LOMWRU.

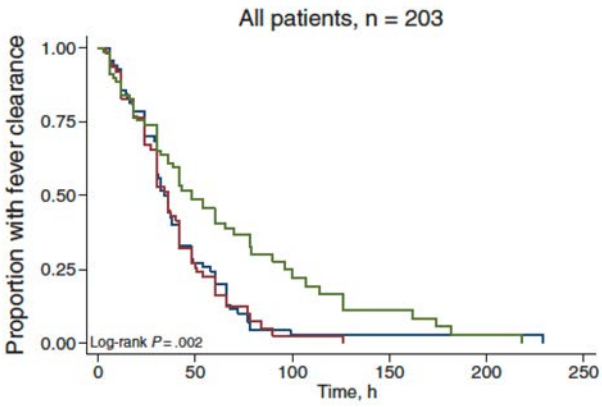
SCIENTIFIC ANNUAL REPORT FOR 2018

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

MORU Tropical Health Network

TO

MINISTRY OF HEALTH
GOVERNMENT OF THE LAO PDR



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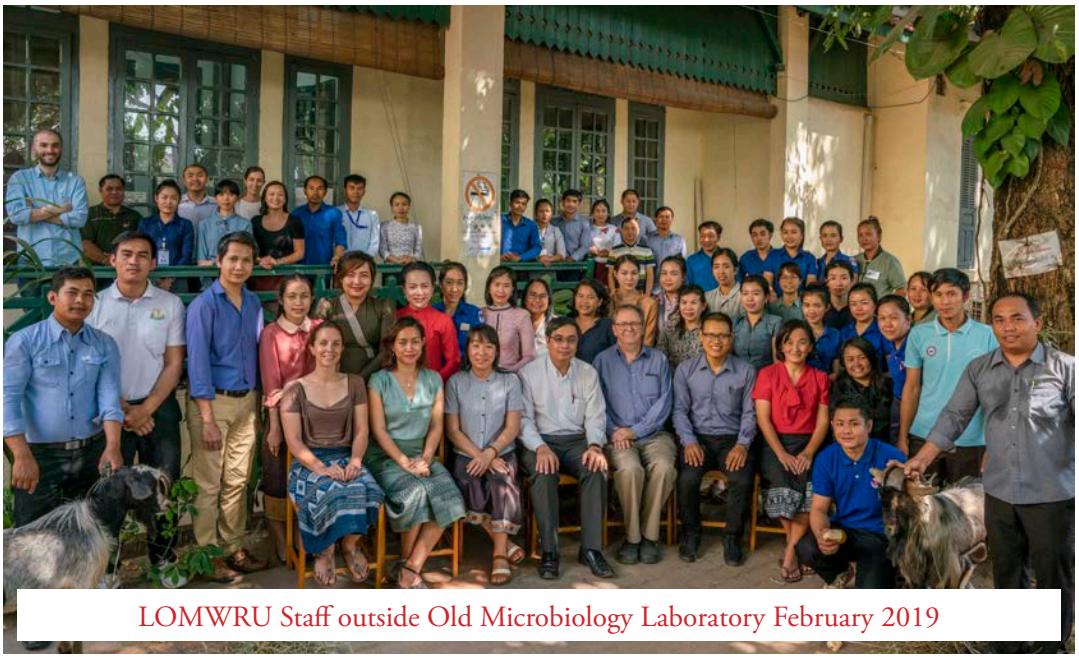


LOMWRU Staff outside Old Microbiology Laboratory February 2019

Assoc. Prof Bounthaphany Bounxouei, Prof Jeremy Farrar, His Excellency Assoc. Prof Dr Bounkong Syhavong, Prof Tobias Bonoeffer & His Excellency Hugh Evans, with colleagues from Wellcome, MORU, LOMWRU and Ministry of Health on the visit of the Wellcome Trust to LOMWRU in February 2018

ບົດສັງລວມຫຍໍ້

ກ. ໂຄງການຄົ້ນຄວ້າພະຍາດເຂດຮ້ອນລະຫວ່າງໂຮງໝໍມະໂຫສົດ-ແອວຄຳຕູ້ສ-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 ປະຈຳບ້ານກອກ (MORU-Bangkok) ແລະ ປະມານ 49% ຂອງວຽກຄົ້ນຄວ້າພວກເຮົາ ຕິດພັນກັບໜ່ວຍງານດັ່ງກ່າວ.

ຂ. ໂຄງການ LOMWRU ໄດ້ຮັບທຶນຊ່ວຍເຫລືອຫລັກ ຈາກທາງແອວຄຳຕູ້ສ ປະເທດ ອັງກິດ ແລະ ທຶນອີກສ່ວນໜຶ່ງແມ່ນໄດ້ຈາກ US Naval Medical Research Centre, the Bill & Melinda Gates Foundation, The European Union, Department for International Development-UK (DFID), New Zealand e-Asia, Fondation Total/Institute Pasteur, Global Good, DTRA, Global Antibiotic Research Partnership ແລະ the Asian Development Bank. ນອກນີ້ ທາງໂຄງການຍັງໄດ້ຮັບການຊ່ວຍເຫລືອເປັນເຄື່ອງອຸປະກອນ ຈາກສະຖາບັນຄົ້ນຄວ້າເພື່ອການພັດທະນາ/ມະຫາວິທະຍາໄລແອ໋ກຊ-ມາກໄຊ ປະເທດຝລັ່ງ ແລະ ໂຄງການຄົ້ນຄວ້າພະຍາດຣິກເກັດເຊຍ ຂອງສູນຄົ້ນຄວ້າທາງການແພດກອງທັບເຮືອ ສະຫະລັດອາເມລິກາ.

ຄ. ພະແນກວິເຄາະຈຸລິນຊີ ມີພະນັກງານ (ພາກລັດ) ທັງໝົດ 27 ຄົນ, ສ່ວນ LOMWRU ມີພະນັກງານໂຄງການ 65 ຄົນ, ໃນນີ້ 84% ແມ່ນຄົນລາວ ແລະ 54% ເປັນເພດຍິງ. ພວກເຮົາມີຫ້ອງວິເຄາະຈຸລະຊີວະວິທະຍາທາງຄລີນິກ, ຫ້ອງວິເຄາະທາງພັນທຸກຳ, ຫ້ອງວິເຄາະເຊໂຮໂລຊີ, ແລະ ຫ້ອງວິເຄາະລະດັບ 3 (BSL3). ການປະຕິບັດງານໃນຫ້ອງວິເຄາະດັ່ງກ່າວ ແມ່ນເປັນໄປຕາມແນວທາງ-ລະບຽບການຄວາມປອດໄພ ຂອງມະຫາວິທະຍາໄລອ໋ອກຟອດ.

ງ. LOMWRU ຊ່ວຍບໍລິການບ່ຽມະຕິພະຍາດຊຶມເຊື້ອພາຍໃນໂຮງມະໂຫສົດ ແລະ ໂຮງໝໍຕ່າງແຂວງ ເຊັ່ນ: ໂຮງໝໍແຂວງ ຫລວງນ້ຳທາ, ສາລະວັນ, ຊຽງຂວາງ ແລະ ແຂວງ ວຽງຈັນ; ເຮັດການຄົ້ນຄວ້າທາງຄລີນິກ; ແລະ ສ້າງຄວາມເຂັ້ມແຂງໃຫ້ແກ່ພະນັກງານພາກລັດໃນດ້ານການບ່ຽມະຕິພະຍາດ ແລະ ການເຮັດຄົ້ນຄວ້າ ໂດຍຜ່ານການຝຶກອົບຮົມ ແລະ ປະຕິບັດງານຕົວຈິງ. LOMWRU ມີການຮ່ວມມືຢ່າງໃກ້ສິດກັບ ມະຫາວິທະຍາໄລວິທະຍາສາດ ສຸຂະພາບ (ມວສ) ໃນຫລາຍໆໂຄງການ, ກັບສູນໄຂ້ຍຸງ, ແມ່ກາຝາກ ແລະ ແມງໄມ້ ເຮັດໂຄງການຄົ້ນຄວ້າຢູ່ 5 ແຂວງພາກໃຕ້, ຮ່ວມມືກັບກົມອາຫານ ແລະ ຢາ ໂດຍສະເພາະພາກສ່ວນກວດກາ ແລະ ສູນວິໄຈອາຫານ ແລະ ຢາ ເພື່ອດຳເນີນໂຄງການຄົ້ນຄວ້າກ່ຽວກັບຄຸນນະພາບຂອງຢາ, ແລະ ມີໂຄງການຮ່ວມມືກ່ຽວກັບການສ້າງຄູ່ມືປິ່ນປົວພະຍາດຊຶມເຊື້ອ ລວມທັງການເຝົ້າລະວັງພະຍາດຊຶມເຊື້ອ ແລະ ການຕ້ານຂອງເຊື້ອຈຸລະຊີບຕໍ່ຢາປິ່ນປົວ ຮ່ວມກັບກົມປິ່ນປົວ ແລະ ຟື້ນຟູໜ້າທີ່ການ + ກົມຄວບຄຸມພະຍາດຕິດຕໍ່ ກະຊວງສາທາລະນະສຸກ; ລວມທັງໂຄງການຄົ້ນຄວ້າກ່ຽວກັບສຸຂະພາບໜຶ່ງດຽວ ຮ່ວມກັບສູນວິເຄາະສຸຂະພາບສັດແຫ່ງຊາດ. ໃນ ສປປ ລາວ ພວກເຮົາຍັງມີໂຄງການຮ່ວມມືຫລັກ ກັບສະຖາບັນປາສະເຕີ້ລາວ ແລະ ສູນພະຍາດຊຶມເຊື້ອຄລິສຕິບ-ເມຣິເອີລາວ.

ຈ. ຈຸດສຸມສຳລັບວຽກຄົ້ນຄວ້າຂອງພວກເຮົາໄດ້ແກ່ ສາເຫດ ແລະ ລະບາດວິທະຍາຂອງໄຂ້, ລັກສະນະການຕ້ານຂອງເຊື້ອພະຍາດຕໍ່ຢາປິ່ນປົວ ລວມທັງການບ່ຽມະຕິ ແລະ ການປິ່ນປົວທີ່ສົມເຫດສົມຜົນ ເຊິ່ງທັງໝົດນີ້ ແມ່ນແນໃສ່ເພື່ອສະໜອງຂໍ້ມູນສຳລັບສ້າງເປັນນະໂຍບາຍດ້ານສາທາລະນະສຸກໃນຂົງເຂດອາຊີ ແລະ ດ້ານຄຸນນະພາບຢາປິ່ນປົວ ໃນລະດັບສາກົນ.

ສ. ໃນປີ 2018 ທາງໂຄງການໄດ້ສະໜັບສະໜູນພະນັກງານລາວຈຳນວນ 16 ຄົນ ເຂົ້າຮ່ວມກອງປະຊຸມ ແລະ ການຝຶກອົບຮົມໄລຍະສັ້ນຢູ່ຕ່າງປະເທດຈຳນວນ 6 ຄັ້ງ ແລະ ໄດ້ໃຫ້ການສະໜັບສະໜູນພະນັກງານພາກລັດ 2 ຄົນ ສຶກສາຕໍ່ໃນລະດັບປະລິຍາເອກ (1 ຄົນ ໄດ້ສຳເລັດການສຶກສາຢູ່ມະຫາວິທະຍາໄລມະຫິດົນ ແລະ ອີກ 1 ຄົນກຳລັງສຶກສາຢູ່ມະຫາວິທະຍາໄລອຳສະເຕີດຳ ປະເທດ ຮອນແລນ), ແລະ ອີກ 1 ຄົນ ກຳລັງສຶກສາໃນ ລະດັບປະລິນຍາຕີ ທີ່ມະຫາວິທະຍາໄລຂອນແກ່ນ.

ຊ. ໃນປີ 2018 ພວກເຮົາໄດ້ຕີພິມ ຫລື ກຳລັງຖືກຮັບຕີພິມເຜີຍແຜ່ຜົນຂອງການຄົ້ນຄວ້າລົງໃນວາລະສານການ ແພດສາກົນ ຈຳນວນ 73 ບົດ, ເຊິ່ງໃນນີ້ ມີ 67 ບົດຖືກພິມເຜີຍແຜ່ໃນວາລະສານທີ່ມີການທົບທວນຄັກແນ່, ເປັນຈົດໝາຍເຫດ 2 ບົດ, ບົດລາຍງານ 1 ບົດ, ບົດພິມລົງໃນປຶ້ມຕຳລາທາງການແພດຈຳນວນ 3 ພາກ. ນັບຕັ້ງແຕ່ໂຄງການຖືກສ້າງຕັ້ງເປັນຕົ້ນມາ, ນັກຄົ້ນຄວ້າຂອງໂຄງການ LOMWRU ມີຜົນງານຕີພິມເຜີຍແຜ່ຜົນ ການຄົ້ນຄວ້າ ທັງໝົດ 392 ບົດ ລວມທັງປຶ້ມຕຳລາຕ່າງໆ.

ຍ. ຜົນການຄົ້ນຄວ້າຜ່ານມາຂອງ LOMWRU ທີ່ຖືກນຳໄປຜັນຂະຫຍາຍ ເປັນແນວທາງນະໂຍບາຍ ດ້ານ ສາທາລະນະສຸກ ພາຍໃນປະເທດ ລວມມີ: ການຈັດຕັ້ງປະຕິບັດການສັກຢາກັນພະຍາດທີ່ເກີດຈາກເຊື້ອ Pneumococcus ແລະ ການສັກຢາກັນພະຍາດອັກເສບສະໝອງຍີ່ປຸ່ນ, ການປ່ຽນແປງແນວທາງການປິ່ນປົວ ພະຍາດໄຂ້ຍູງ, ພະຍາດໄຂ້ທໍລະພິດ (Typhoid) ແລະ ການຈັດພິມເຜີຍແຜ່ແນວທາງການປິ່ນປົວພະຍາດເມລິ ອອຍໂດຊິສ. ນອກນີ້ ພວກເຮົາຍັງໄດ້ຄົ້ນພົບພະຍາດທີ່ສຳຄັນ ບາງຢ່າງເປັນຄັ້ງທຳອິດໃນປະເທດລາວ, ໄດ້ຊີ້ ໃຫ້ເຫັນຄວາມສຳຄັນຂອງພະຍາດໄຂ້ແມງແດງ, ໄຂ້ຢົ່ວໜູ, ໄຂ້ທໍລະພິດ, ເມລິອອຍໂດສຊິສ, ແລະ ອັກເສບ ສະໝອງຍີ່ປຸ່ນ ເຊິ່ງເຮັດໃຫ້ພວກເຮົາເຂົ້າໃຈຢ່າງເລິກເຊິ່ງຕື່ມກ່ຽວກັບລັກສະນະດ້ານລະບາດວິທະຍາ ແລະ ແນວ ທາງການປ້ອງກັນພະຍາດດັ່ງກ່າວ. ທາງໂຄງການຍັງມີບົດຕີພິມເຜີຍແຜ່ທີ່ເປັນຫລັກຖານດ້ານວິທະຍາສາດກ່ຽວ ກັບເຊື້ອຈຸລະຊີບຕ້ານຕ່ຍາປິ່ນປົວພາຍໃນ ສປປ ລາວ ເຊິ່ງຖືເປັນຂໍ້ມູນທີ່ສຳຄັນສຳລັບສ້າງເປັນແຜນຍຸດທະສາດ ແລະ ແນວທາງການປ້ອງກັນສະພາບດັ່ງກ່າວ ແລະ ທາງຄະນະກຳມະການຄວບຄຸມການຕ້ານຂອງເຊື້ອຈຸລະຊີບ ຕ່ຍາປິ່ນປົວຂອງກະຊວງສາທາລະນະສຸກ ກຳລັງນຳໃຊ້ຂໍ້ມູນດັ່ງກ່າວ ມາສ້າງເປັນແນວທາງ ແລະ ແຜນຍຸດ ທະສາດກ່ຽວກັບວຽກງານດັ່ງກ່າວ ລວມທັງນຳມາຊຽນເປັນປຶ້ມຄູ່ມືປິ່ນປົວພະຍາດຊຶມເຊື້ອຂອງລາວ. ເພື່ອເຮັດໃຫ້ ທຸກຄົນສາມາດເຂົ້າເຖິງຂໍ້ມູນດັ່ງກ່າວ, ພວກເຮົາຍັງໄດ້ສ້າງເວັບໄຊ-ຖານຂໍ້ມູນກ່ຽວກັບເຊື້ອຈຸລະຊີບຕ້ານຕ່ຍາປິ່ນ ປົວ ແລະ ກ່ຽວກັບພະຍາດໄຂ້ຍູງລາຍຕື່ມອີກ. ທາງໂຄງການຍັງເປັນຜູ້ລິເລີ່ມວຽກຄົ້ນຄວ້າກ່ຽວກັບການກະຈາຍ ຂອງເຊື້ອ *B. pseudomallei* ໃນດິນ ແລະ ນ້ຳ ພາຍໃນ ສປປ ລາວ ເພື່ອເຮັດໃຫ້ພວກເຮົາເຂົ້າໃຈກ່ຽວກັບ ການກະຈາຍຂອງເຊື້ອພະຍາດທີ່ມີຄວາມອັນຕະລາຍຮ້າຍແຮງດັ່ງກ່າວ ລວມທັງຄວາມສ່ຽງຂອງຄົນທີ່ຈະຕິດເຊື້ອ ນີ້. ທາງໂຄງການຍັງໄດ້ສະໜອງຂໍ້ມູນ-ຫລັກຖານ ສຳລັບການກໍ່ສ້າງຄວາມອາດສາມາດດ້ານການບິ່ງມະຕິທາງ ຫ້ອງວິເຄາະທີ່ເໝາະສົມ, ແມ່ນຍຳ ແລະ ແບບຍືນຍົງ ໃນ ສປປ ລາວ ໂດຍສະເພາະໃນລະດັບໂຮງໝໍແຂວງ ແລະ ຍັງຊີ້ໃຫ້ເຫັນວ່າ ພະຍາດຊຶມເຊື້ອໃນແຕ່ລະພື້ນທີ່ຂອງປະເທດ ແມ່ນມີຄວາມແຕກຕ່າງກັນ. ນອກນີ້ ທາງໂຄງການ ຍັງເປັນຜູ້ລິເລີ່ມວຽກໂຄສະນາເຜີຍແຜ່ຜົນການຄົ້ນຄວ້າ ໃຫ້ສາທາລະນະຊົນໄດ້ຮັບຮູ້ ແລະ ຍັງ ເປັນເຈົ້າການ-ນຳໜ້າວຽກຄົ້ນຄວ້າກ່ຽວກັບບັນຫາຜະລິດຕະພັນຢາທີ່ບໍ່ໄດ້ຄຸນນະພາບ ຫລື ຢາປອມ.

ດ. ສະຫລຸບຜົນຂອງການຄົ້ນຄວ້າທີ່ມີຄວາມໝາຍສຳຄັນຕໍ່ປະເທດລາວ ທີ່ໄດ້ຕີພິມເຜີຍແຜ່ ຫລື ກຳລັງຈະຖືກ ຕີພິມເຜີຍແຜ່ ພາຍໃນປີ 2018 ທີ່ຜ່ານມາ ມີດັ່ງຕໍ່ໄປນີ້ (ກະລຸນາເບິ່ງລາຍລະອຽດຕື່ມໃນບົດລາຍງານ):

*** ລັກສະນະການຕ້ານຂອງເຊື້ອຈຸລະຊີບຕ່ຍາປິ່ນປົວ ກຳລັງເປັນບັນຫາສຳຄັນດ້ານສາທາລະ ນະສຸກ ແລະ ກໍ່ໃຫ້ເກີດຄວາມກັງວົນນັບມື້ຫລາຍຂຶ້ນໃນ ສປປ ລາວ ກໍ່ຄືໃນທົ່ວໂລກ.**

- ບັນຫາການຕ້ານ (ການດື້) ຂອງເຊື້ອ Enterobacteriaceae ຕ່ຍາຕ້ານເຊື້ອໃນກຸ່ມ Beta-lactamin (ESBL) ແລະ ຕ້ານຕ່ຍາຕ້ານເຊື້ອຫລາຍຕົວ ເປັນສາເຫດການຊຶມເຊື້ອສຳຄັນ ທີ່ພົບເຫັນ ນັບມື້ຫລາຍ

ຂຶ້ນໃນໂຮງໝໍມະໂຫສິດ. ອັນນີ້ຖືເປັນບັນຫາເຊື້ອຈຸລິນຊີຕ້ານຕ່ຍາຕ້ານເຊື້ອທີ່ພົບເຫັນເລື້ອຍທີ່ສຸດ ໃນຫ້ອງວິເຄາະຈຸລິນຊີວິທະຍາຂອງພວກເຮົາ ເນື່ອງຈາກອັດຕາການກວດພົບໃນໂຮງໝໍ ແມ່ນເພີ່ມຂຶ້ນເລື້ອຍໆ ແລະ ເຊື້ອດັ່ງກ່າວກໍຕ້ານຕ່ຍາຕ້ານເຊື້ອທີ່ມັກໃຊ້ເປັນປະຈຳ ເຊັ່ນ cephalosporins and penicillins ແລະ ຍັງພົບວ່າ ມີການຕ້ານຕ່ຍາ gentamicin and ciprofloxacin/ofloxacin ເລື້ອຍໆ. ໃນລະຫວ່າງປີ 2010-2014 ເຊື້ອ *E. coli* and *Klebsiella pneumoniae* ພົບໄດ້ປະມານ 24.8% ຂອງຄົນເຈັບທັງໝົດທີ່ກວດພົບເຊື້ອໃນກະແສເລືອດ. ໃນນີ້ 20% ແມ່ນເປັນ ເຊື້ອ ESBL (ເຊື້ອ *E. coli* ກວມ 56% ແລະ ເຊື້ອ *K. pneumoniae* ກວມ 24%). ການຊຶມເຊື້ອເລືອດຈາກເຊື້ອ ESBL-producing *E. coli* ນັບມື້ເພີ່ມຂຶ້ນເລື້ອຍໆໃນຊ່ວງທີ່ທຳການສຶກສາ. ປະຫວັດການໃຊ້ຢາຕ້ານເຊື້ອໃນອາທິດຜ່ານມາຂອງຄົນເຈັບ ແມ່ນມີຄວາມສຳພັນກັບການກວດພົບເຊື້ອ ESBL. ເຖິງຈະຕ້ານຕ່ຍາຫລາຍຊະນິດ, ເຊື້ອ ESBL-producing *E. coli* and *K. pneumoniae* ສ່ວນໃຫ່ຍຍັງຖືກກັບຢາ meropenem and amikacin ຢູ່. ການເພີ່ມຂຶ້ນຂອງກໍລະນີເຊື້ອ ESBL-E ມີຄວາມໝາຍສຳຄັນທີ່ສຸດສຳລັບການປິ່ນປົວຊຶມເຊື້ອເລືອດ ໃນ ສປປ ລາວ ລວມທັງການເຝົ້າລະວັງທີ່ກຳລັງດຳເນີນໄປໃນຂະນະນີ້. ປະຈຸບັນ ໃນ ສປປ ລາວ ກໍຄືໃນທົ່ວໂລກ ກຳລັງປະເຊີນກັບບັນຫາດັ່ງກ່າວ ເຊິ່ງເຊື້ອຈຸລິນຊີໄດ້ຕ້ານຕ່ຍາຕ້ານເຊື້ອເກືອບທຸກຕົວ ເຮັດໃຫ້ພວກເຮົາກັງວົນວ່າ ພວກເຮົາຈະບໍ່ມີຢາທີ່ສາມາດປິ່ນປົວ-ຂ້າເຊື້ອໄດ້ໃນອະນາຄົດ ຫລື ຖ້າມີ ກໍຕ້ອງໃຊ້ຢາທີ່ມີລາຄາແພງທີ່ສຸດ ເຊິ່ງຄົນເຈັບອາດບໍ່ສາມາດຈ່າຍໄດ້.

- ທີ່ໜ້າແປກໃຈກໍຄືວ່າ ESBL bacteria ຍັງພົບເຫັນເປັນປະຈຳໃນລຳໃສ້ຂອງຄົນ ແລະ ສັດ ທີ່ມີສຸຂະພາບແຂງແຮງພາຍໃນບ້ານແຫ່ງໜຶ່ງ ຢູ່ເຂດຫ່າງໄກສອກຫລີກຂອງແຂວງຊຽງຂວາງ ເຊິ່ງຂໍ້ມູນນີ້ຊີ້ໃຫ້ເຫັນວ່າ ESBL ເປັນບັນຫາຢູ່ຊຸມນະບົດຂອງລາວເຮົາແລ້ວ ແລະ ກໍຈະເປັນບັນຫາທີ່ໃຫ່ຍຫລວງໃນອະນາຄົດຖ້າເຮົາບໍ່ມີມາດຕະການຫຍັງຕື່ມ. ສິ່ງໜຶ່ງທີ່ໜ້າແປກໃຈກໍຄືວ່າ ປະຊາຊົນພາຍໃນບ້ານດັ່ງກ່າວຈຳນວນຫລາຍສົມຄວນ (13.4%) ໄດ້ຮັບຢາຕ້ານເຊື້ອພາຍໃນ 2 ອາທິດ ກ່ອນໜ້າທີ່ມງານລົງໄປເຮັດການສຳຫລວດພາຍໃນບ້ານ.
- ນອກຈາກນີ້ ພວກເຮົາຍັງພົບວ່າ ຄົນຕ່າງປະເທດທີ່ມາຮ່ວມສຳມະນາທາງການແພດ ໃນນະຄອນຫລວງວຽງຈັນ (ໄດ້ເອົາຕົວຢ່າງອາຈົມໄປກວດທັນທີ ທີ່ມາເຖິງ ແລະ 3 ອາທິດຕໍ່ມາ) ແມ່ນພົບມີເຊື້ອຈຸລິນຊີທີ່ຕ້ານຕ່ຍາ cephalosporin ເຊິ່ງຂໍ້ມູນນີ້ຊີ້ໃຫ້ເຫັນວ່າ ເຊື້ອ ESBL ແມ່ນພົບໄດ້ຫລາຍໃນຄົນ ແລະ ສິ່ງແວດລ້ອມຢູ່ວຽງຈັນ, ແລະ ກໍຊີ້ໃຫ້ເຫັນວ່າ ແຂກທີ່ມາຢ້ຽມຢາມ ກໍມີການຕິດເຊື້ອເຂົ້າໃນຕົວຢ່າງໄວວາ ເຊິ່ງເຮັດໃຫ້ມີຄວາມສ່ຽງຕໍ່ສຸຂະພາບຂອງເຂົາເຈົ້າ ແລະ ສ່ຽງຕໍ່ການແຜ່ກະຈາຍເຊື້ອໄປຫາບ່ອນອື່ນໆ.
- ເຊື້ອ Gram negative bacilli ທີ່ຕ້ານຕ່ຍາ carbapenem ຖືກພົບເປັນຄັ້ງທຳອິດທີ່ໂຮງໝໍມະໂຫສິດ ເຊິ່ງແມ່ນເຊື້ອ *E. coli* and *Acinetobacter baumannii* ທີ່ພົບຈາກຕົວຢ່າງໜອງ, ຈາກລະບົບຖ່າຍເທ, ຈາກລະບົບຫາຍໃຈ ແລະ ຈາກເລືອດ. ເຊື້ອ Acinetobacters ທີ່ຕ້ານຕ່ຍາ Carbapenem ມັກພົບເຫັນເປັນປະຈຳຈາກຕົວຢ່າງທີ່ໄດ້ຈາກເສັ້ນທາງລະບົບຫາຍໃຈຂອງຄົນເຈັບພະແນກມໍລະສູມ ໂຮງໝໍມະໂຫສິດ ແລະ ມາຮອດປະຈຸບັນ ພວກເຮົາພົບເຊື້ອ E. *coli* ທີ່ຕ້ານຕ່ຍາ Carbapenem ຈຳນວນ 4 ເຊື້ອ ຈາກຕົວຢ່າງທີ່ສົ່ງມາປູກ. NDM carbapenemases ຖືກກວດພົບໃນ *E. coli* ໝົດທຸກຕົວທີ່ພົບ ແລະ OXA-23-like acquired carbapenemase ໃນ *A. baumannii* ໝົດທຸກຕົວເຊັ່ນກັນ. ເນື່ອງຈາກວ່າ ກຳລັງມີການລິເລີ່ມໃຊ້ຢາ carbapenems ໃນລາວ, ສະນັ້ນ ການຄົ້ນພົບດັ່ງກ່າວຈຶ່ງເຮັດໃຫ້ພວກເຮົາມີຄວາມກັງວົນຫລາຍທີ່ສຸດ ແລະ ມັນຮຽກຮ້ອງໃຫ້ຕ້ອງມີການຕິດຕາມສຳລັບການນຳໃຊ້

ຢາດັ່ງກ່າວຢ່າງເຂັ້ມງວດ ເພື່ອຫລີກລ້ຽງຄວາມສ່ຽງຕໍ່ທ່າອ່ຽງທີ່ເພີ່ມຂຶ້ນຂອງເຊື້ອອັນຕະລາຍດັ່ງກ່າວ. ສະນັ້ນ ການຄຸ້ມຄອງການນຳໃຊ້ຢາຕ້ານເຊື້ອຢ່າງສົມເຫດສົມຜົນ ຈຶ່ງເປັນປະເດັນທີ່ຮີບຮ້ອນທີ່ສຸດ.

- ເຊື້ອສາເຫດການຊຶມເຊື້ອເລືອດ ໃນກຸ່ມ non-typhoidal Salmonella ທີ່ໂຮງໝໍມະໂຫສິດ ໄດ້ແກ່ ເຊື້ອ *S.* Enteritidis, *S.* Typhimurium and *S.* Choleraesuis ເຊິ່ງສ່ວນໃຫ່ຍມັກຈະຕ້ານຕໍ່ຢາ Ciprofloxacin. ສ່ວນເຊື້ອທີ່ພົບໃນອາຈົມຂອງຄົນເຈັບຖອກທ້ອງແມ່ນມີຫລາຍຊະນິດ ແຕ່ສ່ວນໃຫ່ຍ ແມ່ນພວກ *S.* Typhimurium, *S.* Weltevreden, and *S.* Stanley. ສິ່ງທີ່ໜ້າສົນໃຈກໍ່ຄືວ່າ: ໜຶ່ງໃນ ຈຳນວນເຊື້ອດັ່ງກ່າວ ຄື ເຊື້ອ *S.* Weltevreden ແມ່ນພົບໃນອາຈົມຂອງໂຕກັບແກ້ພາຍໃນເຮືອນຄົນ ເຊິ່ງຊີ້ໃຫ້ເຫັນວ່າ ເຊື້ອດັ່ງກ່າວອາດສາມາດຕິດຕໍ່ລະຫວ່າງສັດກັບຄົນ ຈຶ່ງເປັນໄປໄດ້ວ່າ ເຮົາຄວນຫາ ແນວທາງຫລຸດຜ່ອນການສຳພັດລະຫວ່າງຄົນເຮົາ ກັບສັດດັ່ງກ່າວ.

- ຜົນການທົດສອບເຊື້ອ *N. gonorrhoeae* ໃສ່ຢາຕ້ານເຊື້ອຈຳນວນ 158 ເຊື້ອ ພົບວ່າ: 100% ແມ່ນ ຖືກກັບຢາ ceftriaxone and spectinomycin ແຕ່ພັດມີການຕ້ານໃນລະດັບສູງ ຕໍ່ຢາ ciprofloxacin, penicillin and tetracycline. ຂໍ້ມູນນີ້ ຊີ້ໃຫ້ເຫັນວ່າ ຢາ ceftriaxone and spectinomycin ໜ້າຈະ ມີປະສິດທິພາບສູງຕໍ່ເຊື້ອ *N. gonorrhoeae* ໃນ ສປປ ລາວ. ການຕິດຕໍ່ຫາຄູ່ນອນຂອງຄົນເຈັບທີ່ຕິດ ເຊື້ອ ເພື່ອມາປິ່ນປົວຮ່ວມກັນ ຖືເປັນແນວທາງສຳຄັນສຳລັບການຫລຸດຜ່ອນພະຍາດ ພຕພ.

- ເຊື້ອ *Neisseria meningitidis* ທີ່ຕ້ານຕໍ່ຢາ Chloramphenicol ຖືກກວດພົບໃນລາວ ແລະ ໃນ ຂົງເຂດອາຊີຕາເວັນອອກສ່ຽງໄຕ້, ຂໍ້ມູນນີ້ຊີ້ໃຫ້ເຫັນວ່າ ເຮົາບໍ່ຄວນນຳໃຊ້ Chloramphenicol ປິ່ນປົວການຕິດເຊື້ອດັ່ງກ່າວໃນຂະນະທີ່ລໍຖ້າຜົນການທົດສອບຂອງເຊື້ອຕໍ່ຢາໃນຫ້ອງວິເຄາະ.

- ພວກເຮົາຍັງສືບຕໍ່ເຮັດການສຳຫລວດກ່ຽວກັບຄວາມຊຸກສຳລັບການນຳໃຊ້ຢາຕ້ານເຊື້ອ ແລະ ມາຮອດ ປະຈຸບັນໄດ້ສຳເລັດການເກັບກຳຂໍ້ມູນ 19 ຮອບແລ້ວ ຢູ່ໂຮງໝໍ 5 ແຫ່ງ ຂອງ ສປປ ລາວ. ພວກເຮົາ ຄາດວ່າ ຂໍ້ມູນຈາກການສຳຫລວດນີ້ ຈະເປັນປະໂຫຍດໃຫ້ແກ່ໂຮງໝໍທີ່ກ່ຽວຂ້ອງ ແລະ ກະຊວງສາທາ ລະນະສຸກ ໂດຍສະເພາະໃນເລື່ອງການຄາດຄະເນປະລິມານຢາຕ້ານເຊື້ອທີ່ຖືກນຳໃຊ້ໃນຄົນເຈັບນອນ ໂຮງໝໍ ແລະ ເພື່ອໃຊ້ສຳລັບຕິດຕາມການປ່ຽນແປງສະພາບການນຳໃຊ້ຢາຕ້ານເຊື້ອ ລວມທັງການນຳ ໃຊ້ຂໍ້ມູນເພື່ອປັບປ່ຽນແນວທາງການສັ່ງຢາທີ່ສົມເຫດສົມຜົນຂອງແພດໝໍ. ສປປ ລາວ ເປັນປະເທດ ທຳອິດໃນບັນດາປະເທດອາຊີຕາເວັນອອກຊ່ຽງໄຕ້ ທີ່ເຂົ້າຮ່ວມໂຄງການສຳຫລວດດັ່ງກ່າວ.

- ການແຜ່ຂະຫຍາຍຂອງເຊື້ອຈຸລິນຊີທີ່ຕ້ານຕໍ່ຢາຕ້ານເຊື້ອໃນ ສປປ ລາວ ຈະສົ່ງຜົນສະທ້ອນຢ່າງໃຫ່ຍ ຫລວງຕໍ່ຄົນເຈັບ, ຊຸມຊົນ ແລະ ເສດຖະກິດ. ສະນັ້ນ ຈຶ່ງມີຄວາມຈຳເປັນຢ່າງຮີບດ່ວນ ທີ່ຈະຕ້ອງ ເອົາໃຈໃສ່ວຽກງານຄວບຄຸມການຕິດເຊື້ອ, ການນຳໃຊ້ຢາຕ້ານເຊື້ອຢ່າງສົມເຫດສົມຜົນ, ແລະ ລະບຽບ ຫລັກການຕ່າງໆທີ່ກ່ຽວຂ້ອງ. ບັນຫາເຊື້ອຕ້ານຕໍ່ຢາປິ່ນປົວໃນ ສປປ ລາວ ຍັງບໍ່ຫລາຍເທົ່າກັບປະເທດ ເພື່ອນບ້ານ, ສະນັ້ນ ຈຶ່ງເປັນໂອກາດດີ ທີ່ຈະປ້ອງກັນບໍ່ໃຫ້ບັນຫາຮ້າຍແຮງຂຶ້ນຄືກັບ ປະເທດອື່ນ.

- ເພື່ອຊ່ວຍໃຫ້ມີຂໍ້ມູນ-ຫລັກຖານ ແລະ ແນວທາງປະຕິບັດທີ່ເໝາະສົມ, ພວກເຮົາໄດ້ຮ່ວມມືກັບ ກະ ຊວງສາທາລະນະສຸກ ລວມທັງຄູ່ຮ່ວມງານອື່ນໆໂດຍສະເພາະຄູ່ຮ່ວມງານ GARP (Global Antibiotic Resistance Partnership) ຈັດຕັ້ງທີມງານວິຊາການຄວບຄຸມເຊື້ອຈຸລິນຊີທີ່ຕ້ານຕໍ່ຢາຕ້ານເຊື້ອ ເຊິ່ງ ທີມງານນີ້ ຈະເຮັດວຽກສົມທົບກັບຄະນະກຳມະການຂອງ WHO/FAO/OIE. ຜົນການທົບທວນສະ ພາບການຕ້ານຂອງເຊື້ອຈຸລະຊີບຕໍ່ຢາປິ່ນປົວໃນລາວ (ຈາກເອກະສານທີ່ຖືກຕີພິມເຜີຍແຜ່, ບໍ່ຖືກຕີພິມ

ເຜີຍແຜ່ ແລະ ຂໍ້ມູນຈາກໂຮງໝໍມະໂຫສົດ) ຈະຖືກນຳມາສົນທະນາຫາລືກັນ ໃນຕົ້ນປີ 2019 ແລະ ນຳໃຊ້ເຂົ້າໃນການສ້າງແຜນນະໂຍບາຍດ້ານອງການດັ່ງກ່າວ ແລະ ໃຊ້ສຳລັບການສ້າງຄູ່ມືປິ່ນປົວ ດ້ວຍຢາຕ້ານເຊື້ອແຫ່ງຊາດ ເຊິ່ງກຳລັງຖືກຮ່າງຂຶ້ນຮ່ວມກັບຫລາຍໆພາກສ່ວນ.

- ພວກເຮົາກຳລັງພັດທະນາລະບົບຖານຂໍ້ມູນເປັນພາສາລາວ ແລະ ພາສາອັງກິດ ເພື່ອເຮັດໃຫ້ຜູ້ເຮັດອງການດ້ານນະໂຍບາຍ ແລະ ບຸກຄະລະກອນສາທາລະນະສຸກ ສາມາດເຂົ້າເຖິງຂໍ້ມູນສາເຫດການຊຶມເຊື້ອຈຸລິນຊີ ແລະ ລັກສະນະການຕອບສະໜອງຂອງເຊື້ອຕ່ຢາປິ່ນປົວ ພາຍໃນໂຮງໝໍມະໂຫສົດ.

- ພວກເຮົາກຳລັງເຮັດການທົບທວນຄືນກ່ຽວກັບຄວາມໝາຍ-ຄວາມສຳຄັນຂອງການປ່ຽນແປງວິທີການທົດສອບລັກສະນະການຕອບສະໜອງຂອງເຊື້ອຈຸລິນຊີຕ່ຢາຕ້ານເຊື້ອ ດ້ວຍວິທີ Clinical and Laboratory Standards Institute (CLSI) methods ມາເປັນວິທີ the European Committee on Antimicrobial Susceptibility Testing (EUCAST) methods ໃນ ສປປ ລາວ ເຊິ່ງຂໍ້ມູນທີ່ໄດ້ມາ ຈະຊ່ວຍໃຫ້ກະຊວງສາທາລະນະສຸກສາມາດຕັດສິນໃຈນຳໃຊ້ວິທີ EUCAST ເຂົ້າໃນຫ້ອງວິເຄາະຈຸລິນຊີວິທະຍາພາຍໃນ ສປປ ລາວ ໃນອະນາຄົດ.

