (34%), leptospirosis (27%), followed by dengue (12%), bacteraemia (10%), JEV, scrub typhus (8%) murine typhus (7%) and *Rickettsia* spp. (2%). The main diagnoses at XK were influenza (56%) and leptospirosis (13%), followed by dengue (11%), bacteraemia (10%), scrub typhus (5%) and murine typhus (3%), and JEV (2%),

We plan to restart at the three provincial hospitals in spring 2017 to investigate the aetiology of fever in inpatients. 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).

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.

D. Central nervous system (CNS) infections. We have completed analyzing the data from the first ~1,065 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. Aetiology, based on assays from blood and cerebrospinal fluid (CSF), was confirmed in 42.3% patients; 93.6% of patients had abnormal CSF. Among single infections, the most frequent aetiology was JEV (8.8%), *Cryptococcus* spp. (6.6%), *Orientia tsutsugamushi* (2.9%), *Dengue virus* (2.5%), *Leptospira* spp. (2.3%), *Rickettsia* spp. (2.3%), *S. pneumoniae* (2.1%), *M. tuberculosis* (1.9%), *Herpes simplex virus* (HSV) (1.4%), *Cytomegalovirus* (CMV) 12 (1.1%), *Human Enterovirus* (0.9%), *Varicella-zoster virus* (VZV) (0.6%), *Mumps virus* (0.5%) and *P. falciparum* (0.4%). The mortality was high at 26.3%. Factors that showed strong association with death were higher CSF lactate (p=0.001) and lower Glasgow Coma Score (GCS)(p<0.001). The evidence suggests that brain and meningeal inflammation (encephalitis and meningitis, respectively), have no clear distinguishable clinical manifestation that relates to the responsible pathogens. 'Conventional' bacterial, fungal and viral infections are important but rickettsial and leptospiral infections are also key. Lao data suggest that particular attention must be paid to patients who present with decreased GCS and high CSF lactate, who are more likely to have a poor outcome, and supportive care for unconscious patients, such as high-dependency units (HDU), along with appropriate urgent antimicrobial therapy, may be key factors in improving outcome. This is a collaborative project with multiple partners, especially with IRD/Aix-Marseille University, France.

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

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.

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.

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.* 2016) suggests that common infectious diseases, such as dengue and scrub typhus may be important contributors. This study was 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. The final laboratory assays are being conducted before analysis and paper writing. The Lao data on frequency and impact of rickettsial diseases in pregnancy are also being analysed for publication.

Assoc. Professor Mayfong Mayxay performing karaoke in Nong District restaurant

F. Mapping of fevers. 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). These data will be mapped as a part of the Infectious Diseases Data Observatory.

G. How many human pathogens are there in Laos? Temporal trend analysis using discovery-curves has been used to estimate organism diversity – for example, how many tree species are there globally? For her LSHTM MSc thesis, Madeleine Clarkson, with modelling supervision by Dr Ricardo Aguas in MORU-Bangkok, applied a similar methodology to country-level data from Laos in order to provide an estimated answer to the question 'how many human pathogens are there in Laos'? The Lao dataset was compiled from searches of French and English archival and recent publications, spanning the period 1874 – 2016, and graded according to the level of diagnostic evidence used in pathogen description. Previous models were improved upon by implementing a time-varying discovery rate in the model to account for observed changes in the rate of pathogen discovery. A dataset of 677 data points was collected through an extensive literature search and 148 human pathogens were identified as recorded in Laos by culture and molecular assays. The model estimated that a total of 157 species of human pathogens are available for discovery in Laos. It captured the significant increase in the rate of pathogen discovery experienced within the last ten years. Madeleine Clarkson has recently returned to the French colonial archives in Aix to complete the search within the colonial 'grey literature'.

H. C-Reactive Protein (CRP) assays. With evidence that CRP, a marker of host inflammation, is a promising test as a predictor for the need for antibiotics (see Annual Report 2015) 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.

Lubell *et al.* (2016) modelled the ability of dengue and scrub typhus rapid tests to inform antibiotic treatment, as compared with testing for elevated CRP. Using data on causes of fever in rural Laos, we estimated the proportion of outpatients that would be correctly classified as requiring an antibiotic and the likely cost-effectiveness of the approaches. Use of either pathogen-specific test slightly increased the proportion of patients correctly classified as requiring antibiotics. However, CRP testing was consistently superior to the pathogen specific tests, despite heterogeneity in causes of fever. All testing strategies are likely to result in higher average costs, but only the scrub typhus and CRP tests are likely to be cost-effective when considering direct health benefits, with median cost per disability adjusted life year averted of approximately $48 USD and $94 USD, respectively. They concluded that testing for biomarkers of host inflammation, such as CRP, is likely to be consistently cost-effective and can also offer substantial reductions in overuse of antimicrobials in viral infections.

I. Target Product Profile for diagnostic assays. In order to reduce the unnecessary empirical use of antimicrobial drugs and improve outcomes, it is essential to improve diagnostic capabilities. In the absence of microbiology facilities in low-income settings, an assay to distinguish bacterial from non-bacterial causes would be a critical first step. To ensure that patient and market needs are met, the requirements of such a test should be specified in a target product profile (TPP). To identify minimal/optimal characteristics for a bacterial vs. non-bacterial fever test, experts from academia and international organizations with expertise in infectious diseases, diagnostic test development, laboratory medicine, global health, and health economics were convened (Dittrich *et al.* 2016). The working group defined non-severely ill, non-malaria-infected children as the target population for the desired assay. To provide access to the most patients, the test should be deployable to community health centers and informal health settings, and staff should require <2 days of training to perform the assay. Further, given that the aim is to reduce inappropriate antimicrobial use as well as to deliver appropriate treatment for patients with bacterial infections, the group agreed on minimal diagnostic performance requirements of >90% and >80% for sensitivity and specificity, respectively. Other key characteristics, to account for the challenging environment at which the test is targeted, included: i) time-to-result <10 min (but maximally <2 hrs); ii) storage conditions at 0–40°C, ≤90% non-condensing humidity with a minimal

A cargo ship, carrying sand, on the Mekong River

shelf life of 12 months; iii) operational conditions of 5–40°C, ≤90% non-condensing humidity; and iv) minimal sample collection needs (50–100µL, capillary blood). This expert approach to define assay requirements for a bacterial vs. non-bacterial assay should guide product development, and enable targeted and timely efforts by industry partners and academic institutions.

J. Novel diagnostics tests and equipment. We worked with Global Good on evaluating new incubators for use in the tropics. These were trialled at Mahosot and in Luang Nam Tha and Salavan Provincial Hospitals and the monitoring data are being used by Global Good to optimise them. We are about to start projects to evaluate new simplified systems for molecular diagnostics, including the miniPCR system and novel recombinase polymerase amplification (RPA) assays using lateral flow tests.

K. 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 and disabilities 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).

2. Clinical Bacteriology

A. Extended spectrum beta-lactamase (ESBL) carriage in a remote Lao village. We estimated 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%) self-reported taking 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 relatively isolated community.

B. Extended spectrum beta-lactamase (ESBL) acquisition amongst visitors to Laos. 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 of three weeks. Further characterisation of these isolates and data analysis are underway. These data suggest that ESBL bacteria are abundant in people and the environment in

Vientiane and visitors rapidly become colonised, risking their health and the dissemination of these bacteria elsewhere.

C. Carbapenem resistant Enterobacteriaceae in Laos. We have found the first isolates of carbapenem resistant Enterobacteriaceae in Laos, both *E. coli*, one from pus and one from the urinary tract. With the recent beginning of use of carbapenems in Laos this is extremely worrying and calls for increasing oversight of their use. Further characterization of these isolates and identification of the resistance mechanisms are underway.

D. *Burkholderia pseudomallei* and the environment. We investigated the environmental factors that influence the presence (and absence) of *B. pseudomallei* in a tropical watershed in Salavan, Laos (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*.

Dr Rosalie Zimmerman has been studying the presence of *B. pseudomallei* in tributaries of the Mekong river. Two rounds of sampling were undertaken, in March and June 2016. Samples were processed by culture and PCR. Preliminary results indicate that 1) *B. pseudomallei* was present in 5% of all stations in the dry season and in ~50% of the stations in the rainy season. 2) *B. pseudomallei* was only present in sediments where the water was *B. pseudomallei*-positive. 3) *B. pseudomallei* was only present in Southern and Central Laos, not in the North. It thus appears that rivers are not reservoirs of *B. pseudomallei*, but rather become contaminated with *B. pseudomallei* which is washed out with eroded soil in the rainy season. Further work is underway using a variety of different molecular methods to detect *B. pseudomallei*, to analyse geochemical factors associated with the presence of *B. pseudomallei*, and to assess microbial diversity in the samples.

After extensive analysis, the manuscript describing the IFMT Master's project undertaken by Dr Loungnilanh Manivanh has been submitted for publication. She studied the presence of *B. pseudomallei* in a rice paddy in Vientiane Province at different depths during different seasons. Overall, 195 of 653 samples (29.7%) yielded *B. pseudomallei*. A higher prevalence of *B. pseudomallei* was found at soil depths greater than the 30 cm currently recommended for *B. pseudomallei* environmental sampling. *B. pseudomallei* was associated with a high soil water content and low total nitrogen, total carbon and organic matter content. The results suggest that a sampling grid of 25 five metre square quadrats (i.e. 25 x 25 m) should be sufficient to detect *B. pseudomallei* at a given location if samples are taken at a soil depth of at least 60 cm. However, it also found that enrichment culture, as used in the recommended international consensus method, may miss *B. pseudomallei* in environmental samples, so molecular approaches should be the mainstay of future studies.

An old porcelain electrical insulator found during renovation of the Old Microbiology Laboratory

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.

E. *Burkholderia pseudomallei* and clinical microbiology. We are evaluating a new rapid diagnostic test (RDT) for *B. pseudomallei* antigen detection in body fluids and blood cultures using the same principle as the work on the detection of typhoid (see Annual Report 2015). This has been found to be highly specific, but lacks sensitivity when used directly on clinical samples, including urine.

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 (Rachlin *et al.* 2016). Ms Rachlin is continuing her work on melioidosis in a PhD at the Menzies School of Health Research, Australia, and will be returning to conduct further work on the sources of *B. pseudomallei* infection in Lao patients.

David Dance worked with the team in COMRU, Siem Reap, to analyse data on melioidosis in children presenting at the Angkor Hospital for Children, 2009-2013. One hundred seventy-three evaluable patients were identified, presenting from eight provinces. Three quarters (131/173) of the children had localised infection, most commonly skin/soft tissue infection (60 cases) or suppurative parotitis (51 cases). There were 39 children with *B. pseudomallei* bacteraemia: 29 (74.4%) of these had clinical and/or radiological evidence of pneumonia. Overall mortality was 16.8% (29/173) with mortality in bacteraemic cases of 71.8% (28/39). At least seven children did not receive an antimicrobial with activity against *B. pseudomallei* prior to death. Given the high mortality associated with bacteraemic infection, there is an urgent need for greater awareness amongst healthcare professionals in Cambodia and other countries where melioidosis is known or suspected to be endemic. Empiric treatment guidelines should ensure suspected cases are treated early with appropriate antimicrobials.

We contributed to a large study, using whole genome sequences of 469 *B. pseudomallei* isolates from 30 countries, including Laos, to explore its geographic distribution and transmission (Chewapreecha *et al.* 2017). The data point to Australia as an early reservoir, with transmission to Southeast Asia followed by onward transmission to South Asia and East Asia. The data support an African origin of the Central and South American isolates with introduction of *B. pseudomallei* into the Americas between 1650 and 1850. The study also identified geographically distinct genes/variants in Australasian or Southeast Asian isolates alone, with virulence-associated genes being among those over-represented.

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. pseudomallei* in human cells, and are collaborating with groups in both Myanmar and Bangladesh in elucidating the burden of melioidosis in those countries.

F. 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 (NTS), as well as *Shigella* species. For typhoid, these data show that, with the exception of the rapidly disseminating H58 subclade (now designated genotype 4.3.1), the global *S.* Typhi population is highly structured and includes dozens of subclades that display geographical restriction.

Dr Rattanaphone Phetsouvanh & Professor Sasithon Pukrittayakamee after Dr Rattanaphone's PhD viva

We are also working with 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*, with the Murdoch Children's Research Institute in Australia on the epidemiology *Streptococcus pyogenes* infection in Laos, and with Public Health England on the genomics of *Staphylococcus aureus*.

The work on the group A streptococcus (GAS, *Streptococcus pyogenes*) characterized emm types, emm clusters of 124 isolates from Laos 2004– 2013 (Rattanavong *et al.* 2016). Considerable differences in emm -type distribution between Laos, Thailand and Cambodia were found. However, vaccine coverage for the Lao isolates was high for the J8 vaccine candidate. Antibiotic resistance was moderate to erythromycin and chloramphenicol (8% and 7%, respectively) and low to ofloxacin (<1%). No isolate was resistant to penicillin and these data suggest that penicillin but not erythromycin or chloramphenicol should be used for *Streptococcus pyogenes* infections in Laos.

G. 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

Matt Robinson, Céline Caillet & Anisone Chanthongthip at the Oxford Tropical Network meeting in Oxford

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 the community in Vientiane that has been extended for a further year.

H. *Clostridium difficile*. We have looked for *Clostridium difficile* in the stools of patients at Mahosot Hospital and have found it. The occurrence of this organism is not unexpected with the high cephalosporin use in Vientiane hospitals and argues for enhanced antibiotic stewardship. Otherwise it risks large outbreaks of hospital-associated diarrhoea.

I. Gonorrhoea. Dr Savandalath Phouangsouvanh, a Lao internal medicine resident, conducted her thesis research to describe the antibiotic susceptibility patterns of *N. gonorrhoeae* in samples cultured at the Microbiology Laboratory, Mahosot Hospital during 2011-2015. A total of 12,281 genital samples were received during this period and 165 (1.3%) grew *N. gonorrhoeae*. Of 158 isolates with antibiotic susceptibility data, all were susceptible to ceftriaxone and spectinomycin but 84.8% were resistant to ciprofloxacin, 89.9% to penicillin and 99.3% to tetracycline. These data suggest that ceftriaxone and spectinomycin are likely to be efficacious against *N. gonorrhoeae* in Laos. Contact tracing and treatment of partners will be a key intervention to reduce the burden of STIs.

J. *Streptococcus suis*. We are working with OUCRU-Ho Chi Minh City on analysing a case series of Lao *S. suis* isolates, including their genomics and how differences in pig farming between Vietnam (where *S. suis* is a more frequent cause of meningitis) and Laos may influence this.

K. Antibiotic resistance & GARP. With increasing concern globally and in Laos about the public health consequences of antibiotic resistance, we have joined with the Ministry of Health and key stakeholders to form an AMR Working Group/Committee that will work synergistically with the WHO/FAO/OIE AMR committee to accumulate and analyse the current scientific evidence for AMR in Laos. As well as describing the antibiotic susceptibility patterns of common Lao bacteria and antibiotic availability and use, these will be compared with their frequency in adjacent counties. This work is with the Global Antibiotic Resistance Partnership (GARP; www.cddep.org/garp/home) and with OUCRU-Hanoi.

L. Health behaviour and antibiotic use. We are also working with the Global Point Prevalence Survey of Antimicrobial Consumption and Resistance (www. http://www.global-pps.com/) and plan to conduct this survey in 2017. Led by Marco Haenssgen from Oxford, we will

participate in a study in 2017 to improve our understanding of patients' antibiotic-related health behaviour, in Laos and Thailand, to inspire more targeted and unconventional interventions. The project tackles three research questions - What are the manifestations and determinants of problematic antibiotic use in patients' healthcare-seeking pathways? Will people's exposure to a behavioural health systems intervention diffuse or dissipate within a network of competing healthcare practices? Which proxy indicators facilitate the detection of problematic antibiotic behaviours across and within communities.

3. Leptospirosis

A. Leptospiral DNA detection in blood cultures. Despite being a global problem, laboratory diagnosis of leptospirosis remains difficult with culture results taking up to 3 months, paired 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 leptospiral diagnosis. Despite initial promising results, a subsequent large prospective evaluation showed very low sensitivity compared with PCR of venous blood samples (Dittrich *et al.* 2016).

B. Leptospirosis 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. Genomics of *Leptospira* spp. We are working with Institut Pasteur-Paris to conduct whole genome sequencing (WGS) on the leptospires cultured from patients in the Microbiology Laboratory with comparison with data from other Asian countries and examining the relationship between genomes and antibiotic susceptibility.

D. Susceptibility testing of *Leptospira* spp. We are working on a project to conduct antibiotic susceptibility testing of these isolates sent for WGS.

4. Rickettsiology and related pathogens

A. Rapid diagnostic tests for scrub typhus. We conducted 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 the diagnosis of this important disease in rural Asia. We are conducting an interim analysis. We are also working on determining the optimal fraction of blood for the molecular diagnosis of rickettsial diseases.

Global Good team, Michael Friend & Simon Ghionea with their incubator and Sengkham Symanivong and Viengmon Davong

B. *Orientia tsutsugamushi* grows in conventional blood cultures. Dittrich *et al.* (2016) demonstrated that *O. tsutsugamushi* numbers increased for up to 5 days in conventional haemocultures. Performing such a culture step before molecular testing could increase the sensitivity of *O. tsutsugamushi* molecular diagnosis.

C. Scrub typhus genotypes. Collaboration with the 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 and antibiotic susceptibility are associated with particular *Orientia tsutsugamushi* genotypes. We are also working with the Oxford Centre for Human Genetics (OCHG) for the WGS of a diversity of *Orientia tsutsugamushi* and *R. typhi.*

D. Revisiting the natural history of scrub typhus. Dr Ivo Elliott has been awarded a Wellcome Trust Fellowship to work in LOMWRU, 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. This is in collaboration with many partners including OCHG.

Ivo Elliott extracting blood from a rodent during scrub typhus fieldwork

E. Scrub typhus in Chile. Endemic scrub typhus was originally thought to be confined to the so called "tsutsugamushi triangle" within the Asia–Pacific region but there are recent reports from the Middle East and some evidence from Kenya. We supported German & Chilean colleagues in the molecular diagnosis of *Orientia tsutsugamushi* in patients samples from Chilo Island in southern Chile, which demonstrates this pathogen in South America (Weitzel *et al.* 2016). This dramatically extends the known distribution of scrub typhus and leads to many questions as to how common *Orientia tsutsugamushi* is in South America and how it is transmitted.

F. Ticks and potential human pathogens. We worked with the Institut Pasteur du Laos and the US Navy on looking for potential human pathogens, *Rickettsia, Bartonella, Orientia, Anaplasma* and *Ehrlichia* species, in a large collection of ticks from Khammouane Province. A significant proportion contained *Rickettsia* spp. DNA, including three novel genotypes that might be new species, *Candidatus* Rickettsia laoensis, *Candidatus* Rickettsia mahosotii and *Candidatus* Rickettsia khammouanensis. In addition, evidence for *Ehrlichia* spp., *Coxiella burnetii, Anaplasma* spp. and *Borellia* spp. were identified. This should help us to narrow down what tick-borne bacterial pathogens we may find in patients in Laos (Taylor *et al.* 2016). This work is continuing with the Institut Pasteur du Laos and the US Navy with further tick collections.

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

H. Bartonella. With support from the US Navy we have been examining the seroprevalence of *Bartonalla* spp. antibodies in Laos and plan to start molecular diagnosis work in 2017. We have described *Bartonella* spp. DNA in Lao rural rodent liver & spleens (Angelakis *et al.* 2009) and human *Bartonella henselae* endocarditis (Rattanavong *et al.* 2014). These findings have prompted us to look in more detail at which *Bartonella* species may be human pathogens in Laos and looking for One Health risks.

I. Antibiotic susceptibility of rickettsial species. The project, forming Weerawat Phuklia's PhD, is examining the antibiotic susceptibility of diverse isolates of *O. tsutsugamushi* is progressing well and we expect to have more information later in 2017. We plan to expand this to *R. typhi*.

J. Trials of the antibiotic therapy of uncomplicated murine typhus and scrub typhus. We are 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.

Phonepasith Panyanouvong, Viengmon Davong, Sabine Dittrich, Anisone Chanthongthip, Assoc. Professor Bounthapany Bounxuai & Dr Manivanh Vongsouvath opposite the Houses of Parliament, London

5. Virology

The virology work of LOMWRU is strongly supported by IRD/ Aix-Marseille University, France. Virological aspects of CNS infections are discussed above.

A. Dengue epidemiology. Thankfully 2016, like 2015, had a low incidence of dengue in Laos, unlike 2013. We are analyzing the changes through time and space of *Dengue virus* serotypes in Vientiane 2006-2010 and in Luang Nam Tha and Salavan 2008-2010.

B. Dengue RDTs. In many endemic areas, including the Lao PDR, inadequate access to dengue diagnostic facilities is a major obstacle to surveillance and study of dengue epidemiology. Filter paper is widely used for blood collection for subsequent laboratory testing for antibody and nucleic acid detection. For the first time, we demonstrated that *Dengue virus* RNA can be extracted from dengue rapid diagnostic tests (RDT) for real-time RT-PCR for serotyping.

We evaluated the Standard Diagnostics (SD) Bioline Dengue Duo RDT, a commonly used test in Laos. *Dengue virus* RNA was extracted from the sample pad of the NS1 RDT. There was good agreement between dengue RT-PCR from NS1 RDT with RT-PCR performed on RNA extracted from patient sera, either using RDT loaded with blood (82.8% and 91.4%, in Vientiane and Salavan, respectively) or serum (91.9% and 93.9%). There was 100% concordance between RDT and serum RTPCR of infecting *Dengue virus* serotype. Hence, the collection of NS1 positive RDTs, which do not require cold storage, may be a novel approach for *Dengue virus* serotyping by RT-PCR and offers promising prospects for the collection of epidemiological data from previously inaccessible tropical areas to aid surveillance and public health interventions. This could facilitate the Lao national monitoring of *Dengue virus* serotypes by the shipping of RDTs from the provinces to Vientiane for dengue RT-PCR of NS1 positive RDTs. Dr Manivanh Vongsouvath performed this research for her successful Mahidol University MSc thesis (Vongsouvath *et al.* 2016)

We continued the evaluation of the thermal stability of dengue RDTs (see Phommasone *et al.* 2015, in 2015 Annual Report) in tropical temperatures using patient sera strengthening the evidence of the robustness of the NS1 cassette after storage (Sengvilaipaseuth *et al.* 2017).

Dr Rosalie Zimmerman's leaving ceremony

C. Filter paper CSF and JEV diagnosis. The use of filter paper as a simple, inexpensive tool for storage and transportation of blood, 'Dried Blood Spots' or Guthrie cards, for diagnostic assays is well-established. In contrast, there is a paucity of diagnostic evaluations of dried cerebrospinal fluid (CSF) spots. CSF filter paper spots have potential applications in low-resource settings, such as Laos, where laboratory facilities for central nervous system (CNS) diagnostics are only available in Vientiane. In Laos, a major cause of central nervous system infection is the *Japanese encephalitis virus*. Bharucha *et al.* (2016) 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. The diagnostic accuracy of the optimised protocol was compared with routine, neat CSF in a pilot, retrospective study of JEV MAC-ELISA on consecutive CSF samples from three Lao hospitals. In comparison to neat CSF, 132 CSF samples stored as dried CSF spots for one month at 25–30°C showed 81.6% positive agreement, 96.8% negative agreement, with a kappa coefficient of 0.81. The novel design of pre-cut filter paper saturated with CSF could provide a useful tool for JEV diagnostics in settings with limited laboratory access. It has the potential to improve national JEV surveillance and inform vaccination policies. The saturation of filter paper has potential use in the wider context of pathogen detection, including dried spots for detecting other analytes in CSF, and other body fluids.

D. 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 EV-A71 disease for when the expected epidemic occurs.

E. *Zika virus* infection. With current global concern of the public health impact of this emerging pathogen and association of infection with microcephaly we are working with partners to build diagnostic capacity at Mahosot Hospital. We are expanding the work on PCR detection of *Dengue virus* from RDTs (see above) to detect *Zika virus* and *Chikungunya virus* to facilitate national surveillance of these pathogens, with the US Navy.

F. JEV patient clinical follow up. Dr Phouvieng Douangdala successfully completed his UHS thesis on the disability associated with CNS JEV infection, using the Liverpool Outcome Score. Of all patients assessed, 1/5 died during hospitalization or after discharge. Although the mortality was similar between children and adults, the neurological sequelae were more serious in children. During the ~ 60 months follow up the proportion of the patients who completely recovered (without neurological sequelae) at the last follow up was 38.3% and this figure was significantly higher in adults (48.9%) than in children (27.7%).

G. Acute Respiratory Illness. In 2012, the World Health Organization (WHO) launched the Battle against Respiratory Viruses (BRaVe) initiative in response to increasing evidence that viruses play an important role in acute respiratory infections (ARI). WHO emphasized the need to prioritize research to gain a better understanding of the epidemiology, pathogenesis, prevention and clinical management of respiratory virus infections across different populations and resource settings. In the framework of ARIVI study (see above), we showed that the *Human respiratory syncytial virus* (RSV) is an important cause of acute respiratory illness in children less than 2-year-old, admitted to Mahosot Hospital, with a peak of RSV infection detected between June and September in 2014.

The Old Old Microbiology Laboratory 2002

6. One Health

We participate in two One Health projects in addition to the studies above on scrub typhus and leptospirosis. LACANET (http://www.onehealthsea.org/lacanet) is a European Union-funded, binational collaboration between Lao PDR and Cambodia in association with the Wildlife Conservation Society (WCS) to conduct field surveillance of zoonotic diseases at human-wildlife interfaces. COMACROSS (http://www.onehealthsea.org/comacross) is also a EU funded project, led by CIRAD and linked to the SEAe project (above). Steven Prigent led a study on Japanese Encephalitis: an ethnographic Approach (Cambodia and Laos).

A. Perception of health risk due food sold by vegetable, domestic meat and wildlife vendors in Lao markets. As part of LACANET and her successful IFMT MSc thesis, Dr Chanfong Philavong investigated vendors' perception of health risk due to food sold in markets. Wild and domestic animals are potential carriers of diverse diseases, and may be potential sources of contamination not only to the consumers, but also to the butcher and the vendors. A descriptive cross-sectional study in markets with traders selling wildlife meat was conducted. Every vegetable, domestic meat and wildlife meat vendor in three major markets (one in the north, one in the center and one in the south) were solicited to participate to the study. In total 177 persons consented, consisted of 85 vegetable vendors, 57 domestic meat vendors and 35 wildlife meat vendors. Nearly all vendors had a very low perception of risk for health due to the food sold. More detailed investigation of these risks and how to engage with market vendors and their customers is needed.

B. Pathogens in wildlife in markets. Also as part of LACANET, collections of samples from wildlife are being made at wildlife trade markets across Laos by the WCS. We are testing these samples for the presence of a number of key pathogen species. During January 2015 to April 2016, 400 specimens from 248 animals were sent to LOMWRU for lab analysis. We found that 44 animals contained *Leptospira* spp. and six animals contained *Rickettsia* spp., including a confirmed *R. felis*. During the testing process, we also identified samples containing *L. garvieae*, *Kurthia* spp., *Ehrlichia* spp. TC251-2, and *A. marginale*. Our findings suggest that *Leptospira* spp. may be the most frequently identified pathogen in tha Lao wildlife trade. The discovery of *R. felis*, an emerging rickettsial pathogen, is the first reported case in a squirrel species. We found Pallas's, Grey-bellied, and Red-cheeked squirrels to be the most common wildlife species sampled in markets. Our observations indicated their potential as multiple disease reservoirs, with a total of four different pathogen species being identified in squirrels. The Champasak and Bolikhamxay Provinces are potential hotspots for wildlife trade as 28.6% and 29.8%, respectively, of specimens we received were from markets located in these regions. The study is continuing.