- ພວກເຮົາກຳລັງພັດທະນາລະບົບຕາຕະລາງ ແລະ ແຜນທີ່ສຳລັບຂໍ້ມູນຄຸນນະພາບຂອງຢາຕ້ານເຊື້ອໃນທົ່ວໂລກ ເຊິ່ງຈະຊ່ວຍສຳລັບການສົນທະນາຫາລື ແລະ ເຮັດ modelling ເພື່ອຫາຄວາມສຳພັນລະຫວ່າງຄຸນນະພາບຂອງຢາ ແລະ ລັກສະນະການຕ້ານຂອງເຊື້ອຈຸລິນຊີຕ່ຢາຕ້ານເຊື້ອ.

* ໄຂ້ມາລາເຣຍ ແລະ ຄວາມສຳຄັນໃນການກຳຈັດພະຍາດດັ່ງກ່າວອອກຈາກ ສປປ ລາວ

- ພວກເຮົາກຳລັງສືບຕໍ່ເຝົ້າລະວັງ ລັກສະນະການຕ້ານຂອງເຊື້ອກາຝາກມາລາເຣຍ ທາງດ້ານພັນທຸກຳຮ່ວມກັບສູນໄຂ້ຍຸງ, ແມ່ກາຝາກ ແລະ ແມງໄມ້ ແລະ MORU-Bangkok ໃນແຂວງພາກໃຕ້ຂອງ ລາວ. ຂໍ້ມູນຈາກການເຝົ້າລະວັງ ພົບເຫັນການຕ້ານຕ່ຢາອາກເຕມີຊີນິນຂອງເຊື້ອຟານຊີປາຣອມ ໃນລະດັບສູງຫລາຍ ເຊິ່ງຂໍ້ມູນນີ້ມີຄວາມໝາຍສຳຄັນຕໍ່ນະໂຍບາຍປິ່ນປົວພະຍາດດັ່ງກ່າວໃນລາວ. ນອກນີ້ ຍັງກວດພົບ Molecular markers ທີ່ຕ້ານຕ່ຢາ piperaquine ຢູ່ທາງພາກໃຕ້ຂອງລາວ ຄືກັບຢູ່ ປະເທດໄທ ແລະ ກຳປູເຈຍ ເຊິ່ງນຳໄປສູ່ຄຳຖາມທີ່ວ່າ: ປະສິດທິພາບຂອງຢາປິ່ນປົວ DHA-piperaquine ໃນຄົນເຈັບທີ່ເປັນມາລາເຣຍຟານຊີປາຣອມຈະເປັນແນວໃດ. ປະຈຸບັນ ຍັງມີຄວາມກັງວົນກ່ຽວກັບການແຜ່ກະຈາຍຂອງເຊື້ອຟານຊີປາຣອມທີ່ຕ້ານຕ່ຢາອາກເຕມີຊີນິນ ຢູ່ທາງພາກໃຕ້ຂອງ ລາວ ແລະ ມີຄວາມຈຳເປັນຈະຕ້ອງໄດ້ຫາລືກັນວ່າ ເຮົາຈະໃຊ້ຢາປິ່ນປົວ ຊະນິດໃດ ຖ້າຢາປະສົມ ACTs ສອງຕົວຫາກໃຊ້ບ່ໄດ້ຜົນແລ້ວ. ສະນັ້ນ ພວກເຮົາກຳລັງຮ່ວມມື ກັບສູນໄຂ້ຍຸງ, ແມ່ກາຝາກ ແລະ ແມງໄມ້ ເຮັດການຄົ້ນຄວ້າແບບຫລາຍສູນ (TRAC-2) ເພື່ອສຶກສາ ການຕ້ານຂອງເຊື້ອຟານຊີປາຣອມຕ່ຢາອາກເຕມີຊີນິນ ຢູ່ທີ່ໂຮງໝໍແຂວງເຊກອງ ໂດຍແມ່ນໜ່ວຍງານຄົ້ນຄວ້າ MORU ຢູ່ບາງກອກເປັນຜູ້ປະສານງານ. ການສຶກສານີ້ ເປັນການທົດລອງທາງຄລີນິກແບບຊຸ່ມ ເພື່ອປຽບທຽບໄລຍະເວລາທີ່ເຊື້ອໜີດໄປຈາກກະແສເລືອດ ລະຫວ່າງຢາ artemether-lumefantrine ແລະ artemether-lumefantrine plus amodiaquine. ພວກເຮົາຄາດວ່າ ຂໍ້ມູນ ທີ່ໄດ້ຈາກການສຶກສານີ້ ຈະເປັນປະໂຫຍດສຳລັບການວາງແນວທາງນະໂຍບາຍປິ່ນປົວ ພະຍາດໄຂ້ມາລາເຣຍ ຂອງ ສປປ ລາວ ໃນຕໍ່ໜ້າ.

- ພວກເຮົາໄດ້ຈັດຕັ້ງປະຕິບັດໂຄງການທົດລອງກໍຈັດມາລາເຣຍຢູ່ແຂວງສະຫວັນນະເຂດ ຮ່ວມກັບສູນໄຂ້ຍູງ, ແມ່ກາຝາກ ແລະ ແມງໄມ້ ແລະ ພົບວ່າ ການປຸກລະດົມຂົນຂວາຍໃຫ້ປະຊາຊົນເຂົ້າຮ່ວມໂຄງການແມ່ນມີຄວາມສຳຄັນທີ່ສຸດ ແລະ ຍັງພົບວ່າ ຄົນທີ່ຖືເຊື້ອມາລາເຣຍໂດຍບໍ່ມີອາການສະແດງອອກ ແມ່ນພົບໄດ້ຫລາຍ ເຊິ່ງເຂົາເຈົ້າອາດເປັນແຫລ່ງສິ່ງຕໍ່ພະຍາດທີ່ສຳຄັນ. ຜົນການທົດລອງເບື້ອງຕົ້ນພົບວ່າ ການຢາຍຢາ dihydroartemisinin-piperaquine ສົມທົບກັບຢາ primaquine ໃນປະລິມານຕ່ຳ ໃຫ້ກິນທົ່ວປ່ອງຊົນ (MDA) ແມ່ນມີຄວາມເປັນໄປໄດ້, ເປັນທີ່ຍອມຮັບຂອງປະຊາຊົນ ແລະ ມີຄວາມປອດໄພສູງ, ແຕ່ຜົນກະທົບຂອງການກິນຢາຕໍ່ລະດັບການແຜ່ເຊື້ອ ຈະຕ້ອງໄດ້ສຶກສາຕື່ມອີກ. ປະມານ 84% ຂອງປະຊາຊົນທັງໝົດ ເຂົ້າຮ່ວມກິນຢາ ເຊິ່ງໃນຈຳນວນນີ້ 90% ກິນຢາຄົບ 3 ຮອບ. ໃນບ້ານທີ່ປະຊາຊົນກິນຢາ, ເປີເຊັນຄວາມຊຸກຂອງການຕິດເຊື້ອມາລາເຣຍຟານຊີປາຣອມທີ່ບໍ່ມີອາການສະແດງອອກຫລຸດລົງຢ່າງມີຄວາມສຳຄັນທາງສະຖິຕິ ທຽບໃສ່ບ້ານທີ່ບໍ່ໄດ້ກິນຢາ ແລະ ເປີເຊັນການຕິດເຊື້ອຍັງຫລຸດລົງຢ່າງຫລວງຫລາຍພາຍຫລັງການກິນຢາໃນຮອບທຳອິດ ແລະ ຫລຸດລົງໃນກຸ່ມຄົນທີ່ກິນຢາຄົບ 3 ຮອບ. ເມື່ອວິເຄາະເບິ່ງວ່າ ເປັນຫຍັງປະຊາຊົນຈຶ່ງເຂົ້າຮ່ວມກິນຢາ, ພວກເຮົາພົບວ່າ ຄວາມເຂົ້າໃຈຂອງເຂົາເຈົ້າຕໍ່ກັບການສຶກສາຄົ້ນຄວ້າ, ການບໍລິການປິ່ນປົວສຸຂະພາບບໍ່ເສຍຄ່າ, ການຮ່ວມມືຂອງອາສາສະມັກບ້ານ, ການສະໜັບສະໜູນຈາກການຈັດຕັ້ງ ແລະ ຊຸມຊົນ ລ້ວນແຕ່ເປັນປັດໄຈທີ່ພົວພັນກັບການເຂົ້າຮ່ວມກິນຢາຂອງປະຊາຊົນ.

- ພວກເຮົາໄດ້ເຮັດການສຳຫລວດອັດຕາຊຸກຊຸມຂອງພາວະຂາດອັງຊົມ G6PD ໃນລາວ ເພື່ອເປັນຂໍ້ມູນສຳລັບການປິ່ນປົວມາລາເຣຍຊະນິດວິວັກຊ໌ຢ່າງປອດໄພ (ການໃຊ້ຢາ primaquine) ແລະ ພົບວ່າ ພາວະຂາດອັງຊົມດັ່ງກ່າວແມ່ນພົບເຫັນໄດ້ເລື້ອຍເຊັ່ນກັນໃນຂົງເຂດນີ້ ໂດຍພົບປະມານ 8.1% ໃນລາວ. ພາວະຂາດອັງຊົມຊະນິດ Mahidol and Viangchan ຖືກພົບຫລາຍກວ່າໝູ່ ໃນ 9 ບ້ານທີ່ເຮັດການສຳຫລວດ. ການປະເມີນວິທີການກວດພາວະຂາດອັງຊົມດັ່ງກ່າວ ແບບ fluorescent spot test and a rapid diagnostic test (RDT) ທຽບກັບວິທີມາດຕະຖານ (spectrophotometry) ພົບວ່າ 2 ວິທີດັ່ງກ່າວແມ່ນມີປະໂຫຍດໃນການກວດພາກສະໜາມ ແຕ່ ການກວດແບບ RDT ແມ່ນຕ້ອງມີການຝຶກອົບຮົມຫລາຍກວ່າ.

* ສາເຫດ, ການບ່ຽງເບ ແລະ ການປິ່ນປົວໄຂ້ ໃນເຂດຊົນນະບົດຂອງ ສປປ ລາວ.

- ພາຍໄຕ້ການສະໜັບສະໜູນຂອງ US Naval Medical Research Centre-Asia ພວກເຮົາໄດ້ຂະຫຍາຍໂຄງການຄົ້ນຄວ້າຫາສາເຫດຂອງໄຂ້ ໃນຄົນເຈັບເຂດນອກ ຂອງໂຮງໝໍແຂວງຊຽງຂວາງ, ສາລະວັນ ແລະ ຫລວງນ້ຳທາ. ສາເຫດຕົ້ນຕໍຂອງໄຂ້ທີ່ພົບໃນໄລຍະ 1 ປີ ໄດ້ແກ່: ພະຍາດໄຂ້ຫວັດ (60%), ໄຂ້ຍ່ຽວໜູ (15%), ໄຂ້ຍູງລາຍ (10%), Scrub typhus (5%), ຊຶມເຊື້ອເລືອດ (5%), murine typhus (3%), ອັກເສບສະໝອງຍີ່ປຸ່ນ (1%) ແລະ *Rickettsia* spp. (1%). ພະຍາດ Melioidosis ພົບເຫັນປະມານ 2.5% ໃນຄົນເຈັບຈາກແຂວງສາລະວັນ ແຕ່ບໍ່ພົບໃນແຂວງຫລວງນ້ຳທາເລີຍ. ພວກເຮົາໄດ້ດຳເນີນການສຶກສາໃນລັກສະນະນີ້ອີກໃນຄົນເຈັບນອນ ນັບແຕ່ເດືອນສິງຫາ 2017 ເປັນຕົ້ນມາ ແລະ ຄາດວ່າຜົນຈາກການຄົ້ນຄວ້ານີ້ ຈະມີຄວາມສຳຄັນສຳລັບການສົນທະນາເພື່ອວາງເປັນແນວທາງການປິ່ນປົວໃນອະນາຄົດ.
- ໂຄງການສຶກສາຄົ້ນຄວ້າຫາສາເຫດຂອງໄຂ້ໃນຫລາຍປະເທດ (FIEBRE) ໄດ້ເລີ່ມຂຶ້ນໃນເດືອນຕຸລາ 2018 ທີ່ໂຮງໝໍແຂວງວຽງຈັນ ໂດຍມີຈຸດປະສົງເພື່ອຊອກຫາສາເຫດຂອງໄຂ້ ລວມທັງສຶກສາເບິ່ງ biomarkers ແນໃສ່ເພື່ອປັບປຸງການປິ່ນປົວໃຫ້ດີຂຶ້ນກວ່າເກົ່າ. ການສຶກສານີ້ ພວກເຮົາໄດ້ຮ່ວມມື ກັບ

London School of Hygiene and Tropical Medicine ພາຍໄຕ້ທຶນຊ່ວຍເຫລືອຈາກທາງ UK Department for International Development (DFID) ປະເທດອັງກິດ.

- ເມລິອອຍໂດຊິສ ເປັນສາເຫດທີ່ສຳຄັນຂອງການຊຶມເຊື້ອເລືອດທີ່ເຮົາມັກຈະບໍ່ຄິດຫາ ໃນ ສປປລາວ ແລະ ໃນຂົງເຂດປະເທດເຂດຮ້ອນ. ນັບແຕ່ປີ 1999 ມາຮອດປະຈຸບັນ ພວກເຮົາໄດ້ບົ່ງມະຕິຄົນເຈັບເປັນພະຍາດເມລິອອຍ ຈຳນວນ 1,354 ຄົນ (ສະເພາະໃນປີ 2018 ພົບ 120 ຄົນ) ແລະ ພວກເຮົາກັງວົນວ່າ: ຍັງມີຄົນເຈັບພະຍາດດັ່ງກ່າວອີກຫລາຍໆຄົນທີ່ບົ່ງມະຕິບໍ່ໄດ້ ແລະ ອາດເສຍຊີວິດ ຈາກການຕິດເຊື້ອພະຍາດດັ່ງກ່າວ ໂດຍສະເພາະໃນເຂດພາກໄຕ້ຂອງລາວ. ພວກເຮົາຍັງໄດ້ເຮັດການປະເມີນຊຸດການບົ່ງມະຕິແບບໄວທີ່ໃຊ້ກັບຕົວຢ່າງຈາກຄົນເຈັບໂດຍກົງ ແລະ ພົບເບື້ອງຕົ້ນພົບວ່າ ມັນມີຄວາມຈຳເພາະສູງ ແຕ່ຄວາມແມ່ນຍຳບໍ່ຄ່ອຍດີ.
- ພວກເຮົາໄດ້ເຮັດວິເຄາະຫາຄວາມສຳພັນລະຫວ່າງປັດໄຈທາງດ້ານດິນຟ້າອາກາດ ກັບການເກີດພະຍາດເມລິອອຍໂດຊິສ ໃນຄົນເຈັບ ສປປ ລາວ ແລະ ປະເທດກຳປູເຈຍ ແລະ ພົບວ່າ: ຄວາມຊຸ່ມ, ທັດສະນະວິໄສທີ່ຕ່ຳ, ແລະ ຄວາມໄວຂອງລົມ ເປັນປັດໄຈທີ່ສຳພັນກັບການເກີດພະຍາດດັ່ງກ່າວ ໃນ ສປປ ລາວ, ສ່ວນ ຢູ່ປະເທດກຳປູເຈຍ ປັດໄຈທີ່ສຳຄັນໄດ້ແກ່ຄວາມຊຸ່ມ, ລະດູຝົນ ແລະ ຄວາມໄວຂອງລົມ. ເດັກນ້ອຍມັກຈະສ່ຽງຕໍ່ການຕິດເຊື້ອພະຍາດດັ່ງກ່າວໃນເດືອນທີ່ມີຄວາມຊຸ່ມສູງ, ສ່ວນຄົນເຈັບທີ່ມີບັນຫາທາງປອດ ແລະ ຕິດເຊື້ອທາງກະແສເລືອດ ມັກຈະພົບຫລາຍໃນເດືອນທີ່ມີລົມພັດຫລາຍ ເຊິ່ງຂໍ້ມູນນີ້ຊີ້ໃຫ້ເຫັນເຖິງການຕິດເຊື້ອຜ່ານທາງລະບົບຫາຍໃຈ. ໄລຍະບົ່ມເຊື້ອຂອງພະຍາດນີ້ແມ່ນ ປະມານ 1 ອາທິດ. ພວກເຮົາກຳລັງມີແຜນຮ່ວມກັບກະຊວງສາທາລະນະສຸກ ຈັດກອງປະຊຸມສຳມະນາ ກ່ຽວກັບພະຍາດດັ່ງກ່າວ ໃນປີ 2019.
- ພວກເຮົາພົບວ່າ ເຊື້ອ *Burkholderia pseudomallei* ຖືກພົບເຫັນຢູ່ໄຕ້ພື້ນດິນຂອງທົ່ງນາ ແລະ ຍັງສາມາດພົບເຫັນໃນລະດັບຄວາມເລິກເຖິງ 290 ແມັດຈາກໜ້າດິນ. ການຄົ້ນພົບນີ້ ມີຄວາມໝາຍ ສຳຄັນເພື່ອຊອກຫາມາດຕະການຫລຸດຜ່ອນຄວາມໜາແໜ້ນຂອງເຊື້ອດັ່ງກ່າວໃນດິນ ແນໃສ່ເພື່ອປ້ອງ ກັນຊາວນາໂດຍສະເພາະ.
- ສປປ ລາວ ເລີ່ມນຳໃຊ້ວັກແຊງ 13-valent pneumococcal vaccine (PCV13) ໃນປີ 2013. ພາຍຫລັງການນຳໃຊ້ວັກແຊງດັ່ງກ່າວ, ອັດຕາການຖືເຊື້ອ PCV13-type ໃນເດັກອາຍຸ 12-23 ເດືອນ ຫລຸດລົງປະມານ 23% ສ່ວນເດັກທີ່ຖືເຊື້ອ non-PCV13 serotype ແມ່ນບໍ່ມີການປ່ຽນແປງ. ຂໍ້ມູນນີ້ ຊີ້ໃຫ້ເຫັນວ່າ ຕົວເລກຂອງຜູ້ຖືເຊື້ອ PCV13 serotype ທີ່ຫລຸດລົງໃນກຸ່ມເດັກທີ່ໄດ້ຮັບວັກແຊງນີ້ ອາດ ສົ່ງຜົນເຮັດໃຫ້ການຕິດຕໍ່ ຫລື ແຜ່ກະຈາຍຂອງເຊື້ອ ຫລື ພະຍາດທີ່ເກີດຈາກເຊື້ອ pneumococcal ຫລຸດລົງໃນ ສປປ ລາວ.
- ການບົ່ງມະຕິພະຍາດໄຂ້ຍ່ຽວໜູ ຍັງມີຄວາມທ້າທາຍຫລາຍໃນຂົງເຂດອາຊີ ໂດຍສະເພາະໃນເຂດຊົນ ນະບົດຫ່າງໄກສອກຫລີກ. ຈາກການຄົ້ນຄວ້າໃນໂຮງໝໍມະໂຫສົດພວກເຮົາພົບວ່າ: ມາຮອດປະຈຸບັນ ແມ່ນຍັງບໍ່ທັນມີຊຸດການບົ່ງມະຕິແບບໄວສຳລັບກວດຫາຫາດກາຍຕ້ານຕໍ່ເຊື້ອ *Leptospira* spp. ທີ່ ມີຄວາມແມ່ນຍຳ ແລະ ຄວາມຈຳເພາະສູງ ເພື່ອຈະນຳມາໃຊ້ໄດ້ເທື່ອ. ແຕ່ພວກເຮົາມີຂໍ້ມູນຊີ້ໃຫ້ເຫັນ ວ່າ: ການກວດດ້ວຍເຕັກນິກທາງພັນທຸກຳ (PCR) ໃນຕົວຢ່າງນ້ຳຍ່ຽວ ແລະ ເຊຣຮມຂອງຄົນເຈັບ ເປັນເຕັກນິກທີ່ມີປະໂຫຍດ ແຕ່ຍັງມີຄວາມຈຳເປັນຕ້ອງເຮັດການຄົ້ນຄວ້າຫາວິທີການກວດທີ່ມີລາຄາຖືກ ເຊິ່ງຈະສາມາດນຳໃຊ້ໃນລະດັບໂຮງໝໍແຂວງໄດ້. ຈາກການສຶກສາຄົນເຈັບທີ່ເປັນໄຂ້ຍ່ຽວໜູໃນລາວ ຍັງບໍ່ພົບ ຫລັກຖານການຕ້ານຕໍ່ຢາປິ່ນປົວ azithromycin, ceftriaxone, ciprofloxacin, doxycycline, gentamicin and penicillin G ເທື່ອ.

- ໃນ ສປປ ລາວ ມີເຊື້ອພະຍາດຈັກຊະນິດ? ໃນ 15 ປີຜ່ານມາ ປະກົດວ່າໄດ້ມີຈຳນວນເຊື້ອພະຍາດທີ່ຖືກກວດພົບຫລາຍຂຶ້ນຢ່າງໄວວາ. ແລ້ວຍັງຈະຖືກຄົ້ນພົບອີກບໍ່? ຜົນການຊອກຄົ້ນຫາໃນບົດຕີພິມເຜີຍແຜ່ແຕ່ປີ 1874 ຫາ ປີ 2016 ພົບວ່າ: ມີເຊື້ອພະຍາດໃນຄົນຈຳນວນ 159 ຊະນິດ ທີ່ ຖືກ ບັນທຶກໃນ ສປປ ລາວ ບໍ່ວ່າຈະດ້ວຍເຕັກນິກການປູກເຊື້ອ ຫລື ເຕັກນິກທາງພັນທຸກຳ. ຜົນຈາກ ການໃຊ້ mathematical model ແບບໃໝ່ ໂດຍພິຈາລະນາອັດຕາການຄົ້ນພົບຕາມການເວລາ ໄດ້ ຄາດຄະເນໄວ້ວ່າ: ໃນ ສປປ ລາວ ຍັງມີເຊື້ອພະຍາດຂອງຄົນປະມານ 10 - 11 ຊະນິດ ທີ່ຍັງຈະ ຕ້ອງໄດ້ຄົ້ນຫາຕື່ມອີກ.

- ຂໍ້ມູນສາເຫດຂອງການຊຶມເຊື້ອລະບົບປະສາດສູນກາງ ໃນຄົນເຈັບຈຳນວນ 1,065 ຄົນ ທີ່ໄດ້ຮັບການ ແທງນ້ຳໄຂສັນຫລັງໄປກວດ ທີ່ໂຮງໝໍມະໂຫສົດ ຊ່ວງປີ 2003-2011 ຖືການຳມາວິເຄາະ ແລະ ພົບ ວ່າ: ປະມານ 42% ຂອງຄົນເຈັບ ແມ່ນຮູ້ສາເຫດຈາກການກວດນ້ຳໄຂສັນຫລັງ ແລະ ຈາກການ ກວດເລືອດ. ໃນຈຳນວນຄົນເຈັບທີ່ຕິດເຊື້ອພຽງຊະນິດດຽວ, ສາເຫດທີ່ພົບຫລາຍກວ່າໝູ່ແມ່ນ ອັກເສບ ສະໝອງຍີ່ປຸ່ນ (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%). ອັດຕາການຕາຍແມ່ນສູງເຖິງ 26.3%. ຄົນເຈັບທີ່ຕິດເຊື້ອໄວຣັສມັກຈະມີອາການຄໍແຂງຫລາຍກວ່າກຸ່ມທີ່ບໍ່ຕິດເຊື້ອໄວຣັສ, ສ່ວນຄົນເຈັບທີ່ຕິດ ເຊື້ອຈຸລິນຊີມັກຈະມີປະຫວັດເປັນເບົາຫວານຫລາຍກວ່າກຸ່ມຄົນທີ່ບໍ່ຕິດເຊື້ອຈຸລິນຊີ. ປັດໄຈທີ່ ພົວພັນກັບ ສາເຫດການຕາຍຢ່າງມີຄວາມສຳຄັນດ້ານສະຖິຕິໄດ້ແກ່ ການທີ່ມີລະດັບ Lactate ສູງໃນນ້ຳໄຂສັນ ຫລັງ ແລະ ຄະແນນ GCS ຕ່ຳ. ຂໍ້ມູນນີ້ ຊີ້ໃຫ້ເຫັນວ່າ: ເຮົາຕ້ອງເອົາໃຈໃສ່ເປັນພິເສດຕໍ່ຄົນເຈັບ ທີ່ມີຄະແນນ GCS ຕ່ຳ ເຊິ່ງຄົນເຈັບກຸ່ມນີ້ມີແນວໂນ້ມທີ່ຈະເສຍຊີວິດສູງ - ສະນັ້ນ ການປິ່ນປົວປະຄັບ ປະຄອງຄົນເຈັບທີ່ສະຕິບໍ່ດີ ເຊັ່ນ ການຕິດຕາມໃນຫ້ອງມໍລະສຸມ ສົມທົບກັບການໃຫ້ຢາຕ້ານເຊື້ອ ທີ່ເໝາະສົມໃນທັນທີ ອາດເປັນແນວທາງຫລັກໃນການຫລຸດຜ່ອນອັດຕາການຕາຍລົງ. ເນື່ອງຈາກວ່າ ສາເຫດທີ່ເກີດຈາກການຕິດເຊື້ອຣິກເກັດເຊັຍຂ້ອນຂ້າງສູງ ສະນັ້ນ ຄວນພິຈາລະນາປິ່ນປົວດ້ວຍ ຢາຕ້ານເຊື້ອ Ceftriaxone + doxycycline ໃນຂະນະທີ່ລໍຖ້າຜົນກວດທາງຫ້ອງວິເຄາະ.

- ພວກເຮົາຍັງພົບວ່າ ສ່ວນໃຫ່ຍຂອງນ້ຳໄຂສັນຫລັງທີ່ສົ່ງກວດ (94%) ໃນນະຄອນຫລວງວຽງຈັນ ແມ່ນມີຄວາມຜິດປົກກະຕິ ເຊິ່ງຊີ້ໃຫ້ເຫັນວ່າ: ເຮົາຄວນເອົາໃຈໃສ່ໃຫ້ມີການເຈາະນ້ຳໄຂສັນຫລັງ ໃນ ຄົນເຈັບທີ່ສົງໃສວ່າມີການຊຶມເຊື້ອລະບົບປະສາດສູນກາງທຸກກໍລະນີ ເພື່ອຫລີກເວັ້ນການພາດໂອກາດ ປິ່ນປົວຄົນເຈັບດັ່ງກ່າວ.

- ພວກເຮົາກຳລັງວິເຄາະຜົນຈາກການສຶກສາຄົ້ນຄວ້າຂະໜາດໃຫ່ຍ ເພື່ອຫາສາເຫດ ແລະ ຜົນກະທົບ ຂອງໄຂ້ໃນແມ່ຍິງຖືພາ ທີ່ເມືອງປາກງື່ມ, ນະຄອນຫລວງວຽງຈັນ. ອັດຕາການຕາຍຂອງແມ່ທີ່ສູງ ໃນ ສປປ ລາວ (ສູງກວ່າໝູ່ໃນອາຊີຕາເວັນອອກຊ່ຽງໄຕ້) ເຮັດໃຫ້ພວກເຮົາເຮັດການສຶກສາຄັ້ງນີ້. ໃນ ຈຳນວນແມ່ມານ 1,000 ຄົນ ທີ່ເຮັດການສຶກສາ, ມີ 110 ຄົນ ທີ່ມີອາການໄຂ້ໃນລະຫວ່າງຖືພາ ຫລື ຫລັງເກີດລູກ. ໃນຈຳນວນນີ້ 18 ຄົນ ລູກ, ເດັກຕາຍຮອບເກີດມີ 6 ຄົນ, 3 ຄົນ ຕາຍຫລັງເກີດມາ ໃໝ່ໆ, ແມ່ 1 ຄົນເສຍຊີວິດ (ຍ້ອນຖືພາລູກນອກທ້ອງ) ແລະ ມີເດັກຈຳນວນ 11 ຄົນ ທີ່ເກີດມາຜິດ ປົກກະຕິ. ປະຈຸບັນ ພວກເຮົາກຳລັງວິເຄາະຫາສາເຫດຂອງໄຂ້ ແລະ ຫາຄວາມສຳພັນລະຫວ່າງ ອາການໄຂ້ ກັບພາວະອື່ນໆ ເຊັ່ນ ນ້ຳໜັກເກີດຕ່ຳ ແລະ ເດັກເກີດມາເສຍຊີວິດ.

- ຜົນການສຶກສາທົດລອງທາງຄລີນິກສຳລັບການປິ່ນປົວພະຍາດໄຂ້ໝັດໝູ (Murine typhus – ທີ່ເກີດຈາກເຊື້ອ *Rickettsia typhi*) ຢູ່ທີ່ໂຮງໝໍມະໂຫສົດ ຊີ້ໃຫ້ເຫັນວ່າ ພວກເຮົາບໍ່ຄວນນຳໃຊ້ຢາ Azithromycin ເພື່ອປິ່ນປົວພະຍາດດັ່ງກ່າວ ແລະ ຄວນໃຊ້ Doxycycline 3 ຫລື 7 ມື້ ເຊິ່ງມີປະສິດທິພາບບໍ່ຕ່າງກັນ.
- ລະບາດວິທະຍາທາງດ້ານ Serotype ຂອງໄຂ້ຍູງລາຍແມ່ນມີຄວາມສັບສົນ, ສະນັ້ນ ພວກເຮົາກຳລັງສ້າງລະບົບຖານຂໍ້ມູນເປັນພາສາລາວ ແລະ ອັງກິດ ທີ່ສາມາດບອກເຖິງອັດຕາການຕິດເຊື້ອໃໝ່ ແລະ ການກະຈາຍຂອງ Serotype ໂດຍອີງຕາມພື້ນທີ່ ແລະ ເວລາຂອງພະຍາດ ທີ່ໄດ້ຈາກຄົນເຈັບໂຮງໝໍມະໂຫສົດ ແລະ ໂຮງໝໍແຂວງອື່ນໆ. ທັງນີ້ກໍ່ເພື່ອເຮັດໃຫ້ຜູ້ທີ່ເຮັດວຽກກ່ຽວພັນກັບນະໂຍບາຍ ແລະ ພະນັກງານແພດໝໍທຸກຄົນສາມາດເຂົ້າເຖິງຂໍ້ມູນດັ່ງກ່າວ ລວມທັງຈະສາມາດບອກເຕືອນໃຫ້ເຮົາຮູ້ທ່າອ່ຽງການລະບາດຂອງພະຍາດ.
- ພວກເຮົາສາມາດກວດພົບ RNA ຂອງເຊື້ອໄວຣັສອັກເສບສະໝອງຍີ່ປຸ່ນໃນຕົວຢ່າງທີ່ຕ້ອຍຈາກຮູຄໍຂອງຄົນ ເປັນເທື່ອທຳອິດໃນປະຫວັດສາດ ເຊິ່ງຂໍ້ມູນນີ້ຊີ້ໃຫ້ເຫັນວ່າ ເຮົາຄວນກວດຕົວຢ່າງທີ່ຕ້ອຍຈາກຮູຄໍຄົນເຈັບເພື່ອຊອກຫາ RNA ຂອງເຊື້ອໄວຣັສອັກເສບສະໝອງຍີ່ປຸ່ນໃນວົງກ້ວ້າງເພື່ອສຶກສາດ້ານລະບາດວິທະຍາຂອງພະຍາດດັ່ງກ່າວ.
- ຈາກການນຳໃຊ້ເຄື່ອງມືການກວດແບບພົກພາ MinION next generation sequencing platform ຢູ່ LOMWRU, ພວກເຮົາສາມາດເຮັດ sequencing ຈີໂນມຂອງເຊື້ອ *Rickettsia typhi* ເຊິ່ງເປັນເຊື້ອສາເຫດຂອງພະຍາດ murine typhus ທີ່ໂຮງໝໍມະໂຫສົດ. ເປັນຄັ້ງທຳອິດທີ່ມີການ Sequence ຈີໂນມທັງໝົດຂອງເຊື້ອດັ່ງກ່າວ ເຊິ່ງສາມາດເຮັດໄດ້ໃນ ສປປ ລາວ ແລະ ຄາດວ່າໃນອະນາຄົດ ເຮົາຈະສາມາດສ້າງຄວາມອາດສາມາດໃຫ້ແກ່ຄົນລາວໃນການນຳໃຊ້ເຕັກນິກດັ່ງກ່າວເປັນເຄື່ອງມື ເຂົ້າໃນການສຶກສາດ້ານລະບາດວິທະຍາ, ການຕອບສະໜອງຕໍ່ຢາປິ່ນປົວ ແລະ ການລະບາດຂອງພະຍາດ.

* ຄວາມສຳຄັນຂອງສຸຂະພາບໜຶ່ງດຽວ

- ຕະຫລາດໃນ ສປປ ລາວ ເປັນສູນລວມຂອງຄົນໃນສັງຄົມ ແຕ່ພວກເຮົາຍັງບໍ່ທັນມີຂໍ້ມູນກ່ຽວກັບຄວາມຮັບຮູ້ຂອງຜູ້ຄ້າຂາຍ ຕໍ່ກັບຄວາມສ່ຽງດ້ານສຸຂະພາບທີ່ອາດເກີດຈາກສິ່ງທີ່ເຂົາເຈົ້າຂາຍ. ເນື່ອງຈາກວ່າ ສັດປ່າ ແລະ ສັດບ້ານ ມີໂອກາດເປັນຕົວການນຳເຊື້ອພະຍາດມາສູ່ຄົນ, ສະນັ້ນ ພວກເຮົາຈຶ່ງໄດ້ເຮັດການຄົ້ນຄວ້າໃນຕະຫລາດກັບຊາວຄ້າຂາຍສັດປ່າ ແລະ ພົບວ່າ: ເກືອບທັງໝົດຂອງຜູ້ຄ້າຂາຍດັ່ງກ່າວ ແມ່ນມີຄວາມຮັບຮູ້ທີ່ຕ່ຳ ກ່ຽວກັບກັບຄວາມສ່ຽງຕໍ່ສຸຂະພາບຂອງເຈົ້າເຈົ້າ ໃນສິ່ງທີ່ເຂົາເຈົ້າຂາຍ. ຍັງມີຄວາມຈຳເປັນຕ້ອງເຮັດການຄົ້ນຄວ້າດ້ານສຸຂະພາບໜຶ່ງດຽວຕື່ມອີກ ເພື່ອໃຫ້ເຂົ້າໃຈກ່ຽວກັບຄວາມສ່ຽງ ແລະ ໃຫ້ໂຄສະນາສຸຂະສຶກສາແກ່ຊາວຄ້າຂາຍ ແລະ ຜູ້ທີ່ມາຊື້ສິນຄ້າ.
- ເຊື້ອພະຍາດໃນຊີ້ນສັດປ່າ. ການວິໄຈຕົວຢ່າງຊີ້ນສັດປ່າທາງພັນທຸກຳຈຳນວນ 717 ຕົວຢ່າງ ຈາກສັດປ່າທີ່ມີກະດູກສັນຫລັງ (ສ່ວນໃຫ່ຍແມ່ນກະຮອກ-ກະແຕ) ຈຳນວນ 359 ໂຕ ທີ່ໄດ້ມາຈາກຕະຫລາດ ພົບວ່າ: ມີສັດຈຳນວນ 469 ໂຕ ທີ່ມີເຊື້ອ *Leptospira* spp., 19 ໂຕ ມີເຊື້ອ *Rickettsia* spp. (ລວມທັງເຊື້ອ *R. felis* 1 ເຊື້ອ). ການກວດພົບເຊື້ອ *Leptospira* spp. ໃນອັດຕາທີ່ສູງຈາກຕົວຢ່າງທີ່ຕ້ອຍມາຈາກທ່ຍ່ຽວ-ອະໄວຍະວະເພດຂອງສັດ ຊີ້ໃຫ້ເຫັນເຖິງຄວາມເປັນໄປໄດ້ສຳລັບການສິ່ງຕໍ່ພະຍາດໄຂ້ຍ່ຽວໝູ ຈາກໂຕກະຮອກ-ກະແຕ ໄປສູ່ຄົນໃນຕະຫລາດ. ນອກນີ້ຍັງກວດພົບບາງຕົວຢ່າງ ທີ່ມີເຊື້ອ *O. tsutsugamushi*, *L. garvieae*, *Kurthia* spp., *Ehrlichia* spp.TC251-2, *Anaplasma marginale*, *A. phagocytophilum*, and *A. bovis*. ພ້ອມນີ້ ພວກເຮົາຍັງກວດພົບເຫັນເຊື້ອ

Lactococcus garvieae, Kurthia spp. ແຕ່ຍັງບໍ່ຮູ້ວ່າມັນໃຫ້ພະຍາດຫລືບໍ່. ຂໍ້ມູນນີ້ ສະໜັບສະໜູນ ຄວາມພະຍາຍາມທີ່ຈະຕ້ອງ ເອົາໃຈໃສ່ຄວບຄຸມການຄ້າຂາຍສັດປ່າຕື່ມອີກ. ການກວດ ພົບ *R. felis* ເຊິ່ງເປັນເຊື້ອ rickettsial pathogen ທີ່ເກີດຂຶ້ນໃໝ່ ແລະ ການກວດພົບເຊື້ອ *O. tsutsugamushi* ໃນໂຕມະຮອກ-ກະແຕ ຖືເປັນການຄົ້ນພົບຄັ້ງທຳອິດໃນປະຫວັດສາດ.

- ຈາກການສຶກສາເບິ່ງທາດກາຍຕ້ານໃນເລືອດຂອງຊາວຄ້າຂາຍໃນທ້ອງຕະຫລາດຂອງ ສປປ ລາວ ຕໍ່ເຊື້ອພະຍາດ murine typhus, scrub typhus and leptospirosis ພົບວ່າ ຜູ້ທີ່ຄ້າຂາຍຊີ້ນສັດປ່າ ອາດມີທາດກາຍຕ້ານຊະນິດ IgG ຕໍ່ພະຍາດ Scrub typhus ທີ່ເພີ່ມຂຶ້ນ. ພວກເຮົາກຳລັງສືບ ຕໍ່ການວິເຄາະຕົວຢ່າງເລືອດທີ່ໄດ້ຈາກກຸ່ມຄົນດັ່ງກ່າວອີກ 2 ຮອບ ເພື່ອເບິ່ງການປ່ຽນແປງຂອງທາດ ກາຍຕ້ານດັ່ງກ່າວຕາມການເວລາ.
- ນັບແຕ່ປີ 2000 ເປັນຕົ້ນມາ ທາງໂຄງການ LOMWRU ສາມາດຄົ້ນພົບເຊື້ອ *Streptococcus agalactiae* (Group B Streptococcus - GBS) ທີ່ມີລັກສະນະທາງພັນທຸກຳຄືກັບເຊື້ອທີ່ພົບເຫັນ ໃນ ລະຫວ່າງການລະບາດຂອງພະຍາດທີ່ເກີດຈາກປາ ທີ່ປະເທດສິງກະໂປໃນປີ 2015 (ST283) ເຊິ່ງ ພວກເຮົາກວດພົບໃນຄົນເຈັບຈຳນວນ 28 ຄົນ ຈາກທັງໝົດ 38 ກໍລະນີທີ່ມີການຊຶມເຊື້ອ GBS ຮຸນແຮງ. ເນື່ອງຈາກວ່າເຊື້ອສາຍພັນດັ່ງກ່າວພົບໃນຄົນທີ່ຕິດເຊື້ອ ແລະ ປາ ໃນຂົງເຂດອາຊີຕາເວັນ ອອກຊ່ຽງໄຕ້ ສະນັ້ນ ປາອາດເປັນແຫລ່ງຂອງພະຍາດທີ່ເກີດຈາກເຊື້ອ GBS ໃນຂົງເຂດນີ້ທີ່ເຮົາຍັງ ມອງຂ້າມຢູ່.