7. Malaria

A. Malaria diagnosis – positive control wells. Rapid diagnostic tests (RDTs) are widely used for malaria diagnosis, but lack of quality control at point of care may restrict trust in test results. Prototype positive control wells (PCW) containing recombinant malaria antigens have been developed to identify poor-quality RDT lots. We worked with the Foundation for Innovative New Diagnostics to assess community and facility health workers' (HW) ability to use PCWs to detect degraded RDTs in Laos and Uganda (Bell *et al.* 2016). A total of 557 HWs participated in Laos (267) and Uganda (290). After training, most (88% to 99%) participants correctly performed the six key individual PCW steps; performance was generally maintained during the 6-month study period. Nearly all (97%) reported a correct action based on PCW use at routine work sites. PCW availability can improve HWs' confidence in RDT results, and benefit malaria diagnostic programs. These data support the implementation of PCWs in RDTs.

B. Malaria diagnosis – scanning RDTs. Drs Rosalie Zimmerman and Rene Niehus are working on new mathematical calculations for examining the relationship between HRP-2 band intensity, as determined by Image J analysis, on malaria RDTs and blood HRP-2 quantification performed by MORU-Bangkok.

C. Artemisinin resistance – clinical aspects. With the spread of *Plasmodium falciparum* 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 parasite clearance in uncomplicated falciparum malaria between artemether-lumefantrine and artemether-lumefantrine plus amodiaquine. We hope that these data will be useful for informing optimal future ACT Government policy. With the decline in *Plasmodium falciparum* malaria in Laos, patient recruitment has been slow with 11 patients recruited by December 2016. The trial will restart with the rains in 2017.

D. Molecular markers of antimalarial resistance. We have been collecting filter paper blood spots from malaria patients all over southern Laos, where malaria is more prevalent, for the last ten years, with the Centre for Malariology, Parasitology & Entomology, to examine how the frequency of molecular markers of anti-malarial resistance, including those of artemisinin 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.

Artemisinin-resistant *Plasmodium falciparum* malaria parasites are now present across much of mainland Southeast Asia. Grist *et al.* (2016) proposed a generic 'smart surveillance' methodology to identify optimal locations for future sampling and thus map the distribution of artemisinin resistance most efficiently. The approach uses the 'uncertainty' map generated iteratively by a geostatistical model to determine optimal locations for subsequent sampling and could improve the quality and efficiency of drug resistance mapping and thereby guide practical operations to eliminate malaria in affected areas.

The markers pfmdr1 and pfcrt were genotyped in parasite samples obtained in 2011–2014 at 14 TRAC (Tracking Resistance to Artemisinin Collaboration) sites in mainland Southeast Asia, including from Attapeu, Laos, using a combination of PCR and next-generation sequencing methods (Srimuang *et al.* 2016). Pfmdr1 amplification, a marker of mefloquine and lumefantrine resistance, was highly prevalent at Mae Sot on the Thailand–Myanmar border (59.8% of isolates) and common (more than 10%)

Phonepasith Boupha & Kem Boutsamay evaluating a medicine quality screening device

at sites in central Myanmar, eastern Thailand and western Cambodia. However, this was not found in Laos. The pfcrt mutation K76T associated with chloroquine resistance was found in 98.2% of isolates. The CVIET haplotype made up 95% or more of isolates in western Southeast Asia while the CVIDT haplotype was common (30–40% of isolates) in north and northeastern Cambodia, southern Laos, and southern Vietnam. The absence of resistance-conferring pfmdr1 mutations and SVMNT pfcrt haplotypes suggests that amodiaquine could be an efficacious component of anti-malarial regimens in SEA. Amodiaquine, in combination with artemether-lumefantrine, is being trialed in the TRAC-2 study, above.

As part of the MalariaGEN (2016) project, we participated in a large global survey, finding that *kelch13* mutations associated with artemisinin resistance in Southeast Asia are present at low frequency in Africa. This showed that African *kelch13* mutations have originated locally, and that *kelch13* shows a normal variation pattern relative to other genes in Africa, whereas in Southeast Asia there is a great excess of non-synonymous mutations, many of which cause radical amino-acid changes. Thus, *kelch13* is not currently undergoing strong selection in Africa, despite a deep reservoir of variations that could potentially allow resistance to emerge rapidly. The practical implications are that public health surveillance for artemisinin resistance should not rely on *kelch13* data alone, and interventions to prevent resistance must account for local evolutionary conditions, shown by genomic epidemiology to differ

Rodent catching team setting off for scrub typhus research

greatly between geographical regions. We also participated in a global study on copy number variants in *Plasmodium falciparum* (Cheeseman *et al*. 2016) and a comparison of the distribution of amino acid mutations in DHFR and DHPS in *Plasmodium vivax* isolates from Laos, India and Colombia (Saralamba *et al*. 2016).

E. Pharmacokinetics. We participated in a comparison, on the Thai/Myanmar border, between the pharmacokinetics of oral syrup and intramuscular paracetamol given to patients with acute falciparum malaria and high body temperature (Wattanakul *et al.* 2016). A randomized, open-label, two-treatment, crossover, pharmacokinetic study of paracetamol dosed orally and intramuscularly was conducted. Paracetamol plasma concentrations after oral syrup and intramuscular administration in patients with acute falciparum malaria were described successfully by a two-compartment disposition model. Relative oral bioavailability compared to intramuscular dosing was estimated as 84.4 % (95 % CI 68.2–95.1 %). Dosing simulations showed that a loading dose followed by six-hourly dosing intervals reduced the time delay to reach therapeutic drug levels after both routes of administration. The safety and efficacy of loading dose paracetamol antipyretic regimens now needs to be established in larger studies.

F. Malaria immunology. An effective antibody response can assist drug treatment to contribute to better parasite clearance in malaria patients. To examine this Goh *et al.* (2016) obtained sera from two groups of adult patients with acute falciparum malaria, prior to drug treatment: patients who (1) had subsequent recrudescent infection, or (2) were cured by Day 28 following treatment. Using a *Plasmodium falciparum* antigen library, the antibody specificities in these sera were examined. While the antibody repertoire of both sera groups was extremely broad and varied, there was a differential antibody profile between the two groups of sera. The proportion of cured patients with antibodies against EXP1, MSP3, GLURP, RAMA, SEA and EBA181 was higher than the proportion of patients with recrudescent infection. The presence of these antibodies was associated with higher odds of treatment cure. Sera containing all six antibodies impaired the invasion of *P. falciparum* clinical isolates into erythrocytes. These results suggest that antibodies specific against EXP1, MSP3, GLURP, RAMA, SEA and EBA181 in *P. falciparum* infections could assist anti-malarial drug treatment and contribute to the resolution of the malarial infection.

G. 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, SMRU and Institut de Recherche pour le Développement (IRD). A G6PD deficiency survey conducted in six randomly selected villages of two districts of Sekong province demonstrated that, using the Trinity fluorescence spot test, the frequency of people with phenotypic G6PD deficiency was ~ 4% (70/1,897). Molecular G6PD analysis is continuing.

Dr Rattanaphone Phetsouvanh and laboratory team at the Microbiology Laboratory Reopening 15 November 2002

H. Gametocyte carriage. We participated in an individual patient clinical data meta-analysis to identify the determinants of gametocyte carriage and the comparative effects of four ACTs: artemether-lumefantrine (AL), artesunate/amodiaquine (AS-AQ), artesunate/mefloquine (AS-MQ), and dihydroartemisinin-piperaquine (DP) (WWARN Gametocyte Study Group 2016). This concluded that AS-MQ and AL are more effective than DP and AS-AQ FDC in preventing gametocytaemia shortly after treatment, suggesting that the non-artemisinin partner drug or the timing of artemisinin dosing are important determinants of post-treatment gametocyte dynamics.

I. Malaria in pregnancy. There are no data on the burden of malaria in pregnancy (MiP) in Laos, where malaria still remains prevalent in the south. Two cross-sectional surveys were conducted in 2014 to assess the prevalence of MiP in Vapi District, Salavan Province, led by CMPE and Institut de Recherche pour le Développement (IRD). The first consisted of screening 204 pregnant women during pregnancies living in 30 randomly selected villages in Vapi District. The second was conducted among 331 pregnant women who delivered during the study period in Vapi and Toumlane District Hospitals and in Salavan Provincial Hospital (Briand *et al.* 2016). Peripheral and placental malaria was detected using rapid diagnostic tests (RDT), thick blood smears (TBS) and real-time quantitative polymerase chain reactions (RT-qPCR). In the villages, 12/204 women (5.9 %) were infected with malaria as determined by RTqPCR: 11 were *Plasmodium vivax* infections and 1 was mixed *Plasmodium vivax*/ *Plasmodium falciparum* infection, among which 9 were sub-microscopic (as not detected by TBS). At delivery, two *Plasmodium falciparum* submicroscopic infections (one peripheral and one placental) were detected (4.5 %; 0.6–15.5) in Vapi District. The majority of infections (94 %) were asymptomatic and half of them were associated with anaemia. 24 % of women had LBW newborns, associated with tobacco use and pre-term delivery. Factors associated with a higher risk of maternal anaemia were no iron supplementation during pregnancy, Lao Theung ethnicity and place of residence. The prevalence of MiP in this population was noticeable. Most infections were asymptomatic and submicroscopic vivax malaria, which raises the question of reliability of recommended national strategies for the screening and prevention of MiP in Laos.

J. Asymptomatic malaria prevalence. A fascinating recent development has been the realization that there are foci in rural Asia of populations with high frequencies of *Plasmodium falciparum* asymptomatic malaria carriage that persist at densities in blood that are not detectable by microscopy or rapid diagnostic tests. These infections may be critical as a transmission reservoir in areas of low malaria endemicity, such as Laos. Cross-sectional surveys in Thapangthong and Nong Districts of Savannakhet Province, determined the prevalence of parasitaemia. A total of 888 blood samples were collected from afebrile volunteers aged ≥15 years in 18 villages during March and July 2015. *Plasmodium* infections were diagnosed by high volume, ultra-sensitive quantitative polymerase chain reaction (uPCR). uPCR detected *Plasmodium* infections in 175 of 888 samples (20 %). The species distribution was *Plasmodium falciparum* 3.6 % (32/888), *Plasmodium vivax* 11.1 % (99/888), mixed infections with *P. falciparum* and *P. vivax* 1.6 % (14/888) and *Plasmodium* of undetermined

species 3.4 % (30/888). RDT identified only 2 % (18/888) positive cases. The K13 kelch propeller domain C580Y mutation, associated with reduced susceptibility to artemisinin derivatives, was found in 75 % (12/18) of *P. falciparum* isolates from Thapangthong and in 7 % (2/28) from Nong ($p < 0.001$). Artemisinin-resistant *P. falciparum* strains form an increasing proportion of the parasite population in Thapangthong District and are already present in the more remote Nong District. The high prevalence of asymptomatic malaria and artemisinin resistance has wider implications for Laos and could reverse the gains achieved by the successful control of malaria in Laos and the Greater Mekong Sub-region (GMS). Considering the rapid elimination of *P. falciparum* should be a top priority in Laos as well as in the wider GMS.

K. Targeted malaria elimination. With these high frequencies of apparently asymptomatic *P. falciparum,* trials of Targeted Malaria Elimination (TME) with DHA-piperaquine in Nong District, Savannakhet Province, with key public engagement actions, started in 2016. This is funded by the Bill and Melinda Gates Foundation with CMPE and MORU-Bangkok.

Three rounds of mass drug administration (DHA-piperaquine) have been completed in the two intervention villages and comparisons of *P. falciparum* and *P. vivax epidemiology* in the communities is continuing. Intensive public engagement programs have been implemented and their impact studied. We expect that trial to be completed late in 2017. Dr Koukeo Phommasone, Dr Tiengkham Pongvongsa and Dr Bipin Adhikari are conducting different aspects of this work for their PhD theses.

L. Vivax malaria treatment. The clinical trial of the efficacy of chloroquine in *P. vivax* malaria is continuing in Sekong. As part of TME study, we have also recently started, with CMPE and MORU-Bangkok, a randomised, single-blinded controlled treatment trial of subclinical vivax infections with primaquine in Nong District, Savannakhet Province. This compares dihydroartemisinin-piperaquine therapy plus 14 days of supervised primaquine (7mg/kg total dose) versus dihydroartemisinin-piperaquine therapy plus 14 days placebo not containing primaquine. The primary objective is to determine whether a 14 day course of 0.5 mg/kg/day primaquine can eliminate subclinical *P. vivax* infections detected by high volume ultra-sensitive PCR (uPCR).

8. Medicine quality & pharmacy

We expanded the work of the group in 2016, with funding from the Wellcome Trust and the Asian Development Bank.

A. The Worldwide Antimalarial Resistance Network (WWARN) & Infectious Disease Data Observatory (IDDO). 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). WWARN is being subsumed with the Infectious Diseases Data Observatory (IDDO; https://www.iddo.org/medicine-quality) and the expanded medicine quality work will be hosted within IDDO. We are tabulating the accessible data on the quality of maternal health medicines, antibiotics, antidiabetics, anti-retrovirals and veterinary medicines and plan to map these in 2017. They will also be analysed for reviews on the quality of these essential medicines in 2017. We are working with HealthMap (http://www.healthmap.org/en/) on trawling the lay literature in multiple languages for reports of poor quality medicines and using text mining to automate searches.

Lychee giant shield bug in garden of Microbiology Laboratory

We are also working with the United States Pharmacopeia to build an individual sample database to map the USP MQDB system (see http://apps.usp.org/app/worldwide/medQualityDatabase/terms.html).

B. History of poor quality medicines. *The Third Man* is a classic 1949 film depicting the trade in fake penicillin in post-war Vienna. Newton & Timmerman (2016) investigated the origins of the story behind the fake penicillin racket portrayed in the film and the illegal trade in penicillin in post-WW2 Europe and links to espionage.

C. Chemistry. We worked with the Georgia Institute of Technology, USA, (Bernier *et al.* 2016) to examine the use of Direct Analysis in Real Time (DART) ionization coupled with a portable low-resolution single-quadrupole instrument in chemical 'fingerprinting' anti-malarial tablets. Using single quadrupole DART-MS, the same sample components were detected as with a high-resolution instrument, while needing significantly less consumables and power, and with the additional advantages of increased

portability and ease of use. Using Principal Component Analysis (PCA) of DART data, specific classes of falsified ACTs were identified, providing a more straightforward method for sourcing counterfeits and assessing their similarities.

D. Evaluation of new diagnostic devices. We have been working with Global Good, pharmacists at Mahosot Hospital, the Faculty of Pharmacy of the UHS and the Bureau of Food and Drug Inspection of Laos to evaluate the diagnostic accuracy of a small Near-Infrared device for screening tablet quality.

With funding from the Asian Development Bank we are investigating the comparative diagnostic accuracy and cost-effectiveness of a diversity of portable and handheld medicine quality screening devices. We also work with Dr Fred Behringer (Surveillent LLC, USA) on FITR techniques for evaluating the quality of anti-tuberculosis medicines.

E. Falsified diazepam – Democratic Republic of the Congo. We worked with Médecins sans Frontières (MSF), Switzerland, on a large epidemic of dystonic reactions in NE Democratic Republic of the Congo (DRC) (Peyraud *et al.* 2017). Over 1,000 patients developed dystonic reactions after taking 'diazepam' that actually contained large amounts of haloperidol.

F. *Hepatitis C virus* (HCV) Treatment. The high prices of innovator products for HCV ('one thousand dollar pills') and the 'alternative' routes to access treatment have quickly reached patients with HCV worldwide. These therapies are not affordable to most of those with HCV and there is growing concern that the online purchase of HCV therapies, with or without medical prescription will result in an unregulated market constituting the only 'accessible' opportunity for patients (Ravinetto *et al.* 2016). With circulation of falsified sofosbuvir and pegylated interferon and ribavirin, patients are at risk of poor quality HCV therapy. Ravinetto *et al.* (2016) argue that market incentives should be in place to push the manufacturers of generic HCV therapy to obtain either WHO Prequalification or Stringent Regulatory Authority (SRA) registration. This has important relevance to Laos as sofosbuvir is available and has been demonstrated to be falsified in Myanmar.

G. Guidelines for medicine quality surveys. We have revised the MEDQUARG guidelines (Newton *et al.* 2009; *PLoS Medicine* 6, e1000052) on conducting and reporting surveys for the quality of medicines for the WHO. These have now formally published as the Guidelines on the Conduct of Surveys of the Quality of Medicines in WHO Technical Report Series, No. 996, 2016, Annex 7 (2016; 36 pages). See:

http://apps.who.int/medicinedocs/en/m/abstract/Js22404en/

The key work of Patricia Tabernero, with Sue Lee, Kasia Stepniewska and Paul Newton, in these guidelines will be acknowledged and corrected by WHO in 2017.

H. 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.

I. 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 for Laos or too small a font to read!

J. 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.

K. Access to Medicines Index. The global reporting of poor quality medicines between stakeholders 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

Sengmany Symanivong & Céline Caillet with a dinosaur in Oxford !

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 Rapid Alert system. The first inclusion of these data in the AMI were published in 2016 – see: http://accesstomedicineindex.org/media/atmi/Access-to-Medicine-Index-2016.pdf.

L. Modelling of the impact of poor quality medicines. There is little objective information on the consequences of poor quality medicines on patient outcomes and, for anti-infectives, on drug resistance (Newton *et al.* 2016). We plan to use the accumulated data on the quality of antimalarials and antibiotics to inform models of the consequences of poor quality antibiotics on patient outcome and drug resistance, working with the mathematical modellers and PK-PD scientists in the MORU Network.

M. 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 (Tabernero *et al.* 2016).

N. Pharmacovigilance & medicine quality. Caillet & Newton (in press) describe the public health issues of poor quality medicines and the factors that facilitate their existence and those that impede action to ensure that patients take good quality medicines. They discuss the role of pharmacovigilance in detecting poor quality medicines.

O. Medicine quality intelligence reports. We wrote reports every two months on medicine quality problems, from a public health perspective, for the International Criminal Police Organization (INTERPOL).

P. Reporting. If we find any evidence of poor quality medicines in Laos through our work we report these findings asap to the Food and Drug Department, Government of the Lao PDR.

Q. Adverse drug reaction (ADR)-related hospitalizations. For her PhD research, Céline Caillet investigated the frequency of serious adverse drug reaction-related hospitalizations in Mahosot Hospital. The frequency of hospitalizations related to an ADR was 5.1% during a 7-week period, similar to that observed for diabetes or hypertension-related complications in Mahosot during the same period. In addition, we observed that more than one-quarter of the ADR-related hospitalization was caused by medicines that had been repackaged in individual bags without labelling of their identity, a practice that was identified as a factor associated with serious ADR.

9. Modelling and public health

Under the leadership of Profs Lisa White and Ben Cooper in MORU-Bangkok, a small public health modelling group is developing in Laos, with Drs Rene Niehus, Khansoudaphone Phakhounthong, Phetsavanh Chanthavilay, Olivier Celhay & Tamalee Roberts, working on a series of projects on antimicrobial resistance, malaria epidemiology and typhus antibody responses. A course in Vientiane on modeling and public health policy, run by MORU-Bangkok, is planned for 2017.

ENGAGEMENT

Pii Mai Lao Celebrations

Community perceptions and engagement

Now that there are more data on infectious disease epidemiology in Laos, we are planning 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. Excitingly, we helped with the first Science Café in Laos that we hope will become a regular feature of the Vientiane scientific 'scene'.

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 - see: (http://www.uhs.edu.la/elibrary/Elibrary.php). If you have any open access papers relevant to public health in Laos please submit them 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 cafés are now popular in other Asian cities, such as Ho Chi Minh City and Bangkok. On July 31st 2016, the first Science café was held in Laos, organised by the University of Health Sciences, Ministry of Health, in collaboration with MORU-Bangkok (Cheah et al. 2016). More than 50 students and staff of the Faculties of Medicine, Pharmacy, Dentistry, Nursing Sciences, Medical Technology, Basic Sciences, and Public Health attended the 2 h long event on Medical and Research Ethics. Lively discussions included what makes a research study ethical, what makes consent valid, and whether children should be involved as participants in clinical research. We hope to collaborate on arranging these as regular events in 2017.

E. London School of Hygiene and Tropical Medicine Short Course on Medicine Product Quality & Public Health. We organised the second course on medicine quality and public health at the LSHTM in July 2016, 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. This will move to Boston University for the 2017 course and then hopefully to West Africa.

F. Medicine Quality. As part of the MAPQAMP project within IDDO we are developing a system for engaging with stakeholders, especially medicine regulatory authorities in LMICs.

Lab Blood donor goats in the garden

Renovation of the Old Microbiology Laboratory roof

OTHER ACTIVITIES

International New Year Party at Wattay Airport buffet

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 and has frequent talks by academic visitors. LOMWRU has contributed to the monthly scientific talks of Mahosot Hospital and the annual Lao Internal Medicine and Paediatric CME Conferences. We also participate regularly via Webex in seminars at MORU in Bangkok.

D. Pathogen Asset Control System (PACS). With the kind support of DTRA of the USA, LOMWRU, along with other medical organisations in Vientiane, has a new Pathogen Asset Control System (PACS) for the barcoding and cataloguing of samples so that they can accurately stored and located. Implementation for our collection of bacterial isolates is nearing completion and we plan to extend this during 2017 to other samples.

E. LIMS. With the considerable help of MORU-Bangkok and COMRU, we have installed a Laboratory Information Management System (LIMS) in the Microbiology Laboratory that went live in January 2017 and we hope will improve the efficiency and accuracy of the microbiological service, and greatly facilitate data retrieval.

Geographic Information System Training graduates with the tutor Dr Daniel Parker

KEY COLLABORATIONS

Targeted Malaria Elimination Team in Nong District

Within Lao PDR

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

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, Jayasree Iyer & Danny Edwards, 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 Richard Laing, Assoc. Prof Veronika Wirtz and Dr Erin Hasselberg, School of Public Health, Boston University, Boston, USA

Professor Sharon Peacock, University of Cambridge, UK

Dr Mike Green, CDC, Atlanta, Georgia, USA

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 Hellen Gelband, Global Antibiotic Resistance Partnership, Washington DC, 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, Unité de Biologie des Spirochètes, Institut Pasteur, Paris

Dr Alain Pierret, Institut de recherche pour le développement, Laos

Dr Guillaume Lacombe, International Water Management Institute, Laos

Dr Olivier Ribolzi, Géosciences Environnement Toulouse, Université de Toulouse, France

Dr Emma Rochelle-Newall, iEES-Paris, Université Pierre et Marie-Curie, Paris, 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, Dr Shunmay Yeung and Dr Harparkash Kaur, London School of Hygiene and Tropical Medicine, London, UK

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

Dr Nicolas Peyraud, Médecins sans Frontières (MSF), 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

Dr Wei-Mei Ching, Viral and Rickettsial Diseases Department,Naval Medical Research Center, USA

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

Professor Tim Anderson, Southwest Foundation for Biomedical Research, San Antonio, Texas, USA

Dr Damien Chaussabel, Sidra Medical and Research Center, Qatar

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

Dr Fred Behringer, Surveillant LLC, Old Lyme, USA

Dr Esther Kuenzli, Dr Rosalie Zimmermann, Dr Kerstin Kling, Dr Andreas Neumayr and colleagues, Swiss Tropical and Public Health Institute, Basel

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

Dr Souly Phanouvong, Dr Lukas Roth, Dr Victor Pribluda & Dr Mustapha Hajjou, United States Pharmacopeia, Rockville, Virginia, USA

Dr Todd French and Philip Bulterys, University of California-Los Angeles, USA

Prof Ivo Steinmetz, University of Graz, Austria

Professor KT Wong, Faculty of Medicine, University of Malaya, Malaysia

Professor Xavier Nicolas de Lamballerie, UMR "Emergence des Pathologies Virales" (EPV: Aix-Marseille university - IRD 190 - Inserm 1207 - EHESP), Marseille, France.

Professors Didier Raoult, Pierre-Edouard Fournier, Jean-Marc Rolain, Philippe Parola, Rickettsial Reference Laboratory, Aix-Marseille University, France

Dr Fiona Russell and Prof Kim Mullholland, Murdoch Childrens Research Institute (MCRI), University of Melbourne, Victoria, Australia

Dr Andrew Steer and Pierre Smeesters, Murdoch Childrens Research Institute (MCRI), University of Melbourne, Victoria, Australia

Professor Marya Lieberman, Department of Chemistry and Biochemistry, University of Notre Dame, USA

Professor Amir Attaran, Faculties of Law and Medicine, University of Ottawa, Ontario, Canada

Dr Nicole Stoesser and Prof Derrick Crook, Nuffield Department of Medicine, University of Oxford, UK

Professor Philippe Guérin and the Infectious Diseases Data Observatory, Centre for Tropical Medicine & Global Health, University of Oxford, UK

Dr Rory Bowden and Dr Liz Batty, Wellcome Trust Centre for Human Genetics, University of Oxford

Dr David AuCoin, University of Nevada School of Medicine, Reno, Nevada, USA

Drs Paul Brett and Mary Burtnick, University of South Alabama, USA

Dr Aleisha Brock and Prof Adrian Esterman, University of South Australia, Adelaide, Australia

Professor Albert Ko, Yale School of Public Health, USA

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

Michael Deats, Pernette Bourdillon-Esteves & Diana Lee, WHO, Geneva, Switzerland

Sabine Dittrich receiving a thank you certificate from Mahosot Hospital Director Assoc. Professor Bounthapany Bounxuai

TITLES AND ABSTRACTS OF PAPERS PUBLISHED OR IN PRESS 2016

include an abstract a brief summary is given in [ ].

1. 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* 374:542-554.

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.

2. Bell D, Bwanika JB, Cunningham J, Gatton M, González IJ, Hopkins H, Kibira SP, Kyabayinze DJ, Mayxay M, Ndawula B, Newton PN, Phommasone K, Streat E, Umlauf R (2016) Prototype Positive Control Wells for Malaria Rapid Diagnostic Tests: Prospective Evaluation of

Céline Caillet & Rene Neihus at OTN

Implementation among Health Workers in Lao People's Democratic Republic and Uganda. *Am J Trop Med Hyg.* 2016 Nov 28. pii: 16-0498.

Abstract. Rapid diagnostic tests (RDTs) are widely used for malaria diagnosis, but lack of quality control at point of care restricts trust in test results. Prototype positive control wells (PCW) containing recombinant malaria antigens have been developed to identify poor-quality RDT lots. This study assessed community and facility health workers' (HW) ability to use PCWs to detect degraded RDTs, the impact of PCW availability on RDT use and prescribing, and preferred strategies for implementation in Lao People's Democratic Republic (Laos) and Uganda. A total of 557 HWs participated in Laos (267) and Uganda (290). After training, most (88% to 99%) participants correctly performed the six key individual PCW steps; performance was generally maintained during the 6-month study period. Nearly all (97%) reported a correct action based on PCW use at routine work sites. In Uganda, where data for 127,775 individual patients were available, PCW introduction in health facilities was followed by a decrease in antimalarial prescribing for RDT-negative patients 5 years of age (4.7–1.9%); among community-based HWs, the decrease was 12.2% (P < 0.05) for all patients. Qualitative data revealed PCWs as a way to confirm RDT quality and restore confidence in RDT results. HWs in malaria endemic areas are able to use prototype PCWs for quality control of malaria RDTs. PCW availability can improve HWs' confidence in RDT results, and benefit malaria diagnostic programs. Lessons learned from this study may be valuable for introduction of other point-of-care diagnostic and quality-control tools. Future work should evaluate longer term impacts of PCWs on patient management.

3. Bernier MC, Li F, Musselman B, Newton PN, Fernández FM (2016) Fingerprinting of Falsified Artemisinin Combination Therapies via Direct Analysis in Real Time Coupled to a Compact Single Quadrupole Mass

Dr Manivanh Vongsouvath, Weerawat Phuklia, Anisone Chanthongthip & Viengmon Davong at Keble College, Oxford

Spectrometer. *Analytical Methods* 8, 6616.