* ຄວາມສຳຄັນຂອງຄຸນນະພາບຢາ

- ບັນຫາຄຸນນະພາບຂອງຢາປິ່ນປົວຫລາຍຊະນິດ ຍັງເປັນເລື່ອງທີ່ໜັກໜ່ວງ ແລະ ໜ້າກັງວົນໃນທົ່ວ ໂລກ. ປະຈຸບັນຍັງພົບວ່າ ມີການນຳໃຊ້ຢາ Artemisinin ຊະນິດດຽວ ເຂົ້າໃນການປິ່ນປົວມາລາເຣຍ ຢູ່ ແລະ ຍັງມີຢາປະສົມ ACTs ທີ່ບໍ່ຮັບປະກັນຄຸນນະພາບຢູ່ ເຊິ່ງອັນນີ້ຍັງຖືເປັນບັນຫາທີ່ສຳຄັນ ໃນພາກພື້ນນີ້.
- ໂດຍການສະໜັບສະໜູນທຶນຮອນຈາກ Wellcome Trust, ພວກເຮົາກຳລັງສ້າງຖານຂໍ້ມູນເປັນຕາຕະ ລາງ ແລະ ແຜນທີ່ແຈກຢາຍດ້ານຄຸນນະພາບຂອງຢາພື້ນຖານ ຈຳນວນໜຶ່ງ ທັງນີ້ກໍ່ເພື່ອເຮັດໃຫ້ທຸກ ຄົນສາມາດເຂົ້າເຖິງຂໍ້ມູນດັ່ງກ່າວ. ໃນນີ້ ກຳລັງສ້າງຖານຂໍ້ມູນກ່ຽວກັບຄຸນນະພາບຂອງຢາໃນຂົງເຂດ ສຸຂະພາບແມ່, ຢາຕ້ານເຊື້ອ, ຢາປົວເບົາຫວານ, ຢາຕ້ານໄວຣັສ, ຢາວັນນະໂລກ ແລະ ຢາທີ່ໃຊ້ໃນ ວົງການສັດຕະວະແພດ ເຊິ່ງທັງໝົດນີ້ຈະຮວບຮວມເອົາເຂົ້າໃນລະບົບຂໍ້ມູນເຝົ້າລະວັງພະຍາດຊຶມເຊື້ອ. ພວກເຮົາກຳລັງຢູ່ໃນຂັ້ນຕອນທົດສອບລະບົບດັ່ງກ່າວ ແລະ ຈະນຳອອກມາເຜີຍແຜ່ພາຍໃນລະດູບານ ໃໝ່ນີ້. ພ້ອມນີ້ ກໍ່ຈະມີການວິເຄາະຂໍ້ມູນດ້ານຄຸນນະພາບຂອງຢາພື້ນຖານດັ່ງກ່າວພາຍໃນປີ 2019.
- ໂດຍການຮ່ວມມືກັບ ກອງກວດກາອາຫານ ແລະ ຢາ ແລະ ພາຍໄຕ້ການສະໜັບສະໜູນດ້ານທຶນ ຮອນຈາກທະນະຄານພັດທະນາອາຊີ, ພວກເຮົາໄດ້ປະເມີນເຄື່ອງກວດຄຸນນະພາບຂອງຢາທີ່ສາມາດ ພົກພາໄປ-ມາໄດ້ຫລາຍໆຍີ່ຫໍ້ ເບິ່ງວ່າມັນມີຄວາມແມ່ນຍຳຫລາຍໜ້ອຍປານໃດ ແລະ ຄຸ້ມຄ່າຫລາຍ ປານໃດ. ຜົນຈາກການປະເມີນຄັ້ງນີ້ຈະຊ່ວຍໃຫ້ເຮົາຮູ້ວ່າ ເຄື່ອງຊະນິດໃດທີ່ຈະເໝາະສົມສຳລັບປະເທດ ເຮົາຫລາຍທີ່ສຸດ. ພວກເຮົາໄດ້ຈັດກອງປະຊຸມຫາລືກ່ຽວກັບຜົນຈາກການປະເມີນນີ້ ທີ່ວຽງຈັນ ໃນລະ ຫວ່າງວັນທີ 9-10 ເມສາ 2018. ເຖິງຈະມີເຄື່ອງກວດຄຸນນະພາບຂອງຢາທີ່ສາມາດພົກພາໄປ-ມາ ຫລາຍໆຍີ່ຫໍ້, ແຕ່ກໍ່ຍັງບໍ່ທັນມີການປະເມີນໃນພາກສະໜາມ ເພື່ອເບິ່ງວ່າ ຍີ່ຫໍ້ໃດຈະໄດ້ຮັບຜົນດີ. ສະນັ້ນ ຈຶ່ງຈຳເປັນຕ້ອງມີການສຶກສາຄົ້ນຄວ້າຢ່າງຮີບດ່ວນ ເພື່ອຈະເອົາຂໍ້ມູນໄປໃຫ້ພາກສ່ວນທີ່ກ່ຽວ

ຂ້ອງໃນລະດັບຊາດຕັດສິນໃຈວ່າຈະເລືອກໃຊ້ເຄື່ອງຍີ່ຫໍ້ໃດດີເຂົ້າໃນວຽກງານສະກັດກັ້ນຢາ ທີ່ບໍ່ໄດ້ຄຸນນະພາບ. ໃນລະດັບຊາດເຫັນວ່າ ເຄື່ອງກວດແບບ spectrometers ທັງ 5 ຊະນິດ ແມ່ນໃຫ້ຜົນຄຸ້ມຄ່າໃນບ່ອນທີ່ມີຄວາມຊຸກຂອງຢາປອມ ແລະ ຢາບໍ່ໄດ້ມາດຕະຖານໃນລະດັບສູງ ແລະ ລະດັບຕ່ຳ, ແຕ່ກໍ່ມີພຽງ 3 ຊະນິດເທົ່ານັ້ນ ທີ່ໃຫ້ຜົນຄຸ້ມຄ່າໃນບ່ອນທີ່ມີຄວາມຊຸກຕ່ຳ. ເຖິງຢ່າງໃດກໍ່ຕາມ ການສຶກສາຄົ້ນຄວ້າພົບວ່າ ຍັງມີຄວາມກັງວົນວ່າ ຜູ້ທີ່ໃຊ້ເຄື່ອງມືດັ່ງກ່າວອາດມີຄວາມໝັ້ນໃຈ ໃນເຄື່ອງມືຫລາຍເກີນໄປ ຈົນເຮັດໃຫ້ການກວດສອບຢາດ້ວຍຕາເປົ່າຫລຸດລົງໄປເລື້ອຍໆ. ຈາກຫລັກຖານທີ່ມີໃນປະຈຸບັນພົບວ່າ ເຄື່ອງມືຍີ່ຫໍ້ໃດກໍ່ສາມາດກວດສອບຄຸນນະພາບຂອງຢາໄດ້ຢ່າງມີປະສິດທິຜົນ. ເຖິງວ່າ ເຄື່ອງມືບາງຊະນິດ ສາມາດກວດສອບຢາປອມ ທີ່ບໍ່ມີສ່ວນປະສົມຂອງຕົວຢາ ຫລື ມີບໍ່ພຽງພໍ ໄດ້ຢ່າງ ແມ່ນຢຳກໍ່ຕາມ, ຍັງບໍ່ທັນມີເຄື່ອງໃດທີ່ສາມາດບອກເປີເຊັນປະລິມານ API ໄດ້ເຊິ່ງຖືເປັນຕົວຊີ້ວັດຕົ້ນຕໍ ຂອງຢາທີ່ບໍ່ໄດ້ມາດຕະຖານຫລາຍໆຊະນິດ.

- ຍັງຈະຕ້ອງມີຄວາມພະຍາຍາມຢ່າງຫລວງຫລາຍ ເພື່ອເຮັດປະເມີນເຄື່ອງມືທີ່ມີຢູ່ຫລາຍຕົວໃນຂະນະນີ້ ໂດຍຈະຕ້ອງທົດສອບກັບຢາຫລາກຫລາຍຊະນິດ ແລະ ຈະຕ້ອງຂະຫຍາຍວຽກການປະເມີນດັ່ງກ່າວ ຢ່າງເປັນອິດສະລະກັບຜູ້ຜະລິດ ທັງນີ້ກໍ່ເພື່ອປະເມີນເຄື່ອງມືດັ່ງກ່າວ ກັບຕົວຢ່າງ ແລະ ດ້ວຍຂັ້ນຕອນ ທີ່ເປັນມາດຕະຖານຄັກແນ່.
- ພວກເຮົາເຮັດການສຳຫລວດແບບຊຸ່ມຫລາຍຂັ້ນຕອນ ເພື່ອປະເມີນຄຸນນະພາບຂອງຢາຕ້ານເຊື້ອ ໂດຍໃຊ້ວິທີການລູກຄ້າປອມ ເຂົ້າໄປຊື້ຢາຕາມຮ້ານຂາຍຢາໃນ 5 ແຂວງພາກໃຕ້ຂອງລາວ ເຊິ່ງການສຳຫລວດນີ້ ແມ່ນເຮັດຮ່ວມກັບກົມອາຫານ ແລະ ຢາ. ການສຶກສາຄັ້ງນີ້ ໄດ້ຊື້ຕົວຢ່າງຢາທັງໝົດ 909 ຕົວຢ່າງ ເຊິ່ງລວມມີ ຢາ amoxicillin, ampicillin, ceftriaxone, ciprofloxacin, doxycycline, ofloxacin, sulfamethoxazole, tetracycline and trimethoprim, ເຊິ່ງຕໍ່ມາກໍ່ໄດ້ນຳໄປວິເຄາະ ດ້ວຍເຄື່ອງ High-Performance Liquid Chromatography (HPLC). ທັງໝົດຕົວຢ່າງຢາທີ່ຊື້ມາ ແມ່ນມີ ສ່ວນປະກອບຕົວຢາຕາມ API ທີ່ກຳນົດໄວ້ ໂດຍບໍ່ມີຫລັກຖານວ່າເປັນຢາປອມ. ແຕ່ພວກເຮົາ ພົບຢາ ບໍ່ໄດ້ມາດຕະຖານ ຄື: 19.6% ຂອງຢາທັງໝົດທີ່ຊື້ມາ ມີປະລິມານຕົວຢາຢູ່ນອກເກນ 90-110% ແລະ 60.2% ຂອງຕົວຢ່າງທັງໝົດ ແມ່ນມີປະລິມານຕົວຢາຢູ່ນອກເກນທີ່ຕັ້ງໄວ້ຂອງ International Pharmacopoeia uniformity. ຢາຕ້ານເຊື້ອທີ່ບໍ່ໄດ້ມາດຕະຖານ ຈະເຮັດໃຫ້ປະສິດທິ ພາບການປິ່ນປົວຫລຸດລົງ ເຊິ່ງຈະສົ່ງຜົນສະທ້ອນຕໍ່ລະບົບສາທາລະນະສຸກ ແລະ ຕໍ່ການຄວບຄຸມການ ຊຶມເຊື້ອຍ້ອນເຊື້ອຈຸລິນຊີ. ເຖິງວ່າເຮົາຍັງບໍ່ທັນເຂົ້າໃຈຢ່າງຈະແຈ້ງວ່າ ຢາບໍ່ໄດ້ຄຸນນະພາບ ມີສ່ວນເຮັດ ໃຫ້ເຊື້ອພະຍາດຕ້ານຕໍ່ຢາປິ່ນປົວຫລາຍໜ້ອຍປານໃດກໍ່ຕາມ, ແຕ່ຢາຕ້ານເຊື້ອທີ່ບໍ່ໄດ້ມາດຕະຖານ ອາດເຮັດໃຫ້ສະພາບການຕ້ານຂອງເຊື້ອພະຍາດຕໍ່ຢາປິ່ນປົວ ໜັກໜ່ວງຂຶ້ນກວ່າເກົ່າ.
- ພວກເຮົາຄົ້ນພົບວ່າ ການນຳໃຊ້ຢາທີ່ບໍ່ມີສະຫລາກໃນນະຄອນຫລວງວຽງຈັນ ແມ່ນມີຄວາມສຳພັນກັບ ອັດຕາການເຂົ້ານອນໂຮງໝໍຍ້ອນການຳມະຜົນສຳຮອງຂອງຢາ ທີ່ກຳລັງເພີ່ມຂຶ້ນເລື້ອຍໆ ເຊິ່ງຂໍ້ມູນນີ້ ຊີ້ໃຫ້ເຫັນວ່າ ມັນມີຄວາມຈຳເປັນທີ່ຈະຕ້ອງເອົາໃຈໃສ່ກ່ຽວກັບວຽກງານການຕິດສະຫລາກໃສ່ຫໍ່ຢາ ໃນ ເວລາຈ່າຍຢາໃຫ້ຄົນເຈັບ.

- ພວກເຮົາໄດ້ຈັດກອງປະຊຸມສາກົນເປັນຄັ້ງທຳອິດ ກ່ຽວກັບຄຸນນະພາບຂອງຢາ ແລະ ສາທາລະນະສຸກ ສາດ ໃນ ເດືອນກັນຍາທີ່ຜ່ານມາ ທີ່ Keble College, Oxford ໂດຍມີຜູ້ເຂົ້າຮ່ວມປະຊຸມຫລາຍກວ່າ 200 ຄົນ ຈາກ 50 ກວ່າປະເທດ.

- ພວກເຮົາຍັງໄດ້ຈັດການອົບຮົມຄັ້ງທີ 4 ກ່ຽວກັບຄຸນນະພາບຂອງຢາ ແລະ ສາທາລະນະສຸກສາດ ທີ່ ລອນດອນ ແລະ ບອສຕັນ ໃນປີທີ່ຜ່ານມາ ແລະ ຢູ່ທີ່ອ໋ອກຟອດ ກ່ອນມີການຈັດປະຊຸມ. ມີຜູ້ເຂົ້າຮ່ວມ ທັງໝົດ 25 ຄົນ ຈາກ 16 ປະເທດເຂົ້າຮ່ວມ.

* ຄວາມສຳຄັນຂອງວຽກງານໂຄສະນາ-ເຜີຍແຜ່ຂໍ້ມູນການສຶກສາຄົ້ນຄວ້າ

- Science Café. ປະຈຸບັນ ສປປ ລາວ ມີຂໍ້ມູນດ້ານສຸຂະພາບຫລາຍຂຶ້ນກວ່າເກົ່າ, ສະນັ້ນ ການເຜີຍແຜ່ຂໍ້ມູນດັ່ງກ່າວໃຫ້ແກ່ຜູ້ສ້າງນະໂຍບາຍ, ພະນັກງານສາທາລະນະສຸກ, ແລະ ປະຊາຊົນທົ່ວໄປ ຈຶ່ງມີຄວາມສຳຄັນເປັນຢ່າງຍິ່ງ. ພວກເຮົາໄດ້ຮ່ວມມືກັບ ມະຫາວິທະຍາໄລວິທະຍາສາດສຸຂະພາບ ຈັດການສົນທະນາ ທີ່ເອີ້ນວ່າ Science Café ເປັນຄັ້ງທຳອິດໃນ ສປປ ລາວ ແລະ ພວກເຮົາຄາດວ່າ ຈຸດເລີ່ມຕົ້ນນີ້ ຈະກາຍເປັນສິ່ງທີ່ຄວນເຮັດຢ່າງເປັນປະຈຳໃນອະນາຄົດ ໃນຮູບແບບຂອງການສົນທະນາໃນຫົວຂໍ້ວິທະຍາສາດ. ຫົວຂໍ້ທີ່ນຳມາສົນທະນາທີ່ຜ່ານມາລວມມີ: ອຸປະຕິເຫດທ້ອງຖະໜົນ, ພະຍາດເບົາຫວານ ແລະ ການຄະລຳອາຫານໃນແມ່ຍິງເກີດລູກໃໝ່.

- ການເຜີຍແຜ່ຜົນການຄົ້ນຄວ້າໃຫ້ຜູ້ຮັບຜິດຊອບນະໂຍບາຍສາທາລະນະສຸກ. ພວກເຮົາໄດ້ເຜີຍແຜ່ໃຫ້ຫລາຍໆກົມກອງຂອງກະຊວງສາທາລະນະສຸກ ແລະ ອົງການອະນາໄມໂລກ ຮັບຊາບ ກ່ຽວກັບການສ້າງຖານຂໍ້ມູນດ້ານລະບາດວິທະຍາຂອງໄຂ້ຍຸງລາຍ ແລະ ສະພາບການຕ້ານຕ່ຍາຂອງເຊື້ອຈຸລະຊີບ ໃນ ສປປ ລາວ ເພື່ອໃຫ້ທຸກຄົນສາມາດເຂົ້າເຖິງຂໍ້ມູນດັ່ງກ່າວ ແລະ ຍັງໄດ້ຮ່ວມມືກັບຫລາຍພາກສ່ວນເພື່ອພັດທະນາຄູ່ມືປິ່ນປົວພະຍາດຊຶມເຊື້ອໃນລາວ. ພວກເຮົາຈະຈັດກອງປະຊຸມເຜີຍແຜ່ຜົນຂອງການຄົ້ນຄວ້າຈາກໂຄງການຂອງພວກເຮົາໃນປີ 2018 ທີ່ຜ່ານມາ ໃຫ້ທາງກົມຄວບຄຸມພະຍາດຕິດຕໍ່ ແລະ ກົມກອງ-ພາກສ່ວນອື່ນໆ ອ້ອມຂ້າງກະຊວງສາທາລະນະສຸກ ໄດ້ຮັບຊາບ ໃນລະດູບານໃໝ່ 2019 ນີ້ ພ້ອມທັງຈະນຳສະເໜີຄວາມໝາຍ-ຄວາມສຳຄັນຂອງຜົນການຄົ້ນຄວ້າ ຕໍ່ກັບນະໂຍບາຍດ້ານສາທາລະນະສຸກໃນ ສປປ ລາວ. ນອກນີ້ ພວກເຮົາກຳລັງຫາລືກ່ຽວກັບການສ້າງໜ່ວຍງານແປຜົນການຄົ້ນຄວ້າທີ່ເຮັດໃນລາວ ແລະ ສາກົນ ເພື່ອນຳມາເປັນຂໍ້ມູນ ຫລື ຫລັກຖານວິທະຍາສາດສຳລັບສ້າງເປັນນະໂຍບາຍດ້ານສາທາລະນະສຸກໃນລາວເຮົາຕື່ມອີກ.

Assoc. Prof Bounthaphany Bounxouei, Assoc. Prof Valy Keoluangkhot, Prof Jeremy Farrar, His Excellency Assoc. Prof Dr Bounkong Syhavong, Prof Tobias Bonoeffer & His Excellency Hugh Evans on the visit of the Wellcome Trust to LOMWRU in February 2018

SUMMARY

Lunch in the garden Wellcome Trust visit February 2018

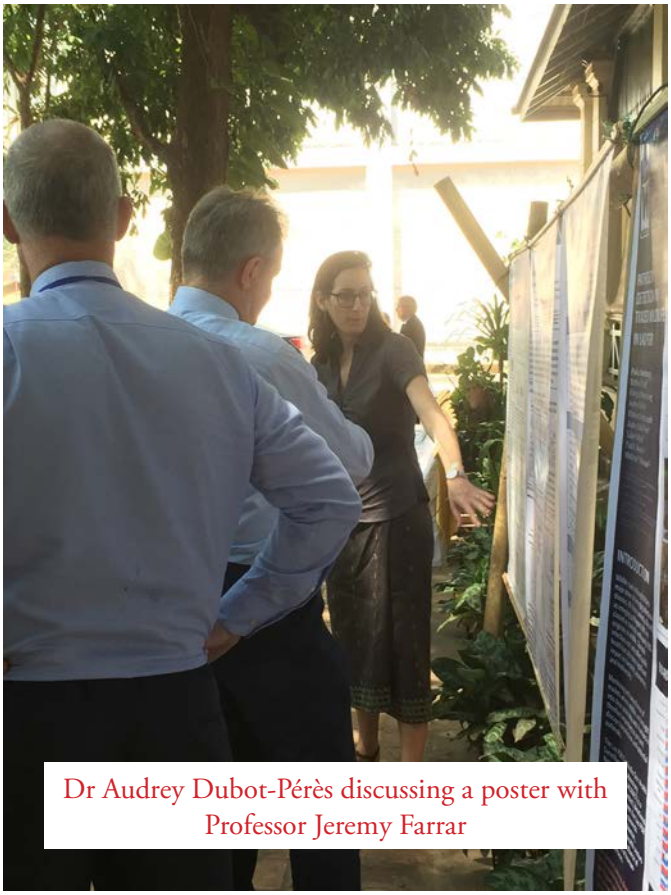
Pii Mai Lao 2018

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 which ~49% of our studies are conducted.

B. LOMWRU is core funded by the Wellcome Trust of Great Britain, with significant additional recent support from the US Naval Medical Research Centre, the Bill & Melinda Gates Foundation, the European Union, Department for International Development-UK (DFID), New Zealand e-Asia, Fondation Total/Institut Pasteur, Global Good, DTRA, Global Antibiotic Research Partnership, 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, including LOMWRU, is composed of 27 Lao Government staff and 65 project-funded staff; 84% are Lao nationals and 54% 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, Xieng Khouang and Vientiane Provinces, performs clinical research and builds diagnostic and research human capacity through training and active participation. LOMWRU also works closely with the University of Health Sciences on multiple projects, with the Centre for Malariology, Parasitology and Entomology on malaria projects in the five southern provinces, with the Lao Food and Drug Department (FDD), Bureau of Food and Drug Inspections (BFDI) and the Food & Drug Quality Control Centre (FDQCC) on the quality of medicines and with the Lao Ministry of Health Department of Health Care and Rehabilitation



Dr Audrey Dubot-Pérès discussing a poster with Professor Jeremy Farrar

LCDR Jeff Hertz (NMRC-A), Dr Matt Robinson, Lt Joey Garcia (NMRC-A), Dr Manivanh and Paul at Jar Site 1 in the Plain of Jars on visit to Xieng Khuang Provincial Hospital July 2018

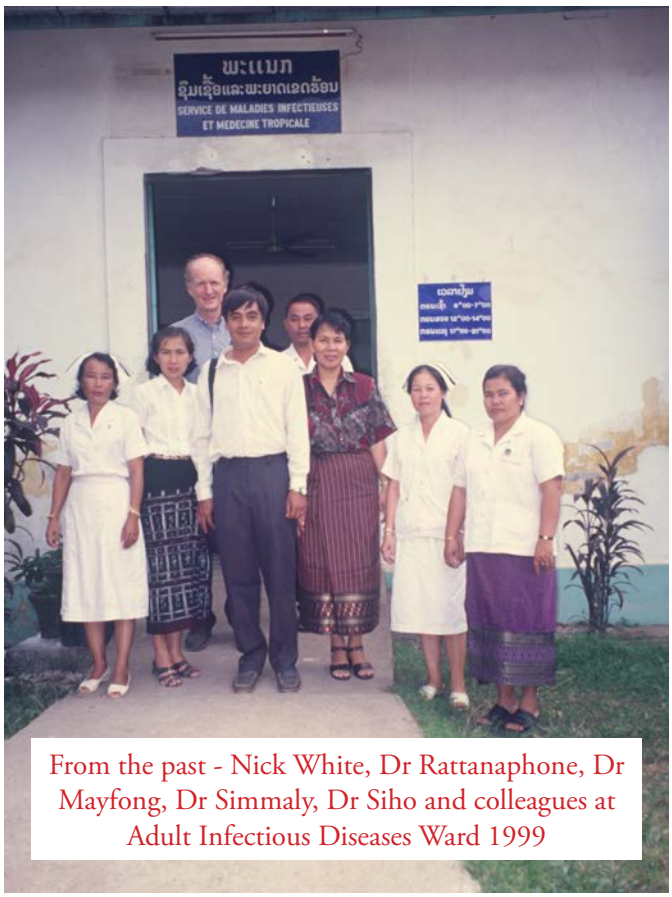
and Department of Communicable Disease Control (DCDC) on infectious disease guidelines, surveillance and antibiotic resistance, and the National Laboratory for Animal Health on 'One Health' research. Within Laos we have key collaborative projects with the Institut Pasteur du Laos and the Centre d'Infectiologie Christophe Mérieux du Laos.

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. In 2018 we supported 16 Lao staff to attend 6 international meetings and short courses and supported one Lao staff to successfully complete his PhD degree at Mahidol University, one in progress at the University of Amsterdam and one to read the BSc in Medical Technology at Khon Kaen University.

G. In 2018 we published or have in press 73 publications, including 67 peer-reviewed papers, two letters, one report and three book chapters. Since LOMWRU was founded, its staff has published 392 papers and book chapters.

H. LOMWRU research translated into policy in Laos includes the implementation of vaccination against the pneumococcus and the *Japanese encephalitis virus* (JEV) and the change in national antimalarial and typhoid treatment policies and the production of melioidosis treatment guidelines. It has also demonstrated the presence of numerous important pathogens for the first time in Laos, and highlighted the local and global importance of scrub typhus, leptospirosis, typhoid, melioidosis and JEV, providing evidence on their epidemiology and prompting interventions. It has yielded the majority of published evidence on antimicrobial resistance (AMR) in Laos, vital for informing strategy and implementation. These data are being used by the MOH AMR Committee to decide policy and implementation plans and in the drafting of Lao national infectious disease treatment guidelines. In order to ensure that data are more accessible we have developed AMR and dengue dashboards (see page 65 of this report for an example). It has conducted pioneering work on the distribution of *B. pseudomallei* in Lao soil and water to inform understanding of distribution of this dangerous pathogen and variation in risks to humans across the country. It has provided evidence to inform appropriate, accurate and sustainable laboratory capacity in Laos, especially for provincial hospitals, and demonstrated that infectious disease varies across the wide latitudinal and geographical variation of the



From the past - Nick White, Dr Rattanaphone, Dr Mayfong, Dr Simmaly, Dr Siho and colleagues at Adult Infectious Diseases Ward 1999

country. It has pioneered health public engagement interventions. It has led key research on the global problem of substandard and falsified medical products.

I. The main findings, in brief, from work published, in press or in preparation in 2018 of immediate relevance to Laos (please see caveats in the 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, multi-drug resistant Enterobacteriaceae (ESBL-E) are increasingly important causes of infection in Mahosot Hospital. This is 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, and frequently to others such as gentamicin and ciprofloxacin/ ofloxacin. Between 2010-2014 *E. coli* and *K. pneumoniae* were found in 34.8 % of all patients with clinically significant bacteraemia. Twenty percent of isolates produced ESBL; *E. coli* accounted for 76% and *K. pneumoniae* 24%, respectively. The incidence of ESBL-producing *E. coli* bacteraemia increased steadily during the study period. Antibiotic use in the previous week was significantly associated with ESBL-positivity. Although multi-resistant, the majority of ESBL-producing *E. coli* and *K. pneumoniae* remained susceptible to meropenem and amikacin. This alarming increase in the incidence of ESBL-E has implications for empiric therapy of sepsis in Laos and ongoing surveillance is essential. Laos, as elsewhere, risks requiring extremely limited, often expensive, options for therapy and we are greatly concerned that soon we will commonly see untreatable infections and infections that will require unaffordable antibiotics.

❖ ESBL-producing bacteria are also surprisingly common in the intestines of healthy people and animals in a remote village of Xieng Khouang Province, suggesting that ESBL have already spread to rural Laos and will become a greater problem unless action is taken. A surprisingly high proportion (13.4%) of the human population of a remote village had taken antibiotics in the preceding 2 weeks.

❖ 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, risking their health and the dissemination of these bacteria elsewhere.

❖ The first isolates of carbapenem resistant Gram negative bacilli have been found in Mahosot Hospital, *E. coli* and *Acinetobacter baumannii*,

Lunch for Wellcome Trust visit February 2018

David Dance and colleagues after leaving basci

from pus, the urinary tract, the respiratory tract and blood. Carbapenem-resistant Acinetobacters are seen regularly in respiratory tract samples from the Intensive Care Unit at Mahosot Hospital and four carbapenem resistant *E. coli* have also been isolated from clinical samples. NDM carbapenemases were detected in all *E. coli* isolates, and the OXA-23-like acquired carbapenemase in all of the *A. baumannii*. With the recent beginning of the use of carbapenems in Laos, this is extremely worrying and calls for increasing oversight of their use to reduce the risk of increase in these extremely difficult-to-treat bacteria. Enhanced antibiotic stewardship of these vital agents is urgently needed.

- The main causes of non-typhoid Salmonella bacteraemia at Mahosot Hospital are *S.* Enteritidis, *S.* Typhimurium and *S.* Choleraesuis. The majority of bloodstream isolates are non-susceptible to ciprofloxacin. A greater diversity was found in diarrhoeal stools, mainly *S.* Typhimurium, *S.* Weltevreden, and *S.* Stanley. Of interest some of these species, such as *S.* Weltevreden have also been found in house gecko faeces, suggesting possible zoonotic transmission and possibilities for interventions to reduce human exposure.

- 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 still 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 sexually transmitted diseases.

- Chloramphenicol-resistant *Neisseria meningitidis* has been detected in Laos and elsewhere in SE Asia, suggesting that chloramphenicol should not be used for this infection until antibiotic susceptibility data are available.

The Triumphant Chiggers

- We continue the Global Point Prevalence Survey of Antimicrobial Consumption (PPS), completing 19 rounds of data collection at five hospitals in Laos. We hope that these data will be very helpful for individual hospitals and the Lao Ministry of Health, as the first estimates of hospital inpatient antibiotic use (AMU) in Laos and to monitor changes and use the data to inform optimal prescribing policy. Laos was the first mainland SE Asian country to participate in this program.

- 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 of antibiotic sales are urgently needed. Laos has not yet reached the levels of resistance in some neighbouring countries and thus has a (short) window of opportunity to intervene.

- To assist with providing an evidence base for appropriate action/intervention, we have joined with the Ministry of Health and key stakeholders, especially the Global Antibiotic Resistance Partnership, to form an AMR Technical Working Group that will be synergistic with the WHO/FAO/OIE AMR committee. The review of the AMR situation in Laos, based on published scientific and grey literature and unpublished data from Mahosot Hospital will be discussed further in early 2019 and used to inform national AMR policy and the Lao national antimicrobial treatment guidelines that are also being drafted in collaboration with diverse stakeholders.

- English and Lao language dashboard systems have been developed so that the data on the aetiology of bacterial infections and their susceptibility profile from Mahosot Hospital can be made easily available to policy makers and health workers.

- A review of the implications of changing from the Clinical and Laboratory Standards Institute (CLSI) methods for antibiotic susceptibility testing to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) methods for Laos has been conducted and the evidence informed the Lao Ministry of Health decision to use EUCAST for microbiology laboratories in Laos in future.

- We are working on a tabulation and mapping system for the quality of antibiotics globally, that will help inform discussions and modelling of the relationship between medicine quality and AMR

Malaria and the Importance of Elimination for Laos

- Malaria molecular marker surveillance continues, with CMPE and MORU-Bangkok, in southern Laos. This demonstrates very high frequencies of falciparum malaria markers of artemisinin resistance that has important implications for Lao national antimalarial treatment policy. Molecular markers of piperaquine resistance have been found in southern Laos, as in adjacent Thailand and Cambodia, calling into question the efficacy of DHA-piperaquine in falciparum malaria treatment. There remain serious concerns about the spread of artemisinin resistant *P. falciparum* parasites in southern Laos and discussions are needed as to what therapy will be recommended if double-therapy ACTs fail. We have participated in the multicentre TRAC-2 study, with CMPE and 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.

- A large Targeted Malaria Elimination trial in Savannakhet Province, was conducted with CMPE & MORU-Bangkok, in four Lao villages, that has demonstrated the importance of targeted public engagement and that asymptomatic malaria is very common and probably facilitates malaria transmission. The preliminary results suggest that the mass drug administration (MDA) of dihydroartemisinin-piperaquine plus a single low dose of primaquine is feasible, acceptable and safe but its impact on malaria transmission needs to be further investigated. Eighty-four percent of the village residents participated in the MDAs, of whom 90% completed 3 rounds of MDA. In the intervention villages, the prevalence of asymptomatic *P. falciparum* infections fell by more than in the control villages. *P. falciparum* infections were significantly reduced with early MDA and completion of 3 MDA rounds. Analysis of why

people participated in MDA suggested that factors such as understanding the concept and rationale of the study, free health care, collaboration with the village volunteers, support from authorities and cohesive communities contributed in building trust in MDA.

❖ Surveys of the prevalence of phenotypic and genotypic markers of G6PD deficiency in Laos were conducted to inform safer use of 8-aminoquinolines (e.g. primaquine) in vivax malaria. G6PD deficiency was found to be common in the region with an overall mean prevalence of deficient or mutated hemizygous males of 8.1% in Laos. Mahidol and Viangchan mutations were the most common and widespread variants found among the nine investigated. An evaluation of the fluorescent spot test and a rapid diagnostic test (RDT) against reference spectrophotometry for G6PD deficiency, suggested that both were useful field techniques but that the RDT requires more training for optimal use.

▪ **The causes, diagnosis and treatment of fevers in rural Laos**

❖ The Expanded Fever Surveillance (EFS) project, with the support of the US Naval Medical Research Centre-Asia, investigated the aetiology of fever in outpatients attending Xieng Khouang, Salavan and Luang Nam Tha Provincial Hospitals. The main diagnoses over one year were influenza (60%), followed by leptospirosis (15%), dengue (10%), scrub typhus and bacteraemia (both 5%), murine typhus (3%), JEV and *Rickettsia* spp. (both 1%). Melioidosis was found in 2.5% of patients in Salavan but in none in Xieng Khouang and Luang Nam Tha. This surveillance restarted in August 2017 for inpatients, and these data will be vital for informing treatment algorithm discussions.

❖ The multi-country FIEBRE project started patient recruitment, in October 2018, to investigate the causes of fever and investigate biomarkers for optimizing patient management at Vientiane Provincial Hospital, Phonghong District, Vientiane Province. This is in collaboration with the London School of Hygiene and Tropical Medicine through the generous support of the UK Department for International Development (DFID).

❖ Melioidosis, caused by *Burkholderia pseudomallei*, is an important and under-recognised cause of sepsis in Laos, as well as other tropical regions. We have diagnosed 1,354 culture-positive patients since 1999 (120 in 2018) and are concerned that there is substantial unrecognised but potentially treatable mortality due to *B. pseudomallei* elsewhere, especially in southern Laos. A rapid diagnostic test that can be performed directly on clinical samples has shown good specificity but only moderate sensitivity in preliminary evaluations.

❖ Analysis of the association between climatic factors and the occurrence of melioidosis in patients from

Microbiology Laboratory Staff training on new BD Bactec blood culture machine July 2018

Laos and Cambodia, suggested that humidity, low visibility and maximum wind speeds were important in Laos, and humidity, rainy days and maximum wind speed in Cambodia. Children were at significantly higher risk of infection during humid months and pulmonary and disseminated infections were more common during windy months, suggesting a role for inhalation in initiating infection. The likely mean incubation period was estimated at 1 week. With the Ministry of Health, we are organising a workshop on this important pathogen in Laos in 2019.

- *Burkholderia pseudomallei* does occur below the topsoil in rice fields, being detected at 290cm below the soil surface. This has implications for any interventions for reducing the density of this important pathogen in soil to protect rice farmers in particular.

- In 2013 Laos introduced the 13-valent pneumococcal vaccine (PCV13). After PCV13 introduction there was a 23% relative reduction in PCV13-type carriage in children aged 12-23 months, and no significant change in non-PCV13 serotype carriage. In infants too young to be vaccinated, there was no significant change in carriage of PCV13 serotypes or non-PCV13 serotypes. These data suggest that the reductions in PCV13 serotype carriage in vaccine-eligible children are likely to result in reductions in pneumococcal transmission and disease in Laos.

- The diagnosis of leptospirosis remains challenging in rural Asia. Data from a diagnostic evaluation at Mahosot Hospital suggest that there are no anti-*Leptospira* spp. antibody detecting RDTs with good sensitivity and specificity for local use. However, data suggest that PCR of urine and serum is a useful technique, but that more research on low-cost PCR systems that could be used at the provincial level is needed. In *Leptospira* spp. from Lao patients, no evidence of resistance to azithromycin, ceftriaxone, ciprofloxacin, doxycycline, gentamicin and penicillin G was found.

- 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 from 1874 to 2016 yielded evidence that 159 human pathogens have been recorded in Laos by culture and molecular assays. A novel mathematical model, incorporating a time-varying discovery rate, estimated that some further 10-11 species of human pathogens are currently waiting to be discovered in Laos.

- The causes of central nervous system infections for the first 1,065 lumbar punctures at Mahosot Hospital, 2003-2011, have been 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%. Patients with viral infection more frequently had neck stiffness than those without. Patients with bacterial infection were more likely to have a history of diabetes than those without. Factors that showed strong association with death were higher CSF lactate and lower Glasgow Coma Score (GCS). The Lao data suggest that particular attention should be paid to patients presenting with decreased GCS and the provision of supportive care for unconscious patients, such as high-dependency units (HDU), along with appropriate urgent antimicrobial therapy may be key factors in improving outcome. Given the high frequency of rickettsial infections, empirical therapy with ceftriaxone plus doxycycline should be considered.

- The majority of laboratory cerebrospinal fluid examinations (94%) in Vientiane are abnormal, suggesting that lumbar punctures in inpatients with suspected central nervous system (CNS) infections should be facilitated, with a lower threshold for performing lumbar puncture, 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 is being analysed. Laos has the highest estimated maternal mortality in SE Asia and this 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.

- A clinical trial of the treatment of uncomplicated murine typhus (*Rickettsia typhi*) at Mahosot & Setthathirat Hospitals suggests that oral azithromycin should not be used for therapy but that three and seven days oral doxycycline have similar efficacy.

- The serotype epidemiology of dengue in Laos is very complicated and we are working on an English and Lao language dashboard system so that the data on incidence and spatial and temporal changes in serotype distribution of dengue, from Mahosot and provincial Hospitals, can be made easily available to policy makers and health workers and give warning of impending outbreaks.

- We have found *Japanese encephalitis virus* (JEV) RNA in human throat samples, apparently for the first time, suggesting that testing patients' throat swabs for JEV RNA should be performed on a larger scale to investigate the epidemiology of JEV.

- Using the portable MinION next generation sequencing platform in LOMWRU, whole genome sequencing of *Rickettsia typhi,* the agent of murine typhus, was performed at Mahosot Hospital. This was first ever whole genome sequencing performed in Laos and yields hope for future national capabilities to perform these techniques as an aid to epidemiological, AMR and outbreak investigations.

The Importance of One Health

- 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 from 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 717 specimens from 359 wild vertebrates (mostly squirrels) collected at markets demonstrated that 69 animals contained *Leptospira* spp. and 19 contained *Rickettsia* spp. (including a confirmed *R. felis*). The high frequency of *Leptospira* spp. detected in urogenital swabs suggests a potential risk of squirrel-human transmission of leptospirosis in markets. We also identified samples containing *Orientia tsutsugamushi*, *Ehrlichia* spp. TC251-2, *Anaplasma marginale*, *A. phagocytophilum*, and *A. bovis*. We also identified *Lactococcus garvieae*, *Kurthia* spp. but remain uncertain of their pathogenicity in this context. These data support the public heath interest in controlling the commercial trade in bushmeat. The discovery of *R. felis*, an emerging rickettsial pathogen, and *O. tsutsugamushi* are the first reported detections in squirrels.

- In a study of the serology of Lao market vendors, we evaluated their immunological status regarding murine typhus, scrub typhus and leptospirosis**.** Sellers of wildlife meat had an increased probability of having anti-scrub typhus IgG. The study has been continued with two more sampling rounds in order to investigate changes in vendor serostatus through time.

- *Streptococcus agalactiae* (Group B Streptococcus - GBS) with the same sequence type as that which caused a unique fish-borne outbreak in Singapore in 2015 (ST283) accounted for 29 of 38 cases of invasive GBS infection diagnosed in LOMWRU since 2000. As this sequence type has been found in both human infections and fish in other countries in SE Asia, it is possible that fish may represent an under-recognised source of GBS infection regionally.