Abstract. Falsified anti-malarial treatments continue to constitute a major health crisis, especially in malarious Africa. Even after detection of poor quality pharmaceuticals, it is critical that they be fully analyzed to determine their components, in order to assess their health effects and ultimately allow forensic tracing of their sources of production and distribution. Timely assessment requires robust and complete field-testing, or at the very least timely analysis after seizure or purchase. Ideally, low-cost and simple analytical equipment such as portable mass spectrometry (MS) is the best approach for achieving this quick and informative analysis. To date, Direct Analysis in Real Time (DART) MS has been successfully implemented to rapidly analyze falsified artemisinin-based combination therapies (ACTs) in laboratory settings, but this approach typically translates into high-cost and the need for high-resolution instrumentation. Here, we examine the use of DART ionization coupled with a portable low-resolution single-quadrupole instrument, and compare its success in fingerprinting anti-malarial tablets with higher resolution instrumentation. Using single quadrupole DART-MS, the same sample components were detected as with the high-resolution instrument, while needing significantly less consumables and power, and the additional advantages of increased portability and ease of use. Using Principal Component Analysis (PCA) of DART data, specific classes of falsified ACTs were identified, providing a more straightforward method for sourcing counterfeits and assessing their similarities.

4. Bharucha T, Chanthongthip A, Phuangpanom S, Phonemixay O, Sengvilaipaseuth O, Vongsouvath M, Lee S, Newton PN, Dubot-Pérès A (2016) Pre-cut filter paper for detecting anti-Japanese Encephalitis Virus IgM from Dried Cerebrospinal Fluid Spots. *PLOS Neglected Tropical Diseases* 10(3):e0004516.

Abstract. Background. The use of filter paper as a simple, inexpensive tool for storage and transportation of blood, 'Dried Blood Spots' or Guthrie cards, for diagnostic assays is well-established. In contrast, there are a paucity of diagnostic evaluations of dried cerebrospinal fluid (CSF) spots. These have potential applications in low-resource settings, such as Laos, where laboratory facilities for central nervous system (CNS) diagnostics are only available in Vientiane. In Laos, a major cause of CNS infection is Japanese encephalitis virus (JEV). We aimed to develop a dried CSF spot protocol and to evaluate its diagnostic performance using the World Health Organisation recommended anti-JEV IgM antibody capture enzyme-linked immunosorbent assay (JEV MAC-ELISA). **Methodology and Principal Findings.** Sample volumes, spotting techniques and filter paper type were evaluated using a CSF substitute of anti-JEV IgM positive serum diluted in Phosphate Buffer Solution (PBS) to end-limits of detection by JEV MAC-ELISA. A conventional protocol, involving eluting one paper punch in 200µl PBS, did not detect the end-dilution, nor did multiple punches utilizing diverse spotting techniques. However, pre-cut filter paper enabled saturation with five times the volume of CSF-substitute, sufficiently improving sensitivity to detect the end-dilution. The diagnostic accuracy of this optimised

protocol was compared with routine, neat CSF in a pilot, retrospective study of JEV MAC-ELISA on consecutive CSF samples, collected 2009–15, from three Lao hospitals. In comparison to neat CSF, 132 CSF samples stored as dried CSF spots for one month at 25–30°C showed 81.6%(65.7–92.3 95%CI) positive agreement, 96.8% (91.0–99.3 95%CI) negative agreement, with a kappa coefficient of 0.81 (0.70–0.92 95%CI). **Conclusions/Significance.** The novel design of pre-cut filter paper saturated with CSF could provide a useful tool for JEV diagnostics in settings with limited laboratory access. It has the potential to improve national JEV surveillance and inform vaccination policies. The saturation of filter paper has potential use in the wider context of pathogen detection, including dried spots for detecting other analytes in CSF, and other body fluids.

5. Briand V, Hesran J-Y, Mayxay M, Newton PN, Bertin G, Houzé S, Keomany S, Inthavong Y, Vannavong N, Chindavongsa K, Hongvanthong B, Fievet N (2016) Prevalence of malaria in pregnancy in southern Laos: a cross-sectional survey. *Malaria Journal* 15:436.

Abstract. Background. There are no data on the burden of malaria in pregnancy (MiP) in Laos, where malaria still remains prevalent in the south. **Methods**: Two cross-sectional surveys were conducted in 2014 to assess the prevalence of MiP in Vapi District, Salavan Province, southern Laos: the first consisted of screening 204 pregnant women during pregnancies [mean (95 % CI) gestational age: 23 (22–25) weeks] living in 30 randomly selected villages in Vapi District; the second was conducted among 331 pregnant women, who delivered during the study period in Vapi and Toumlane District Hospitals and in Salavan Provincial Hospital. Peripheral and placental malaria was detected using rapid diagnostic tests (RDT), thick blood smears (TBS) and real-time quantitative polymerase chain reactions (RT-qPCR). Factors associated with low birth weight (LBW) and maternal anaemia were assessed. **Results**: In the villages, 12/204 women (5.9 %; 95 % CI 3.1–10.0) were infected with malaria as determined by RTqPCR: 11 were *Plasmodium vivax* infections and 1 was mixed *Plasmodium vivax/Plasmodium falciparum* infection, among which 9 were sub-microscopic (as not detected by TBS). History of malaria during current pregnancy tended to be associated with a higher risk of MiP (aIRR 3.05; 95 % CI 0.94–9.88). At delivery, two *Plasmodium falciparum* submicroscopic infections (one peripheral and one placental) were detected (4.5 %; 0.6–15.5) in Vapi District. In both surveys, all infected women stated they had slept under a bed net the night before the survey, and 86 % went to the forest for food-finding 1 week before the survey in median. The majority of infections (94 %) were asymptomatic and half of them were associated with anaemia. Overall, 24 % of women had LBW newborns. Factors associated with a higher risk of LBW were tobacco use (aIRR 2.43; 95 % CI 1.64–3.60) and pre-term delivery

Italian Lunch with Dr Rattanaphone in Bangkok June 2016

(aIRR 3.17; 95 % CI 2.19–4.57). Factors associated with a higher risk of maternal anaemia were no iron supplementation during pregnancy, Lao Theung ethnicity and place of living. **Conclusions**: The prevalence of MiP in this population was noticeable. Most infections were asymptomatic and submicroscopic vivax malaria, which raises the question of reliability of recommended national strategies for the screening and prevention of MiP in Laos.

6. Caillet C & Newton PN (in press) The case of falsified and substandard medicines in resource-limited countries. IN: Special Issues in Pharmacovigilance in Resource-Limited Countries, edited by Syed Rizwanuddin Ahmad, Springer.

Abstract. Poor quality medicines have been described as a global pandemic that threatens the lives of millions of people. The problem is much more severe in poor-resource countries where pharmaceutical legislation and regulation are limited. Medicines may be of poor quality if they are falsified, substandard or degraded. Few objective data on their prevalence exist but surveys suggest that an alarming proportion of anti-infectives in much of the developing world are of poor quality. The use of poor quality medicines may lead to severe complications not just for the individual but also for the community. Falsified, substandard or degraded drugs with subtherapeutic concentrations of the active ingredient or the wrong active ingredient are likely to engender the emergence and spread of resistance to anti-infectives, putting affordable treatments at risk. Those with excessive amounts of active ingredient or containing wrong harmful active ingredients may induce adverse drug reactions. Furthermore, poor quality medicines lead to a loss of faith of the patients in essential medicines and in health systems. To detect poor quality medicines at different levels of the pharmaceutical supply chain, different techniques have been developed, each with advantages and limits. This chapter describes these aspects of poor quality medicines and also discusses the factors that facilitate their existence and those that impede action to ensure that patients take good quality medicines. We discuss the role of pharmacovigilance in detecting poor quality medicines.

7. Chansamouth V, Thammasack S, Phetsouvanh R, Keoluangkot V, Moore CE, Blacksell SD, Castonguay-Vanier J, Dubot-Pérès AD, Tangkhabuanbutra J, Tongyoo N, Phommasone K, Vongsouvath M, Sengdethka D, Seurbsanith A, Souphaphonh P, Sengvilaipaseuth O, Craig S, Hermann L, Strobel M, Newton PN (2016) The aetiologies and impact of fever in pregnant inpatients in Vientiane, Laos. *PLoS NTD* 10(4):e0004577.

Abstract. Introduction. Laos has the highest maternal mortality ratio in mainland Southeast Asia and a high incidence of infectious diseases. Globally, malaria has been the pathogen most intensively investigated in relation to impact on pregnancy, but there has been relatively little research on the aetiology and impact of other diseases. We therefore aimed to determine the causes and impact of fever in pregnant women admitted to two central hospitals in Vientiane City, Lao PDR (Laos).
Materials and Methods. This hospital-based prospective study was conducted in Mahosot Hospital and the Mother and Child Hospital, Vientiane, between 2006 and 2010, with the aim to recruit 250 consenting pregnant women admitted with tympanic temperature >37.5°C. Primary outcome was the cause of fever and secondary outcomes were pregnancy outcomes. Specific investigations (culture, antigen, molecular and serological tests) were performed to investigate causes of fever. After discharge, all pregnant women were asked to return for review and convalescence serum on day 10–14 and were monitored until delivery.
Principle findings. 250 pregnant women were recruited to this study between February 2006 and November 2010. Fifty percent were pregnant for the first time. Their median (range) gestational age on admission was 24 (4–43) weeks. The median (range) tympanic admission temperature was 38.5°C (37.5–40.5°C). Fifteen percent of patients stated that they had taken antibiotics before admission. Headache, myalgia, back pain and arthralgia were described by >60% of patients and 149 (60%) were given a laboratory diagnosis. Of those with confirmed diagnoses, 132 (53%) had a single disease and 17 (7%) had apparent mixed diseases. Among those who had a single disease, dengue fever was the most common diagnosis, followed by pyelonephritis, scrub typhus, murine typhus and typhoid. Patients were also diagnosed with tuberculosis, appendicitis, *Staphylococcus aureus* septicemia, leptospirosis, Japanese encephalitis virus infection and *Plasmodium falciparum* malaria. Severe consequences, including maternal death, miscarriage, stillbirth, low birth weight and preterm birth, were found among 28 (78%) mothers with dengue fever, rickettsioses and typhoid.
Conclusion. Fevers other than malaria, such as dengue, pyelonephritis, rickettsioses and typhoid are common causes of fever during pregnancy in the Asian tropics. Further investigations of their impact in the community on maternal death, fetal loss, vertical transmission, low birth weight and preterm birth are needed.

8. Cheah PY, Newton PN, Mayxay M (2016) The first Science Café in Laos. *The Lancet*, 388 October 1, 2016.
[This article describes the first Science Café in Laos. This was organised by the University of Health Sciences, Ministry of Health, in collaboration with the MORU Network. More than 50 students and staff of the Faculties of Medicine, Pharmacy, Dentistry, Nursing Sciences, Medical Technology, Basic Sciences, and Public Health attended the 2 h long event on Medical and Research Ethics. Lively discussions included what makes a research stud y ethical, what makes consent valid, and whether children should be involved as participants in clinical research]. We hope to collaborate on arranging these as regular events in 2017.

9. 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 (2016) Population Structure Shapes Copy Number Variation in Malaria Parasites. *Mol Biol Evol.* 33(3):603-20.

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 (Ne) than from populations with a small Ne. 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 FST 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.

10. Chewapreecha C, Holden MT, Vehkala M, Välimäki N, Yang Z, Harris SR, Mather AE, Tuanyok A, De

Viengmon Davong inspecting agar plates

Smet B, Le Hello S, Bizet C, Mayo M, Wuthiekanun V, Limmathurotsakul D, Phetsouvanh R, Spratt BG, Corander J, Keim P, Dougan G, Dance DA, Currie BJ, Parkhill J, Peacock SJ (2017) Global and regional dissemination and evolution of *Burkholderia pseudomallei*. *Nat Microbiol.* 2:16263.

Abstract. The environmental bacterium *Burkholderia pseudomallei* causes an estimated 165,000 cases of human melioidosis per year worldwide and is also classified as a biothreat agent. We used whole genome sequences of 469 *B. pseudomallei* isolates from 30 countries collected over 79 years to explore its geographic transmission. Our data point to Australia as an early reservoir, with transmission to Southeast Asia followed by onward transmission to South Asia and East Asia. Repeated reintroductions were observed within the Malay Peninsula and between countries bordered by the Mekong River. Our data support an African origin of the Central and South American isolates with introduction of *B. pseudomallei* into the Americas between 1650 and 1850, providing a temporal link with the slave trade. We also identified geographically distinct genes/variants in Australasian or Southeast Asian isolates alone, with virulence-associated genes being among those over-represented. This provides a potential explanation for clinical manifestations of melioidosis that are geographically restricted.

11. Crump JA, Newton PN, Baird SJ, Lubell Y (in press) Febrile illness in adolescents and adults. *DCP3 Volume 6 HIV/AIDS*

[A review of what we know and the gaps in our knowledge of the aetiology and impact of diverse fevers in adolescents and adults in the tropics]

12. Currie BJ, Dance DAB (2016) Melioidosis and glanders. *BMJ Best Practice* http://bestpractice.bmj.com/best-practice/monograph/1601/resources/credits.html

[An on-line module summary of these diseases with associated learning module]

13. Dance D (2016) Letter: Melioidosis parotitis in children. *Journal of Venomous Animals and Toxins including Tropical Diseases* 22:33

Abstract. A recent paper published in Journal of Venomous Animals and Toxins including Tropical Diseases reporting a child in Hainan with parotitis caused by *Burkholderia pseudomallei* misleadingly described parotitis as a rare manifestation of melioidosis. In fact, it is one of the commonest forms of paediatric melioidosis seen in other parts of Southeast Asia, although interestingly not in Australia.

Stata Workshop class with Dr Mavuto and Kib

14. Dance DAB (2016) Challenges in diagnosis and management of melioidosis. *International Journal of Infectious Diseases* 45: 30.

[Abstract of presentation given at the International Congress on Infectious Diseases in Hyderabad, India]

15. Dance DAB, Limmathurotsakul D, Currie, BJ (in press) *Burkholderia pseudomallei*: challenges for the clinical microbiology laboratory - a response from the front line. *Journal of Clinical Microbiology*

[Letter in response to a minireview contrasting the US perspective on melioidosis with that in endemic areas]

16. Day N, Newton PN (2016) 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]

17. Dittrich S, Card E, Phuklia W, Rudgard W, Silousok J, Phoumin P, Bouthasavong L, Azarian S, Davong V, Dance D, Vongsouvath M, Phetsouvanh R, Newton PN (2016) Survival and growth of *Orientia tsutsugamushi* in conventional hemocultures. *Emerging Infectious Diseases* 22, 1460-1463.

Abstract. *Orientia tsutsugamushi,* which requires specialized facilities for culture, is a substantial cause of disease in Asia. We demonstrate that *O. tsutsugamushi* numbers increased for up to 5 days in conventional hemocultures. Performing such a culture step before molecular testing could increase the sensitivity of *O. tsutsugamushi* molecular diagnosis.

18. Dittrich D, Tadesse BT, Moussy F, Chua A, Zorzet A, Tängdén T, Dolinger DL, Page AL, Crump JA, D'Acremont V, Bassat Q, Lubell Y, Newton PN, Heinrich N, Rodwell T, González IJ (2016) Target Product Profile for a diagnostic assay to differentiate between bacterial and non-bacterial infections and reduce antimicrobial overuse in resource-limited settings: An expert consensus. *PloS One* DOI:10.1371/journal.pone.0161721.

Abstract. Acute fever is one of the most common presenting symptoms globally. In order to reduce the empiric use of antimicrobial drugs and improve outcomes, it is essential to improve diagnostic capabilities. In the absence of microbiology facilities in low-income settings, an assay to distinguish bacterial from non-bacterial causes would be a critical first step. To ensure that patient and market needs are met, the requirements of such a test should be specified in a target product profile (TPP). To identify minimal/optimal characteristics for a bacterial vs. non-bacterial fever test, experts from academia and international organizations with expertise in infectious diseases, diagnostic test development, laboratory medicine, global health, and health economics were convened. Proposed TPPs were reviewed by this working group, and consensus characteristics were defined. The working group defined non- severely ill, non-malaria infected children as the target population for the desired assay. To provide access to the most patients, the test should be deployable to community health centers and informal health settings, and staff should require <2 days of training to perform the assay. Further,

Pornjarus Sukhapiwat & Anisone Chanthongthip at Keble College for OTN

given that the aim is to reduce inappropriate antimicrobial use as well as to deliver appropriate treatment for patients with bacterial infections, the group agreed on minimal diagnostic performance requirements of >90% and >80% for sensitivity and specificity, respectively. Other key characteristics, to account for the challenging environment at which the test is targeted, included: i) time-to-result <10 min (but maximally <2 hrs); ii) storage conditions at 0–40°C, ≤90% non-condensing humidity with a minimal shelf life of 12 months; iii) operational conditions of 5–40°C, ≤90% non-condensing humidity; and iv) minimal sample collection needs (50–100µL, capillary blood). This expert approach to define assay requirements for a bacterial vs. non-bacterial assay should guide product development, and enable targeted and timely efforts by industry partners and academic institutions.

19. 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 (2016) The utility of blood culture fluid for the molecular diagnosis of *Leptospira*: a prospective evaluation. *American Journal of Tropical Medicine & Hygiene* 94(4):736-40.

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 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

20. Goh YS, Peng K, Chia WN, Siau A, Chotivanich K, Gruner AC, Preiser P, Mayxay M, Pukrittayakamee S, Sriprawat K, Nosten F, White NJ, Renia L (2016) Neutralizing Antibodies against *Plasmodium falciparum* Associated with Successful Cure after Drug Therapy. *PLoS One.* 11(7):e0159347.

An effective antibody response can assist drug treatment to contribute to better parasite clearance in malaria patients. To examine this, sera were obtained from two groups of adult patients with acute falciparum malaria, prior to drug treatment: patients who (1) have subsequent recrudescent infection, or (2) were cured by Day 28 following treatment. Using a *Plasmodium falciparum* antigen library, we examined the antibody specificities in these sera. While the antibody repertoire of both sera groups was extremely broad and varied, there was a differential antibody profile between the two groups of sera. The proportion of cured patients with antibodies against EXP1, MSP3, GLURP, RAMA, SEA and EBA181 was higher than the proportion of patients with recrudescent infection. The presence of these antibodies was associated with higher odds of treatment cure. Sera containing all six antibodies impaired the invasion of *P. falciparum* clinical isolates into erythrocytes. These results suggest that antibodies specific against EXP1, MSP3, GLURP, RAMA, SEA and EBA181 in *P. falciparum* infections could assist anti-malarial drug treatment and contribute to the resolution of the malarial infection.

21. Grist EP, Flegg JA, Humphreys G, Mas IS, Anderson TJ, Ashley EA, Day NP, Dhorda M, Dondorp AM, Faiz MA, Gething PW, Hien TT, Hlaing TM, Imwong M, Kindermans JM, Maude RJ, Mayxay M, McDew-White

Chiggers in the ear of a Thai rodent collecting during Ivo Elliott's scrub typhus fieldwork

M, Menard D, Nair S, Nosten F, Newton PN, Price RN, Pukrittayakamee S, Takala-Harrison S, Smithuis F, Nguyen NT, Tun KM, White NJ, Witkowski B, Woodrow CJ, Fairhurst RM, Sibley CH, Guerin PJ (2016) Optimal health and disease management using spatial uncertainty: a geographic characterization of emergent artemisinin-resistant *Plasmodium falciparum* distributions in Southeast Asia. *Int J Health Geogr.* 15(1):37.

Abstract. BACKGROUND: Artemisinin-resistant *Plasmodium falciparum* malaria parasites are now present across much of mainland Southeast Asia, where ongoing surveys are measuring and mapping their spatial distribution. These efforts require substantial resources. Here we propose a generic 'smart surveillance' methodology to identify optimal candidate sites for future sampling and thus map the distribution of artemisinin resistance most efficiently. METHODS: The approach uses the 'uncertainty' map generated iteratively by a geostatistical model to determine optimal locations for subsequent sampling. RESULTS: The methodology is illustrated using recent data on the prevalence of the K13-propeller polymorphism (a genetic marker of artemisinin resistance) in the Greater Mekong Subregion. CONCLUSION: This methodology, which has broader application to geostatistical mapping in general, could improve the quality and efficiency of drug resistance mapping and thereby guide practical operations to eliminate malaria in affected areas.

22. 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* 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.

23. Lubell Y, Althaus T, Blacksell SD, Paris DH, Mayxay M, Pan-Ngum W, White L, Day N, Newton PN (2016) Modelling the impact and cost-effectiveness of biomarker tests as compared with pathogen-specific diagnostics in the management of undifferentiated fever in remote tropical settings. *PloS One* 11(3): e0152420.

Abstract. Background. Malaria accounts for a small fraction of febrile cases in increasingly large areas of the malaria endemic world. Point-of-care tests to improve the management of non-malarial fevers appropriate for primary care are few, consisting of either diagnostic tests for specific pathogens or testing for biomarkers of host response that indicate whether antibiotics might be required. The impact and cost-effectiveness of these approaches are relatively unexplored and methods to do so are not well-developed. **Methods**. We model the ability of dengue and scrub typhus rapid tests to inform antibiotic treatment, as compared with testing for elevated C-Reactive Protein (CRP), a biomarker of host inflammation. Using data on causes of fever in rural Laos, we estimate the proportion of outpatients that would be correctly classified as requiring an antibiotic and the likely cost-effectiveness of the approaches.
Results. Use of either pathogen-specific test slightly increased the proportion of patients correctly classified as requiring antibiotics. CRP testing was consistently superior to the pathogenspecific tests, despite heterogeneity in causes of fever. All testing strategies are likely to result in higher average costs, but only the scrub typhus and CRP tests are likely to be cost- effective when considering direct health benefits, with median cost per disability adjusted life year averted of approximately $48 USD and $94 USD, respectively. **Conclusions**. Testing for viral infections is unlikely to be cost-effective when considering only direct health benefits to patients. Testing for prevalent bacterial pathogens can be cost-effective, having the benefit of

informing not only whether treatment is required, but also as to the most appropriate antibiotic; this advantage, however, varies widely in response to heterogeneity in causes of fever. Testing for biomarkers of host inflammation is likely to be consistently cost-effective despite high heterogeneity, and can also offer substantial reductions in overuse of antimicrobials in viral infections.

24. MalariaGEN (2016) *Plasmodium falciparum* Community Project. Genomic epidemiology of artemisinin resistant malaria. *Elife.* 5. pii: e08714.

The current epidemic of artemisinin resistant *Plasmodium falciparum* in Southeast Asia is the result of a soft selective sweep involving at least 20 independent *kelch13* mutations. In a large global survey, we find that *kelch13* mutations which cause resistance in Southeast Asia are present at low frequency in Africa. We show that African *kelch13* mutations have originated locally, and that *kelch13* shows a normal variation pattern relative to other genes in Africa, whereas in Southeast Asia there is a great excess of non-synonymous mutations, many of which cause radical amino-acid changes. Thus, *kelch13* is not currently undergoing strong selection in Africa, despite a deep reservoir of variations that could potentially allow resistance to emerge rapidly. The practical implications are that public health surveillance for artemisinin resistance should not rely on *kelch13* data alone, and interventions to prevent resistance must account for local evolutionary conditions, shown by genomic epidemiology to differ greatly between geographical regions.

25. Newton PN, Caillet C, Guerin PJ (2016) A link between poor quality antimalarials and malaria drug resistance? *Expert Rev Anti Infect Ther.* 14(6):531-3.

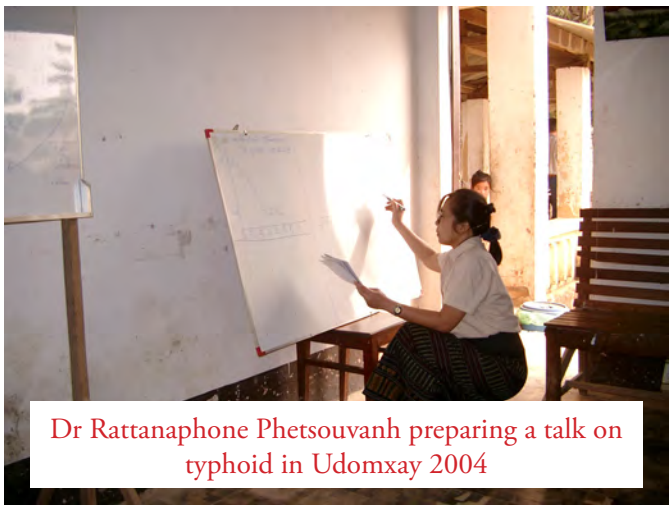
[An editorial discussing what is known about the relationship between antimalarial quality and drug resistance]

26. Newton PN, Timmermann B (2106) Fake penicillin, *The Third Man*, and Operation Claptrap. *BMJ* 355:i6494
[An article investigating the origins of the story behind the fake penicillin racket portrayed in the file *The Third Man* and illegal trade in penicillin in post-WW2 Europe and links to espionage]

27. Newton PN (in press) Medicine Quality, Physicians and Patients. Oxford Textbook of Medicine

[A review of medicine quality and public health]

28. Peyraud N, Rafael F, Parker LA, Quere M, Alcoba G, Korff C, Deats M, Bourdillon Esteve P, Cabrol J-C, Serafini M, Ciglenecki I, Rull M, Amine Larabi I, Baud F, Grandesso F, Ilunga B K, Alvarez J-C, Newton PN (2017) A large epidemic of dystonic reactions in central Africa: the possible role of falsified diazepam containing haloperidol. *Lancet Global Health* 5, 3137-e138.



Dr Rattanaphone Phetsouvanh preparing a talk on typhoid in Udomxay 2004

[In December 2014, patients with suspected meningitis were reported by the Ituri Health District, in the northeast Democratic Republic of Congo. In January 2015, Médecins sans Frontières (MSF) was approached by the Ministry of Health (MoH) to support outbreak investigation and response. At MoH and MSF case-management sites, patients' demographic characteristics, clinical features, and outcome were described. Cerebrospinal fluid was analysed for evidence of bacterial meningitis, and urine and 39 medicine samples underwent toxicological investigations. Initial investigations suggested that bacterial meningitis was not the aetiology. Patients presented with acute dystonic reactions affecting the muscles of the face, eyes, neck, tongue, and upper limbs with Parkinsonism and oculogyric crises. Over eight months, there were over one thousand hospitalisations. The urine from all patient samples (n=9) tested positive for haloperidol. One tablet type sold as 'diazepam' in the community unexpectedly contained haloperidol as the sole active pharmaceutical ingredient, suggesting that this large outbreak was due to haloperidol toxicity from falsified diazepam. How diazepam came to contain haloperidol is under investigation by national/international authorities. This outbreak emphasizes the need for thorough investigation of atypical presentations of common diseases and for considering toxicity from falsified medicines. Increased funding pressure and scarce resources are likely to risk disadvantaged populations accessing unreliable sources of medicine. Strengthening the capacity of medicine regulatory authorities is a key requirement to ensure the quality of medicines, especially for vulnerable populations.]

29. 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.

Mak Bok (*Irvingia malayana*) tree

Abstract. 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 are commercially available; evaluations of their clinical impact and cost-effectiveness at pointof care is warranted.

30. Phommasone K, Adhikari B, Henriques G, Pongvongsa T, Phongmany P, von Seidlein L, White NJ, Day N, Dondorp A, Newton PN, Imwong M, Mayxay M (2016) Asymptomatic malaria prevalence in 18 villages of southern Savannakhet Province, Lao PDR (Laos). *Malaria Journal* 15, 296.