The Importance of Medicine Quality

- There remain severe, focal problems with the quality of diverse medicines globally. There remain significant regional issues with the availability of oral artemisinin derivative monotherapy and non-Quality Assured ACTs.

- We are tabulating and mapping the accessible data on the quality of essential medicines with funding from the Wellcome Trust. This includes the accessible data on the quality of maternal health medicines, antibiotics, antidiabetics, anti-retrovirals, anti-tuberculous, cardiovascular and veterinary medicines and vaccines for inclusion in Infectious Diseases Data Observatory surveyor mapping systems. We are about to Beta test these surveyors for release in the spring. The data will also be analysed for reviews on the quality of these essential medical products in 2019.

- With the Lao Bureau of Food and Drug Inspection, supported by the Asian Development Bank, we evaluated a wide diversity of portable medicine screening devices, their diagnostic accuracy and cost-effectiveness. The results should help inform policy for which devices are best for Laos. A meeting was held in Vientiane on 9-10th April 2018 to discuss these issues. Although a diverse range of portable field detection devices for medicines quality screening is available, there is a vitally important lack of independent evaluation of the majority of devices, particularly in field settings. Intensive research is needed in order to inform national medicines regulatory authorities of the optimal choice of device(s) to combat poor quality medicines. At the country level, all five spectrometers included were found to be cost-effective in settings with 'high' prevalence of falsified and substandard antimalarials but only three were cost-effective in lower prevalence. However, the study raised concerns that those using these devices may develop false confidence in the devices and reduce the vital visual inspection of medicines. With the current evidence, it is unlikely that any one device would be able to effectively monitor the quality of all medicines. Although some devices can accurately detect falsified medicines containing no or incorrect active pharmaceutical ingredients (API), we did not find evidence that they could quantitate % API, a key aspect of many substandard medicines.

- Much more work is needed to evaluate devices for the great diversity of medicines, and to expand our work with a platform, independent from device manufacturers, to evaluate new devices using standard protocols and samples.

- A stratified random survey, using mystery shoppers, of the availability and quality of antibiotics sold to patients in the private sector in five southern Lao provinces was conducted with the Lao Food & Drug Department. This collected 909 samples of amoxicillin, ampicillin, ceftriaxone, ciprofloxacin, doxycycline, ofloxacin, sulfamethoxazole, tetracycline and trimethoprim, that were assayed for content by High-Performance Liquid Chromatography (HPLC). All the analysed samples contained the stated API without evidence for falsification. However, substandard antibiotics were found; 19.6% of samples had quantities outside the 90-110% content of the label claim and 60.2% of the samples had quantities outside the International Pharmacopoeia uniformity of content limit range. Substandard antibiotics will have reduced therapeutic efficacy, impacting public health and control of bacterial infections. Furthermore, although the contribution made by poor quality medicines to the development of antimicrobial resistance (AMR) remains poorly understood, substandard antibiotics are likely to engender AMR.

- The use of medicines without labelling in Vientiane is associated with increased frequency of admission to hospital with adverse drug reactions, suggesting that there is a need to ensure appropriate labelling of medicines at the dispensing point.

- There is limited awareness in Vientiane residents of the risks of medicines and more engagement with the public would make a valuable contribution to the appropriate use of medicines.

- We organised the first international conference on Medicine Quality & Public Health in September at Keble College, Oxford. Some 220 participants from over 50 countries attended.

- We also organised the fourth course on Medicine Quality & Public Health, that has been held in London and Boston in previous years, in Oxford the week before the conference. Twenty-five people from sixteen countries attended.

- **The Importance of Engagement**

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 further Science Cafés in Laos, which we hope will become a regular feature of the Vientiane scientific 'scene'. The topics discussed included road crashes, diabetes and post-partum food avoidance behaviour.

Policy maker engagement. We have been engaging with diverse departments in the Ministry of Health and with WHO on innovative dashboards for the accessible and straightforward representation of data about the epidemiology of dengue and antimicrobial resistance in Laos and also with many additional stakeholders for new infectious disease treatment guidelines for Laos. We will be meeting with the Department of Communicable Disease Control and other departments of the Lao Ministry of Health in spring of 2019 to discuss the work completed in 2018 and the public health policy implications. We are discussing a LOMWRU-linked unit at the University of Health Sciences to facilitate translation of Lao and global scientific public health evidence into health policy in Laos.

Dr Koukeo Phommasone studying for his PhD in Amsterdam

2nd Global Health Bioethics Network Asia Regional Ethics Conference hosted by OUCRU-Nepal, including Assoc Prof Mayfong Mayxay and Dr Vimalay Souvong

INTRODUCTION

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. LOMWRU core 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 of the building was funded by the Wellcome Trust and opened in 2008) contains the Molecular, Serology and BSL3 Laboratories and offices. We are arranged into Administration (including data entry, informatics, finance, samples and supplies), BSL3, Clinical, Clinical Research Support, Conventional Bacteriology, Field Work and Malaria, Medicine Quality & Pharmacy, Molecular Bacteriology, Public Engagement, Health & Safety and Virology Groups.

The Chinese Government is funding the rebuilding of Mahosot Hospital and we expect that the Microbiology Laboratory, including LOMWRU, will move to a new Infectious Disease Centre in 2020 or later.

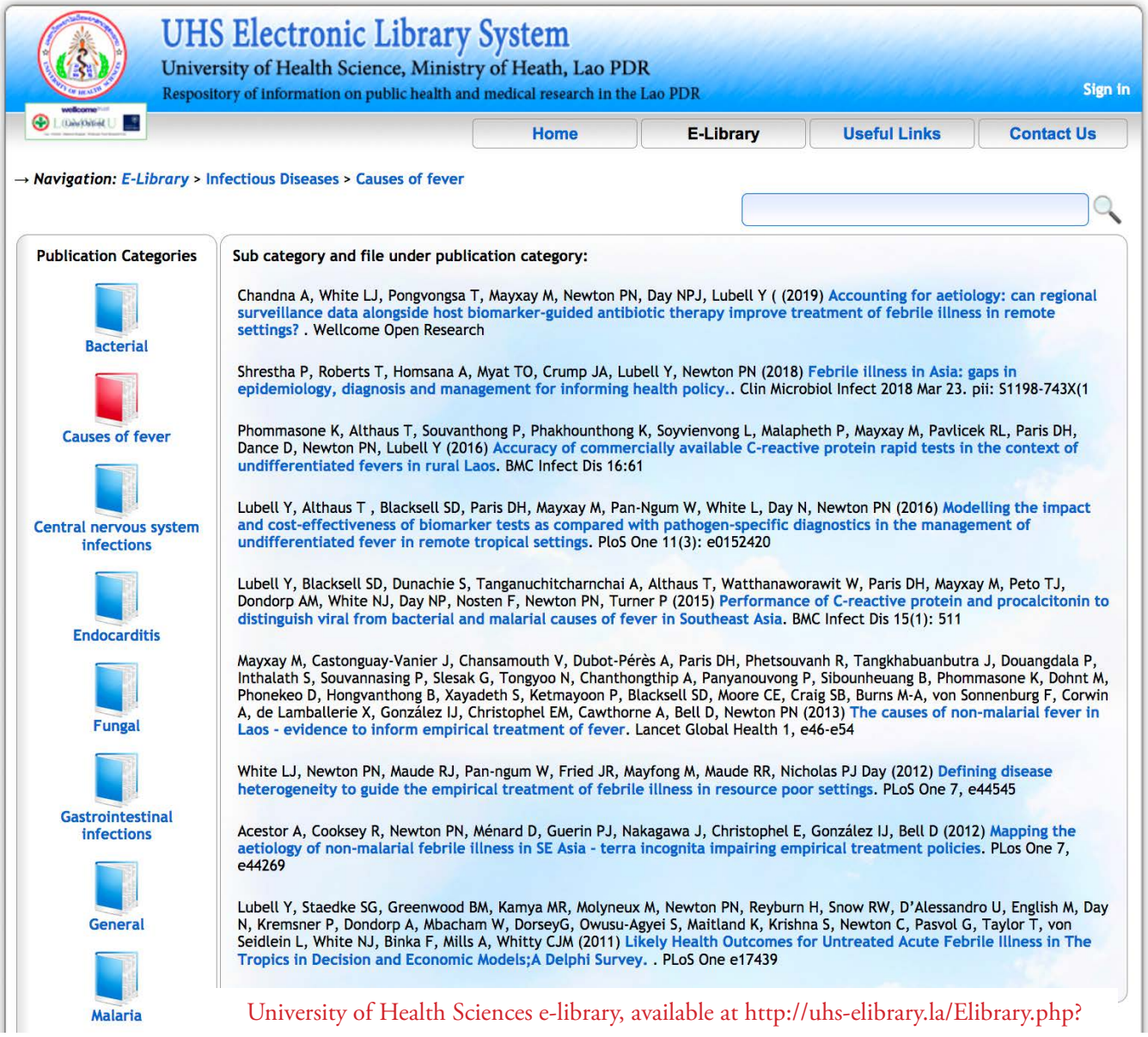
Oxford University headquarters are at the Centre for Tropical Medicine & Global Health, in the Nuffield Department of Medicine on the Churchill Hospital site in Oxford in the United Kingdom. We are a component of the Mahidol-Oxford Research Unit (MORU) Network, with HQ in the Faculty of Tropical Medicine, Mahidol University, Bangkok, and are greatly assisted by the supplies, logistics and accounting staff of MORU. We have many scientific liaisons within MORU; ~49% of our research is conducted jointly with MORU-Bangkok. We are very grateful for all the help of MORU-Bangkok for vital logistical and auditing support for LOMWRU.

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 also have important collaborations with them.

Mr Sengkham Symanivong feeding Mr Rambo a maize lunch

We have been part of the WorldWide Antimalarial Resistance Network (WWARN) for 6 years. WWARN maps evidence on antimalarial resistance and treatment and includes published data from Laos, and at Mahosot Hospital we run the WWARN Antimalarial Quality group and mapping system (http://www.wwarn.org/aqsurveyor/) with the WWARN Informatics team in Oxford. This system is being extended to other infectious diseases under the Infectious Disease 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 are being graded, mapped and released progressively during 2018/19 as part of the Wellcome Trust funded MAPQAMP project.

The Microbiology Laboratory and LOMWRU together are staffed by 27 Lao Government staff and 65 project-funded staff, post-graduate students, contractors and long-term visitors; 84% are Lao nationals and 54% are female. Our Senior Management Meeting Group is 54% Lao and 54% 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



University of Health Sciences e-library, available at http://uhs-elibrary.la/Elibrary.php?

Naval Medical Research Centre, the Bill & Melinda Gates Foundation, the European Union, Department for International Development-UK (DFID), New Zealand e-Asia, Fondation Total/Institute Pasteur, Global Good, DTRA, Global Antibiotic Resistance Partnership, 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 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 the centre (Vientiane Provincial Hospital) and other hospitals and institutions on request, as well as performing clinical research and building diagnostic and research human capacity. In 2018 the Laboratory processed blood cultures from 3,836 patients at Mahosot Hospital and 1,768 for provincial hospital patients, cerebrospinal fluid from 132, urine from 1,656, stool from 602, sputum culture from 232, pus from 835, genital swabs from 3,022, other body fluids from 396, cryptococcal antigen tests from 171 and throat swabs from 1,237 patients. Dengue IgM serum and CSF ELISA were performed for 1,586 patients, NS1 serum and CSF ELISAs were performed for 1,933 patients, dengue serum and CSF PCRs for 2,431, JEV serum and CSF IgM ELISAs for 1,707 patients, scrub typhus and murine typhus rapid diagnostic tests (RDTs)on sera from 1,037 patients and dengue RDTs on sera from 579 patients. LOMWRU also works with the Centre for Malariology, Parasitology and Entomology (CMPE) on malaria projects in the five southern provinces, with the Food and Drug Department on the quality of medicines, with the

Bureau of Food and Drug Inspection & Food and Drug Quality Control Centre on the evaluation of innovative medicine quality screening devices and with the Ministry of Health Department of Communicable Disease Control (DCDC) on antibiotic resistance.

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

In 2018 we published or have in press 73 publications, including 67 peer-reviewed papers, two letters, one report and three book chapters. Since LOMWRU was founded, its staff has published 392 papers and book chapters. Here we describe this work and briefly summarize diverse activities over the past year.

Microbiology Laboratory Meeting

STAFF AND HUMAN CAPACITY BUILDING

FIEBRE Multicountry Meeting Zimbabwe December 2018

New staff and longer-term visitors who joined in 2018 include, in alphabetical order:

Mrs Aphaphone Adsamoud - Laboratory Technician – Molecular Bacteriology Group

Mrs Latsaniphone Boudthasavong - Laboratory Technician – Molecular Bacteriology Group

Dr Phetsavanh Chanvilay - Public Engagement Coordinator

Dr Johannes Doppler – LSHTM MSc student

Mrs Phatsalin Keomoukda - Laboratory Technician

Mr Padthana Kietsathith - Laboratory Technician - Virology Group

Dr Khamfong Kunlaya - Research Physician – FIEBRE

Dr Manh Hung Nguyen – MSc student – LaoTPHI

Dr Chom Phaiphichit - Research Physician

Dr Siribun Panapruksachat - Molecular Bacteriologist

Dr Somvai Singhaxaiyaseng - Research Physician

Dr Vimalay Souvong - Clinical Trials Support Group Coordinator

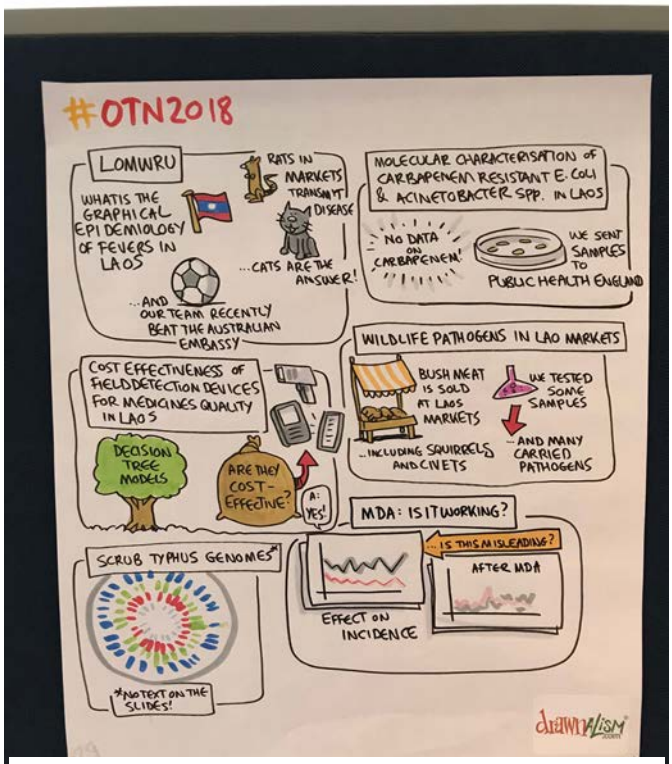
Dr Souphaphone Vannachone - Research Physician

Mr Souksakhone Volavong - Specimens Storage Assistant

Dr Phoudthapanya Xongmixay - Public Engagement Assistant

We congratulate Assoc. Prof Mayfong Mayxay on being appointed Vice-President of the University of Health Sciences

Scientific Conference for 60th Anniversary of University of Health Sciences (UHS). Assoc. Prof. Mayfong Mayxay, Vice-President of UHS, Assoc. Prof Phouthone Meuangpak, Vice-Minister of Health & Dr. Phouthone Vangkonevilay, Interim President of UHS



Drawnalism Artwork LOMWRU Talk Vietnam Oxford Tropical Network Meeting

Dr Tiengkham Pongvongsa was awarded his Mahidol University PhD for his thesis entitled 'Dynamics of asymptomatic *Plasmodium falciparum* parasitaemia during mass drug administration with dihydroartemisinin-piperaquine plus single low dose of primaquine in Savannakhet Province, Laos' and Dr Bipin Adhikari his Oxford University DPhil, for his thesis entitled 'Community engagement for targeted malaria elimination in Lao PDR (Laos)' both as part of the TME project in Savannakhet Province. Congratulations to both !

Mr Weerawat Phuklia will complete his PhD on the antibiotic susceptibility of diverse isolates of *O. tsutsugamushi* in early 2019. Dr Koukeo Phommasone is completing analysis and writing up of his University of Amsterdam PhD thesis, also to finish in 2019.

Dr Nguyen Manh Hung, a Vietnamese Masters student from the Lao Tropical and Public Health Institute successfully completed his Master's thesis 'Screening of ectoparasites from domesticated dogs for bacterial pathogens in Vientiane, Lao PDR'.

Dr Ivo Elliott has completed his fieldwork on scrub typhus in Laos and Thailand for his Oxford DPhil thesis, spending much time trapping rodents and identifying chiggers, and is finalising his thesis for submission in early 2019.

Dr Tehmina Bharucha has started her DPhil on the proteomics of *Japanese encephalitis virus* infection working with Prof Nicole Zitzman of the Department of Biochemistry, Oxford University, Dr Audrey Dubot-Pérès and Paul Newton.

Dr Rebecca Inglis continues her Oxford DPhil mixed methods research on intensive care units (ICU) in Laos to inform the design of a Lao-run sustainable ICU course.

Ms Audrey Rachlin returned to Vientiane for three months to delve in Vientiane ditches looking for *Burkholderia pseudomallei* for her PhD at the Menzies School of Health Research in Darwin, Australia, on 'Environmental sources and distribution of *Burkholderia pseudomallei* infection in northern Australia and Lao People's Democratic Republic'. Dr Johannes Doppler spent two months with us for his successful LSHTM thesis 'A systematic review of the outcomes and untreated mortality of murine typhus'. Mr Theophilus Ndorbor, WHO/TDR Fellow, from the Liberian Medicine Regulatory Authority, spent 2018 with us, working on West African MRA engagement and reviewing the global evidence on the quality of cardiovascular medicines and medicines for neglected tropical diseases.

Dr Vilada Chansamouth has been awarded a Wellcome Trust International Fellowship for her Oxford DPhil project 'Evaluating the impact of a Lao language mobile phone antibiotic guideline application on antibiotic prescribing in Laos' that she will start in the spring.

Dr Audrey Dubot-Pérès, who leads the LOMWRU Virology Group, is based in Marseille but returned for her traditional three months of intensive virology work in LOMWRU in the summer of 2018. Ms Bountoy Sibounheuang continues her studies for the BSc in Medical Technology at Khon Kaen University.

To support the research of the unit, including organising ethical approvals and audits, Dr Vimalay Souvong was appointed CTSG Coordinator, working with LOMWRU and the Clinical Trials Support Group in MORU-Bangkok.

We are fortunate to have strong links with Public Health England (PHE) who have supported Dr

Collage of photographs for FIEBRE Study Training October 2018

Mrs Konnie Bellingham
English Tutor
LOMWRU

Mrs Phouvy Sayyalath
English Tutor
LOMWRU

LOMWRU English Teachers

Tomas-Paul Cusack, a UK microbiology/infectious disease trainee, who extended his stay with us until January 2019.

In 2018 we supported 16 Lao staff to attend 6 international meetings and short courses, including the Oxford Tropical Network Meeting in Ho Chi Minh City and the FIEBRE meeting in Zimbabwe. Mrs Somsavanh Sayalard spent six weeks in Professor Tim Walsh's laboratory in Cardiff, Wales, working on colistin resistance in Enterobacteriaceae. Mr Nilamith Hanthongxay successfully took the course in shipping dangerous goods in Bangkok and Dr Manivanh Vongsouvath, Mrs Viengmon Davong and Ms Amphonesavanh Sengduangphachanh spent two weeks learning about laboratory quality management for ISO 15189 in Khon Kaen.

Numerous students and doctors in diverse health disciplines studied in the Microbiology Laboratory in 2018. The Laboratory staff assisted with the post-graduate internal medicine and paediatric training programme teaching.

Mrs Anisone Chanthongthip and Ms Neeranuch Thangnimitchok taught on the vector-borne disease course organised by Institut Pasteur du Laos and the Lao Ministry of Defence in May 2018.

Regular classes are being held for learning the English language.

Mr Prayoon Yuentrakul, of MORU-Bangkok, ran a Good Clinical Practice course in September 2018 and led an audit of the FIEBRE study with Dr Vimalay Souvong.

In December, the MORU-Bangkok Team for Chemical Safety visited LOMWRU to review our management of chemicals and undertake practical chemical safety training. The team was composed of Phettree Niamyim, Sopida Srichaiwattana, Thananya Naknonhan, Benjamas Sriburin, Chonchawan Jankam, Siribha Apinan, Rommanee Bangphoomi and Markus Winterberg.

Mr Prapass Wannapinij of MORU-Bangkok visited to help with further development of the Laboratory Information Management System that has been a transformative intervention.

We are very grateful for vital statistical analysis support from across the MORU Tropical Health Network, especially from Dr Sue Lee, Dr Mavuto Mukaka & Dr Pimnara Peerawaranun.

As every year, the Vientiane Pompier or Fire Police visited to give fire prevention and action training.

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 Lao Tropical Public Health Institute (Lao TPHI, the successor to the 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 continue to build capacity within the Unit with hands-on training in microbiology, clinical history taking, examination and case presentation, ELISA, molecular diagnostics 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 Hospital 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, and a Lao Laboratory Manager. A Laboratory Management Adviser is co-ordinating a programme of work towards ISO 15189 accreditation for the Microbiology Laboratory, and we are working closely with other laboratories in Laos that are working towards such accreditation.

The Laboratory football team, 'The Chiggers', play weekly in Vientiane. Recent notable victories include playing the Australian Embassy team, winning again 8:6 – congratulations to The (Mitey) Chiggers!

Fire training in Microbiology Laboratory garden

New Staff 2018

Ms Aphaphone Adsamouth
Lab Technician - Molecular
LOMWRU

Ms Latsaniphone Boudthasavong
Lab Technician - Molecular
LOMWRU

Dr Phetsavanh Chanthavilay
Public Engagement
LOMWRU

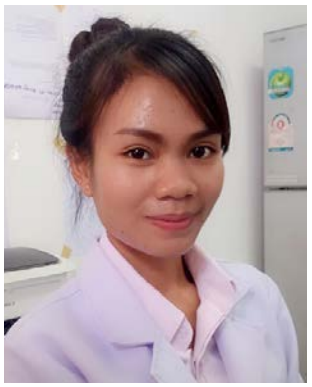
Dr Johannes Doppler
Visiting Research Physician
LOMWRU

Ms Phatsalin Keomoukda
Lab Technician –
EFS Xiengkhuang
LOMWRU

Mr Padthana Kietsathit
Lab Technician - Virology
LOMWRU

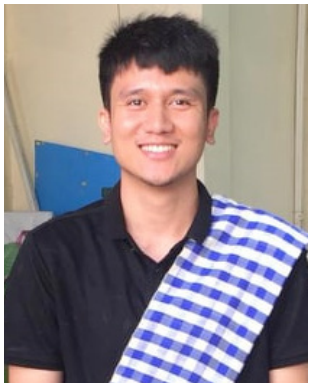
Dr Khamfong Kunlaya
Research Physician
LOMWRU

Dr Manh Hung Nguyen
Masters student Lao TPHI
LOMWRU



Dr Chom Phaiphichit
Research Physician –
EFS Saravane
LOMWRU

Dr Siribun Panapruksachat
Molecular Bacteriologist
LOMWRU



Dr Somvai Singhaxaiyaseng
Research Physician
LOMWRU

Dr Alicia Quach
Research Physician
LOMWRU

Dr Vimalay Souvong
CTSG Coordinator
LOMWRU

Dr Souphaphone Vannachone
Research Physician
LOMWRU

Mr Souksakhone Volavong
Specimens Storage Assistant
LOMWRU

Dr Phoudthapanya Xongmixay
Public Engagement Assistant
LOMWRU

Dr Guillermo Alonso Zabala
Visiting Research Physician
LOMWRU

Ms DO Thi Ngan
Research Assistant
LOMWRU

Ms Tadam Solathtanavong
Finance & HR Officer
LOMWRU

RESEARCH RESULTS AND THEIR PUBLIC HEALTH IMPLICATIONS

Khun Prapass Wannapinij from MORU, visiting for work on the LIMS, with Khun Risara and Ms Sengmany of LOMWRU

1. Infectious Disease Diagnosis, Epidemiology and Treatment

We continue to report every month all clinically significant positive blood, cerebrospinal fluid and genital swab culture results and their antibiotic susceptibility profiles, through the Monthly Microbiology Summary, to the Lao Ministry of Health, National Centre for Epidemiology and Laboratory (NCLE), National Animal Health Laboratory, other hospitals in Vientiane and WHO. We aim to expand this reporting using dashboard systems (see page 65).

A. Fevers in Asia. Shrestha *et al.* (2018) conducted a review of the epidemiology and management of fevers in South and South-East Asia and highlighted gaps in our knowledge that impair evidence-based health policy decisions. They identified 100 studies. Among the 30 studies (30%) including both children and adults that investigated three or more pathogens, the most frequently reported fever aetiology was dengue (reported by 15, 50%), followed by leptospirosis (eight, 27%), scrub typhus (seven, 23%) and *Salmonella enterica* serotype Typhi (six, 20%). Among four studies investigating three or more pathogens in children, dengue and *Staphylococcus aureus* were the most frequent, followed by non-typhoidal *Salmonella* spp, *Streptococcus pneumoniae*, *Salmonella* Typhi, and *Orientia tsutsugamushi*. Increased awareness is needed that rickettsial pathogens are common but do not respond to cephalosporins, and that alternative therapies, such as tetracyclines, are required. Many key gaps remain, and consensus guidelines for study design are needed to aid comparative understanding of the epidemiology of fevers. More investment in developing accurate and affordable diagnostic tests for rural Asia and independent evaluation of those already on the market are needed. Treatment algorithms, including simple biomarker assays, appropriate for empirical therapy of fevers in different areas of rural

Asia should be a major aim of fever research. Enhanced antimicrobial resistance (AMR) surveillance and openly accessible databases of geography-specific AMR data would inform policy on empirical and specific therapy. More investment in innovative strategies facilitating infectious disease surveillance in remote rural communities would be an important component of poverty reduction and improving public health.

B. Fever in rural Laos. The data published by 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 have been expanding this work since December 2014, as the Expanded Fever Surveillance (EFS) project with the support of the US Naval Medical Research Centre, to include Xieng Khouang Provincial Hospital (XK) in the Plain of Jars, 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 outpatients, 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, 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.

LCDR Hertz and Lt Garcia of the Naval Medical Research Centre on a visit to the laboratory of Xieng Khuang Provincial Hospital

LOMWRU FIEBRE Team at Zimbabwe Meeting

Between December 2014 and 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 the outpatient department 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 from 1st of September 2015 until the end of this study, so 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 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%).

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%). The final diagnostic data will soon be ready and the final analysis performed.

Microbiology Laboratory Team en route to the That Luang Festival November 2018

We restarted this study, with continued Naval Medical Research Centre-Asia support, at the three provincial hospitals in August 2017 to investigate the aetiology of fever in inpatients. The blood culture results are reported as soon as we have results in the Microbiology Laboratory to aid patient management and inform AMR issues. Rickettsial, leptospiral and dengue assays are performed in batches multiple times a month. 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).

Between August 2017 and July 2018 (12 months), 1,818 in-patients were recruited (364 from LNT, 663 from XK and 791 from SV). Of the 1,818 patients, 160/1,818 (9%) were given a confirmed laboratory diagnosis (either culture, antigen, nucleic acid assays or seroconversion) in this preliminary analysis, 52/160 (33%) patients were from LNT, 37/160 (23%) were from XK and 71/160 (44%) were from SV. The main overall diagnoses were dengue fever 57 (36%), followed by bacteremia (including melioidosis, typhoid fever, *E. coli* bacteremia, *S. aureus* bacteremia and bacteremia from other pathogens) 56 (35%), leptospirosis 24 (15%), *Rickettsia* spp. infection 9 (6%), scrub typhus 5 (3%), *Japanese encephalitis virus* 1 (0.6%) and flavivirus infection 1 (0.6%). Seven (4%) patients were infected with more than one pathogen.

Working with Professor David Mabey and Dr Heidi Hopkins and colleagues at the London School of Hygiene & Tropical Medicine we recently started patient recruitment with colleagues at Vientiane Provincial Hospital, in Phonghong, for the multi-country FIEBRE study, funded by the UK Department for International Development. This consensus protocol investigation of the aetiology of fever is being conducted in Malawi, Mozambique, Zimbabwe and Laos and another country to be decided upon.

C. 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

LCDR Hertz, Dr Matt Robinson, Lt Garcia, Dr Manivanh Vongsouvath & Paul Newton at Plain of Jars Site 1

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 and whether new blood biomarkers can distinguish bacterial from viral infections.

D. Central nervous system (CNS) infections. Central nervous system (CNS) infections are important causes of mortality and morbidity in Southeast Asia. Little is known about their aetiology and impact in Laos. We have completed analyzing the data from the first 1,065 patients to have a lumbar puncture at Mahosot Hospital since 2003 as part of the CNS Study (Dubot-Pérès *et al.* in press). We recruited patients of all ages admitted to Mahosot Hospital, Vientiane, with suspected CNS infection (2003-2011); 1,065 patients had no contraindications and consented to lumbar puncture. Aetiology was confirmed in 450 (42.3%) patients of whom 93.6% had abnormal CSF. The aetiologies were *Japanese encephalitis virus* in 94 (8.8%) patients, *Cryptococcus* spp. in 70 (6.6%), *O. tsutsugamushi* in 31 (2.9%), *Dengue virus* in 27 (2.5%), *Leptospira* spp. in 25 (2.3%), *Rickettsia* spp. in 24 (2.3%), *Streptococcus pneumoniae* in 22 (2.1%), *Mycobacterium tuberculosis* in 20 (1.9%), *Herpes simplex virus* (HSV) (1.4%), *Human cytomegalovirus* (HCMV) 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). Patients with viral infection more frequently had neck stiffness than those without (AOR 1.9, 95%CI 1.3-2.8). Patients with bacterial infection had higher cerebrospinal fluid lactate (AOR 1.1, 95%CI 1.1-1.2) and serum CRP (AOR 1.0, 95%CI 1.0-1.0) and were more likely to have a history of diabetes (AOR 3.08, 95%CI 1.27-7.46) than those without bacterial infection. The responsible pathogens spanned encephalitis and meningitis without clearly distinguishable clinical manifestations by aetiology.

No clinical/laboratory variables could reliably guide selection of antibiotics. With the importance of rickettsial pathogens, first line treatment with both ceftriaxone and doxycycline should be considered for suspected CNS infection. 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 die, and the provision of supportive care for unconscious patients, such as high-dependency units (HDU), along with appropriate urgent antimicrobial therapy, may be key factors in improving outcome. With the increasing global prevalence of diabetes, further investigation of the relationship between hyperglycaemia and bacterial CNS infection is needed. This is a collaborative project with multiple partners, especially with IRD/ Aix-Marseille University, France.

The majority of CSF samples collected gave abnormal results, suggesting that a higher frequency of lumbar

Naval Medical Research Centre -Asia and LOMWRU team at lunch in Luang Nam Tha

LCDR Hertz, Lt Garcia and LOMWRU Team Xieng Khuang Hospital July 2018 with the Director Dr Komua Nenglao and hospital staff

puncture is needed to ensure that serious CNS infections are not missed, especially in patients at the extremes of age.

We work with the Centre d'Infectiologie Christophe Mérieux du Laos on detection of molecular markers of *M. tuberculosis* drug resistance from patients with TB meningitis. We have been working with Dr Sarosh Irani, Head of the Oxford Autoimmune Neurology Group, who has found evidence of autoimmune central nervous system disease in Lao patients – this will be pursued further in 2019. We have also been working with Professor Nicole Zitzmann and Dr Bevin Gangadharan of the Department of Biochemistry, Oxford, on a search for proteomic signatures in the CSF and sera of patients with Japanese encephalitis infection and clinical encephalitis. This work is the subject of Dr Tehmina Bharucha's DPhil research in Oxford, Marseille and Vientiane.

The CNS work was expanded in 2014 as the 'SEAe project' in collaboration with the Institut Pasteur, Paris, funded by the Fondation Total, 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), Laos (at Mahosot Hospital and the Children's Hospital) and Myanmar (Yangon Children's Hospital) using common study protocols. This allows first line PCR diagnosis of 21 pathogens in the first 24 hours after LP. This project has been completed and the data are being analysed. We have now reverted to the protocol of the original CNS Study.

E. Association between reported aetiology of central nervous system infections and the speciality of study investigators. Conventional descriptions of central nervous system infections are variably categorised into clinical syndromes for patient investigation, management and research. Aetiologies of the most commonly recognised syndromes, encephalitis and meningitis, tend to be attributed predominantly to viruses and bacteria, respectively. We reviewed aetiological studies of CNS syndromes by reported

author specialities (Bharucha *et al.* in 2018). This identified an association between the author's speciality and the CNS syndrome studied, with a tendency for virologists to study encephalitis, and microbiologists to study meningitis, suggesting that stronger multidisciplinary collaboration in CNS infection research is needed.

F. 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 and analysis is progressing. 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.

G. Mapping of fevers. We are working with the London School of Hygiene & Tropical Medicine (LSHTM), the Infectious Diseases Data Observatory (IDDO) and the Foundation for Innovative New Diagnostics (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 are being mapped by global regions by the Infectious Diseases Data Observatory and others, including the partners listed above.

H. 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/Oxford, applied a similar methodology to country-level data from Laos in order to estimate the total diversity of pathogens available for discovery in Laos and answer 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.

A dataset of 5,140 data points was collected through an extensive literature search. The data were graded according to the level of diagnostic evidence used in discovery. Two hundred and thirty-nine species-level data points, with some species repetitions for different grades of evidence, were identified for inclusion in the analysis. The investigation juxtaposed the data with the history of Laos for the 143 year period under investigation prior to modelling to address data gaps and other data novelties. Up to 2016, 159 pathogens have been described in Laos by culture and PCR.

Previous discovery-curve models were improved upon by implementing a time-varying discovery rate in the model to account for observed changes in rate of pathogen discovery. The models estimated between 170 and 196 total species of human pathogens are available for discovery in Laos. This implied that between 10 and 11 new additional species remained available for discovery at the end of 2016. The model also captured a 36-fold increase in the rate of pathogen discovery during the last ten years due to laboratory and technical advances. The changes in rates of pathogen description can be related to the different phases of Lao history over the 142 years of investigation.

I. Novel incubators. We worked with Global Good on evaluating new incubators designed for use in the tropics. These were trialled at Mahosot and in Luang Nam Tha and Salavan Provincial Hospitals. The monitoring data have been used by Global Good to optimise the incubators (Miller *et al.* 2018).

J. Novel and appropriate diagnostics. We are evaluating new simplified systems for molecular diagnostics, including the miniPCR system and novel recombinase polymerase amplification (RPA) assays using lateral flow tests for *O. tsutsugamushi* molecular detection. In 2019 we aim to evaluate these systems in locations outside Vientiane, to assess their impact and usability for the diagnostic of febrile illness. We are also extending our evaluation of the Biofire Travellers Film Array molecular detection system. In early 2019 we will be evaluating a novel multiplexed RDT in collaboration with FIND.

We participated in a project reviewing the infrastructural, technical and behavioural challenges that low resource settings (LRS) face when implementing clinical bacteriology – that is a prerequisite for effective AMR control (Ombelet *et al.* 2018). The majority of

microbiological techniques and equipment have not been developed for the specific needs of LRS and that pending the arrival of a new generation of LRS-friendly diagnostics, improving, adapting and implementing conventional, culture-based techniques are required. LRS priorities should include harmonized, quality-assured and "tropicalized" equipment, consumables and techniques as well as rationalized bacterial identification and AMR testing. Furthermore, diagnostics should be integrated into clinical care and patient management. Clinically relevant specimens must be appropriately selected and prioritized. Open-access training materials and information management tools should be developed. The team advocated on-site validation and field-adoption of diagnostics in LRS, with considerable shortening of the time between development and implementation of diagnostics. Implementing clinical bacteriology in LRS will improve patient management, provide valuable surveillance data for local antibiotic treatment guidelines and national policies, and augment AMR containment and hospital infection prevention and control.

K. CRP-driven antibiotic treatment decisions. Chandna *et al.* (2018) modelled the impact on mortality from febrile illness of using point-of-care C-reactive protein (CRP) testing to inform the decision to prescribe antibiotics and regional surveillance data to inform antibiotic selection in rural Savannakhet Province, Laos. The model simulated 100 scenarios with varying quarterly incidence of six key pathogens known to be common in rural Laos. In the simulations, community health workers either prescribed antibiotics in-line with current practice as documented in health facilities in rural Laos, or with the aid of the two interventions. Cost-effectiveness estimates for each strategy alone and then for an integrated approach using both interventions were made. They concluded that an integrated system incorporating point-of-care host biomarker testing and regional surveillance data appears highly cost-effective, and may warrant piloting in a real-life setting.

L. Diabetes mellitus. Because of the importance of diabetes as a risk for melioidosis and tuberculosis, and the apparent high prevalence in Vientiane, we have started a pilot study to examine the clinical and biochemical phenotype of diabetes in Vientiane in collaboration with Setthathirat Hospital. The biochemical marker assays are being conducted at the Oxford Centre for Diabetes, Endocrinology and Metabolism.

M. Novel data sharing systems. Olivier Celhay has been developing, in R language, dashboard systems for the expression of infectious disease data from Laos that we are discussing with the Ministry of Health and other stakeholders to enable health workers and policy makers to have ready access to latest data from laboratories in Laos in a user-friendly format. These include an AMR dashboard, initiated by Professor Paul Turner of COMRU, expressing data on antimicrobial resistance patterns of key bacterial pathogens causing infections in Laos and a dengue dashboard for the depiction of dengue epidemiology across Laos, graded by strength of evidence and by serotype. Please see page 65 of this report for an example. We intend that this will give early warning of dengue outbreaks in collaboration with Institut Pasteur du Laos. We are also planning point prevalence use of antibiotic survey data dashboards and one for the FIEBRE study (above).

2. Clinical Bacteriology, Melioidosis & AMR

A. Defining System Requirements for Simplified Blood Culture. Dailey *et al.* (2019) created a target product profile for a simplified blood culture system to inform product innovation and development efforts. They enlisted a group of specialists working in Africa and Asia to answer questions to understand challenges and how these constraints inform system requirements. The specialists were infectious disease physicians, public health/clinical microbiologists, clinical researchers, and technology experts with different geographical backgrounds. All suggested that blood cultures should ideally be available at the district hospital level. Many of the same operational challenges, such as limited availability of culture bottles, electricity and internet connectivity, profuse dust, the lack of ambient temperature control, and human capacity constraints were identified across the different regions. Blood cultures, although the accepted gold standard for diagnosis of blood stream infections, are not widely available outside reference/research centres in Africa and Asia. To extend the reach of this important tool, it is crucial to engage product developers and academic research partners to develop accessible alternatives.

B. Antimicrobial Susceptibility testing. Dr Tom Cusack

has been leading a project to compare the antimicrobial susceptibility testing methods and guidelines of The European Committee on Antimicrobial Susceptibility Testing (EUCAST) http://www.eucast.org/ with those of the Clinical and Laboratory Standards Institute https://clsi.org/standards/products/microbiology/ and to provide evidence for the Ministry of Health as to which may be the most appropriate to adopt as the Lao national standard.

C. 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 two weeks. Further data analysis is underway and whole genome sequencing of the isolates is being undertaken in order to investigate the epidemiology of antimicrobial resistance within this relatively isolated community.