Abstract. Background: A large fraction of *Plasmodium* infections do not cause clinical signs and symptoms of disease and persist at densities in blood that are not detectable by microscopy or rapid diagnostic tests. These infections may be critical as a transmission reservoir in areas of low malaria endemicity. Understanding the epidemiology of these infections would be helpful for malaria elimination.
Methods: A cross-sectional survey was conducted in Thapangthong and Nong Districts of Savannakhet Province, Lao PDR, to determine the prevalence of parasitaemia. A total of 888 blood samples were collected from afebrile volunteers aged ≥15 years in 18 villages during March and July 2015. *Plasmodium* infections were diagnosed by rapid diagnostic tests (RDT) and high volume, ultra-sensitive quantitative polymerase chain reaction (uPCR). **Results**: uPCR detected *Plasmodium* infections in 175 of 888 samples (20 %). The species distribution was *Plasmodium falciparum* 3.6 % (32/888), *Plasmodium vivax* 11.1 % (99/888), mixed infections with *P. falciparum* and *P. vivax* 1.6 % (14/888) and *Plasmodium* of undetermined species 3.4 % (30/888). RDT identified only 2 % (18/888) positive cases. Using uPCR as reference, the sensitivity and specificity of RDTs were 28 and 100 %, respectively, in detecting *P. falciparum* infections, and 3 and 99 % in detecting asymptomatic *P. vivax* infections. The K13 kelch propeller domain C580Y mutation, associated with reduced susceptibility to artemisinin derivatives, was found in 75 % (12/18) of *P. falciparum* isolates from Thapangthong and in 7 % (2/28) from Nong ($p < 0.001$). In a multivariate analysis, males were more likely to have *P. vivax* infections [adjusted odds ratio (aOR) 4.76 (95 % CI 2.84–8.00)] while older villagers were at lower risk for parasitaemia [aOR for increasing age 0.98 (95 % CI 0.96–0.99)]. **Conclusion**: There is a high prevalence of asymptomatic *Plasmodium* infections in southern Savannakhet. Artemisinin- resistant *P. falciparum* strains form an increasing proportion of the parasite population in Thapangthong District and are already present in the more remote Nong District. This worrying trend has wider implications for Laos and could reverse the gains achieved by the successful control of malaria in Laos and the Greater Mekong Sub-region (GMS). Rapid elimination of *P. falciparum* has to be a top priority in Laos as well as in the wider GMS.

Mak Bok seeds

31. Rachlin A, Dittrich S, Phommasone K, Douangnouvong A, Phetsouvanh R, Newton PN, Dance D (2016) Investigation of recurrent melioidosis in Laos by multilocus sequence typing. *American Journal of Tropical Medicine & Hygiene* Mar 21,15-0909.

Abstract. Melioidosis is an infectious disease caused by the saprophytic bacterium *Burkholderia pseudomallei*. In northeast Thailand and northern Australia, where the disease is highly endemic, a range of molecular tools have been used to study its epidemiology and pathogenesis. In the Lao People's Democratic Republic (Laos) where melioidosis has been recognized as endemic since 1999, no such studies have been undertaken. We used a multilocus sequence typing scheme specific for *B. pseudomallei* to investigate nine cases of culture-positive recurrence occurring in 514 patients with melioidosis between 2010 and 2015: four were suspected to be relapses while the other five represented reinfections. In addition, two novel sequence types of the bacterium were identified. The low overall recurrence rates (2.4%) and proportions of relapse and reinfection in the Laos are consistent with those described in the recent literature, reflecting the effective use of appropriate antimicrobial therapy.

32. Rattanavong S, Dance DAB, Davong V, Baker C, Frost H, Phetsouvanh R, Vongsouvath M, Newton PN, Steer AC, Smeesters PR (2016) Group A Streptococcal strains isolated in Lao People's Democratic Republic from 2004 to 2013. *Epidemiol Infect* 144: 1770-1773.

Abstract. 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 identifi ed 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%).

33. Ravinetto R, De Weggheleire A, Dorlo TPC, Francque S, Sokkab A, Luhman N, Pouget C, Meessen B, Tabernero P, Newton PN, Lynen L (2016) Predictable threats to public health of delaying universal access to innovative medicines for Hepatitis C**.** A pharmaceutical standpoint. *TMIH* doi:10.1111/tmi.12784

[The high prices of innovator products for HCV ('one thousand dollar pills') and the 'alternative' routes to access treatment have quickly reached patients with HCV worldwide. These therapies are not affordable to most of those with HCV and there is growing concern that the online purchase of generic HCV therapies, with or without medical prescription will result in an unregulated market constituting the only 'accessible' opportunity for patients (Ravinetto *et al.* 2016). With circulation of falsified sofosbuvir and pegylated interferon and ribavirin patients are at risk of poor quality HCV therapy. Ravinetto *et al.* (2016) argue that market incentives should be in place to push the manufacturers of generic HCV therapy to obtain either WHO Prequalification or Stringent Regulatory Authority (SRA) registration.]

34. 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* 23(8):7828-39.

Abstract. *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

LOMWRU team performing at OTN

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.

35. Rongkard P, Hantrakun V, Dittrich S, Srilohasin P, Amornchai P, Langla S, Lim C, Day NP, AuCoin D, Wuthiekanun V, Limmathurotsakul D (2016) Utility of a Lateral Flow Immunoassay (LFI) to Detect *Burkholderia pseudomallei* in Soil Samples. *PLoS Negl Trop Dis.* 10(12):e0005204.

Abstract. BACKGROUND: Culture is the gold standard for the detection of environmental *B. pseudomallei*. In general, soil specimens are cultured in enrichment broth for 2 days, and then the culture broth is streaked on an agar plate and incubated further for 7 days. However, identifying B. pseudomallei on the agar plates among other soil microbes requires expertise and experience. Here, we evaluate a lateral flow immunoassay (LFI) developed to detect *B. pseudomallei* capsular polysaccharide (CPS) in clinical samples as a tool to detect *B. pseudomallei in* environmental samples. METHODOLOGY/PRINCIPAL FINDINGS: First, we determined the limit of detection (LOD) of LFI for enrichment broth of the soil specimens. Soil specimens (10 grams/specimen) culture negative for *B. pseudomallei* were spiked with *B. pseudomallei* ranging from 10 to 105 CFU, and incubated in 10 ml of enrichment broth in air at 40°C. Then, on day 2, 4 and 7 of incubation, 50 **µ**L of the upper layer of the broth were tested on the LFI, and colony counts to determine quantity of *B. pseudomallei* in the broth were performed. We found that all five soil specimens inoculated at 10 CFU were negative by LFI on day 2, but four of those five specimens were LFI positive on day 7. The LOD of the LFI was estimated to be roughly 3.8x106 CFU/ml, and culture broth on day 7 was selected as the optimal sample for LFI testing. Second, we evaluated the utility of the LFI by testing 105 soil samples from Northeast

Thailand. All samples were also tested by standard culture and quantitative PCR (qPCR) targeting orf2. Of 105 soil samples, 35 (33%) were LFI positive, 25 (24%) were culture positive for *B. pseudomallei*, and 79 (75%) were qPCR positive. Of 11 LFI positive but standard culture negative specimens, six were confirmed by having the enrichment broth on day 7 culture positive for B. pseudomallei, and an additional three by qPCR. The LFI had 97% (30/31) sensitivity to detect soil specimens culture positive for *B. pseudomallei.* CONCLUSIONS/ SIGNIFICANCE: The LFI can be used to detect *B. pseudomallei* in soil samples, and to select which samples should be sent to reference laboratories or proceed further for bacterial isolation and confirmation. This could considerably decrease laboratory workload and assist the development of a risk map for melioidosis in resource-limited settings.

36. Saralamba N, Nakeesathit S, Mayxay M, Newton PN, Osorio L, Kim JR, White NJ, Day NP, Dondorp AM, Imwong M (2016) Geographic distribution of amino acid mutations in DHFR and DHPS in *Plasmodium vivax* isolates from Lao PDR, India and Colombia. *Malar J.* 15(1):484.

Abstract. Background. Non-synonymous mutations in *dhfr* and *dhps* genes in *Plasmodium vivax* are associated with sulfadoxine–pyrimethamine (SP) resistance. The present study aimed to assess the prevalence of point mutations in *P. vivax dhfr* (*pvdhfr)* and *P. vivax dhps* (*pvdhps*) genes in three countries: Lao PDR, India and Colombia. Methods. Samples from 203 microscopically diagnosed vivax malaria were collected from the three countries. Five codons at positions 13, 57, 58, 61, and 117 of *pvdhfr* and two codons at positions 383 and 553 of *pvdhps* were examined by polymerase chain reaction-restriction fragment length polymorphism methodology. Results. The largest number of 58R/117 N double mutations in *pvdhfr* was observed in Colombia (94.3 %), while the corresponding wild-type amino acids were found at high frequencies in Lao PDR during 2001–2004 (57.8 %). Size polymorphism analysis of the tandem repeats within *pvdhfr* revealed that 74.3 % of all the isolates carried the type B variant. Eighty-nine per cent of all the isolates examined carried wild-type *pvdhps* A383 and A553. Conclusions. Although SP is not generally used to treat *P. vivax* infections, mutations in *dhfr* and *dhps* that confer antifolate resistance in *P. vivax* are common. The data strongly suggest that, when used primarily to treat falciparum malaria, SP can exert a substantial selective pressure on *P. vivax* populations, and this can lead to point mutations in *dhfr* and *dhps*. Accurate data on the global geographic distribution of *dhfr* and *dhps* genotypes should help to inform anti-malarial drug-use policies.

37. Sengvilaipaseuth O, Phommasone K, de Lamballerie X, Vongsouvath M, Phonemixay O, Blacksell SD, Mayxay M, Keomany S, Souvannasing P, Newton PN, Audrey Dubot-Pérès (2017) Temperature stability of a Dengue Rapid Diagnostic Test under tropical climatic conditions: A follow up study. PloS One 12(1):e0170359.

Abstract. The Dengue Duo Rapid Diagnostic Test (SD Dengue RDT) has good specificity and sensitivity for dengue diagnosis in rural tropical areas. In a previous study, using four control sera, we demonstrated that that the diagnostic accuracy of these RDTs remains stable after long-term storage at high temperatures. We extended this study by testing sera from 119 febrile patients collected between July-November 2012 at Salavan Provincial Hospital (southern Laos) with RDTs stored for 6 months at 4ºC, 35º and in a hut (miniature traditional house) at Lao ambient temperatures. The dengue NS1 antigen results from RDTs stored at 35º C and in the hut demonstrated 100% agreement with those stored at 4ºC. However, lower positive percent agreements, with broad 95%CI, were observed for the tests: IgM, 60% (14.7-94.7) and 40% (5.3-85.3) for RDTs store at 35°C and in the hut, compared to those stored at 4°C, respectively. This study strenghtens the evidence of the robustness of the NS1 antigen detection RDT for the diagnosis of dengue after storage at tropical temperatures.

38. Srimuang K, Miotto O, Lim P, Fairhurst RM, Kwiatkowski DP, Woodrow CJ, Imwong M, Tracking Resistance to Artemisinin Collaboration (2016) Analysis of anti-malarial resistance markers in pfmdr1 and pfcrt across Southeast Asia in the Tracking Resistance to Artemisinin Collaboration. *Malar J.* 15(1):541.

Abstract. Background: Declining anti-malarial efficacy of artemisinin-based combination therapy, and reduced *Plasmodium falciparum* susceptibility to individual anti-malarials are being documented across an expanding area of Southeast Asia (SEA). Genotypic markers complement phenotypic studies in assessing the efficacy of individual anti-malarials. **Methods:** The markers pfmdr1 and pfcrt were genotyped in parasite samples obtained in 2011–2014 at 14 TRAC (Tracking Resistance to Artemisinin Collaboration) sites in mainland Southeast Asia using a combination of PCR and next-generation sequencing methods. **Results:** Pfmdr1 amplification, a marker of mefloquine and lumefantrine resistance, was highly prevalent at Mae Sot on the Thailand–Myanmar border (59.8% of isolates) and common (more than 10%) at sites in central Myanmar, eastern Thailand and western Cambodia; however, its prevalence was lower than previously documented in Pailin, western Cambodia. The pfmdr1 Y184F mutation was common, particularly in and around Cambodia, and the F1226Y mutation was found in about half of samples in Mae Sot. The functional significance of these two mutations remains unclear. Other previously documented pfmdr1 mutations were absent or very rare in the region. The pfcrt mutation K76T associated with chloroquine resistance was found in 98.2% of isolates.

The CVIET haplotype made up 95% or more of isolates in western SEA while the CVIDT haplotype was common (30–40% of isolates) in north and northeastern Cambodia, southern Laos, and southern Vietnam. **Conclusions:** These findings generate cause for concern regarding the mid-term efficacy of artemether–lumefantrine in Myanmar, while the absence of resistance-conferring pfmdr1 mutations and SVMNT pfcrt haplotypes suggests that amodiaquine could be an efficacious component of anti-malarial regimens in SEA.

39. Turner P, Kloprogge S, Miliya T, Soeng S, Tan P, Sar P, Yos P, Moore CE, Wuthiekanun V, Limmathurotsakul D, Turner C, Day NP, Dance DA (2016) A retrospective analysis of melioidosis in Cambodian children, 2009-2013. *BMC Infect Dis.* 16(1):688.

Abstract. Background. Melioidiosis, infection by *Burkholderia pseudomallei*, is an important but frequently under-recognised cause of morbidity and mortality in Southeast Asia and elsewhere in the tropics. Data on the epidemiology of paediatric melioidosis in Cambodia are extremely limited.
Methods. Culture-positive melioidosis cases presenting to Angkor Hospital for Children, a non-governmental paediatric hospital located in Siem Reap, Northern Cambodia, between 1st January 2009 and 31st December 2013 were identified by searches of hospital and laboratory databases and logbooks.
Results. One hundred seventy-three evaluable cases were identified, presenting from eight provinces. For Siem Reap province, the median commune level incidence was estimated to be 28-35 cases per 100,000 children <15 years per year. Most cases presented during the wet season, May to October. The median age at presentation was 5.7 years (range 8 days–15.9 years). Apart from undernutrition, co-morbidities were rare. Three quarters (131/173) of the children had localised infection, most commonly skin/soft tissue infection (60 cases) or suppurative parotitis (51 cases). There were 39 children with *B. pseudomallei* bacteraemia: 29 (74.4%) of these had clinical and/or radiological evidence of pneumonia. Overall mortality was 16.8% (29/173) with mortality in bacteraemic cases of 71.8% (28/39). At least seven children did not receive an antimicrobial with activity against *B. pseudomallei* prior to death. **Conclusions**. This retrospective study demonstrated a considerable burden of melioidosis in Cambodian children. Given the high mortality associated with bacteraemic infection, there is an urgent need for greater awareness amongst healthcare professionals in Cambodia and other countries where melioidosis is known or suspected to be endemic. Empiric treatment guidelines should ensure suspected cases are treated early with appropriate antimicrobials.