D. 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.

E. Extended spectrum beta-lactamase-producing Enterobacteriaceae bacteraemia in Vientiane over 5 years. While there has been an increasing incidence of bacteraemia caused by ESBL-producing Enterobacteriaceae (ESBL-E) across Southeast Asia,

Burkholderia pseudomallei field sampling site at Ban Nabone, Vientiane Province

there are sparse data from the Lao PDR, where laboratory capacity for antimicrobial resistance surveillance is limited. We therefore retrospectively reviewed bacteraemia caused by ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* between 2010-2014 at Mahosot Hospital. Between 2010-2014, 360 patients were identified with *E. coli* and *K. pneumoniae* bacteraemia, representing 34.8 % of all patients with clinically significant bacteraemia. Seventy-two (20%) isolates produced ESBL; *E. coli* accounted for 55 (76%) and *K. pneumoniae* 17 (24%) respectively. The incidence of ESBL-producing *E. coli* bacteraemia increased steadily during the study period. By multiple logistic analysis, antibiotic use in the previous week was significantly associated with ESBL-positivity. Although multi-resistant, the majority of ESBL-producing *E. coli* and *K. pneumoniae* remained susceptible to meropenem and amikacin. This alarming increase in the incidence of ESBL-E has implications for empiric therapy of sepsis in Laos and ongoing surveillance is essential. This analysis is being extended back to 2000 to further model changes over the last 18 years and examine potential drivers.

F. Rapid detection of Extended-spectrum beta-lactamase (ESBL) in Gram negative bacteraemia. We are evaluating the performance of the beta LACTA test (Bio-Rad, Marnes-la-Coquette, France), a 15-minute chromogenic test for the presence of ESBL in blood cultures positive for Gram negative bacilli. It has performed well both on bacterial colonies and directly on blood culture broth, allowing detection of ESBL 24-48 hours earlier than conventional methods. Further analysis of the test accuracy and the impact of a positive test result on antibiotic selection by clinicians is underway.

G. Carbapenem-resistant Gram negative bacilli in Laos. We have described the first isolates of carbapenem-resistant *E. coli* and *Acinetobacter baumannii* in Laos. The *E. coli* included isolates from pus, the urinary tract and one case of bacteraemia, whilst the *A. baumannii* were mainly isolated from respiratory samples from the Intensive Care Unit of Mahosot Hospital. Molecular characterisation demonstrated the presence of the NDM carbapenemase in all *E. coli* isolates, and the OXA-23-like carbapenemase in all of the *A. baumannii*. With the recent beginning of carbapenem use in Laos this is extremely worrying and calls for increasing oversight of their use.

H. Chloramphenicol resistant *Neisseria meningitidis*. *N. meningitidis* is a relatively rare cause of disease in Laos and SE Asia but there is increasing evidence that an important minority of isolates are resistant to chloramphenicol. Further work is being conducted on isolates from Laos, Cambodia, Thailand and Vietnam to understand this better, but these data suggest that chloramphenicol should not be used for this infection until antibiotic susceptibility data are available.

I. Clinical microbiology of *Burkholderia pseudomallei.* By the end of 2018, LOMWRU had diagnosed 1,354 patients with culture-positive *B. pseudomallei* infection since 1999, confirming melioidosis as an important public health problem in Laos, although the number of cases diagnosed during 2018 (120) was slightly lower than in the two previous years. The case series describing the first 1,088 patients, twice as many as the largest case series in the literature, was presented by Dr Manophab Luangraj at the European Melioidosis Congress in Oxford in March 2018, and further analyses are being undertaken with a view to submitting this for publication. Discussions are also underway about holding a Lao national workshop on melioidosis during 2019, supported by DTRA, and a planning meeting was held in January 2019.

A review of melioidosis in Laos was included in a special issue of the journal 'Tropical Medicine and Infectious Disease' devoted to the global burden and challenges of melioidosis, co-edited by David Dance. Twenty-two country- or region-specific papers in this issue have already been published online (Chowdhury *et al.* 2018, Dance & Limmathurotsakul 2018, Dance *et al.* 2018a, Mukhopadhyay et al. 2018, Rolim *et al.* 2018, San Martin *et al.* 2018, Tauran *et al.* 2018, Win *et al.* 2018). This is the most comprehensive collection of papers published to date about the global epidemiology of the disease, and they confirm that it is likely that it is still being grossly under-diagnosed throughout the tropics, due to a lack of laboratories and a lack of awareness amongst healthcare staff. Even where laboratories exist, problems with identifying *B. pseudomallei* persist, as exemplified by a recent paper describing the mis-identification of the organism as *Acinetobacter* species in northern Thailand (Greer *et al.* 2018).

The paper describing our first evaluation of the Active Melioidosis Detect lateral flow immunoassay (AMD, InBios International, USA) was published during 2018 (Woods *et al.* 2018). The results of a second evaluation, undertaken by Maria Chiara Rizzi, who scored the highest possible marks for her project report from the University of Pavia, Italy, were presented at the European Melioidosis Congress in Oxford and a paper is in preparation. As reported last year, a strongly positive AMD result on pus, sputum or urine may help to speed up the diagnosis of melioidosis by at least a day, although the test has problems of both specificity and sensitivity when testing urine. Further work is underway with Dr David Aucoin from the University of Nevada to quantify the amount of extracellular polysaccharide, the antigen detected by the AMD test, in the serum and urine of patients with melioidosis. We are also in discussion with InBios, who are modifying the test with a view to addressing these problems, and hope to conduct further evaluations of a re-formulated kit.

During 2018, David Dance has been extending the work he has done previously on the very early history of melioidosis in Myanmar, Sri Lanka and Malaysia States. He gave a talk on this topic at the HOMSEA meeting in Vientiane in January and will be leaving LOMWRU in 2019 in order to focus exclusively on this project.

J. *Burkholderia pseudomallei* and the environment.

Work on the ecology of *Burkholderia pseudomallei* in collaboration with several organisations, both within Laos (particularly L'Institut de recherche pour le développement - IRD) and internationally, has continued during 2018.

A paper describing the initial results of Rosalie Zimmermann's project on the presence of *B.*

Audrey Rachlin soil sampling in a Vientiane ditch

pseudomallei in tributaries of the Mekong river was published in Nature Scientific Reports (Zimmerman *et al.* 2018). Further analyses of the samples she collected are still underway, including the evaluation of different DNA extraction protocols and quantitative real-time PCR (qPCR) of water filter and sediment samples at the Institute for Hygiene, Microbiology and Environmental Medicine in Graz, Austria, and digital droplet PCR (ddPCR) assay of the TTSS1 target at Laboratory Spiez in Switzerland. Digital droplet PCR is a very sensitive quantitative PCR technique, which has, to our knowledge, not yet been used to detect *B. pseudomallei* in the environment. Preliminary results have confirmed that qPCR without pre-enrichment is less sensitive than PCR after enrichment, but ddPCR has so far given disappointing results, although the assays will be run again again with qPCR-positive DNA samples. Further phylogenetic analyses, including whole-genome sequencing of 16 *B. pseudomallei* culture-positive river samples (water filters and sediment), are also being undertaken. A proposal to build on this work in a comprehensive 5-year long project entitled 'Understanding the environmental determinants of the occurrence and spread of *B. pseudomallei* to predict contamination hazards in the Mekong River Basin' has been prepared in collaboration with IRD and is currently under discussion with the US Defence Threat Reduction Agency (DTRA), who funded the original project.

The detection of *B. pseudomallei* in environmental samples presents considerable methodological difficulties due to the presence of many closely related but non-pathogenic species in soil and water. A paper describing the relative insensitivity of the published consensus *B. pseudomallei* culture method on soil samples from Laos was published (Dance *et al.* 2018c). Currently the most sensitive method for detection of environmental *B. pseudomallei* we have used to date is PCR following broth enrichment culture and this is the method we would use to screen any new location being tested for the presence of *B. pseudomallei*. However, this method is qualitative rather than quantititive, so we will continue to try to develop new and better quantitative methods, as our current culture methods are very laborious and semi-quantitative at best. One possible approach, which has given promising results in initial laboratory experiments by our collaborators at the University of California at Los Angeles (UCLA), is immunomagnetic separation, and we hope to conduct field evaluations of this method in 2019.

Our previous studies of *B. pseudomallei* in soil in Vientiane Province led to the hypothesis that *B. pseudomallei* may occupy specific micro-niches within the highly heterogeneous environment of soil (Manivanh *et al.* 2017 – see 2017 annual Report). Preliminary experiments to investigate this in collaboration with IRD have yielded interesting results but this work is hampered by the lack of a simple and reproducible quantitative detection method as mentioned above. It is hoped that the recent arrival of a specialist soil microbiologist, Dr Anne Pando, seconded to Laos for 2 years, will present an opportunity to undertake further method development and to investigate these micro-niches in more detail. IRD have established a sampling station in a field in Vientiane Province in order to investigate seasonal variations of the soil physico-chemical environment resulting from changes in soil saturation (due to the infiltration of rainwater and wide scale variations of the water table) with the aim of documenting the seasonal pattern of these variations in relation to the detection of *B. pseudomallei*. One early result of these studies was the confirmation that *B. pseudomallei* could be detected 290 cm below the soil surface, deeper than it has ever been found before. We suspect that it can be found at even greater depths and plan to explore this.

The DTRA funded project 'Eco-environmental signatures of danger to identify melioidosis-endemic hotzones' in collaboration with Dr Todd French of the UCLA has been in abeyance this year due to funding and staffing issues. However, discussions are underway with a view to reactivating this project, in collaboration with colleagues in MORU, Bangkok, during 2019. A spin-off from this collaboration was an analysis of the association between climatic factors and the occurrence of melioidosis in 870 patients from Laos and 173 patients from Cambodia (Bulterys *et al*. 2018). Associations were found with humidity, low visibility and maximum wind speeds in Laos, and humidity, rainy days and maximum wind speed in Cambodia. Children were at significantly higher risk of infection during humid months and pulmonary and disseminated infections were more common during windy months, suggesting a role for inhalation in initiating infection. It was also possible to estimate a likely incubation period of 1 week from these data.

Audrey Rachlin from the Menzies School of Health Research in the Northern Territory of Australia visited LOMWRU during the rainy season in June and July 2018 and undertook sampling of soil and water sources in Vientiane, mirroring the sampling she had undertaken in the city of Darwin, as part of her PhD studies. Sampling was performed at 40 different sites distributed across 30 villages within urban Vientiane. In total, 320 samples (120 water and 200 soil) were collected. Water samples were tested by culture and PCR at LOMWRU and 15/120 (12.5%) samples from 8/40 (20%) sites were positive for *B. pseudomallei*, establishing the presence of the bacterium within the environment of urban Vientiane. Direct PCR testing of the water samples is currently underway in Darwin and culture of the 200 soils has commenced. Isolates collected and stored during the survey are also being prepared for whole-genome sequencing with the aim of improving the current knowledge of *B. pseudomallei* sequence types and their distribution in Laos.

A paper describing '*B. thailandensis* capsular variants' (*B. thailandensis* that cross react with *B. pseudomallei* latex agglutination reagent), including one isolate from Laos, was published by Hantrakun *et al.* (2018) and cross-reacting *Burkholderia cepacia* have also been found in both clinical and environmental samples previously. However, no further isolates of cross-reacting *Burkholderia* species were identified during 2018. The work on the genetics and biochemical basis of these cross-reactions in collaboration with colleagues at Mahidol University and the Universities of South Alabama and Nevada has been delayed as a result of the relocation of two of our key collaborators.

In collaboration with colleagues in Yangon, investigation of the epidemiology of melioidosis in Myanmar has continued during 2018. The results of clinical surveillance have so far yielded only 9 cases identified in four hospitals in and around Yangon. However, two papers (Win *et al*. 2019a and Win et al. 2019b) describe the detection of environmental *B. pseudomallei* in townships in Yangon, and Ayeyawaddy and Bago Regions. A more extensive survey of the environment in Myanmar is underway as a collaboration between the Myanmar Oxford Clinical Research Unit, Shoklo Malaria Research Unit and the Department of Medical Research in Yangon. This has shown that the organism is widely but unevenly distributed across Myanmar, reinforcing the view that melioidosis is likely to be greatly underdiagnosed within the country.

K. Respiratory infections. We are continuing a prospective description of the clinical features and aetiologies of respiratory illness in children (ARIVI). This has given the first evidence that *Mycoplasma pneumoniae* does occur in Laos. Within this study, and working with the Murdoch Children's Research Institute, Melbourne, we are also estimating the hospital incidence of *S. pneumoniae* invasive disease, pneumococcal carriage and its serotypes to examine how their frequencies change with the introduction of 13 valent *S. pneumoniae* vaccination in Laos (Chan *et al.* 2018) – the PneuCAPTIVE study is funded by the Bill & Melinda Gates Foundation and has been extended to December 2019.

There are few data on the impact of PCVs in lower income settings, particularly in Asia. In 2013 Lao introduced the 13-valent pneumococcal vaccine (PCV13) as a 3 + 0 schedule (doses at 6, 10 and 14 weeks of age) with limited catch-up vaccination. Satzke *et al.* (2019) conducted two cross-sectional carriage surveys (pre- and two years post-PCV) to assess the impact of PCV13 on nasopharyngeal pneumococcal carriage in 5-8 week old infants (n = 1,000) and 12-23 month old children (n = 1,010) in Vientiane. Pneumococci were detected by quantitative real-time PCR, and molecular serotyping was performed using DNA microarray.

Second Point Prevalence Survey of antimicrobial use planning meeting at Mahosot Hospital Feb 2018

Post PCV13, there was a 23% relative reduction in PCV13-type carriage in children aged 12-23 months, and no significant change in non-PCV13 serotype carriage. In infants too young to be vaccinated, there was no significant change in carriage of PCV13 serotypes or non-PCV13 serotypes. Over 70% of pneumococcal-positive samples contained at least one antimicrobial resistance gene, which were more common in PCV13 serotypes. This provides evidence of PCV13 impact on carriage in a population without prior PCV7 utilisation and suggest that the reductions in PCV13 serotype carriage in vaccine-eligible children are likely to result in reductions in pneumococcal transmission and disease in Laos.

We have completed the LaCoRIS study, which is a large cohort study examining the aetiology of respiratory illness in the community in Vientiane, working with the Centre d'Infectiologie Christophe Mérieux and funded by the US Naval Medical Research Centre-Asia. The most commonly detected potential pathogens were *Streptococcus pneumoniae* and influenza A. A paper describing the findings has been submitted for publication.

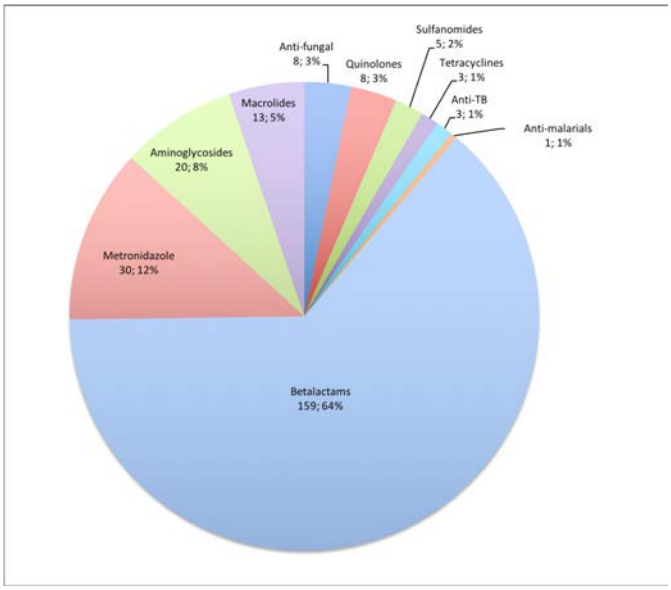
L. Gonorrhoea. Phouangsouvanh *et al.* (2018) described the antibiotic susceptibility patterns of *N. gonorrhoeae* in samples cultured at the Microbiology Laboratory, Mahosot Hospital during 2011-2015. A total of 12,281 genital samples were received during this period and 165 (1.3%) grew *N. gonorrhoeae*. 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. This fortunately means that the latest Lao national guidelines for treating gonorrhoea should still be effective. Contact tracing and treatment of partners will be a key intervention to reduce the burden of STIs.

M. *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 antimicrobial susceptibility, and how differences in pig farming between Vietnam (where *S. suis* is a more frequent cause of meningitis) and Laos may influence this.

N. Azithromycin resistance in *Shigella* spp. Also in collaboration with OUCRU, Darton *et al.* (2018) assessed the frequency and mechanisms of decreased susceptibility to azithromycin in clinical *Shigella* spp. isolates from Vietnam and Laos and used these data to suggest appropriate susceptibility breakpoints. Of 475 available *Shigella* spp. isolated in Vietnam and Laos between 1994 and 2012, 6/181 *S. flexneri* (3.3%, MIC≥16g/L) and 16/294 *S. sonnei* (5.4%, MIC≥32g/L) were phenotypically resistant to azithromycin. None of these were from Laos, although 14 of 45 Lao isolates showed resistance to quinolone antibiotics.

O. Non-typhoidal *Salmonella* serovars. Phuong *et al.* (2018) examined the causative serovars of non-typhoidal *Salmonella* invasive (iNTS) and non-invasive disease in Laos. They performed MLST and antimicrobial susceptibility profiling of 168 NTS (63 blood and 105 faecal) organisms isolated in Laos between 2000 and 2012. Six different serovars were isolated from blood. *S.* Enteritidis, *S.* Typhimurium and *S.* Choleraesuis accounted for >90% of the iNTS cases. In contrast, the isolates from diarrhoeal faeces comprised 18 different serovars, the mostly commonly identified being *S.* Typhimurium, *S.* Weltevreden, and *S.* Stanley. *S.* Enteritidis and *S.* Choleraesuis were significantly more associated with systemic disease than diarrhoeal disease. Organisms isolated from faecal samples were significantly less likely to be susceptible to ampicillin and trimethoprim-sulphamethoxazole than those isolated from blood. The majority of bloodstream isolates were non-susceptible to ciprofloxacin; this proportion was significantly greater than in the organisms isolated from faeces. There is a small but not-insignificant burden of iNTS disease in Laos; further clinical and epidemiological investigations are required to assess mortality and the role of co-morbidities such as HIV. Of interest some of these species, such as *S.* Weltevreden, are found in house gecko faeces, suggesting possible zoonotic transmission and potential opportunities for interventions to reduce human exposure.

P. *Streptococcus agalactiae* (Group B Streptococcus - GBS). Following a unique fish-borne outbreak of GBS serotype III, ST283, in Singapore in 2015, we have collaborated with Dr Tim Barkham at Tan Tock Seng Hospital in investigating the regional epidemiology of this organism. Of 38 patients with invasive GBS infection diagnosed by LOMWRU since 2000, 29 (76%) were infected with ST283. Invasive GBS



Point Prevalence Survey Data on Antimicrobial Use among inpatients at Mahosot Hospital June 2018

ST283 has thus been present in Laos since 2000 and possibly earlier, and appears also to be present in both fish and human infections in neighbouring countries, raising interesting questions about its epidemiology and the role of fish as a source of infection. Further work to understand the epidemiology of this intriguing finding for Laos and the wider SE Asian region, and the relationship between GBS, fish, both farmed and wild, and human infections, is being planned.

Q. Multilocus sequence typing of *Cryptococcus neoformans* var. grubii from Laos. In a project in collaboration with OUCRU, Thanh *et al.* (2018) used the consensus ISHAM Multilocus sequence typing (MLST) scheme to define the population structure of 81 clinical *C. neoformans* var. grubii isolates from Laos, in the global context. They observed a phylogeographical relationship in which the Lao isolates were similar to those in Thailand, being dominated (83%) by Sequence Types (ST) 4 and 6, whereas the population in Vietnam consists of an admixture of isolates dominated by the ST4/ ST6 (35%) and ST5 (48%) lineages. Where the ST5 lineage is present, some disease in HIV-uninfected patients is to be expected. They suggest diversity in the *C. neoformans* var. grubii population across Southeast Asia is driven by ecological rather than human host factors.

R. Antibiotic resistance & GARP. With increasing concern globally and in Laos about the public health consequences of antibiotic resistance, we have joined

3rd Point Prevalence Survey of antimicrobial use Team Mahosot Hospital June 2018

with the Ministry of Health and key stakeholders to form an AMR Technical Working Group that will work synergistically with the WHO/FAO/OIE AMR Committee to accumulate and analyse the current scientific evidence for AMR in Laos. For the report we are describing the antibiotic susceptibility patterns of Lao bacteria and antibiotic availability and use, and these will be compared with their frequency in adjacent countries. This work is with the Global Antibiotic Resistance Partnership (GARP; www.cddep.org/garp/home) and with OUCRU-Hanoi. The review of the AMR situation in Laos, based on published scientific and grey literature and unpublished data from Mahosot Hospital, will be discussed further in early 2019 and used to inform national AMR policy and Lao national antimicrobial treatment guidelines.

S. Health seeking behaviour, PPS and antibiotic use. We have conducted 19 rounds of the Global Point Prevalence Survey of Antimicrobial Consumption and Resistance (PPS) (www. http://www.global-pps.com/) at five hospitals in Laos and will repeat this three times a year to provide the first estimates of hospital inpatient antibiotic use for the country, monitor changes and use the data to inform optimal prescribing policy and antibiotic stewardship interventions.

There are few data describing where and why people seek health care at different levels at different hospitals in Asian cities. As a part of the University of Otago (New Zealand) funded e-Asia project, we have worked with colleagues in Myanmar and New Zealand to compare health-seeking behaviour between Yangon and Vientiane. For Laos we hope that this will increase our understanding of the catchment population of Mahosot Hospital and determinants of health-seeking behaviour in Vientiane and help us estimate the community-incidence of typhoid.

Marco Haenssgen from MORU-Bangkok has completed a study in Laos and Thailand to improve our understanding of patients' antibiotic-related health behaviour, to inspire more targeted and unconventional interventions (Haenssgen *et al.* 2018a, b). The project

Visit of Khun Jiab Sukhapiwat and MORU Supplies Team to LOMWRU

has tackled 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?

The protocol for this study in Chiang Rai (Thailand) and Salavan (Laos) has been published (Haenssgen *et al.* 2018a), with the intention to interview ~4,800 adults to produce district-level representative and social network data. Social research in AMR is nascent, but the detailed data on microlevel treatment-seeking behaviour will contribute an understanding of behaviour beyond awareness and free choice, highlighting, for example, decision-making constraints, problems of marginalisation and lack of access to healthcare, and competing ideas about desirable behaviour. In the first paper from this work (Haenssgen *et al.* 2018b) they describe how they implemented educational activity in two Lao villages to share general antibiotic-related messages and also to learn about people's conceptions and health behaviours. This included 1,130 adults over two rounds, including 58 activity participants and 208 villagers exposed indirectly via conversations in the village. They found that activity-related communication circulated among more privileged groups, which limited its indirect effects. Among participants, the educational activity influenced the awareness and understanding of "drug resistance", whereas the effects on attitudes were minor. Evidence on the behavioural impacts was sparse and mixed, but the range of possible consequences included a disproportionate uptake of antibiotics from formal healthcare providers. This study casts doubt on the continued dominance of awareness raising as a behavioural tool to address antibiotic resistance.

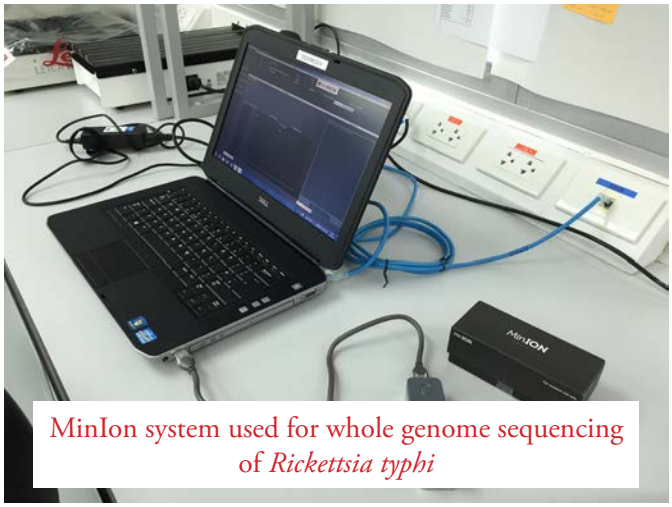
T. A stepped-wedge randomised trial of impact of a Lao language mobile phone prescribing app on antibiotic prescriptions in Laos. Dr Vilada Chansamouth has been awarded a Wellcome Trust International Training Fellowship to evaluate the impact of a Lao language mobile phone antimicrobial use guideline application on antimicrobial prescribing in Laos from 2019. This will be a stepped wedge randomized controlled trial in six central/provincial hospitals for 2 years. We are looking for the changes in proportion of prescribing consistent with guidelines and the cost- effectiveness of the intervention. Point prevalence surveys (PPS) on in- and outpatients antimicrobial usage will be performed every 4 months throughout the trial to collect prescribing data and hospital cost data.

W. Antimicrobial Resistance Networks. Ashley *et al.* (2018a) searched for supranational networks performing AMR surveillance in LMICs and assessed their organisation, methodology, impacts and

Good Clinical Practice (GCP) Training Mahosot Hospital Sept 2018 led by Mr Prayoon Yuentrakul of MORU-Bangkok

challenges. Since 2000, 72 supranational networks for AMR surveillance in bacteria, fungi, HIV, TB and malaria involving LMICs were found, with 34 still active. Networks were categorised as WHO/governmental (n=26), academic (n=24), or pharma initiated (n=22). Funding sources varied, with 30 networks receiving public or WHO funding, 25 corporate, 13 trust or foundation, and four funded from more than one source. The biggest challenges faced by these networks has been achieving high coverage across LMICs and complying with the recommended frequency of reporting. Antibiotic resistance surveillance requires a level of laboratory infrastructure and training which is not widely available in LMICs. The nascent Global Antimicrobial Resistance Surveillance System (GLASS) aims to build up passive surveillance in all member states. Past experience suggests complementary active approaches may be needed in many LMICs if representative, clinically relevant, meaningful data are to be obtained.

X. Grading antimicrobial susceptibility data quality. Ashley *et al.* (2018b) highlighted concerns regarding the reliability of antimicrobial susceptibility data. Assurance of the quality of microbiology data before publication is not based on any objective criteria, and the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) system is not designed to assess them. They propose that additional guidelines are needed to provide quality assurance of microbiological data before publication. The gold standard would be laboratory accreditation by the International Organization for Standardization, but this is unrealistic for many laboratories, especially those in low-income and middle-income countries. Quality improvement initiatives such as Strengthening Laboratory Management Toward Accreditation and WHO's Laboratory Quality Stepwise Implementation Tool are supporting laboratories to raise standards.



MinIon system used for whole genome sequencing of *Rickettsia typhi*

In the meantime, as a minimum they suggest that publication should be conditional on reporting of methods used, laboratory accreditation status, participation in external quality assurance schemes, and verification of adherence to accepted standard methods for establishing antimicrobial susceptibility. Antimicrobial resistance is a complex issue and is widely considered to be getting worse. However, the quality of the microbiology data that are being published to support this position could be substantially improved.

3. Leptospirosis

A. Leptospirosis rapid diagnostic tests. Simple rapid diagnostic tests (RDTs) are needed to enable health care workers, particularly in low resource settings, to diagnose leptospirosis early and give timely targeted treatment. We compared four commercially available RDTs to detect human IgM against *Leptospira* spp. in a head-to-head prospective evaluation in Mahosot Hospital. Patients with an acute febrile illness consistent with leptospirosis (n=695) were included in the study during the 2014 rainy season (Dittrich *et al.* 2018). Samples were tested with 4 RDTs: ('Test-it' (Life Assay, South Africa), 'Leptorapide' (Linnodee, Northern Ireland); 'Dual Path Platform' (DPP) (Chembio, USA) and 'SD-IgM' (Standard Diagnostics, South Korea). Diagnostic performance characteristics were calculated and compared to a composite reference standard combining PCR (*rrs*), microscopic agglutination tests (MAT) and culture.

Of all patients investigated, 39/695 (5.6%) were positive by culture, PCR or MAT. The sensitivity and specificity of the RDTs ranged greatly from 17.9-63.6% and 62.1-96.8%, respectively. None of the investigated RDTs reached a sensitivity and specificity of >90% for detecting *Leptospira* spp. infections on admission. This highlights the challenges associated with *Leptospira* diagnostics, particularly in populations with multiple exposures and emphasizes the need for extensive prospective evaluations in multiple endemic settings to establish the value of rapid tools for diagnosing fever aetiology. The results are disappointing for Laos in that no leptospiral antibody-detecting RDT was found that could fill the current gap in diagnosing leptospirosis in the country.

B. Comparison of PCR assays for detecting *Leptospira* spp. Woods *et al.* (2018) compared two molecular assays (rrs quantitative PCR (qPCR) versus a combined 16SrRNA and LipL32 qPCR) on different sample types for diagnosing leptospirosis in febrile patients presenting to Mahosot Hospital. *Leptospira* spp. culture and microscopic agglutination tests (MAT) were performed as reference standards. Bayesian latent class modelling was performed to estimate sensitivity and specificity of each diagnostic test. 787 patients were included in the analysis: 0.5% were *Leptospira* culture positive, 3.8% were MAT positive, 9.7% were rrs qPCR positive and 2.5% were 16SrRNA/LipL32 qPCR positive for pathogenic *Leptospira* spp. They concluded that serum and urine were better samples for qPCR than buffy coat, and 16SrRNA/*LipL32* qPCR performs better than *rrs* qPCR on urine. Quantitative PCR on admission is a reliable rapid diagnostic tool, performing better than MAT or culture, with significant implications for clinical and epidemiological investigations of this global neglected disease.

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 examination of the relationship between genomes and antibiotic susceptibility (below).

D. Susceptibility testing of *Leptospira* spp. Jennifer Boss from the LSHTM conducted antibiotic susceptibility testing of *Leptospira* spp. isolates for her MSc thesis (Boss *et al.* in press). No evidence of resistance to azithromycin, ceftriaxone, ciprofloxacin, doxycycline, gentamicin and penicillin G was found. As there are no published susceptibility guidelines for the *Leptospira* genus, zone interpretation was subjective. However, the large zone sizes suggest that resistance has not emerged to these six antibiotics in Lao *Leptospira* spp. These isolates have been sent for WGS at Institut Pasteur in Paris to investigate the relationship between susceptibility and genomes (above).

E. MALDI-TOF MS identification of *Leptospira* spp. Sonthayanon *et al.* (in press) used whole cell matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) for identification of *Leptospira* spp. from Thailand and Laos. Although leptospires can be identified by both genotyping and serotyping, they are time-consuming and established in few reference laboratories. The protein spectra of ninety-seven clinical isolates from Thailand and Laos gave 98.9% correct identification when compared

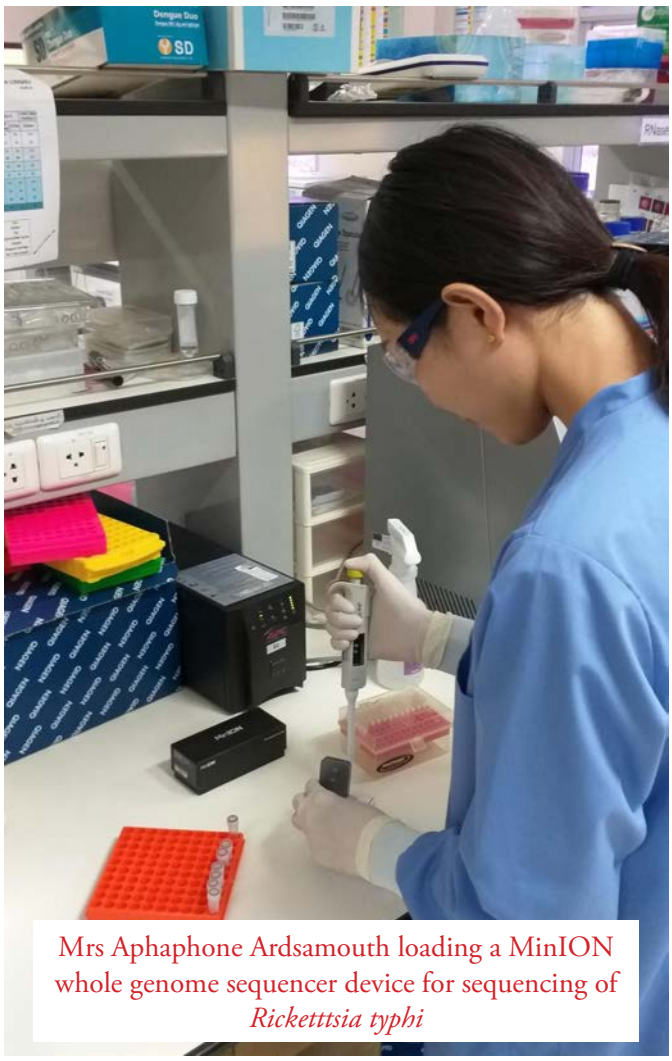
with 16S rRNA gene sequences method. Moreover, MALDI-TOF MS could identify spiked leptospires in urine.

4. Rickettsiology and related pathogens

A. Rapid diagnostic tests for scrub typhus. We have conducted a prospective study from 2016-2018 of the diagnostic accuracy of 5 different scrub typhus RDTs to determine which one(s) are optimal for the diagnosis of this important disease in rural Asia. This has finished in 2018 and immunofluoresence serological assays and PCR are being performed. We intend that these results should provide useful evidence to determine optimal choice of RDTs for use in Laos to diagnose scrub typhus.

B. Scrub typhus genotypes. The 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* isolates to examine whether different genotypes are associated with disease severity. Fleshman *et al.* (2018) performed successful WGS for 20 of these Lao isolates as part of a larger study comparing 40 *O. tsutsugamushi* genomes from diverse countries. They confirmed patterns of extensive homologous recombination likely driven by transposons, conjugative elements and repetitive sequences. Pan-genomic comparisons using 31,082 high-quality bacterial genomes from 253 species suggests that genomic duplication in *O. tsutsugamushi* is almost unparalleled. Unlike other highly recombinant species where the uptake of exogenous DNA largely drives genomic diversity, the pan-genome of *O. tsutsugamushi* is driven by duplication and divergence. Extensive gene innovation by duplication is most commonly attributed to plants and animals and is thought to be only a minor evolutionary mechanism for bacteria. More Lao genotypes will be sequenced by Dr Liz Batty in MORU-Bangkok. These data will help us understand the genetic diversity of *O. tsutsugamushi* in Laos and if severe scrub typhus and antibiotic susceptibility are associated with particular *O. tsutsugamushi* genotypes.

C. Whole genome sequencing of *Rickettsia typhi* using Oxford Nanopore MinION. Dr Ivo Elliott, as part of his Wellcome Trust Fellowship, established the portable MinION next generation sequencing platform in LOMWRU. Together with Drs Matt Robinson and Pruksa Nawtaisong, the first ever whole genome sequencing in Laos was performed.



Mrs Aphaphone Ardsamouth loading a MinION whole genome sequencer device for sequencing of *Ricketttsia typhi*

In collaboration with Dr Liz Batty (MORU), we demonstrated that every step of the process, from DNA extraction to bioinformatic analysis, can be performed locally. Despite the worldwide importance of murine typhus, genome-level analysis has previously been restricted to only a few strains, and none collected more recently than the 1970s. In contrast to *O. tsutsugamushi* (above) the *R. typhi* genome revealed an unprecedented lack of diversity between strains, despite published sequences covering a time period of nearly 90 years.

D. Revisiting the natural history of scrub typhus. Dr Ivo Elliott is completing his Wellcome Trust Fellowship, investigating the clinical epidemiology and ecology of scrub typhus in humans, chiggers and rodents. Most research on the natural history of the disease was performed prior to the 1970s. He is revisiting fundamental aspects of scrub typhus epidemiology using modern techniques including whole genome

sequencing and geographical information systems. His research attempts to begin to understand the complex population biology of the pathogen by linking *O. tsutsugamushi* genotypes to vector/host interactions and geographical locations at different scales. The sequencing of *O. tsutsugamushi* from humans, chiggers and rodents from Laos and Thailand will test the hypothesis that genomes do not differ between *O. tsutsugamushi* in these three niches.

There are many uncertainties about the ecology of scrub typhus and this work will increase our understanding and inform interventions to reduce transmission and link in with development of improved diagnostic assays and vaccines. This is in collaboration with many partners including the Oxford Centre for Human Genetics (OCHG) and the Chiang Rai MORU Unit. He will finish his DPhil thesis early in 2019.

E. Zoonotic disease vectors and potential human pathogens. We continued working with the Institut Pasteur du Laos and the US Naval Medical Research Center-Asia, looking for potential human pathogens, *Rickettsia, Bartonella, Orientia, Anaplasma* and *Ehrlichia* species, in further large collections of ticks from Khammouane Province. A number of samples identified the presence of *Rickettsia* spp. DNA, including *Rickettsia japonica*. In addition, evidence for *Ehrlichia* spp., *Anaplasma* spp. (including *Anaplasma bovis*) and *Leptospira* spp. were identified. This expands our knowledge of tick-borne bacterial pathogens in Laos from previous collections (Taylor *et al.* 2016) and has opened further interesting avenues for investigation, especially regarding the potential carriage of *Leptospira* spp. in ticks which has only been recently suggested in Europe. We have also made the first detection in Laos of *Midichloria mitochondii*, an intracellular pathogen of ticks, and are collaborating with the Institut Pasteur du Laos and researchers in Italy to investigate this further. We are also continuing our zoonotic vector work with the Institut Pasteur du Laos and the US Naval Medical Research Center-Asia with investigation of potential human pathogens in bats from caves in Khammouane Province, and the study of sandfly vectors. We have supported a Masters student, Dr Nguyen Manh Hung from the Lao Tropical and Public Health Institute, who investigated the presence of human pathogens in ticks, fleas and lice collected from domestic dogs in Vientiane. His work was the first such study to be conducted in Vientiane City, and identified a number of potential human pathogens including *Rickettsia felis* and the first detection in Laos of *Rickettsia asembonensis*. Robinson et al. (2018) reviewed the evidence regarding tick-transmitted pathogens in Asia.

F. *Bartonella*. With support from the US Navy we have been examining the seroprevalence of *Bartonella* spp. antibodies in Laos. 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 to identify One Health risks.

G. Antibiotic susceptibility of rickettsial species. There are very few data on the antibiotic susceptibility rickettsial pathogens globally. With historic reports of doxycycline and chloramphenicol resistant *Orientia tsutsugamushi* in northern Thailand, Weerawat Phuklia's PhD research is revisiting phenotypic and genotypic evidence for *O. tsutsugamushi* antibiotic resistance in Laos and Thailand. Phuklia *et al.* (2018) developed a method to enable the large-scale antimicrobial susceptibility screening of *Orientia tsutsugamushi* clinical isolates, using one timepoint and one concentration of antibiotics to considerably speed up the time to result. The Minimum Inhibitory Concentrations (MICs) of azithromycin, chloramphenicol and doxycycline for reference strains and clinical isolates, including isolates that were described as resistant 20 years ago, were determined. The resulting MICs were in line with previously published susceptibility data for all reference strains, except for Karp and AFSC-4. The data do not support the current existence of doxycycline- and chloramphenicol-resistant scrub typhus in northern Thailand and Laos.

We plan to expand this work to *R. typhi* (see H) and the Spotted Fever group of *Rickettsia* spp.

H. Trials of the antibiotic therapy of uncomplicated murine typhus and scrub typhus. Murine typhus, or infection with *Rickettsia typhi*, is a global but neglected disease without randomised clinical trials to guide antibiotic therapy. Doxycycline is commonly used but without objective evidence for optimum treatment duration. Azithromycin is a potential alternative. We conducted a prospective, open, randomised trial in

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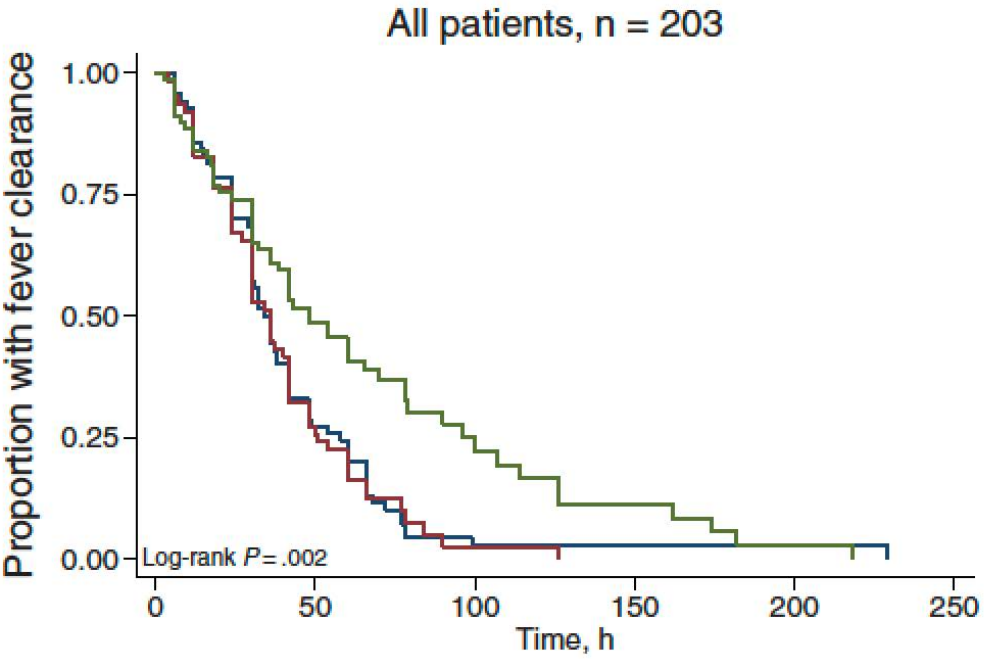
non-pregnant, consenting inpatient adults with rapid diagnostic test evidence for uncomplicated murine typhus at two hospitals in Vientiane, Laos (Newton *et al.* 2018). Patients were randomised to seven (D7)



No. at risk

Doxycycline (7 d) (blue color)	70	19	2	2	1	0
Doxycycline (3 d) (red color)	64	17	1	0	0	0
Azithromycin (3 d) (green color)	69	31	9	4	1	0

Kaplan-Meier plot of fever clearance for all murine typhus RDT positive patients who presented with or developed fever and randomised to either 7 days doxycycline, 3 days doxycycline or 3 days azithromycin

or three days' (D3) oral doxycycline or three days' oral azithromycin (A3). Primary outcome measures were fever clearance time (FCT) and frequencies of treatment failure and relapse.

Between 2004 and 2009, 216 patients (72 per arm) were enrolled; 158 (73.2%) patients had serology/ PCR-confirmed murine typhus; 24.1% were *R. typhi* PCR-positive. All patients survived to discharge. One patient in each treatment group withdrew. Treatment failure risk was greater following regimen A3 (22.5%) compared to D3 (4.2%) or D7 (1.4%) ($p<0.0001$). The area under the time-fever curve and FCT, for *R. typhi* PCR-positive patients, was significantly higher in patients following A3 than D3 (1.8 fold and 1.9 fold, respectively) and D7 (1.5 fold and 1.6 fold, respectively) ($p=0.005$ & $p=0.021$). No patients returned with PCR-confirmed *R. typhi* relapse.

These data suggest that, for Lao adults, azithromycin is inferior to doxycycline for the oral therapy of uncomplicated murine typhus. Three and seven days of doxycycline have similar efficacy. Azithromycin use in murine typhus should be reconsidered. Investigation of genomic and phenotypic markers of *R. typhi* azithromycin resistance is needed.

The parallel scrub typhus clinical trial, with the same treatment groups, is being analysed.

I. Laboratory-acquired scrub typhus and murine typhus infections. Blacksell *et al.* (2018) examined the literature on laboratory-acquired infections (LAIs) associated with scrub typhus (*Orientia tsutsugamushi*) and murine typhus (*Rickettsia typhi*) research to provide an evidence base for biosafety and biocontainment. Scrub typhus LAIs were documented in 25 individuals, with eight (32%) deaths during the pre-antibiotic era. There were 35 murine typhus LAI reports and no deaths. Results indicated that the highest-risk activities were working with infectious laboratory animals involving significant aerosol exposures, accidental self-inoculation, or bite-related infections. A risk-based biosafety approach for *in vitro* and *in vivo* culture of *O. tsutsugamushi* and *R. typhi* would require that only high-risk activities (animal work or large culture volumes) be performed in high-containment biosafety level (BSL) 3. laboratories.

5. Virology

The virology work of LOMWRU is strongly supported by IRD/ Aix-Marseille University, France. Virological aspects of CNS infections are discussed above. All dengue and JEV surveillance data are reported to the Lao Ministry of Health, National Centre for Epidemiology and Laboratory (NCLE) and WHO, and we work closely with NCLE, Institut Pasteur du Laos and WHO on coordinating surveillance. We are planning dashboard systems to make these data more easily accessible.

A. Dengue epidemiology. Thankfully 2018, like 2016 & 2017, had a relatively low incidence of dengue in Laos, unlike 2013. We continue to support, with diagnostic tests, dengue surveillance for Mahosot Hospital, Luang Nam Tha, Xieng Khouang and Salavan Provincial Hospitals.

We described the complexity of dengue epidemiology in Laos, demonstrating dynamic circulation that varies over space and time, according to serotype in Castonguay-Vanier *et al.* (2018). We recruited 1,912 consenting patients presenting with WHO dengue criteria at Mahosot Hospital between 2006 and 2010, and 1,413 patients with undifferentiated fever between 2008 and 2010 at Luang Namtha Provincial Hospital and 555 at Salavan (SV) Provincial Hospital. Peaks of dengue infection were observed in the rainy seasons. Importantly, 11% of confirmed cases in the provinces and 4.6% in the capital were detected during the dry and cool seasons (between December and February).

All four dengue serotypes were detected among the 867 RT-PCR positive patients: 76.9% dengue-1, 9.6% dengue-2, 7.7% dengue-4 and 5.3% dengue-3. Dengue-1 was the predominant serotype throughout, except in Luang Namtha in 2008 and 2009 when it was dengue-2. Phylogenetic analyses of dengue virus envelope sequences suggest concurrent multiple introductions of new strains as well as active dengue circulation throughout Laos and neighbouring countries. It is therefore of great importance to develop and strengthen a year-round nation-wide surveillance network, with coordination with neighbouring countries, in order to collect data that would allow anticipation of public health issues caused by the occurrence of large dengue outbreaks.

B. Hand, Foot and Mouth disease. We continue to support enteroviral RT-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.

C. *Zika virus* infection. With current global concern of the public health impact of this emerging pathogen and association of infection with microcephaly and other neurodevelopmental sequelae, we are working with partners to build diagnostic capacity at Mahosot Hospital. We have expanded the work on RT-PCR detection of *Dengue virus* from RDTs (see 2017 Annual Report) to detect *Zika virus* and *Chikungunya virus* in order to facilitate national surveillance of these pathogens, with the Naval Medical Research Center-Asia. Pastorino *et al.* (2018) conducted a seroprevalence study amongst blood donors in Vientiane, Laos working with the Lao Red Cross. Sera from 359 asymptomatic consenting adult donors in 2003-2004 and 687 in 2015 were screened for anti-ZIKV IgG using NS1 ELISA assay. Non-negative samples were confirmed for anti-ZIKV neutralizing antibodies by Virus Neutralisation Tests. The findings suggest that ZIKV has been circulating in Vientiane over at least the last decade. ZIKV seroprevalence observed in the studied blood donors was low, 4.5% in 2003-2004 with an increase in 2015 to 9.9% ($p = 0.002$), possibly reflecting the increase of ZIKV incidence. With a low herd immunity in Vientiane, ZIKV represents a risk for future large scale outbreaks. Implementation of a nation-wide ZIKV surveillance network is needed.

D. Improved RT-qPCR detection of *Japanese encephalitis virus* (JEV) RNA. Bharucha *et al.* (2018b) performed a systematic review of published JEV RT-PCR protocols, and evaluated them *in silico* and *in vitro* alongside new primers and probes designed using a multiple genome alignment of JEV strains from GenBank. The new assays included pan-genotype and genotype specific assays targeting genotypes 1 and 3. Of ten RT-qPCR assays, they selected three with the lowest limit of detection (LOD). One of the assays, targeting NS2A, showed the best results, with LOD approximately 4 copies/ reaction, and no cross-reaction on testing closely related viruses in the JEV serocomplex, *West Nile virus* and *St. Louis Virus*. The optimised assays were validated in consecutive patients with central nervous system infections in Laos, testing paired CSF and serum samples. A JEV specific RT-qPCR assay with at least 1 $\log_{10}$ improved analytical sensitivity as compared to existing assays was developed.

E. Detection of *Japanese encephalitis virus* RNA in human throat samples and urine. Bharucha *et al.* (2018c) conducted a pilot study among eleven JEV IgM MAC-ELISA positive patients. Although CSF and serum samples were JEV RT-qPCR negative, two had JEV RNA detected in their throat swabs, confirmed by sequencing of RT-qPCR products. As the first apparent report of JEV RNA detection in human throat samples, they suggest that testing patients' throat swabs for JEV RNA should be performed on a larger scale to investigate the epidemiology of JEV in human throats. Throat swabs are an easy and non-invasive tool that could be rolled out to a wider population to improve knowledge of JEV molecular epidemiology. Bharucha *et al.* (in press) also examined for the detection of JEV by RT-qPCR in human urine. However, degradation of JEV RNA occurred in human urine even for short storage periods at 4°C or -80°C and they suggest that more studies are needed with techniques to stabilise JEV RNA in urine.

F. *Japanese encephalitis virus* patient clinical follow up. Dr Phouvieng Douangdala successfully completed his University of Health Sciences thesis on changes through time of 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%).

6. One Health

We participated in two One Health projects, in addition to the studies above on scrub typhus and leptospirosis, that finished in 2018. 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) and Institut Pasteur - Cambodia to conduct field surveillance of zoonotic diseases at human-wildlife interfaces. COMACROSS (http://www.onehealthsea.org/comacross) is also an EU funded project, led by the Centre de coopération internationale en recherche agronomique pour le développement (CIRAD) and linked to the SEAe project (above).

A. Perception of health risk due to 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. Markets selling wildlife are common aspects of Southeast Asian culture and economy. However, their role in circulation and transmission of both endemic and emerging disease is a source of concern in a hotspot of disease emergence. In Laos, live and dead wild animals are also frequently found in markets, despite legislation against the bushmeat trade. This is generally considered to increase the risk of disease transmission and emergence, although whether or not wildlife vendors do indeed have increased incidence of zoonotic disease has rarely been assessed. In preparation for a future longitudinal study of market vendors, we conducted a pilot survey of market vendors of wildlife meat, livestock meat and vegetables, to identify demographic characteristics and potential control groups within the market. We also provided a baseline of the risk perception for infectious diseases among market vendors, and assessed the association between risk perception and risk mitigation behaviours. The surveys conducted among 177 vendors revealed similar age, sex, ethnic background and geographical origin between vendor types, but differences in professional background and history for livestock meat vendors. The perception of disease risk was very low across vendor types, and so was the reported use of protective equipment, and the two appeared uncorrelated. Personal risk discounting and assumptions about likely transmission routes may explain this lack of association. This information is important to the careful development of future research, risk communication and risk mitigation policy.

B. Pathogens in wildlife in markets. Also as part of LACANET, collections of wildlife specimens were made at wildlife trade markets across Laos by the Wildlife Conservation Society. We are testing these samples for the presence of a number of key pathogen species. From January 2015 to October 2017, 717 specimens from 359 animals were sent to LOMWRU for analysis. We found that 69 animals contained *Leptospira* spp. and 19 animals contained *Rickettsia* spp., including *R. typhi* and *R. felis*. During the testing process, we also identified samples containing *O. tsutsugamushi*, *L. garvieae*, *Kurthia* spp., *Ehrlichia* spp. TC251-2, *Anaplasma marginale*, *A. phagocytophilum* and *A. bovis*. Our findings suggest that *Leptospira* spp. are the most frequently identified pathogens, of those we tested for, in wildlife trade. The discovery of *R. felis*, an emerging rickettsial pathogen, and *O. tsutsugamushi* are the first reported cases in a squirrel species.

Pallas's, Grey-bellied, and Red-cheeked squirrels were the most common wildlife species sampled in markets. Our observations indicated their potential as multiple disease reservoirs, with a total of six different pathogen species being identified in squirrels. We received most samples from the markets located in Champasak and Bolikhamxay provinces indicating that these areas may be high volume markets for wildlife trade, with Pha-hom and Thajok serving as possible hotspots for cross transmission. However, the highest positivity rates were found in the samples collected at the Provincial Offices of Forest Inspection (PoFI), especially the Bolikhamxay and Vientiane PoFIs where wildlife were confiscated. Species identification of positive *Leptospira* spp. and *Rickettsia* spp. are in progress to determine whether they are pathogenic strains. We are also investigating the genotype of *O. tsutsugamushi* detected in squirrel samples. We have performed DNA extraction of the ectoparasites collected along with the wildlife samples and they are now being analyzed by PCR as part of the LACANET project.

C. Serology of market vendors to wildlife-borne diseases. Dr Nilandone Senvanpan conducted this study for her IFMT MSc thesis research in 2017, to evaluate the immunological status regarding three wildlife and human pathogens (murine typhus, scrub typhus and leptospirosis) in vendors working in Lao markets**.** Consenting fresh food vendors (150) in major Lao markets in Xiengkhouang, Bolikhamxay and Saravan Provinces were asked for blood samples and a series of questions related to their work. The prevalence of anti-leptospiral antibodies was 11% for IgM and 6% for IgG, 3% for IgM and 25% for IgG for anti-murine typhus antibodies, and 7% for IgM and 19% for IgG for anti-scrub typhus antibodies. Sellers of wildlife meat had an increased probability of having anti-scrub typhus IgG. The study has been continued with two more sampling rounds in order to investigate changes in vendor serostatus through time as part of the LACANET project.

D. Perceptions of risks associated with bats. In order to contribute to recommendations improving the management of risks linked to the potential emergence

of Nipah and other bat-borne infectious diseases in Laos, we conducted a project to understand better bat-human interactions in the framework of daily activities (agriculture, fruit harvesting, hunting, market chain etc.). This was conducted on the periphery of Vientiane, amongst the general population and amongst palm fruit traders as part of the COMACROSS project and the data are being analyzed.

E. *Angiostrongylus cantonensis*. Following from a successful visit by the Worldwide Universities Network-funded *Angiostrongylus* Network Group in late 2018, diagnosis of potential *Angiostrongylus cantonensis* infections has improved and has produced a strong interest between infectious disease clinicians. Applications for funding further work under the One Health framework on this neglected pathogen will be submitted in 2019.

F. Q-fever and Brucellosis in goats. Goat raising is a growing industry in Laos, with minimal disease investigation to date, especially zoonoses. LOMWRU keeps goats for blood agar preparation. Burns *et al.* (2018a) determined the seropositivity of two zoonotic diseases: Q fever (causative agent *Coxiella burnetii*) and Brucellosis (*Brucella* species) in goats across five Lao provinces. Overall seropositivity of *C. burnetii* was 4.1% and *Brucella* spp. was 1.4%. They concluded that Lao goats have been exposed to both *C. burnetii* and *Brucella* spp. However, the risk of clinical disease has not yet been determined and there is an urgent need to determine human and goat health risks and economic losses caused by Q fever and Brucellosis.

7. Malaria

A. 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 artemisinin combination therapies (ACTs). We have participated, with the Centre for Malariology, Parasitology and Entomology, in the multicentre TRAC-2 study, coordinated by MORU-Bangkok, at Sekong Provincial Hospital. This was a randomised clinical trial comparing parasite clearance in uncomplicated falciparum malaria between artemether-lumefantrine and artemether-lumefantrine plus amodiaquine. These data will be useful for informing optimal future ACT Government policy. With the decline in *Plasmodium falciparum* malaria in Laos, patient recruitment was slow with only 11 patients recruited to the trial that is now being analysed.

B. 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 colleagues in MORU-Bangkok, including Professor Mallika Imwong and Professor Olivo Miotto. Working with MORU-Bangkok and the Nanyang Technological University, Singapore and other institutions, Zhu *et al.* (2018) used the TRAC-1 (see Annual Report 2014) data and found the majority of *P. falciparum* parasites are transcriptomically converged within each geographic site with two broader physiological profiles across the Greater Mekong Subregion. Worryingly, working with Professor Olivo Miotto in MORU-Bangkok, we found evidence for molecular markers of piperaquine resistance in southern Laos, as in adjacent Thailand and Cambodia, calling into question the efficacy of DHA-piperaquine in falciparum malaria treatment in Laos.

C. 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, an 8-aminoquinoline, 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, MORU 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).

To further inform safer use of 8-aminoquinolines in the Greater Mekong Subregion, Bancone *et al.* (2019) performed a multi-centre study to assess the prevalence of G6PD deficiency and to identify the main G6PD

Dr Markus Winterberg and team, MORU Bangkok, giving chemical safety training December 2018

variants in samples collected in Cambodia, Lao PDR, Myanmar, Thailand and Vietnam. Blood samples were characterized for G6PD phenotype using the Fluorescent Spot Test and then genotyped for a panel of G6PD mutations. G6PD deficiency was found to be common in the region with an overall mean prevalence of deficient or mutated hemizygous males of 14.0%, ranging from a mean 7.3% in Thailand, 8.1% in Lao PDR, 8.9% in Vietnam, 15.8% in Myanmar and 18.8% in Cambodia. Mahidol and Viangchan mutations were the most common and widespread variants found among the nine investigated. They concluded that owing to the high prevalence of G6PD deficiency in the Greater Mekong Subregion, strategies for vivax malaria elimination should include point-of-care G6PD testing (both qualitative and quantitative) to allow safe and wide treatment with 8-aminoquinolines.

Henriques *et al.* (2018) evaluated the performance of the fluorescent spot test (Trinity, Ireland; FST) and a G6PD RDT (Carestart, USA) against reference spectrophotometry in detecting G6PD deficiency. Participants were enrolled during cross-sectional surveys in Laos and by purposive sampling in Cambodia. Amongst 757 participants in Laos and 505 in Cambodia FST and RDT performed best at 30% cut-off activity and performed significantly better in Laos than in Cambodia. When defining intermediate results as G6PD deficient, the FST had a sensitivity of 100% and specificity of 90% in Laos, and sensitivity of 98% and specificity of 71% in Cambodia. The RDT had sensitivity and specificity of 100% and 99% in Laos, and sensitivity and specificity of and 93% in Cambodia. The RDT performed significantly better than the FST when intermediate FST results were defined as G6PD deficient. They concluded that interpretation of RDT results requires more training but is a good alternative to the FST.

D. Targeted malaria elimination. Recently it has been realised that a significant proportion of apparently well people in rural Asia have *P. falciparum* infections that are not detected by RDTs or microscopy but are evident by high blood volume ultra-sensitive quantitative PCR (uPCR). With these high frequencies of apparently asymptomatic *P. falciparum,* trials of Targeted Malaria Elimination (TME) with DHA-piperaquine and single low dose primaquine in Nong District, Savannakhet Province, with key public engagement actions, started in 2016 and were completed in late 2017. This was funded by the Bill and Melinda Gates Foundation with CMPE and MORU-Bangkok. Dr Koukeo Phommasone, Dr Tiengkham Pongvongsa and Dr Bipin Adhikari have been conducting different aspects of this work for their PhD theses.

Three rounds of mass drug administration (MDA, DHA-piperaquine) with intensive public engagement programs were completed in the two intervention villages, but not in the two control villages that had MDA one year later, with analysis of the comparative epidemiology of *P. falciparum* and *P. vivax*.

Pongvongsa *et al.* (2018) examined the dynamics of asymptomatic *Plasmodium falciparum* infections following mass drug administrations. A total of 1,036 residents were enrolled in early MDA villages and 883 in control villages (deferred-MDA). Tri-monthly parasitaemia surveys using uPCR were conducted for a year in the 4 villages. Eighty-four percent of the residents participated in the MDAs, of whom 90% completed 3 rounds of MDA (9 doses). In the intervention villages, the prevalence of asymptomatic *P. falciparum* infections decreased by 85% after MDA from 4.8% at baseline to 0.7% after one year.

In the control villages there was a decrease of 33% in *P. falciparum* prevalence from 17.5% to 11.6% after one year. In bivariate and multivariate analyses *P. falciparum* infections were significantly reduced with early MDA and completion of 3 MDA rounds. A quarter of participants reported adverse events of which 99% were mild. They found a significant reduction in *P. falciparum* prevalence and incidence following MDA and concluded that MDA was safe, well tolerated, feasible, and achieved high population coverage and adherence. They suggest that MDAs must be integrated in multi-pronged approaches such as vector control and preventive measures with a focus on specific risk groups such as mobile, migrant population and forest goers for a sustained period to eliminate the remaining parasite reservoirs.

Adhikari *et al.* (2018a) investigated why people participate in MDA. Lao Theung communities were cohesive and community members tended to follow each other's behaviour closely including participation in MDA. Factors such as understanding the concept and rationale of the study, free health care, collaboration with the village volunteers, support from authorities and cohesive communities contributed in building trust and high population coverage in MDA.

In a further paper, Adhikari *et al.* (2018b) explored perceptions of asymptomatic malaria infection and their implications for malaria control and elimination in Laos. A minority (14.2%) of respondents agreed that a seemingly healthy person could have malaria parasites in his or her blood. Half (52%) disagreed and one third (33.8%) were unsure. Respondents who responded that "MDA aims to cure everyone", "MDA is to make our community malaria free" and "I will take part in future MDA" were more likely to accept the idea of asymptomatic malaria. They concluded that awareness of asymptomatic malaria infections, and MDA as a tool to eliminate malaria, was low. With the need to target asymptomatic malaria carriers for elimination efforts in the GMS, accompanying community engagement must build trust in interventions through the active collaboration of government stakeholders, key local persons and community members. This entails training and devolving responsibilities to the community members to implement and sustain the control and elimination efforts von Seidlein *et al.* (2019) included the Lao TME data in a report, from 16 remote village populations in four GMS countries with prevalent artemisinin resistance, of a cluster-randomized trial to assess the duration of effectiveness of MDA on falciparum malaria incidence and prevalence. As above, half of villages were selected by restricted random methods to receive early MDA and the other 8 villages served as controls for 12 months after which they received deferred MDA. 8,445 residents (52%) living in 16 villages were randomised. Of 4,135 (52%) residents living in eight villages randomised to early MDA, 3,790 (86%) participated in at least one MDA round and 2,520 (57%) participated in all three rounds. By three months (M3) the *P .falciparum* prevalence had fallen by 92% (from 5.1% to 0.4%) in MDA villages and by 29% (from 7.2% to 5.1%) in control villages. Over the following 9 months the *P. falciparum* prevalence increased in early MDA villages to 3.3% and in control villages to 6.1%. Individual protection was proportional to the number of completed MDA rounds. Of 221 participants with subclinical *P. falciparum* infections who participated in MDA and could be followed up, 207 (94%) cleared their infections including 9 of 10 infections with artemisinin and piperaquine resistance. They concluded that for these 4 countries, adding three rounds of MDA with dihydroartemisinin-piperaquine to community-based basic malaria control measures, reduced the incidence and prevalence of falciparum malaria over a one-year period in areas affected by artemisinin resistance. Malaria was reintroduced, presumably from surrounding areas. MDA deployed in contiguous areas of higher transmission could be a useful additional tool for accelerating malaria elimination in the GMS.

F. Artemether-lumefantrine (AL) dosing for malaria treatment in young children and pregnant women.

Kloprogge *et al.* (2018) conducted a pharmacokinetic-pharmacodynamic meta-analysis of lumefantrine and of its metabolite, desbutyl-lumefantrine, in order to inform optimal dosing regimens in all patient populations. Venous plasma lumefantrine

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concentrations 7 days after starting standard AL treatment were 24.2% and 13.4% lower in children weighing <15 kg and 15–25 kg, respectively, and 20.2% lower in pregnant women compared with non-pregnant adults. They concluded that revised AL dosing regimens for young children and pregnant women would improve drug exposure but would require longer or more complex schedules. These dosing regimens should be evaluated in prospective clinical studies to determine whether they would improve cure rates, demonstrate adequate safety, and thereby prolong the useful therapeutic life of this valuable antimalarial treatment.

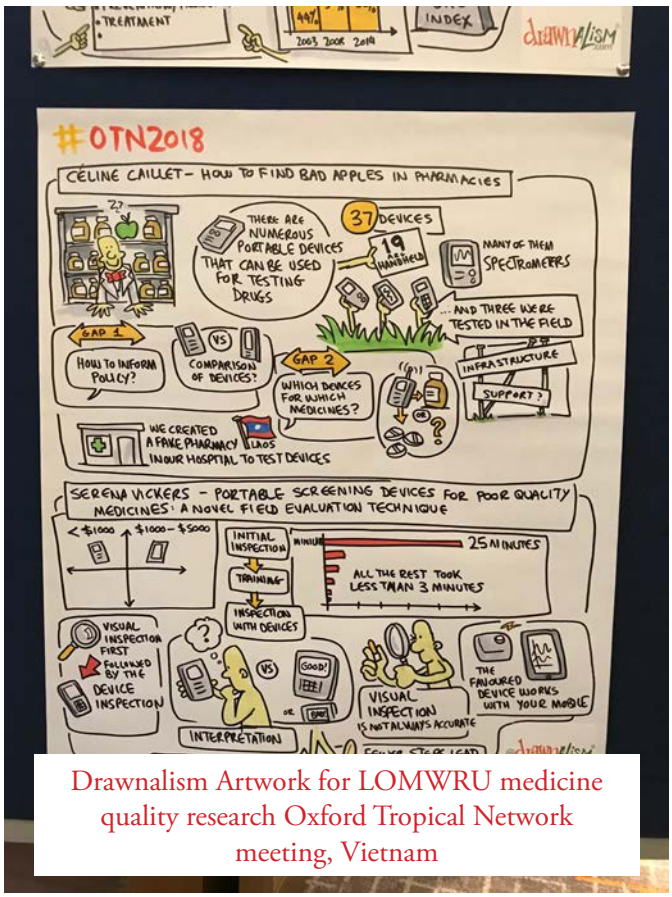
G. Vivax malaria treatment. At the same site as the TME study, we completed, with CMPE and MORU-Bangkok, a randomised, single-blinded controlled treatment trial of subclinical vivax infections with primaquine in Nong District, Savannakhet Province. This compared dihydroartemisinin-piperaquine therapy plus 14 days of supervised primaquine (7mg/kg total dose) versus dihydroartemisinin-piperaquine therapy plus 14 days placebo not containing primaquine. The primary objective was 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). This study has been completed and is being analysed.

H. Genetics of *Plasmodium malariae* from Laos. *Plasmodium malariae* is characterized by its long asymptomatic persistence in the human host. Saralamba *et al.* (2018) characterised the genetic polymorphisms in *pmcsp* of Asian isolates of *P. malariae* from patients in Thailand, Myanmar, Laos, and Bangladesh. *pmcsp* was amplified using semi-nested PCR and the resulting 89 *pmcsp* sequences were analysed together with 58 previously published *pmcsp* sequences from African parasites. Polymorphisms in the central repeat region of *pmcsp* showed association with the geographical origin of *P. malariae* isolates and can be potentially used as a marker for genetic epidemiology of *P. malariae* population.

Srisutham *et al.* (2018) further examined the genetic diversity of the three important antigenic protein genes in *P. malariae* from patients in Thailand, Myanmar and Laos by PCR. Most of the nucleotide substitutions were non-synonymous, which indicated that the genetic variations of these genes were maintained by positive diversifying selection, thus, suggesting their role as a potential target of protective immune response. High mutational diversity was observed in *P. malariae trap* and *ama1* as compared to *p48/45* in *P. malariae* samples isolated from Thailand, Myanmar, and Laos. These results suggested that P48/45 might be a good vaccine candidate against *P. malariae* infection because of its sufficiently low genetic diversity and highly conserved amino acids.

8. Intensive Care Medicine

Dr Rebecca Inglis is continuing her DPhil mixed methods research with Dr Khamsay Detleuxay, Head of Mahosot Hospital Adult Intensive Care Ward, and Prof. Arjen Dondorp in MORU-Bangkok, on three



Drawnalism Artwork for LOMWRU medicine quality research Oxford Tropical Network meeting, Vietnam

ICUs in Laos to investigate the optimal design of a Lao-run ICU training course. Inglis *et al.* (2018) reviewed the literature on the management of acute respiratory failure in low- and middle-income countries. They emphasise that mechanical ventilation is expensive and associated with considerable mortality (36 and 72%) and a high rate of iatrogenic complications in many LMICs. Measures to avert the need for invasive mechanical ventilation in LMICs are showing promise: bubble continuous positive airway pressure has been demonstrated to decrease mortality in children with acute respiratory failure and trials suggest that noninvasive ventilation can be conducted safely in settings where resources are low. They conclude that management of patients with acute respiratory failure in LMICs should focus on avoiding intubation where possible, improving the safety of mechanical ventilation and expediting weaning.

9. Dermatology

Skin disease and quality of life in a rural Lao community. In rural communities where access to healthcare may be limited and individuals rely on farming for food and income, the impact of skin diseases may be especially important. There are very few data from Laos. Wootton *et al.* (2018) assessed skin disease prevalence and impact in a rural village using the Dermatology Life Quality Index amongst 340 participants. Of these 181 (53%) had a skin disease, mostly eczema (22%), dermatophyte infections (19%), acne (10%), scabies infestation (9%), melasma (8%) and pityriasis versicolor (4%). Just over half of those with skin disease (51%) completed the DLQI, with scores ranging from 0 to 24. Those with skin problems on examination were significantly more likely to be farmers, have had a previous skin problem, be older or live in a smaller family. This study established the high rate of skin disease in a rural community and the associated impact these diseases have on health-related quality of life, suggesting the need for enhanced dermatology services in rural areas.

10. Medicine quality & pharmacy

A. The Medicine Quality Research Group. This group is based within LOMWRU and the MORU Tropical Health Network and is linked within the WorldWide Antimalarial Resistance Network (WWARN) & Infectious Disease Data Observatory (IDDO).

Medicine Quality & Public Health Course attendees and tutors Wolfson College, Oxford, September 2018

LOMWRU & IDDO Medicine Quality Group meeting with Dr Sivong Sengaloundeth at the Food & Drug Department, Government of Lao PDR

It continues to tabulate and map reports of the quality of antimalarials (see http://www.wwarn.org/aqsurveyor/). WWARN is now within the Infectious Diseases Data Observatory (IDDO; https://www.iddo.org/medicine-quality) and we are expanding this system, with Wellcome Trust support, for other classes of essential medicines as the MAPQAMP project. We are tabulating the accessible data on the quality of maternal health medicines, antibiotics, antidiabetics, anti-retrovirals, anti-tuberculous, cardiovascular and veterinary medicines and vaccines and are mapping these. We are about to Beta test these surveyors for release in the spring on the IDDO website. They will also be analysed for reviews on the quality of these essential medicines in 2019.

We are working with HealthMap (http://www.healthmap.org/en/) on scouring the lay literature in multiple languages for reports of poor quality medicines and using text mining to automate searches. These have been mapping on a globe system by IDDO and we are about to Beta test this system for release in the spring.

We are also working with the United States Pharmacopeia to build an individual sample database and mapping system, to include data from the USP MQDB system (see http://apps.usp.org/app/worldwide/medQualityDatabase/terms.html). With IDDO we are working on a REDCap system for the straightforward but controlled data entry for medicine quality surveys.

MQPH Course Course Dinner Wolfson College, Oxford, September 2018

B. The unexpected power of toothpicks. Our collaborators Mathew Bernier and Facundo Fernandez of Georgia Institute of Technology in Atlanta, are experimenting with portable and low cost ionization sources for mass spectrometry for analysing medicines, important as mass analyzers become potentially portable. With triboelectric nanogenerators (TENG), the charge required to produce ions needed for sensitive analyte detection can be obtained reproducibly without the need for high voltage electrical circuitry. Experiments performed in this study show that a simple extraction into methanol along with the use of a sliding freestanding (SF)-TENG-powered electrospray from a dry wooden toothpick can provide good detection capabilities, but with much simpler instrumentation (Bernier *et al.* 2018).

C. Benchtop low-field 1H Nuclear Magnetic Resonance detection of falsified medicines. Assemat *et al.* (2018) evaluates the potential of a benchtop low-field (LF) Nuclear Magnetic Resonance (NMR) spectrometer for uncovering drug falsification by focusing on the analysis of fifteen erectile dysfunction and nine antimalarial medicines. After a simple and rapid sample preparation and ≈5 min of spectrum recording, LF 1H NMR allows to conclude on the quality of the medicine: presence or absence of the expected active pharmaceutical ingredient (API), presence of unexpected API, absence of any API.

D. A review of field detection devices for medicines quality screening. As part of the Asian Development Bank project (below), Vickers *et al.* (2018) reviewed the plethora of innovative portable devices to screen for poor quality medicines that lead to hope that they could empower medicine inspectors and enhance surveillance. Information comparing these new technologies is woefully scarce. Scientific studies evaluating the performances/abilities of portable devices to assess any aspect of the quality of pharmaceutical products were included. Forty-one devices, from small benchtop spectrometers to 'lab-on-a-chip' single-use devices, with prices ranging from <US$10 to >US$20 000, were included. Only six devices had been field-tested (GPHF-Minilab, CD3/CD3+, TruScan RM, lateral flow dipstick immunoassay, CBEx and Speedy Breedy). The median (range) number of active pharmaceutical ingredients (APIs) assessed per device was only 2 (1–20). The majority of devices showed promise to distinguish genuine from falsified medicines. However, the 10 spectroscopic devices tested for their abilities to quantitate APIs required processing complex API-specific calibration models. Scientific evidence of the ability of the devices to accurately test liquid, capsule or topical formulations, or to distinguish between chiral molecules, was limited. There was a dire lack of evidence on cost-effectiveness or where in the pharmaceutical supply chain these devices could be best deployed.

They concluded that although a diverse range of portable field detection devices for medicines quality screening is available, there is a vitally important lack of independent evaluation of the majority of devices, particularly in field settings. Intensive research is needed in order to inform national medicines regulatory authorities of the optimal choice of device(s) to combat poor quality medicines.

E. Evaluation of a new NIR medicine quality screening device. We are 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 (NIR) device for screening tablet quality. We are analyzing the results before writing this work up.

F. Evaluation of diverse medicine quality screening devices. With funding from the Asian Development Bank (ADB) we investigated the comparative diagnostic accuracy and cost-effectiveness of a diversity of portable and handheld medicine quality screening devices in the laboratory and in the 'field'. With colleagues at the Georgia Institute of Technology in Atlanta, USA, we evaluated the diagnostic accuracy of a wide diversity of devices in the laboratory. We then worked with the Bureau of Food and Drug Inspection inspectors to understand their advantages and disadvantages in an evaluation pharmacy we created at Mahosot Hospital. Health economists from MORU in Bangkok also analyzed the cost-effectiveness of the devices compared to the current practice in Laos.

The results were discussed at a meeting in Vientiane on 9th and 10th April 2018. with attendees from medicine regulatory authorities of Laos, Thailand, Cambodia, Myanmar, Vietnam, Indonesia and Liberia along with observers from the WHO from the Lao Country Office, WPRO, SEARO and Geneva, the Global Fund Lao country office; the Asian Development

Medicine Quality & Public Health Conference

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Bank, UNDP Geneva, the Wellcome Trust and the US Pharmacopeial Convention. Additional staff from the Lao MRA, including from provinces, and from the Lao University of Health Sciences (UHS) also attended the meeting.

Although a diverse range of portable field detection devices for medicines quality screening is available, there is a vitally important lack of independent evaluation of the majority of devices, particularly in field settings. Intensive research is needed in order to inform national medicines regulatory authorities of the optimal choice of device(s) to combat poor quality medicines. While some devices can accurately detect falsified medicines containing no or incorrect active pharmaceutical ingredients (API) we did not find evidence that they could quantitate % API, a key aspect of many substandard medicines. At the country level, all five spectrometers were found to be cost-effective in settings with 'high' prevalence of falsified and substandard antimalarials but three only were cost-effective in lower' prevalence.

However, the study raised concerns that those using these devices may develop false confidence in the devices and reduce the vital visual inspection of medicines. With the current evidence, it is unlikely that any one device would be able to effectively monitor the quality of all medicines. Much more work is needed to evaluate devices for the great diversity of medicines, and to expand our work with a platform, independent from device manufacturers, to evaluate new devices using standard protocols and samples.

We also work with Dr Fred Behringer (Surveillent LLC, USA) on FTIR techniques for evaluating the quality of anti-tuberculosis medicines.

G. 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. The data are being analysed and will be available in 2019.

H. Packaging. Working with the Institut de la Francophonie pour la Médecine Tropicale (IFMT), we have surveyed the information and language of antimalarial packaging – much of which is in the wrong language for Laos or too small a font to read! This is being analysed for submission.

I. 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.

J. 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

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evaluation of the policies that the pharmaceutical industry have, and their adherence to these, for the rapid reporting of poor quality medicines to national medicine regulatory authorities and the WHO Rapid Alert system. The second inclusion of these data in the AMI were published in 2018. See https://accesstomedicinefoundation.org/access-to-medicine-index. Of twenty innovative pharmaceutical companies examined only **three** companies showed evidence of policies for reporting SF cases within the recommended seven days to the relevant NMRAs and the WHO SF Medical Products Group. **Six** had 'No policy or approach'.

K. 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. We are being funded by the Wellcome Trust to model, with Professors Lisa White and Dr Aronrag Meeyai of MORU-Bangkok, the consequences of poor quality antimalarials, anti-TB drugs and anti-HCV drugs on patient outcome and drug resistance. The initial results were presented at the Conference on Medicine Quality & Public Health in Oxford in September 2018.

Medicine Quality & Public Health Conference

L. 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. Publication of this book has been delayed.

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

N. Course on Medicine Quality & Public Health. In September 2018, with Centre of Tropical Medicine & Global Health, we ran the annual Course on Medicine Quality & Public Health at Wolfson College, Oxford. This has previously held at the London School of Hygiene and Tropical Medicine and Boston University School of Public Health. Twenty -five people from sixteen countries attended. Thirteen LMIC attendees were funded by the generosity of the Bill & Melinda Gates Foundation. We hope to organise a fifth annual course in Africa in 2020.

O. Medicine Quality Side Meetings. We organized, with the United States Pharmacopeia, the annual side meeting on medicine quality at the American Society of Tropical Medicine & Hygiene Conference in New Orleans in November 2018.

P. Conference on Medicine Quality & Public Health. We organized this conference in Oxford in September 2018 (See: http://www.tropicalmedicine.ox.ac.uk/medicinequality2018). Two hundred and twenty people from over 50 countries attended. LMIC attendees were funded by the Bill & Melinda Gates

Foundation, Wellcome Trust, Medicines for Malaria Venture, United States Pharmacopeial Convention, Concept Foundation and the East African Community (EAC) Regional Centre of Excellence for Vaccines, Immunisation and Health Supply Chain Management /German Development Bank.

The conference discussed the latest evidence on the epidemiology of substandard and falsified (SF) medical products, their health, economic, social, legal and ethical implications, and debated interventions to ensure that all the world's population have access to affordable and quality-assured medical products.