40. Tabernero P, Parker M, Ravinetto R, Phanouvong S, Yeung S, Kitutu FE, Cheah PY, Mayxay M, Guerin PJ, Newton PN (2016) Ethical challenges in designing and conducting medicine quality surveys. *Trop Med Int Health* 21(6):799-806.

Dr Rattanaphone Phetsouvanh (right) with the first scrub typhus patient diagnosed at Mahosot Hospital 2002

Abstract. Objectives In this paper we discuss the main ethical challenges related to the conduct of medicine quality surveys and make suggestions on how to address them. **Methods**. Most evidence-based information regarding medicine quality derives from surveys. However, existing research ethical guidelines do not provide specific guidance for medicine quality surveys. Hence, those conducting surveys are often left wondering how to judge what counts as best practice. A list of the main ethical challenges in the design and conduct of surveys is presented.
Results and conclusions. It is vital that the design and conduct of medicine quality surveys uphold moral and ethical obligations and analyse the ethical implications and consequences of such work. These aspects include the impact on the local availability of and access to medicines; the confidentiality and privacy of the surveyors and the surveyed; questions as to whether outlet staff personnel should be told they are part of a survey; the need of ethical and regulatory approvals; and how the findings should be disseminated. Medicine quality surveys should ideally be conducted in partnership with the relevant national Medicine Regulatory Authorities. An international, but contextually sensitive, model of good ethical practice for such surveys is needed.

41. Taylor A, Vongphayloth K, Vongsouvath M, Grandadam M, Brey PT, Newton PN, Sutherland IW, Dittrich S (2016) Large scale survey of ticks from Khammouan Province, Lao PDR. *EID* 22(9):1635-9

Abstract. We screened 768 tick pools containing 6,962 ticks from Khammouan Province, Laos, by using quantitative real-time PCR and identified *Rickettsia* spp., *Ehrlichia* spp., and *Borrelia* spp. Sequencing of *Rickettsia spp.* positive and *Borrelia spp.* positive pools provided evidence for distinct genotypes. Our results identified bacteria with human disease potential in ticks in Laos.

Market seller, Pakse

42. Vongsouvath M, Phommasone K, Sengvilaipaseuth O, Kosoltanapiwat N, Narisara Chantratita N, Blacksell SD, Lee SJ, Lamballerie X, Mayxay M, Keomany S, Newton PN, Audrey Dubot-Pérès A (2016) Using Rapid Diagnostic Tests as a Source of Viral RNA for Dengue Serotyping by RT-PCR - a Novel Epidemiological Tool. *PloS NTD* 10(5): e0004704.

Abstract. Background. Dengue virus infection causes major public health problems in tropical and subtropical areas. In many endemic areas, including the Lao PDR, inadequate access to laboratory facilities is a major obstacle to surveillance and study of dengue epidemiology. Filter paper is widely used for blood collection for subsequent laboratory testing for antibody and nucleic acid detection. For the first time, we demonstrate that dengue viral RNA can be extracted fromdengue rapid diagnostic tests (RDT) and then submitted to real-time RT-PCR for serotyping. **Methodology/Principal Findings**. We evaluated the Standard Diagnostics (SD) Bioline Dengue Duo RDT, a commonly used test in dengue endemic areas. First, using the QIAamp RNA kit, dengue RNA was purified from the sample pad of the NS1 RDT loaded with virus isolates of the four serotypes, then quantified by RT-PCR. We observed greater recovery of virus, with a mean of 27 times more RNA recovered from RDT, than from filter paper. Second, we evaluated dengue NS1 RDTs from patients at Mahosot Hospital, Vientiane, (99 patients) and from rural Salavan Provincial Hospital (362 patients). There was good agreement between dengue RT-PCR from NS1 RDT with RT-PCR performed on RNA extracted from patient sera, either using RDT loaded with blood (82.8% and 91.4%, in Vientiane and Salavan, respectively) or serum (91.9% and 93.9%). There was 100% concordance between RDT and serum RTPCR of infecting dengue serotype.
Conclusions/Significance. Therefore, the collection of NS1 positive RDTs, which do not require cold storage, may be a novel approach for dengue serotyping by RT-PCR and offers promising prospects for the collection of epidemiological data from previously inaccessible tropical areas to aid surveillance and public health interventions.

43. Wattanakul T, Teerapong P, Plewes K, Newton PN, Chierakul W, Silamut K, Chotivanich K, Ruengweerayut R, White NJ, Dondorp AM, Tarning J (2016) Pharmacokinetic properties of intramuscular versus oral syrup paracetamol in *Plasmodium falciparum* malaria. *Malar J.* 15(1):244.

Abstract. Background: Fever is an inherent symptom of malaria in both adults and children. Paracetamol (acetaminophen) is the recommended antipyretic as it is inexpensive, widely available and has a good safety profile,

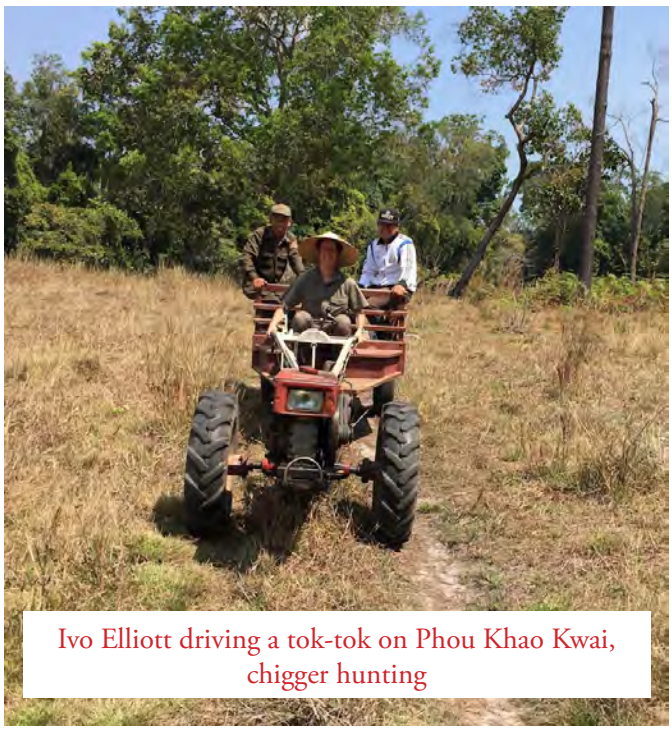
Jana Lai leading a Mug Cake cookery demonstration

but patients may not be able to take the oral drug reliably. A comparison between the pharmacokinetics of oral syrup and intramuscular paracetamol given to patients with acute falciparum malaria and high body temperature was performed.

Methods: A randomized, open-label, two-treatment, crossover, pharmacokinetic study of paracetamol dosed orally and intramuscularly was conducted. Twenty-one adult patients with uncomplicated falciparum malaria were randomized to receive a single 600 mg dose of paracetamol either as syrup or intramuscular injection on day 0 followed by a single dose administered by the alternative route on day 1. Paracetamol plasma concentrations were quantified frequently and modelled simultaneously using nonlinear mixed-effects modelling. The final population pharmacokinetic model was used for dose optimization simulations. Relationships between paracetamol concentrations with temperature and parasite half-life were investigated using linear and non-linear regression analyses.

Results: The population pharmacokinetic properties of paracetamol were best described by a two-compartment disposition model, with zero-order and first-order absorption for intramuscular and oral syrup administration, respectively. The relative bioavailability of oral syrup was 84.4 % (95 % CI 68.2–95.1 %) compared to intramuscular administration. Dosing simulations showed that 1000 mg of intramuscular or oral syrup administered six-hourly reached therapeutic steady state concentrations for antipyresis, but more favourable concentration–time profiles were achieved with a loading dose of 1500 mg, followed by a 1000 mg maintenance dose. This ensured that maximum therapeutic concentrations were reached rapidly during the first 6 h. No significant relationships between paracetamol concentrations and temperature or parasite half-life were found. **Conclusions**: Paracetamol plasma concentrations after oral syrup and intramuscular administration in patients with acute falciparum malaria were described successfully by a two-compartment disposition model. Relative oral bioavailability compared to intramuscular dosing was estimated as 84.4 % (95 % CI 68.2–95.1 %). Dosing simulations showed that a loading dose followed by six-hourly dosing intervals reduced the time delay to reach therapeutic drug levels after both routes of administration. The safety and efficacy of loading dose paracetamol antipyretic regimens now needs to be established in larger studies.

44. Weitzel T, Dittrich S, López J, Phuklia W, Martinez-Valdebenito C, Velásquez K, Blacksell SD, Paris DH, Abarca K (2016) Endemic Scrub Typhus in South America. *N Engl J Med*. 375(10):954-61.



Ivo Elliott driving a tok-tok on Phou Khao Kwai, chigger hunting

Abstract. Scrub typhus is a life-threatening zoonosis caused by *Orientia tsutsugamushi* organisms that are transmitted by the larvae of trombiculid mites. Endemic scrub typhus was originally thought to be confined to the so called "tsutsugamushi triangle" within the Asia–Pacific region. In 2006, however, two individual cases were detected in the Middle East and South America, which suggested that the pathogen was present farther afield. Here, we report three autochthonous cases of scrub typhus caused by *O. tsutsugamushi* acquired on Chilo Island in southern Chile, which suggests the existence of an endemic focus in South America.

45. Wong VK, Baker S, Connor TR, Pickard D, Page AJ, Dave J, Murphy N, Holliman R, Sefton A, Millar M, Dyson ZA, Dougan G, Holt KE; International Typhoid Consortium (2016) An extended genotyping framework for *Salmonella enterica* serovar Typhi, the cause of human typhoid. *Nat Commun* 7:12827.

Abstract. The population of *Salmonella enterica* serovar Typhi (*S.* Typhi), the causative agent of typhoid fever, exhibits limited DNA sequence variation, which complicates efforts to rationally discriminate individual isolates. Here we utilize data from whole-genome sequences (WGS) of nearly 2,000 isolates sourced from over 60 countries to generate a robust genotyping scheme that is phylogenetically informative and compatible with a range of assays. These data show that, with the exception of the rapidly disseminating H58 subclade (now designated genotype 4.3.1), the global *S.* Typhi population is highly structured and includes dozens of subclades that display geographical restriction. The genotyping approach presented here can be used to interrogate local *S.* Typhi populations and help identify recent introductions of *S.* Typhi into new or previously endemic locations, providing information on their likely geographical source. This approach can be used to classify clinical isolates and provides a universal framework for further experimental investigations.

46. World Health Organisation (2016) Guidelines on the Conduct of Surveys of the Quality of Medicines in WHO Technical Report Series, No. 996, 2016, Annex 7 (2016; 36 pages). See:

http://apps.who.int/medicinedocs/en/m/abstract/Js22404en/

WHO on methods for designing, conducting and reporting surveys of medicine quality, building on the MEDQUARD guidelines published in 2009

[NB. The key work of Patricia Tabernero, with Sue Lee, Kasia Stepniewska and Paul Newton, in these guidelines will be acknowledged and corrected by WHO in 2017]

47. WWARN Gametocyte Study Group (2016) Gametocyte carriage in uncomplicated *Plasmodium falciparum* malaria following treatment with artemisinin combination therapy: a systematic review and meta-analysis of individual patient data. *BMC Medicine* 14:79.

Abstract. Background: Gametocytes are responsible for transmission of malaria from human to mosquito. Artemisinin combination therapy (ACT) reduces post-treatment gametocyte carriage, dependent upon host, parasite and pharmacodynamic factors. The gametocytocidal properties of antimalarial drugs are important for malaria elimination efforts. An individual patient clinical data meta-analysis was undertaken to identify the determinants of gametocyte carriage and the comparative effects of four ACTs: artemether-lumefantrine (AL), artesunate/amodiaquine (AS-AQ), artesunate/mefloquine (AS-MQ), and dihydroartemisinin-piperaquine (DP). Methods: Factors associated with gametocytaemia prior to, and following, ACT treatment were identified in multivariable logistic or Cox regression analysis with random effects. All relevant studies were identified through a systematic review of PubMed. Risk of bias was evaluated based on study design, methodology, and missing data. Results: The systematic review identified 169 published and 9 unpublished studies, 126 of which were shared with the WorldWide Antimalarial Resistance Network (WWARN) and 121 trials including 48,840 patients were included in the analysis. Prevalence of gametocytaemia by microscopy at enrolment was 12.1 % (5887/48,589), and increased with decreasing age, decreasing asexual parasite density and decreasing haemoglobin concentration, and was higher in patients without fever at presentation. After ACT treatment, gametocytaemia appeared in 1.9 % (95 % CI,

Stuart Blacksell, Paul Newton, HE Prof Dr Bounkong Syhavong, Dr Rattanaphone Phetsouvanh, Assoc Prof Mayfong Mayxay & Shunmay Yeung in Chinatown, London, 2003

1.7-2.1) of patients. The appearance of gametocytaemia was lowest after AS-MQ and AL and significantly higher after DP (adjusted hazard ratio (AHR), 2.03; 95 % CI, 1.24-3.12; P = 0.005 compared to AL) and AS-AQ fixed dose combination (FDC) (AHR, 4.01; 95 % CI, 2.40-6.72; P < 0.001 compared to AL). Among individuals who had gametocytaemia before treatment, gametocytaemia clearance was significantly faster with AS-MQ (AHR, 1.26; 95 % CI, 1.00-1.60; P = 0.054) and slower with DP (AHR, 0.74; 95 % CI, 0.63-0.88; P = 0.001) compared to AL. Both recrudescent (adjusted odds ratio (AOR), 9.05; 95 % CI, 3.74-21.90; P < 0.001) and new (AOR, 3.03; 95 % CI, 1.66-5.54; P < 0.001) infections with asexual-stage parasites were strongly associated with development of gametocytaemia after day 7. Conclusions: AS-MQ and AL are more effective than DP and AS-AQ FDC in preventing gametocytaemia shortly after treatment, suggesting that the non-artemisinin partner drug or the timing of artemisinin dosing are important determinants of post-treatment gametocyte dynamics.

That Luang Festival