On the final day an Oxford statement was discussed on the global negative health and economic impact of substandard and falsified (SF) medical products and what policy changes, implementation and interventions are needed. This led to a declaration about the need to eliminate poor-quality medicines that undermine public health and waste precious resources, as well as the importance of making quality medical products affordable and accessible to all. This is available on the Medicines We Can Trust website at: https://medswecantrust.org/oxford-statement

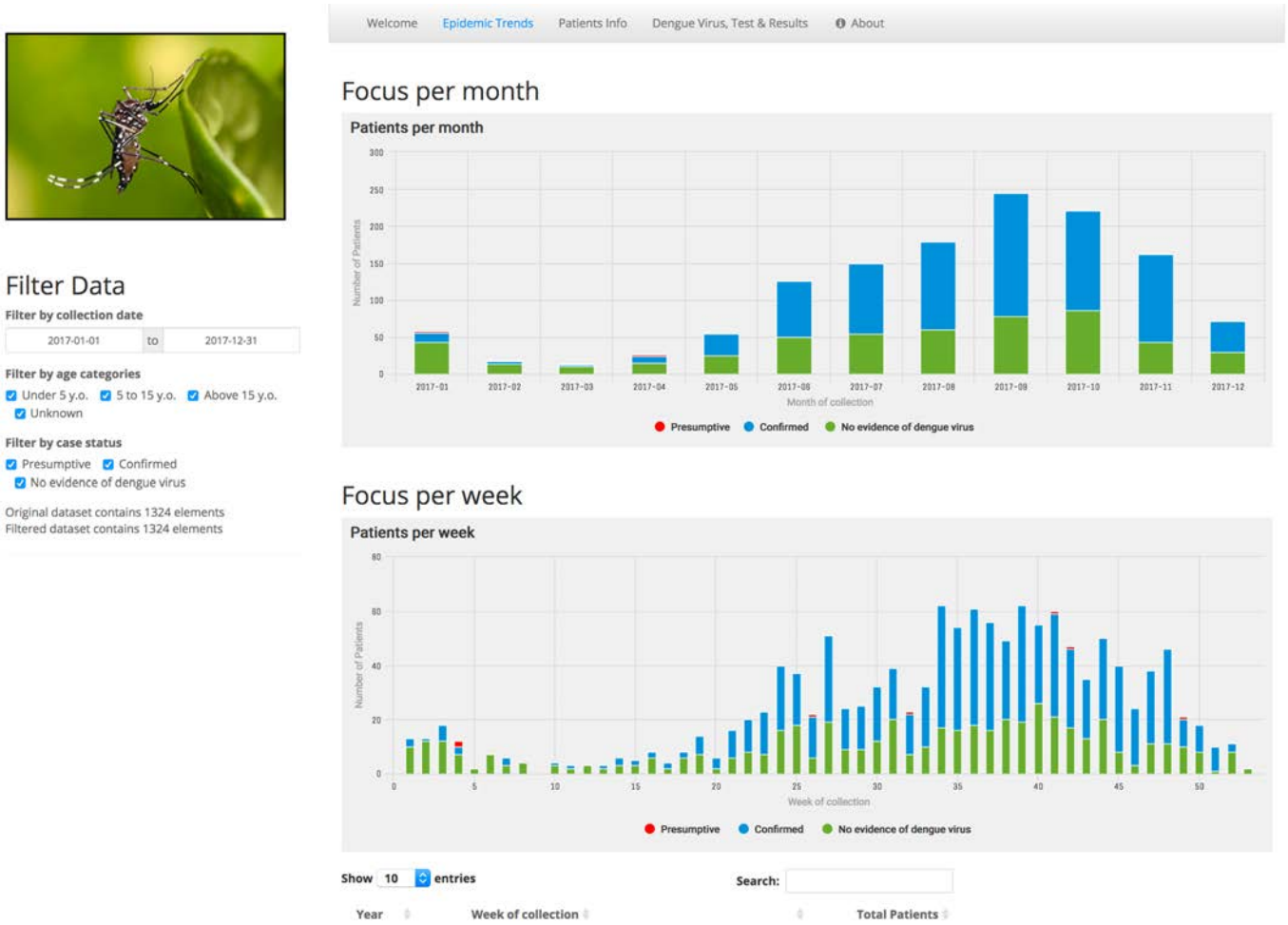
and at:

https://www.iddo.org/news/mqph-short-statement-2018

A wide diversity of 41 organisations, including international organisations, NGOs, universities and funders have signed up. A further separate longer statement is being drafted. We hope to organise a second conference on this neglected topic in 2021.

11. 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 Phetsavanh Chanthavilay, Olivier Celhay & Tamalee Roberts, working on a series of projects on antimicrobial resistance, malaria epidemiology and murine typhus antibody responses. A course in Vientiane on modeling and public health policy, run by MORU-Bangkok, is planned for 2019.



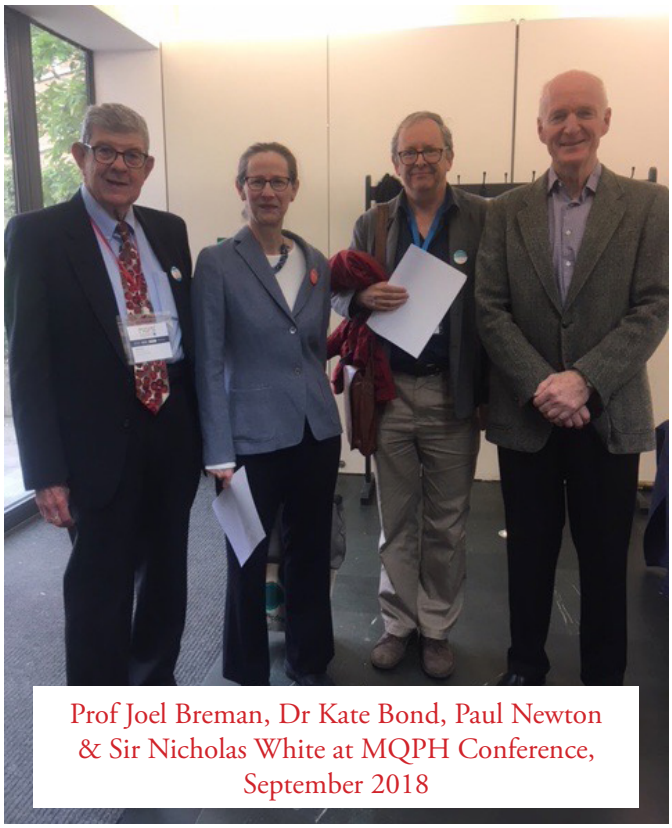
Example of dashboard page for representation of dengue epidemiology in Laos developed by Olivier Celhay. A similar system has been developed for bacterial antibiotic susceptibility data from Mahosot Hospital. We hope that such systems will facilitate easy data sharing.

CLINICAL RESEARCH SUPPORT

Mr Prayoon Yuentrakul and Dr Vimalay Souvong auditing FIEBRE Study documents

Dr Vimalay Souvong started in 2018 as LOMWRU clinical research support coordinator working with the Clinical Trial Support Group in MORU-Bangkok to conduct audits of research work and coordinate ethical review applications in Laos and Oxford.

Science Cafe Public Engagement session on post-partum food avoidance traditions, November 2018, in Vientiane



Prof Joel Breman, Dr Kate Bond, Paul Newton & Sir Nicholas White at MQPH Conference, September 2018

PUBLIC ENGAGEMENT

Community perceptions and engagement

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

A. The Science Café project has continued with sessions on post-partum food avoidance behaviours, road crashes and diabetes with lively discussions on both topics. We plan to hold these every 2 months.

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://uhs-elibrary.la/Elibrary.php?&parentID=0&CatID=10

C. LOMWRU website. The LOMWRU aspects of the www.tropmedres.ac website have been updated with more details of LOMWRU PIs. See http://www.tropmedres.ac/lomwru-laos

D. 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.

E. Pint of Science – this engagement system has started in Thailand led by Dr Matthew Robinson of LOMWRU.

POLICY ENGAGEMENT

A. We have numerous meetings during the year with diverse departments of the Ministry of Health and WHO National office to discuss translation of evidence into policy, especially for AMR, dengue, melioidosis, central nervous system infections, malaria and forthcoming Lao antimicrobial treatment guidelines.

B. E-Library. The University of Health Sciences (UHS) website e-library is up and running as a repository of published and grey literature information about Lao public health - see: http://uhs-elibrary.la/index.php? If anyone has any open access papers relevant to public health in Laos they are encouraged to submit them by sending the pdfs to elibrary.uhs@gmail.com. We hope very much that this will become a Lao national resource for health workers and policy makers.

C. Unit of Health Evidence and Policy. We are discussing a LOMWRU-linked unit at the University of Health Sciences to facilitate translation of Lao and global scientific public health evidence into health policy in Laos.

Public Engagment Science Cafe Meeting


Science Cafe July 2018 in Vientiane

OTHER ACTIVITIES

A. External quality assurance. We participate in the UK National External Quality Assessment Service (NEQAS) scheme for general bacteriology, antimicrobial susceptibility testing, AAFB microscopy, cryptococcal antigen, Molecular detection of viruses in CSF 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, planning multi-centre studies and for laboratory safety. This has been expanded to include virology.

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. 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 has already improved the efficiency and accuracy of the microbiological service, and greatly facilitated data retrieval. It will also put us in a good position to act as a sentinel site for the provision of data to the WHO Global Antimicrobial Resistance Surveillance system (GLASS).

F. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF). In January 2018 we were fortunate to receive a MALDI-TOF machine that will be used for identification of otherwise hard to identify pathogens and research on the identification of Lao vectors, especially ticks and which pathogens they carry.

G. Rickettsial Coordination Meetings. The Network holds these every twelve months, organised by Dr Matthew Robinson.

H. Recycling. We have developed a recycling scheme for paper, bottles and cans etc and have a policy to avoid using water bottles for meetings.

MQPH Conference Keble College Oxford September 2018

KEY COLLABORATIONS

Within Lao PDR

Centre for Malariology, Parasitology & Entomology, Ministry of Health
National Centre for Laboratory & Epidemiology, Ministry of Health
Department of Communicable Disease Control (DCDC), Ministry of Health
Department of Health Care and Rehabilitation, Ministry of Health
Food and Drug Department, Ministry of Health
University of Health Sciences, Ministry of Health
Lao Tropical and Public Health Institute
Provincial Hospitals of Luang Nam Tha, Xieng Khouang, Salavan, Phonhong (Vientiane Province) 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
Food & Drug Quality Control Laboratory, Ministry of Health

Lao Red Cross, Vientiane
World Health Organisation Lao Country Office, Vientiane
Institut de Recherche pour le Développement
Centre d'Infectiologie Christophe Mérieux du Laos
Institut Pasteur du Laos
Health Frontiers, Vientiane
US CDC, US Embassy
International Water Management Institute

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

Jayasree Iyer, Danny Edwards, Beth Boyer & Wim Leereveld, Access to Medicine Foundation, Haarlem, The Netherlands

Professor Xavier Nicolas de Lamballerie, UMR «Unité des Virus Emergents» (UVE: Aix-Marseille university - IRD 190 - Inserm 1207 – IHU Méditerranée Infection), Marseille, France.

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

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

Dr Mike Green, CDC, Atlanta, Georgia, USA

Professor Richard Laing, Dr Veronika Wirtz and Dr Erin Hasselberg, School of Public Health, Boston University, Boston, USA

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

Dr Cecilia Kato, Rickettsial Zoonoses Branch (RZB) , CDC, Atlanta, Georgia, USA

Dr Serge Morand, CIRAD, Bangkok

Dr Aurelie Binot, CIRAD, France

Ms Fiona Theunissen, Concept Foundation, Geneva

Professor Adrian Linacre, Flinders University, Australia

Mrs Aline Plançon, FMEDS, Paris, France

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

Prof Clark Freifeld, Boston Children's Hospital, Harvard University, Boston, USA

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

Dr Jyoti Joshi & Professor Ramanan Laxminarayan, Global Antibiotic Resistance Partnership, Washington DC, USA

Dr Dallas Mildenhall, GNS Science, New Zealand

Infectious Diseases Centre team farewell lunch for Ms Neeranuch Thangnimitchok

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 - Cambodia, Phnom Penh, Cambodia

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

Dr Mathieu Picardeau, Unité de Biologie des Spirochètes, Institut Pasteur, Paris, France

Dr Alain Pierret, Institut de recherche pour le développement, 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 Lee Smythe & Scott Craig, Leptospiral Reference Laboratory, Coopers Plains, Australia

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

Professor Sharon Peacock, London School of Hygiene and Tropical Medicine, UK

Dr Martin Cinnamond, GHAP, Geneva, Switzerland

Dr Nicolas Peyraud, Médecins sans Frontières (MSF), Geneva, Switzerland

Ms Christa Cepuch & Mr Alain Alsalhani, MSF Access Campaign, Geneva, Switzerland

Ms Isabel Lucas Manzano, 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

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

Captain Patrick Blair, LCDR Jeff Hertz & Lt Josey Garcia, Naval Medical Research Centre- Asia, Singapore

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

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

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

Professor Eric Morgan, Queens University, Belfast, Northern Ireland, UK
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

Professor Daniel Paris, Dr Esther Kuenzli, Dr Rosalie Zimmermann, Dr Jakob Zopfi and colleagues, Swiss Tropical and Public Health Institute, Basel/University of Basel, Switzerland

Dr Tim Barkham, Tan Tock Seng Hospital, Singapore

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

Prof Joost Wiersinga, University of Amsterdam, The Netherlands

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

Dr David & Rachel Dance leaving party with Dr Matthew Robinson and his melioidosis themed cake !

Dr Daniel Parker, University of California-Irvine, USA

Professor William Horsnell, University of Cape Town

Dr Francis Anto, University of Ghana , Ghana

Prof Ivo Steinmetz, University of Graz, Austria

Dr Steve Sait, University of Leeds, UK

Dr Pierre-Yves Sacré, University of Liège, Belgium

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

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 John Crump, University of Otago, New Zealand

Professor Nicole Zitzmann & Dr Bevin Gangadharan Department of Biochemistry, University of Oxford, UK

Dr Nicole Stoesser and Professor 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, Wellcome Trust Centre for Human Genetics, University of Oxford, UK

Dr Catherine Moyes, Big Data Institute, University of Oxford, UK

Drs David AuCoin, Paul Brett and Mary Burtnick, University of Nevada School of Medicine, Reno, Nevada, USA

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

Dr Richard Malik, University of Sydney, Australia

Prof Lutz Heide, University of Tubingen, Germany

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

Professor Albert Ko, Yale School of Public Health, USA

Michael Deats, Pernette Bourdillon-Esteves & Diana Lee, Substandard and Falsified Medical Products Group WHO, Geneva, Switzerland

Dr Mathieu Pruvot & Khonsy Khammavong, Wildlife Conservation Society, Laos

Dr Sayaphet Rattanavong, Deputy Director of the Microbiology Laboratory discussing the work of the Clinical Team at the board round with the Master's Mekong Pharma students from University of Health Sciences December 2018

TITLES AND ABSTRACTS OF PAPERS PUBLISHED OR IN PRESS 2018

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

1. Adhikari B, Phommasone K, Kommarasy P, Soundala X, Souvanthong P, Pongvongsa T, Henriques G, Newton PN, White NJ, Day NPJ, Dondorp AM, von Seidlein L, Mayxay M, Cheah PY, Pell C (2018a) Why do people participate in mass antimalarial administration? Findings from a qualitative study in Nong District, Savannakhet Province, Lao PDR (Laos). *Malar J* 17: 15.

Abstract. BACKGROUND. As a part of targeted malaria elimination (TME) in the Greater Mekong Sub-region (GMS), mass drug administration (MDA) with anti-malarials was conducted in four villages in Nong District, Savannakhet Province, Lao PDR (Laos). A high proportion of the target population participated in the MDA, with over 87% agreeing to take the anti-malarial. Drawing on qualitative data collected alongside the MDA, this article explores the factors that led to this high population coverage. METHODS. Qualitative data collection methods included observations, which were recorded in field notes, focus group discussions (FGDs), and semi-structured interviews (SSIs). Data were collected on local context, MDA-related knowledge, attitudes and perceptions. FGDs and SSIs were audio-recorded, transcribed and translated to English. All transcriptions and field notes underwent qualitative content analysis using QSR NVivo. RESULTS. Respondents recognized malaria as a health concern and described the need for a malaria control program. The risk of malaria including asymptomatic infection was explained in terms of participants' work in forest and fields, and poor hygiene. During the MDA rounds, there was an improvement in knowledge on the concept of asymptomatic malaria, the rationale of MDA and the blood test. In all four villages, poverty affected access to healthcare and the provision of free care by TME was highly appreciated. TME was jointly undertaken by research staff and local volunteers. Authorities were involved in all TME activities. Lao Theung communities were cohesive and community members tended to follow each other's behaviour closely including participation in MDA. Factors such as understanding the concept and rationale of the study, free health care, collaboration with the village volunteers, support from authorities and cohesive communities contributed in building trust and high population coverage in MDA.

CONCLUSION. Future malaria control programmes can become successful in achieving the high coverage in MDAs drawing from the success of TME in Laos. A high population coverage in TME was a combination of various factors that included the community engagement to promote the concept and rationale of MDA for asymptomatic malaria in addition to their baseline understanding of malaria as a health concern, provision of free primary health care, partnering of the research with local volunteers and authorities, building social relationship with community members and the cohesive nature of the communities boosted the trust and participation in MDA.

2. Adhikari B, Phommasone K, Pongvongsa T, Soundala X, Koummarasy P, Henriques G, Peto TJ, von Seidlein L, White NJ, Day NPJ, Dondorp AM, Newton PN, Cheah PY, Mayxay M, Pell C (2018b) Perceptions of asymptomatic malaria infection and their implications for malaria control and elimination in Laos. *PLoS One* 13(12): e0208912.

Abstract. BACKGROUND. In the Greater Mekong Sub-region (GMS), malaria elimination efforts are targeting the asymptomatic parasite reservoirs. Understanding community perceptions about asymptomatic malaria infections and interventions that target this reservoir is critical to the design of community engagement. This article examines knowledge, attitudes, perceptions and practices related to asymptomatic malaria infections and mass drug administration (MDA) in malaria-endemic villages in southern Savannakhet Province, Laos. METHODS. A questionnaire consisting of questions on socio-demographic characteristics, knowledge, attitudes, perceptions and practices on malaria and MDA was administered to each household head or representative (n = 281) in four villages. These topics were also further discussed in 12 single-gender focus group discussions (FGDs). The FGDs were conducted in all four villages and consisted of eight to 10 participants.

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RESULTS. A minority (14.2%; 40/281) of respondents agreed that a seemingly healthy person could have malaria parasite in his or her blood. Half (52%; 146/281) disagreed and one third (33.8%, 95/281) were unsure. Respondents who responded that "MDA aims to cure everyone" [AOR = 4.6; CI: 1.6–13.1], "MDA is to make our community malaria free" [AOR = 3.3; CI: 1.3–8.1] and "I will take part in future MDA" [AOR = 9.9; CI: 1.2–78.8] were more likely to accept the idea of asymptomatic malaria. During FGDs, respondents recalled signs and symptoms of malaria (fever, chills and headache), and described malaria as a major health problem. Symptomatic and asymptomatic malaria infections were associated with their work in the forest and living conditions. Measures described to eliminate malaria included using mosquito nets, wearing long-sleeved clothes and taking medicine when symptomatic. Most respondents were unaware of MDA as a tool to eliminate malaria. CONCLUSIONS. Awareness of asymptomatic malaria infections, and MDA as a tool to eliminate malaria, was low. With the need to target asymptomatic malaria carriers for elimination efforts in the GMS, as well as informing target groups about asymptomatic infection, accompanying community engagement must build trust in interventions through the active collaboration of government stakeholders, key local persons and community members. This entails training and devolving responsibilities to the community members to implement and sustain the control and elimination efforts.

3. Ashley E, Recht J, Chua A, Dance D, Dhorda M, Thomas N, Ranganathan N, Turner P, Guerin P, White NJ, Day NJP (2018a) An inventory of supranational antimicrobial resistance surveillance networks involving low- and middle-income countries since 2000. *J Antimicrobial Chemo* 73(7): 1737–1749.

Abstract. Low- and middle-income countries (LMICs) shoulder the bulk of the global burden of infectious diseases and drug resistance. We searched for supranational networks performing AMR surveillance in LMICs and assessed their organisation, methodology, impacts and challenges. Since 2000, 72 supranational networks for AMR surveillance in bacteria, fungi, HIV, TB and malaria have been created which have involved LMICs, of which 34 are ongoing. The median [range] duration of the networks was 6 years [1-70] and number of LMICs included was 7 [1-67]. Networks were categorised as WHO/governmental (n=26), academic (n=24), or pharma initiated (n=22). Funding sources varied, with 30 networks receiving public or WHO funding, 25 corporate, 13 trust or foundation, and four funded from more than one source. The leading global programmes for drug resistance surveillance in tuberculosis, malaria and HIV gather data in LMICs through periodic active surveillance efforts or combined active and passive approaches. The biggest challenges faced by these networks has been achieving high coverage across LMICs and complying with the

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recommended frequency of reporting. Obtaining high quality, representative surveillance data in LMICs is challenging. Antibiotic resistance surveillance requires a level of laboratory infrastructure and training which is not widely available in LMICs. The nascent Global Antimicrobial Resistance Surveillance System (GLASS) aims to build up passive surveillance in all member states. Past experience suggests complementary active approaches may be needed in many LMICs if representative, clinically relevant, meaningful data are to be obtained. Maintaining an up-to-date registry of networks would promote a more coordinated approach to surveillance.

4. Ashley EA, Dance DAB, Turner P (2018b) Grading antimicrobial susceptibility data quality: room for improvement. *Lancet Infect Dis* 18(6):603-604.

[The authors highlight specific concerns regarding the reliability of antimicrobial susceptibility data. Assurance of the quality of microbiology data before publication is not based on any objective criteria, and the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) system is not designed to assess them. They propose that additional guidelines are needed to provide quality assurance of microbiological data before publication. The gold standard would be laboratory accreditation by the International Organization for Standardization, but this is unrealistic for many laboratories, especially those in low-income and middle-income countries. Quality improvement initiatives such as Strengthening Laboratory Management Toward Accreditation and WHO's Laboratory Quality Stepwise Implementation Tool are supporting laboratories to raise standards. In the meantime, as a minimum they suggest that publication should be conditional on reporting of methods used, laboratory accreditation status, participation in external quality assurance schemes, and verification of adherence to accepted standard methods for establishing antimicrobial susceptibility (e.g. Clinical and Laboratory Standards Institute and The European Committee on Antimicrobial Susceptibility Testing). Antimicrobial resistance is a complex issue and is widely considered to be getting worse. However, the quality of the microbiology data that are being published to support this position could be substantially improved.]

5. Assemat C, Balayssac S, Gerdova A, Gilard V, Caillet C, Williamson D, Malet-Martino M (2019) Benchtop low-field 1H Nuclear Magnetic Resonance for detecting falsified medicines. *Talanta* 196, 163–173.

Abstract. Falsified medicines represent a serious threat to public health. Among the different measures to effectively combat this scourge, analytical methods play a key role in their detection and removal from the market before they reach patients. The present study

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evaluates for the first time the potential of a benchtop low-field (LF) Nuclear Magnetic Resonance (NMR) spectrometer for uncovering drug falsification by focusing on the analysis of fifteen erectile dysfunction and nine antimalarial medicines, the most commonly reported falsified medicines in developed and developing countries respectively. After a simple and rapid sample preparation and ≈5 min of spectrum recording, LF 1H NMR allows to conclude on the quality of the medicine: presence or absence of the expected active pharmaceutical ingredient (API), presence of unexpected API, absence of any API. Some 2D experiments are also described but although conclusive they are hampered by the duration of the experiments. The LF 1H NMR assay, based on the internal standard method, is validated by the determination of its accuracy, repeatability, limits of detection (LOD) and quantification (LOQ), and by comparison of the data obtained on some medicines after 45 min of spectrum recording to those measured with high-field 1H NMR. Because of its saving capabilities (cost, space, user experience), LF 1H NMR spectroscopy might become a routine screening tool in laboratories in charge of detecting falsified medicines.

6. Bancone G, Menard D, Khim N, Kim S, Canier L, Nguong C, Phommasone K, Mayxay M, Dittrich S, Vongsouvath M, Fievet N, Le Hesran JY, Briand V, Keomany S, Newton PN, Gorsawun G, Tardy K, Chu CS, Rattanapalroj O, Dong LT, Quang HH, Tam-Uyen N, Thuy-Nhien N, Hien TT, Kalnoky M, Nosten F (2019) Molecular characterization and mapping of glucose-6-phosphate dehydrogenase (G6PD) mutations in the Greater Mekong Subregion. *Malar J.* 18(1):20.

Abstract. BACKGROUND. *Plasmodium vivax* malaria elimination can only be achieved by the deployment of 8-aminoquinolines (primaquine and tafenoquine) in combination with ACT to kill both blood and liver-stage parasites. However, primaquine and the other 8-aminoquinolines cause dose-dependent haemolysis in subjects with G6PD deficiency, an X-linked disorder of red blood cells that is very common in populations living in tropical and subtropical areas. In order to inform safer use of 8-aminoquinolines in the Greater Mekong Subregion, a multi-centre study was carried out to assess the prevalence of G6PD deficiency and to identify the main G6PD variants in

samples collected in Cambodia, Lao PDR, Myanmar, Thailand and Vietnam. METHODS. Blood samples were collected in the five countries during National Malaria Surveys or during Population Surveys. During Population Surveys samples were characterized for G6PD phenotype using the Fluorescent Spot Test. Samples were then genotyped for a panel of G6PD mutations. RESULTS. G6PD deficiency was found to be common in the region with an overall mean prevalence of deficient or mutated hemizygous males of 14.0%, ranging from a mean 7.3% in Thailand, 8.1% in Lao PDR, 8.9% in Vietnam, 15.8% in Myanmar and 18.8% in Cambodia. Mahidol and Viangchan mutations were the most common and widespread variants found among the nine investigated. CONCLUSIONS. Owing to the high prevalence of G6PD deficiency in the Greater Mekong Subregion, strategies for vivax malaria elimination should include point-of-care G6PD testing (both qualitative and quantitative) to allow safe and wide treatment with 8-aminoquinolines.

7. Bernier MC, Li A, Winalski L, Zi Y, Li Y, Caillet C, Newton PN, Wang ZL, Fernández FM (2018) Triboelectric nanogenerator (TENG) mass spectrometry of falsified antimalarials. *Rapid Commun Mass Spectrom*. 32(18): 1585–1590.

Abstract. RATIONALE. An epidemic of low-quality medicines continues to endanger patients worldwide. Detection of such 'medicines' requires low cost, ambient ionization sources coupled to fieldable mass spectrometers for optimum sensitivity and specificity. With the use of triboelectric nanogenerators (TENGs), the charge required to produce gas-phase ions for mass analysis can be obtained without the need for high-voltage electrical circuitry, simplifying and lowering the cost of next-generation mass spectrometry instruments. METHODS. A sliding freestanding (SF) TENG was coupled to a toothpick electrospray setup for the purposes of testing if falsified medicines could be fingerprinted by this approach. Extracts from both genuine and falsified medicines were deposited on the toothpick and the SF TENG actuated to generate electrical charges, resulting in gas-phase ions for both active pharmaceutical ingredients and excipients. RESULTS. Our previous work had shown that direct analysis in real time (DART) ambient mass spectrometry can identify the components of multiple classes of falsified antimalarial medicines. Experiments performed in this study show that a simple extraction into methanol along with the use of a SF TENG-powered toothpick electrospray can provide similar detection capabilities, but with much simpler and rugged instrumentation, and without the need for compressed gases or high-voltage ion source power supplies. CONCLUSIONS. TENG toothpick MS allows for rapid analyte ion detection in a safe and low-cost manner, providing robust sampling and ionization capabilities.

8. Bharucha T, Vickers S, Ming D, Lee SJ, Dubot-Peres A, de Lamballerie X, Newton PN (2018a) Association between Reported Aetiology of Central Nervous System Infections and the Speciality of Study Investigators - A Bias Compartment Syndrome? *Trans Royal Soc Trop Med Hyg*. 111(12): 579–583.

Abstract. BACKGROUND: Conventional descriptions of central nervous system (CNS) infections are variably categorised into clinical syndromes for patient investigation, management and research. Aetiologies of the most commonly recognised syndromes, encephalitis and meningitis, tend to be attributed predominantly to viruses and bacteria, respectively. METHODS: A systematic review was performed of aetiological studies of CNS syndromes, and data extracted on reported author specialities. RESULTS: The analysis identified an association between the author's speciality and the CNS syndrome studied, with a tendency for virologists to study encephalitis, and microbiologists to study meningitis. DISCUSSION: We suggest there is bias in study design. Stronger multidisciplinary collaboration in CNS infection research is needed.

9. Bharucha T, Sengvilaipaseuth O, Vongsouvath M, Vongsouvath M, Davong V, Panyanouvong P, Piorkowski G, Garson JA, Newton PN, de Lamballerie X, Dubot-Pérès A (2018b) Development of an improved RT-qPCR Assay for detection of *Japanese encephalitis virus* (JEV) RNA including a systematic review and comprehensive comparison with published methods. *PLoS One* 13(3):e0194412.

Abstract. BACKGROUND. *Japanese encephalitis virus* (JEV) is a major cause of encephalitis in Asia, and the commonest cause of mosquito-borne encephalitis worldwide. Detection of JEV RNA remains challenging due to the characteristic brief and low viraemia, with 0–25% of patients positive, and the mainstay of diagnosis remains detection of anti-JEV IgM antibody. METHODS. We performed

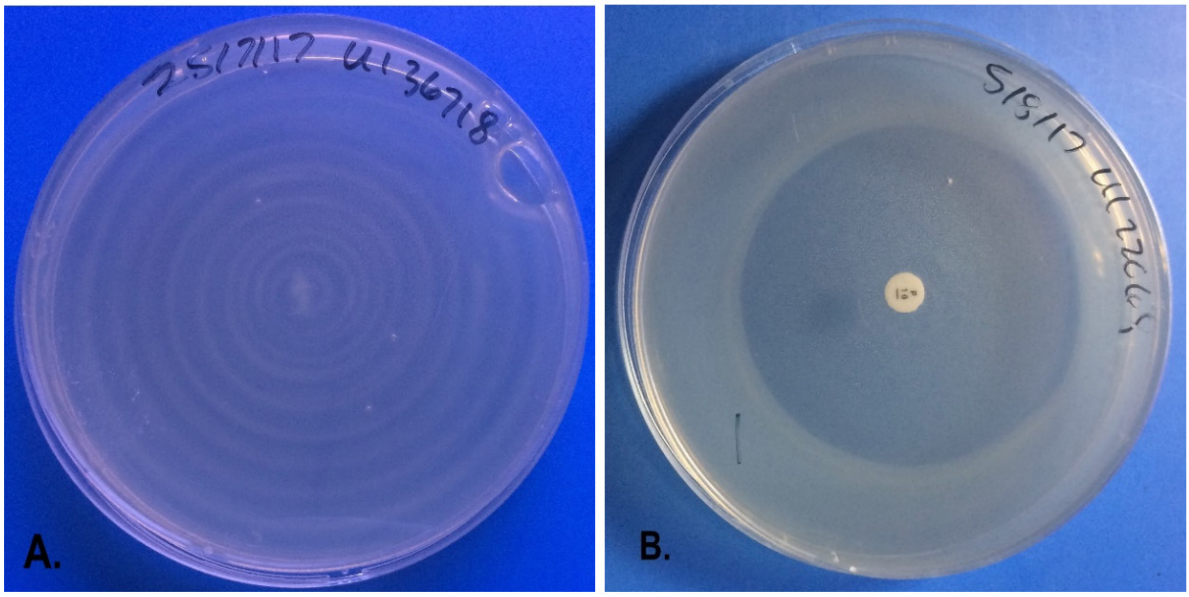
a systematic review of published RT-PCR protocols, and evaluated them *in silico* and *in vitro* alongside new primers and probes designed using a multiple genome alignment of all JEV strains >9,000nt from GenBank, downloaded from the NCBI website (November 2016). The new assays included pan-genotype and genotype specific assays targeting genotypes 1 and 3. RESULTS. Ten RT-qPCR assays were compared, a pre-existing in-house assay, three published assays and six newly designed assays, using serial RNA dilutions. We selected three assays, one published and two novel assays, with the lowest limit of detection (LOD) for further optimisation and validation. One of the novel assays, detecting NS2A, showed the best results, with LOD approximately 4 copies/ reaction, and no cross-reaction on testing closely related viruses in the JEV serocomplex, *West Nile Virus* and *St. Louis Virus*. The optimised assays were validated in consecutive patients with central nervous system infections admitted to hospitals in Laos, testing paired CSF and serum samples. CONCLUSIONS. We succeeded in developing a JEV specific RT-qPCR assay with at least 1 $\log_{10}$ improved sensitivity as compared to existing assays. Further evaluation is required, field-testing the assay in a larger group of patients.

10. Bharucha T, Sengvilaipaseuth O, Seephonelee M, Vongsouvath M, Vongsouvath M, Rattanavong S, Piorkowski G, Lecuit M, Gorman C, Pommier JD, Newton PN, de Lamballerie X, Dubot-Pérès A (2018c) Detection of Japanese Encephalitis Virus RNA in Human Throat Samples in Laos – A Pilot study. *Sci Rep* 8: 8018.

Abstract. *Japanese encephalitis virus* (JEV) is the most commonly identified cause of acute encephalitis syndrome (AES) in Asia. The WHO recommended test is anti-JEV IgM-antibody-capture-enzyme-linked-immunosorbent-assay (JEV MAC-ELISA). However, data suggest this has low positive predictive value, with false positives related to other *Flavivirus* infections and vaccination. JEV RT-PCR in cerebrospinal fluid (CSF) and/or serum is highly specific, but is rarely positive; 0–25% of patients that fulfil the WHO definition of JE (clinical Acute Encephalitis Syndrome (AES) and JEV MAC-ELISA positive). Testing other body fluids by JEV RT-qPCR may improve the diagnosis. As a pilot study thirty patients admitted to Mahosot Hospital 2014–2017, recruited to the South-East-Asia-Encephalitis study, were tested by JEV MAC-ELISA and two JEV real-time RT-PCR (RT-qPCR) assays (NS2A and NS3). Eleven (36.7%) were JEV MAC-ELISA positive. Available CSF and serum samples of these patients were JEV RT-qPCR negative but 2 (7%) had JEV RNA detected in their throat swabs. JEV RNA was confirmed by re-testing, and sequencing of RT-qPCR products. As the first apparent report of JEV RNA detection in human throat samples, the provides new perspectives on human JEV infection, potentially informing improving JEV detection. We suggest that testing patients' throat swabs for JEV RNA is performed, in combination with molecular and serological CSF and serum investigations, on a larger scale to investigate the epidemiology of the presence of JEV in human throats. Throat swabs are an easy and non-invasive tool that could be rolled out to a wider population to improve knowledge of JEV molecular epidemiology.

11. Bharucha T, Sengvilaipaseuth O, Seephonelee M, Vongsouvath M, Vongsouvath M, Rattanavong S, Piorkowski G, Lecuit M, Gorman C, Pommier J-D, Garson JA, Newton PN, de Lamballerie X, Dubot-Pérès A (in press) Viral RNA degradation makes urine a challenging specimen for detection of *Japanese encephalitis virus* in patients with suspected CNS infection. *Open Forum Infectious Diseases*

Abstract. *Japanese encephalitis virus* (JEV) is the most common identified cause of central nervous system (CNS) infections in Asia, and results in significant morbidity and mortality. JEV RNA is rarely detected in serum or cerebrospinal fluid (CSF), and a diagnosis of JEV is based on serological tests that are notoriously difficult to interpret. Urine is a non-invasive sample, relatively easy to obtain, and plentiful. To date, there has been minimal investigation into the role of testing urine for JEV RNA. We investigated optimal urine storage conditions for detection of JEV RNA by reverse-transcription real-time polymerase chain reactions (RT-qPCR), and evaluated this method in a study of consecutive patients admitted to hospital with suspected CNS infections in Laos. Experimentation demonstrated degradation of JEV RNA in urine even for short storage periods at 4°C or -80°C. Although there was no advantage of using a Microsep concentration device, immediate addition of lysis buffer to fresh urine improved the detection of JEV RNA at the limit of detection. In two studies of 41 patients with acute encephalitis syndrome, 11 (27%) were positive for JEV IgM in CSF and/or serum and two were JEV RT-qPCR positive from throat swabs. JEV RNA was not detected in any of these patients' urine samples. However, only 17/41 (41%) urine samples were stored in lysis buffer. Our findings suggest a need for larger



Growth of *Leptospira* spp. isolates on LVW agar. **A.** Growth control of isolate UI3618 with no antimicrobial disk. **B.** Zone of inhibition (55 mm) of isolate UI22068 penicillin G.

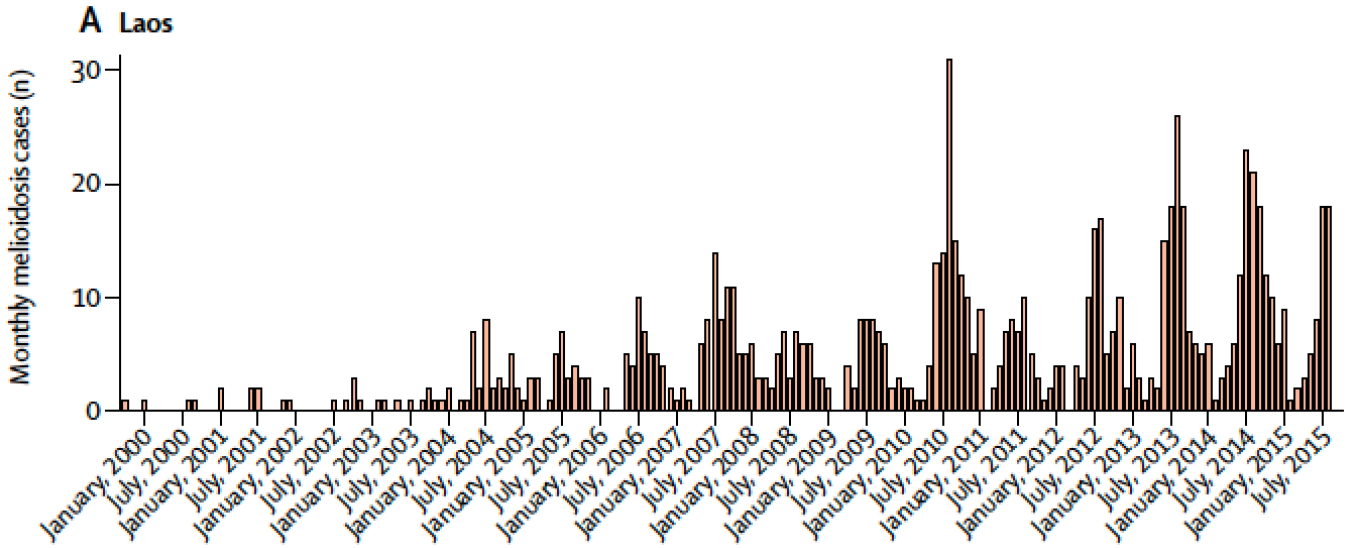
rigorous studies testing urine for JEV RNA, with urine collected at different times of onset, and using Buffer AVL-carrier RNA for storage.

12. Blacksell SD, Robinson MT, Newton PN, Day NPJ (2018) Laboratory-acquired scrub typhus and murine typhus infections: The argument for risk-based approach to biosafety requirements for *Orientia tsutsugamushi* and *Rickettsia typhi* laboratory activities. *Clin Infect Dis* 2018 Aug 10.

Abstract. This study examined the literature on laboratory-acquired infections (LAIs) associated with scrub typhus (*Orientia tsutsugamushi*) and murine typhus (*Rickettsia typhi*) research to provide an evidence base for biosafety and biocontainment. Scrub typhus LAIs were documented in 25 individuals, from 1931 to 2000 with 8 (32%) deaths during the preantibiotic era. There were 35 murine typhus LAI reports and no deaths. Results indicated that the highest-risk activities were working with infectious laboratory animals involving significant aerosol exposures, accidental self-inoculation, or bite-related infections. A risk-based biosafety approach for in vitro and in vivo culture of *O. tsutsugamushi* and *R. typhi* would require that only high-risk activities (animal work or large culture volumes) be performed in high-containment biosafety level (BSL) 3 laboratories. We argue that relatively low-risk activities including inoculation of cell cultures or the early stages of in vitro growth using low volumes/low concentrations of infectious materials can be performed safely in BSL-2 laboratories within a biological safety cabinet.

13. Boss J, Dance DAB, Chanthongthip A, Newton PN, Wuthiekanun V, Robinson MT (in press) Antimicrobial susceptibility testing of *Leptospira* spp. in the Lao People's Democratic Republic using disk diffusion. *Am J Trop Med Hyg*

Abstract. Leptospirosis is a global zoonotic disease caused by pathogenic bacteria of the *Leptospira* genus which are fastidious, slow growing organisms. Antimicrobial susceptibility data of leptospires are limited as they traditionally have not been culturable on solid media. However, the recent development of *Leptospira* Vanaporn Wuthiekanun (LVW) agar, which facilitates rapid growth of *Leptospira* spp., provides the opportunity for antimicrobial susceptibility testing. Eighty-three clinical isolates of *Leptospira* spp. originating from leptospirosis patients in Laos between 2006 and 2016 were tested against six antimicrobials (azithromycin, ceftriaxone, ciprofloxacin, doxycycline, gentamicin and penicillin G) using disk diffusion on LVW agar. Quality control was undertaken using American Type Culture Collection (ATCC) reference strains with known susceptibilities on both standard media and LVW agar. All *Leptospira* spp. isolates produced large zones of inhibition around each of the six antimicrobials. All zones were greater than 25



Monthly changes in diagnosis of *Burkholderia pseudomallei* culture positive infections at Mahosot Hospital

mm, with gentamicin producing the smallest zones (median 35 mm; interquartile range 30 mm to 37 mm) and azithromycin producing the largest zones (median 85 mm; interquartile range 85 mm to 85 mm). Zones produced by ATCC reference strains on LVW agar were within 2 mm of the accepted strain-specific quality control range on standard media. Antimicrobial activity on LVW agar appears to be similar to that on standard media. As there are no published susceptibility guidelines for the *Leptospira* genus, zone interpretation was subjective. However, the large zone sizes suggest that resistance has not emerged to these six antibiotics in Lao *Leptospira* spp.

14. Bulterys P, Bulterys M, Phommasone K, Luangraj M, Mayxay M, Kloprogge S, Miliya T, Vongsouvath M, Newton PN, Phetsouvanh R, French CT, Miller JF, Turner P, Dance DA (2018) Climatic drivers of melioidosis in Laos and Cambodia. *Lancet Planetary Health* 2(8):e334-e343.

Abstract. BACKGROUND. *Burkholderia pseudomallei* is the cause of melioidosis, a serious and difficult to treat infection that is endemic throughout the tropics. Melioidosis incidence is highly seasonal. We aimed to identify the climatic drivers of infection and to shed light on modes of transmission and potential preventive strategies. METHODS. We examined the records of patients diagnosed with melioidosis at the Microbiology Laboratory of Mahosot Hospital in Vientiane, Laos, between October, 1999, and August, 2015, and all patients with culture-confirmed melioidosis presenting to the Angkor Hospital for Children in Siem Reap, Cambodia, between February, 2009, and December, 2013. We also examined local temperature, humidity, precipitation, visibility, and wind data for the corresponding time periods. We estimated the *B pseudomallei* incubation period by examining profile likelihoods for hypothetical exposure-to-presentation delays. FINDINGS. 870 patients were diagnosed with melioidosis in Laos and 173 patients were diagnosed with melioidosis in Cambodia during the study periods. Melioidosis cases were significantly associated with humidity ($p<0{\cdot}0001$), low visibility ($p<0{\cdot}0001$), and maximum wind speeds ($p<0{\cdot}0001$) in Laos, and humidity ($p=0{\cdot}010$), rainy days ($p=0{\cdot}015$), and maximum wind speed ($p=0{\cdot}0070$) in Cambodia. Compared with adults, children were at significantly higher odds of infection during highly humid months (odds ratio 2·79, 95% CI 1·83–4·26). Lung and disseminated infections were more common during windy months. The maximum likelihood estimate of the incubation period was 1 week (95% CI 0–2). INTERPRETATION. The results of this study demonstrate a significant seasonal burden of melioidosis among adults and children in Laos

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Dr Sayaphet Rattanavong encouraging recycling

and Cambodia. Our findings highlight the risks of infection during highly humid and windy conditions, and suggest a need for increased awareness among at-risk individuals, such as children.

15. Burns RJL, Douangngeun B, Theppangna W, Khounsy S, Mukaka M, Selleck PW, Hansson E, Wegner MD, Windsor PA, Blacksell SD (2018a) Serosurveillance of Coxiellosis (Q-fever) and Brucellosis in goats in selected provinces of Lao People's Democratic Republic. *PLoS Negl Trop Dis* 12(4): e0006411.

Abstract. Goat raising is a growing industry in Lao People's Democratic Republic, with minimal disease investigation to date, especially zoonoses. This study determined the proportional seropositivity of two zoonotic diseases: Q fever (causative agent *Coxiella burnetii*) and Brucellosis (*Brucella* species) in goats across five provinces (Vientiane Capital, Xayaboury, Xiengkhuang, Savannakhet and Attapeu). A total of 1458 goat serum samples were tested using commercial indirect ELISA for both pathogens, plus Rose Bengal agglutination test for Brucellosis. Overall individual seropositivity of *C. burnetii* was 4.1% and *Brucella* spp. was 1.4%. A multiple logistic regression model identified that province (Vientiane Capital, p = 0.05), breed (introduced Boer mixed breed, p = 0.006) and age (goats >= 3 years old, p = 0.014) were significant risk factors for *C. burnetii* seropositivity. The results of the survey indicated that province (Vientiane Capital, p<0.001), breed (introduced Boer mixed breed, p<0.001), production system (commercial, p<0.001), age (adult, p = 0.004), and farm size (large, 0.001) were all significant risk factors seropositivity for Brucella spp. It was concluded that Lao goats have been exposed to both *C. burnetii* and *Brucella* spp. however the risk of clinical disease has not yet been determined and there is an urgent need to determine human health risks and economic losses caused by Q fever and Brucellosis.

16. Burns RJL, Douangngeun B, Theppangna W, Mukaka M, Wegner MD, Windsor PA, Blacksell SD (2018b) Peste des Petits Ruminants (PPR) virus serological surveillance in goats in Lao PDR: Issues for disease eradication in a low-resource disease-free setting. *Transbound Emerg Dis*. 2018;00:1–9.

Abstract. Peste des Petits ruminants (PPR) is an economically important transboundary viral disease of goats. This study aimed to determine a baseline of serological evidence for Peste des petits ruminants virus (PPRV) in Lao goats. A total of 1,072 sera samples were collected by convenience sampling across five provinces in Laos and tested for antibody response to PPRV using a commercially available competitive ELISA. Positive antibody responses were found in 2.2% (95% CI 1.4, 3.2) of the samples. True prevalence calculations indicated a total overall sample prevalence of 1.7% (95% CI 0.9, 2.8). The highest provincial seroprevalences were Xiangkhouang (3.5%, 95% CI 1.6, 6.9) and Xayaboury (2.9% (95% CI 1.3, 5.7). There was no association between antibody response and each of the following factors: location, breed, gender or age. Considering apparent absence of disease manifestation of PPR in Laos, likely explanations of antibody response could be cross reaction to other Morbilliviruses such as Measles or Canine Distemper, importation of pre-vaccinated goats, need for test cut-

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off re-evaluation to be region specific, or a subclinical and a less virulent circulating virus. This study highlights that the sampled Lao goat population is highly likely to be naïve to PPRV and therefore at risk of an outbreak, possibly by transboundary incursion of livestock from PPR endemic China. Further work is required in the testing of small ruminants in Laos that may eventually provide evidence for a status of freedom from disease, particularly in support of programs aimed at global PPR eradication.

17. Caillet C, Vickers S, Zambrzycki S, , Vidhamaly V, Boutsamay K, Boupha P, Luangasanatip N, Lubell Y, Fernández FM, Newton PN (2018) An evaluation of portable screening devices to assess medicines quality for national Medicines Regulatory Authorities. Asian Development Bank (2018) Results for Malaria Elimination and Control of Communicable Disease Threats in Asia and the Pacific. Consultant's report. Manila (RETA 8763). Available at:

https://www.iddo.org/document/evaluation-portable-screening-devices-assess-medicines-quality-national-medicines

with policy brief drafted by ADB based on this report at:

https://www.adb.org/publications/portable-screening-devices-medicine-quality

[Medicines Regulatory Authorities (MRAs) are the keystone for the majority of interventions to prevent, detect and remove poor quality medicines before they reach patients. Innovative portable devices hold promise for empowering medicine inspectors in screening medicine quality in supply systems. However, regulators lack information on their performance, limitations and cost-effectiveness. This project was undertaken as an independent evaluation and comparison of devices to provide evidence to allow MRAs to decide whether these new technologies are appropriate for screening of medicines quality in their countries.

With funding from the Asian Development Bank we investigated the comparative diagnostic accuracy and cost-effectiveness of a diversity of portable and handheld medicine quality screening devices in the laboratory and in the 'field'. With colleagues at the Georgia institute of Technology in Atlanta, USA, we evaluated the diagnostic accuracy of a wide diversity of devices in the laboratory. We then worked with the Bureau of Food and Drug Inspection (BFDI) inspectors to understand their advantages and disadvantages in an evaluation pharmacy we created at Mahosot Hospital. Health economists from MORU-Bangkok also analyzed the cost-effectiveness of the devices compared to the current practice in Laos.

We included 11 devices, of which four were included in

Pii Mai Lao. Who can drink the fastest?

a laboratory evaluation only and seven (**in bold**), were also tested by 16 medicine inspectors from the BFDI in a field evaluation study: four handheld spectrometers using infrared (**MicroPHAZIR RX, NIRScan**) or Raman (**Progeny**, **Truscan RM**); five portable devices using infrared (**4500 aFTIR**, Neospectra 2.5), liquid chromatography (C-Vue), thin-layer chromatography (**Minilab**), microfluidic technology with luminescence detection (PharmaChk); and two single-use disposable devices: one using paper-based colour test (**PADs)** and one using lateral flow immunoassay technology (RDTs).

In the laboratory evaluation, all devices tested on simulated and field-collected branded medicines containing seven different anti-infectives (within each device's capabilities to detect certain APIs) showed 100% sensitivities to correctly identify samples with 0% and wrong API after removal from their packaging except the NIRScan (91.5%). Specificities of 100% were observed for all devices, except for the C-Vue (60.0%), PharmaChk (50.0%) and Progeny (95.5%). The two devices with stated abilities to quantitate APIs showed high sensitivities to correctly identify 50%/80% API samples in a pass/fail configuration (C-Vue : 100% and PharmaChk : 83.3%) whereas the RDTs, able to identify samples containing lower API than stated, showed a sensitivity of 17%. Spectrometers included in the evaluation were not stated to have the ability to identify medicines with lower API than stated using the device stock built-in algorithms available. Accordingly, the mentioned spectrometers showed limited sensitivities (from 6% to 50%). Of the field-evaluated devices the Minilab was the most sensitive to correctly identify 50%/80% API samples in the laboratory evaluation (59.5%), with significantly higher sensitivity than other devices ($p<0.05$), except the MicroPHAZIR (50%). The NIRScan was the fastest of the field-evaluated devices to test one sample, followed by the MicroPHAZIR RX whilst the PADs and the Minilab were the slowest devices. The time spent to inspect the pharmacy was significantly longer when using the devices compared to visual inspection only, for all the devices except the NIRScan and Truscan RM. The main errors made by medicine inspectors were the selection of the wrong reference library while using the Truscan RM, NIRScan, MicroPHAZIR RX (Truscan RM seemed to be less prone to this error)

and wrong user interpretation of the PADs and 4500a FTIR results. When testing a set of samples, the PADs showed lower accuracy than other devices to correctly identify samples as poor or good quality, except the Progeny and the Minilab [no significant ($p>0.05$) statistical difference observed]. An under-development web-based reader of the results of the PADs could reduce sample misclassification. The Truscan RM had the highest fixed total costs over a 5-years period, followed by the Progeny, MicroPHAZIR, 4500a FTIR, NIRScan, and PADs. At the country level, all spectrometers were found to be cost-effective in settings with 'high' and 'lower' prevalence of falsified and substandard antimalarials and all were cost-effective compared with the baseline of visual inspections alone. The NIRScan, that had the lowest initial cost per device (below US$5,000), was the most cost-effective in the two prevalence scenarios. Difficulties to assemble batches of quality-assured genuine medicines to create and update reference libraries, high costs of most devices, maintenance/calibration and low sensitivity to identify substandard medicines without highly trained operators using complex API-specific models were perceived as the main obstacles for the implementation of the field-evaluated spectrometers. Sample preparation and sourcing of consumables (for the Minilab only), level of training and results that were felt too user-dependent (for the PADs only) were the main barriers to the use of PADs and Minilab. Although we provide general recommendations of the best strategy to choosing devices adapted to different settings, major gaps of evidence were identified by our work: the lack of knowledge about the level of training required; the effect of the potential 'false confidence' on the device versus visual inspection of medicines; the best sampling strategies for field testing (standard operating procedures are required in different contexts in the absence of manufacturer guidelines); the APIs and medicines formulation each device is able to test (except for a few devices such as the Minilab or the PADs); at which level of the supply chain they would be best used (we believe this is highly setting dependent) and how the health system should adapt to optimise their use; the impact of tablet coatings, packaging and capsule shells on the performance of spectrometers.

With the current evidence, it is unlikely that any one device would be able to effectively monitor the quality of all medicines. Much more work is needed to evaluate devices for the great diversity of medicines, and to expand our work with a platform, independent from device manufacturers, to evaluate new devices using standard protocols and samples.]

18. 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.

19. Castonguay-Vanier J, Klitting R, Sengvilaipaseuth O, Piorkowski G, Baronti C, Sibounheuang B, Vongsouvath M, Chanthongthip A, Thongpaseuth S, Mayxay M, Phommasone K, Douangdala P, Inthalath S, Souvannasing P, Newton PN, de Lamballerie X, Dubot-Pérès A (2018) Molecular epidemiology of dengue viruses in three provinces of Lao PDR, 2006-2010. *PLoS Negl Trop Dis* 12(1):e0006203.

Abstract. Few data on dengue epidemiology are

available for Lao PDR. Here, we provide information on the complexity of dengue epidemiology in the country, demonstrating dynamic circulation that varies over space and time, according to serotype. We recruited 1,912 consenting patients presenting with WHO dengue criteria at Mahosot Hospital, Vientiane (central Laos), between 2006 and 2010. Between 2008 and 2010, 1,413 patients with undifferentiated fever were also recruited at Luang Namtha (LNT) Provincial Hospital (northern Laos) and 555 at Salavan (SV) Provincial Hospital (southern Laos). We report significant variations in Dengue virus (DENV) circulation between the three sites. Peaks of DENV infection were observed in the rainy seasons, although 11% of confirmed cases in the provinces and 4.6% in the capital were detected during the dry and cool seasons (between December and February). Four DENV serotypes were detected among the 867 RT-PCR positive patients: 76.9% DENV-1, 9.6% DENV-2, 7.7% DENV-4 and 5.3% DENV-3. DENV-1 was the predominant serotype throughout the study except in LNT in 2008 and 2009 when it was DENV-2. Before July 2009, DENV-2 was not detected in SV and only rarely detected in Vientiane. DENV-3 and DENV-4 were commonly detected in Vientiane, before 2008 for DENV-4 and after 2009 for DENV-3. The phylogenetic analyses of DENV envelope sequences suggest concurrent multiple introductions of new strains as well as active DENV circulation throughout Laos and with neighboring countries. It is therefore of great importance to develop and strengthen a year-round nation-wide surveillance network in order to collect data that would allow anticipation of public health issues caused by the occurrence of large dengue outbreaks.

20. Chan J, Nguyen CD, Lai JYR, Dunne EM, Andrews R, Blyth CC, Datta S, Fox K, Ford R, Hinds J, La Vincente S, Lehmann D, Lim R, Mungun T, Newton PN, Phetsouvanh R, Pomat WS, Xeuatvongsa A, von Mollendorf C, Dance DAB, Satzke C, Muholland K, Russell FM (2018) Determining the pneumococcal conjugate vaccine coverage required for indirect protection against vaccine-type pneumococcal carriage in low and middle-income countries: a protocol for a prospective observational study. *BMJ Open* 8(5): e021512.

Abstract. INTRODUCTION. Pneumococcal conjugate vaccines (PCVs) prevent disease through both direct protection of vaccinated individuals and indirect protection of unvaccinated individuals by reducing nasopharyngeal (NP) carriage and transmission of vaccine-type (VT) pneumococci. While the indirect effects of PCV vaccination are well described, the PCV coverage required to achieve the indirect effects is unknown. We will investigate the relationship between PCV coverage and VT carriage among undervaccinated children using hospital-based NP pneumococcal carriage surveillance at three sites in Asia and the Pacific. METHODS and ANALYSIS. We are recruiting cases, defined as children aged 2–59 months admitted to participating hospitals with acute respiratory infection in Lao People's Democratic Republic, Mongolia and Papua New Guinea. Thirteen-valent PCV status is obtained from written records. NP swabs are collected according to standard methods, screened using *lytA* qPCR and serotyped by microarray. Village-level vaccination coverage, for the resident communities of the recruited cases, is determined using administrative data or community survey. Our analysis will investigate the relationship between VT carriage among undervaccinated cases (indirect effects) and vaccine coverage using generalised estimating equations. ETHICS AND DISSEMINATION. Ethical approval has been obtained from the relevant ethics committees at participating sites. The results are intended for publication in open-access peer-reviewed journals and will demonstrate methods suitable for low- and middle-income countries to monitor vaccine impact and inform vaccine policy makers about the PCV coverage required to achieve indirect protection.

21. Chandna A, White LJ, Pongvongsa T, Mayxay M, Newton PN, Day NPJ, Lubell Y (2019) Accounting for aetiology: can regional surveillance data alongside host biomarker-guided antibiotic therapy improve treatment of febrile illness in remote settings? *Wellcome Open Research*

Abstract. BACKGROUND. Across Southeast Asia, declining malaria incidence poses a challenge for healthcare providers, in how best to manage the vast majority of patients with febrile illnesses who have a negative malaria test. In rural regions, where the majority of the population reside, empirical treatment guidelines derived from central urban hospitals are often of limited relevance. In these settings, relatively untrained health workers deliver care, often without any laboratory diagnostic support. In this paper, our aim was to model the impact on mortality from febrile illness of using point-of-care C-reactive protein testing to inform the decision to prescribe antibiotics and regional surveillance data to inform antibiotic

Dr Céline Caillet and Ms Vayouly Vidhamaly discussing medicine quality with pharmacy students from the University of Health Sciences

selection, rooted in the real-world context of rural Savannakhet province, southern Laos. METHODS. Our model simulates 100 scenarios with varying quarterly incidence of six key pathogens known to be prevalent in rural Laos. In the simulations, community health workers either prescribe antibiotics in-line with current practice as documented in health facilities in rural Laos, or with the aid of the two interventions. We provide cost-effectiveness estimates for each strategy alone and then for an integrated approach using both interventions. RESULTS. We find that each strategy alone is predicted to be highly cost-effective, and that the combined approach is predicted to result in the biggest reduction in mortality (averting a predicted 510 deaths per year in rural Savannakhet, a 28% reduction compared to standard practice) and is highly cost-effective, with an incremental cost-effectiveness ratio of just $66 per disability-adjusted life year averted. CONCLUSIONS. Substantial seasonal variation in the predicted optimal empirical antibiotic treatment for febrile illness highlights the benefits of up-to-date information on regional causes of fever. In this modelling analysis, an integrated system incorporating point-of-care host biomarker testing and regional surveillance data appears highly cost-effective, and may warrant piloting in a real-life setting.

22. Chowdhury FR, Jilani MSA, Barai L, Rahman T, Saha MR, Amin MR, Fatema K, Islam KMS, Faiz MA, Dunachie SJ, Dance DAB (2018) Melioidosis in Bangladesh: A Clinical and Epidemiological Analysis of Culture-Confirmed Cases. *Trop Med Infect Dis* 3(2): 40.

Abstract. Melioidosis is known to occur in Bangladesh, but there are few reports about the condition in the published international literature. We set out to review all known cases of melioidosis in the country to date, using both retrospective and prospective data. A web-based literature search was conducted to identify all published case reports, original articles and conference abstracts. Cases were also included from a prospective study conducted in 2017. Fifty-one cases were identified between 1961 and 2017. Cases have been reported from sixteen out of the 64 districts of Bangladesh. The median age of the patients at presentation was 45 years (IQR 37–52), with a significant male (77%) predominance. Many patients

David Burton - MORU Network COO with LOMWRU staff for dinner overlooking the Mekong River, May 2018

(14/39; 36%) were farmers and 83% had diabetes mellitus. A skin/soft tissue abscess was the most common primary clinical presentation (13/49; 27%), followed by septic arthritis (10/49; 20%), pneumonia, and a deep-seated abscess/organ abscess (7/49; 14%). The major challenges to the diagnosis and treatment of melioidosis in Bangladesh are the lack of resources and the lack of awareness of melioidosis. Capacity development programs are urgently required to define the burden of disease and to tackle the mortality rates.

23. Dailey PJ, Osborn J, Ashley EA, Baron EJ, Dance DAB, Fusco D, Fanello C, Manabe YC, Mokomane M, Newton PN, Tessema B, Isaacs C, Dittrich S (2019) Defining System Requirements for Simplified Blood Culture to Enable Widespread Use in Resource-Limited Settings. *Diagnostics* 9, 10; doi:10.3390/diagnostics9010010.

Abstract: Bacterial blood stream infections (BSI) are a common cause of mortality and morbidity globally. As the causative agents and the resulting treatment decisions vary, near-patient testing and surveillance tools are necessary to monitor bacterial causes and resistance to antimicrobial agents. The gold standard to identify BSIs is blood culture (BC), a methodology not widely available in resource-limited settings. The aim of the study was to map out a target product profile of a simplified BC system (SBCS) to inform product development efforts. To identify the desired characteristics of a SBCS, we enlisted a small group of specialists working in Africa and Asia. Questions were used to understand challenges and how these constraints inform system requirements. The specialists were infectious disease physicians, public health/clinical microbiologists, clinical researchers, and technology experts with different geographical backgrounds. All suggested that BC should ideally be available at the district hospital level. Many of the same operational challenges, such as limited availability of culture bottles, electricity and internet connectivity, profuse dust, the lack of ambient temperature control, and human capacity constraints were identified across the different regions. BCs, although the accepted gold standard for diagnosis of BSIs, are not widely available outside of reference/research centers in Africa and Asia. To extend the reach of this important tool, it is crucial to engage product developers and academic research partners to develop accessible alternatives.

24. Dance DAB, Limmathurotsakul D (2018) Global Burden and Challenges of Melioidosis. *Trop Med Infect Dis* 3(1), 13.

[Introduces melioidosis as a neglected disease, summaries history of research on this important pathogen and sets the scene for a large series of articles from countries and regions around the world that summarise the current status, including what is known locally about the burden of melioidosis, and the key challenges facing local clinicians, laboratory staff and public health and policy makers, in relation to this elusive but common and fatal disease. They hope that this will become a key source of information for those who share our concern and are taking actions against this disease]

25. Dance DAB, Luangraj M, Rattanavong S, Sithivong N, Vongnalaysane O, Vongsouvath M, Newton PN (2018a) Melioidosis in the Lao People's Democratic Republic. *Trop Med Infect Dis* 3(1): 21.

Engineer Keith Trotter with QMS ISO9001 Lead Auditor certificate

Abstract. Melioidosis is clearly highly endemic in Laos, although the disease has only been diagnosed regularly in humans (1359 cases) since 1999, and only a single animal case has been microbiologically confirmed. *Burkholderia pseudomallei* is extensively and abundantly present in soil and surface water in central and southern Laos, but the true distribution of the disease across the country remains to be determined. Surveillance is almost non-existent and diagnostic microbiology services are not yet well established, whilst awareness of melioidosis is low amongst policy-makers, healthcare providers, and the public. It is hoped that this situation will improve over the next decade as the country rapidly develops, especially as this is likely to be accompanied by a further increase in the prevalence of diabetes, meaning that more people in this predominantly agricultural population will be at risk of contracting melioidosis.

26. Dance DAB, Sarovich D, Price EP, Limmathurotsakul D, Currie BJ (2018) Human Infection with *Burkholderia thailandensis*, China, 2013. *Emerg Infect Dis* 24(5): 953–954.

[A letter suggesting that a report describing a patient in China with *Burkholderia thailandensis* infection gave insufficient evidence to prove that this was *B. thailandensis* and that the presented data suggest that this isolate was, in fact, *B. pseudomallei*]

27. Dance DAB, Knappik M, Dittrich S, Davong V, Silisouk J, Vongsouvath M, Rattanavong S, Pierret A, Newton PN, Amornchai P, Wuthiekanun V, Langla S, Limmathurotsakul D (2018c) Evaluation of consensus method for the culture of *Burkholderia pseudomallei* in soil samples from Laos. Version 2. *Wellcome Open Res* 3: 132.

Abstract. BACKGROUND**.** We have previously shown that PCR following enrichment culture is the most sensitive method to detect *Burkholderia pseudomallei* in environmental samples. Here we report an evaluation of the published consensus method for the culture of *B. pseudomallei* from Lao soil in comparison with our conventional culture method and with PCR with or without prior broth enrichment. METHODS**.** One hundred soil samples were collected from a field known to contain *B. pseudomallei* and processed by: (i) the conventional method, (ii-iii) the consensus method using media prepared in either Laos or Thailand, and (iv) the consensus method performed in Thailand, as well as by (v) PCR following direct extraction of DNA from soil and (vi) PCR following broth pre-enrichment. RESULTS**.** The numbers of samples in which *B. pseudomallei* was detected were 42, 10, 7, 6, 6 and 84, respectively. However, two samples were positive by the consensus method but negative by conventional culture, and one sample was negative by PCR following enrichment although *B. pseudomallei* was isolated by the conventional culture method. CONCLUSIONS/DISCUSSION**.** The results show that no single method will detect all environmental samples that contain *B. pseudomallei.* People conducting environmental surveys for this organism should be aware of the possibility of false-negative results using the consensus culture method. An approach that entails screening using PCR after enrichment, followed by the evaluation of a range of different culture methods on PCR-positive samples to determine which works best in each setting, is recommended.

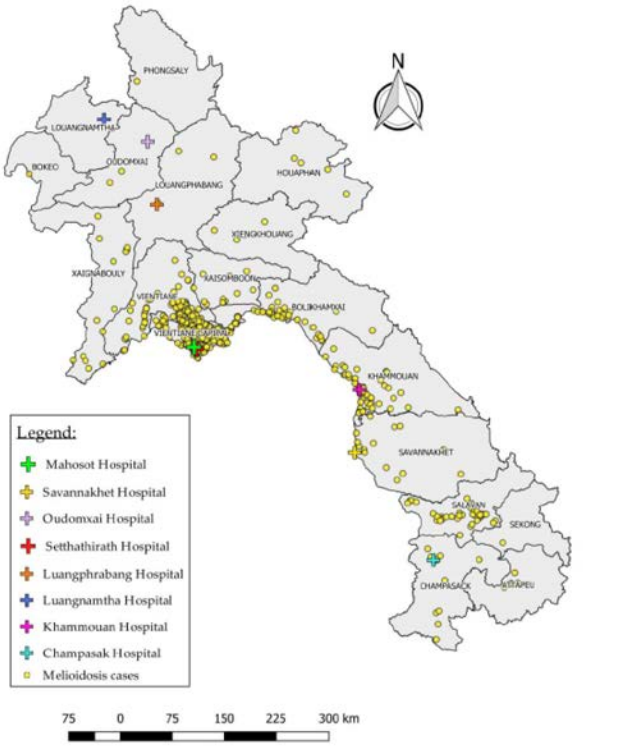

Figure 1. Location of homes of 1310 cases of melioidosis and hospital laboratories capable of making a diagnosis of melioidosis in Laos (data only available for 1310 of 1359 cases).

28. Darton TC, Tuyen HT, The HC, Newton PN, Dance DAB, Campbell JI, Thwaites GE, Parry CM, Thanh DP Baker S (2018) Azithromycin resistance in *Shigella* spp. in Southeast Asia. *Antimicrobial Agents & Chemo* 62(4): e01748-17.

Abstract. Infection by *Shigella* spp. is a common cause of dysentery in Southeast Asia. Antimicrobials are thought to be beneficial for treatment, however

antimicrobial resistance in *Shigella* spp. is becoming widespread. We aimed to assess the frequency and mechanisms associated with decreased susceptibility to azithromycin in Southeast Asian *Shigella* isolates and use these data to assess appropriate susceptibility breakpoints. *Shigella* isolated in Vietnam and Laos were screened for susceptibility against azithromycin (15µg) by disc diffusion and minimum inhibitory concentration (MIC). Phenotypic resistance was confirmed by PCR amplification of macrolide resistance loci. We compared the genetic relationships and plasmid contents of azithromycin resistant *S. sonnei* using whole genome sequences. From 475 available *Shigella* spp. isolated in Vietnam and Laos between 1994 and 2012, 6/181 *S. flexneri* (3.3%, MIC≥16g/L) and 16/294 *S. sonnei* (5.4%, MIC≥32g/L) were phenotypically resistant to azithromycin. PCR amplification confirmed a resistance mechanism in 22/475 (4.6%) isolates (19 mphA and 3 ermB). Susceptibility data demonstrated the acceptability of *S. flexneri* (MIC≥16g/L, zone≤15mm) and *S. sonnei* (MIC≥32g/L, zone≤11mm) breakpoints with <3% discrepancy. Phylogenetic analysis demonstrated that decreased susceptibility has arisen sporadically in Vietnamese *S. sonnei* on at least seven occasions between 2000 and 2009, but failed to become established. While the proposed susceptibility breakpoints may allow better recognition of resistant isolates, additional studies are required to assess the impact on clinical outcome. The potential emergence of azithromycin resistance highlights the need for alternative management options for *Shigella* infections in endemic countries.

29. Dittrich S, Boudthasavong L, Keokhamhoung D, Phuklia W, Craig S, Tulsiani S, Burns M-A, Weier S, Dance D, Davone V, Vongsouvath M, Mayxay M, Phetsouvanh R, Newton PN, Woods K (2018) A prospective hospital study to evaluate the diagnostic accuracy of rapid diagnostic tests for the early detection of leptospirosis in Laos. *Am J Trop Med Hyg* 98(4): 1056–1060.

Abstract. Leptospirosis is a globally important cause of acute febrile illness, and a common cause of non-malarial fever in Asia, Africa and Latin America. Simple rapid diagnostic tests (RDTs) are needed to enable health care workers, particularly in low resource settings, to diagnose leptospirosis early and give timely targeted treatment. This study compared four commercially available RDTs to detect human IgM against *Leptospira* spp. in a head-to-head prospective evaluation in Mahosot Hospital, Lao PDR. Patients with an acute febrile illness consistent with leptospirosis (n=695) were included in the study during the 2014 rainy season. Samples were tested with 4 RDTs: ('Test-it' (Life Assay, South Africa; n=418); 'Leptorapide' (Linnodee, Northern Ireland; n=492); 'Dual Path Platform' (DPP) (Chembio, USA; n=530); and 'SD-IgM' (Standard Diagnostics, South Korea; n=481)). Diagnostic performance characteristics were calculated and compared to a composite reference standard combining PCR (rrs), microscopic agglutination tests (MAT) and culture. Of all patients investigated, 39/695 (5.6%) were positive by culture, PCR or MAT. The sensitivity and specificity of the RDTs ranged greatly from 17.9-63.6% and 62.1-96.8%, respectively. None of the investigated RDTs reached a sensitivity or specificity of >90% for detecting *Leptospira* infections on admission. In conclusion, our investigation highlights the challenges associated with *Leptospira* diagnostics, particularly in populations with multiple exposures. These findings emphasize the need for extensive prospective evaluations in multiple endemic settings to establish the value of rapid tools for diagnosing fevers to allow targeted antibiotics.

30. Dubot-Pérès A, Mayxay M, Phetsouvanh R, Lee SJ, Rattanavong S, Vongsouvath M, Davong M, Chansamouth V, Phommasone K, Moore C, Dittrich S, Lattana O, Sirisouk J, Phoumin P, Panyanivong P, Sengduangphachanh A, Sibounheuang B, Chanthongthip A, Simmalavong M, Sengdatka D, Seubsanith A, Keoluangkot V, Phimmasone P, Sisout K, Detleuxay K, Luangxay K, Phouangsouvanh I, Craig SB, Tulsiani SM, Burns M-A, Dance DAB, Blacksell SD, de Lamballerie X, Newton PN (in press) Revisiting management of central nervous system infections in Southeast Asia. *Emerging Infect Dis*

Abstract. The disease burden of central nervous system (CNS) infections is important in Laos and globally. We recruited 1,065 patients of all ages admitted to Mahosot Hospital, Vientiane, with suspected CNS infection (2003-2011). Aetiology was laboratory confirmed for 42.3% of patients, mostly with emerging pathogens (viral in 16.2% -mainly *Japanese encephalitis* virus (8.8%); bacterial in 16.4% -including *Orientia tsutsugamushi* (2.9%), *Leptospira* spp. (2.3%) and *Rickettsia* spp. (2.3%); *Cryptococcus* spp. in 6.6%). No significant differences were observed in distribution of clinical encephalitis/meningitis syndromes by

bacterial/viral aetiologies. However, patients with bacterial infection were more likely to have a history of diabetes than others. Mortality (26.3%) was associated with low Glasgow Coma Scale and was higher in bacterial than viral infections. No clinical/laboratory variables could guide antibiotic selection. We conclude that strengthening dependency units and implementing first-line treatment combining both ceftriaxone and doxycycline for suspected CNS infections would improve patient survival in Laos.

31. Fleshman A, Mullins K, Sahl J, Hepp C, Nieto N, Wiggins K, Hornstra H, Kelly D, Chan TC, Phetsouvanh R, Dittrich S, Panyanivong P, Paris D, Newton PN, Richards A, Pearson T (2018) Comparative pan-genomic analyses of *Orientia tsutsugamushi* reveal an exceptional model of bacterial evolution driving genomic diversity. *Microb Genom* 4(9): e000199.

Abstract. *Orientia tsutsugamushi*, formerly *Rickettsia tsutsugamushi*, is an obligate intracellular pathogen that causes scrub typhus, an underdiagnosed acute febrile disease with high morbidity. Scrub typhus is transmitted by the larval stage (chigger) of *Leptotrombidium* mites and is irregularly distributed across endemic regions of Asia, Australia and islands of the western Pacific Ocean. Previous work to understand population genetics in *O. tsutsugamushi* has been based on sub-genomic sampling methods and whole-genome characterization of two genomes. In this study, we compared 40 genomes from geographically dispersed areas and confirmed patterns of extensive homologous recombination likely driven by transposons, conjugative elements and repetitive sequences. High rates of lateral gene transfer (LGT) among *O. tsutsugamushi* genomes appear to have effectively eliminated a detectable clonal frame, but not our ability to infer evolutionary relationships and phylogeographical clustering. Pan-genomic comparisons using 31082 high-quality bacterial genomes from 253 species suggests that genomic duplication in *O. tsutsugamushi* is almost unparalleled. Unlike other highly recombinant species where the uptake of exogenous DNA largely drives genomic diversity, the pan-genome of *O. tsutsugamushi* is driven by duplication and divergence. Extensive gene innovation by duplication is most commonly attributed to plants and animals and, in contrast with LGT, is thought to be only a minor evolutionary mechanism for bacteria. The near unprecedented evolutionary characteristics of *O. tsutsugamushi*, coupled with extensive intra-specific LGT, expand our present understanding of rapid bacterial evolutionary adaptive mechanisms.

One of Dr Serena Vickers Farewell Cakes !
May 2018

32. Greer RC, Wangrangsimakul T, Amornchai P, Wuthiekanun V, Laongnualpanich A, Dance DAB, Limmathurotsakul D (2018) Misidentification of *Burkholderia pseudomallei* as *Acinetobacter* species in northern Thailand. *Trans R Soc Trop Med Hyg.* 2018 Oct 5

Abstract. *Burkholderia pseudomallei* is the causative agent of melioidosis, a disease endemic throughout the tropics. A study of reported *Acinetobacter* spp. bacteraemia was performed at Chiang Rai provincial hospital from 2014 to 2015. Isolates were collected and tested for confirmation. A total of 419 putative *Acinetobacter* spp. isolates from 412 patients were re-identified and 5/419 (1.2%) were identified as *B. pseudomallei*. Four of the five patients with melioidosis died. An estimated 88/419 (21%) isolates were correctly identified as Acinetobacter spp. Misidentification of *Acinetobacter* spp. as *B. pseudomallei* or other bacteria is not uncommon and programmes to address these shortfalls are urgently required.

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33. Hantrakun V, Thaipadungpanit J, Rongkard P, Srilohasin P, Amornchai P, Langla S, Mukaka M, Chantratita N, Wuthiekanun V, Dance DAB, Day NPJ, Peacock SJ, Limmathurotsakul D (2018) Presence of *B. thailandensis* and *B. thailandensis* expressing *B. pseudomallei*-like capsular polysaccharide in Thailand, and their associations with serological response to *B. pseudomallei*. *PLoS Negl Trop Dis*. 12(1):e0006193.

Abstract. BACKGROUND. *Burkholderia pseudomallei* is an environmental Gram-negative bacillus and the cause of melioidosis. *B. thailandensis*, some strains of which express a *B. pseudomallei*-like capsular polysaccharide (BTCV), is also commonly found in the environment in Southeast Asia but is considered non-pathogenic. The aim of the study was to determine the distribution of *B. thailandensis* and its capsular variant in Thailand and investigate whether its presence is associated with a serological response to *B. pseudomallei*. METHODOLOGY/PRINCIPAL FINDINGS: We evaluated the presence of *B. pseudomallei* and *B. thailandensis* in 61 rice fields in Northeast (n = 21), East (n = 19) and Central (n = 21) Thailand. We found BTCV in rice fields in East and Central but not Northeast Thailand. Fourteen fields were culture positive for *B. pseudomallei* alone, 8 for *B. thailandensis* alone, 11 for both *B. pseudomallei* and *B. thailandensis*, 6 for both B. thailandensis and BTCV, and 5 for *B. pseudomallei, B. thailandensis* and BTCV. Serological testing using the indirect hemagglutination assay (IHA) of 96 farmers who worked in the study fields demonstrated that farmers who worked in *B. pseudomallei*-positive fields had higher IHA titers than those who worked in *B. pseudomallei*-negative fields (median 1:40 [range: <1:10-1:640] vs. <1:10 [range: <1:10-1:320], p = 0.002). In a multivariable ordered logistic regression model, IHA titers were significantly associated with the presence of *B. pseudomallei* (aOR = 3.7; 95% CI 1.8-7.8, p = 0.001) but were not associated with presence of *B. thailandensis* (p = 0.32) or BTCV (p = 0.32). One sequence type (696) was identified for the 27 BTCV isolates tested. CONCLUSIONS/ SIGNIFICANCE: This is the first report of BTCV in Thailand. The presence of *B. pseudomallei* and *B. thailandensis* in the same field was not uncommon. Our findings suggest that IHA positivity of healthy rice farmers in Thailand is associated with the presence of *B. pseudomallei* in rice fields rather than *B. thailandensis* or BTCV.

34. Haenssgen MJ, Charoenboon N, Zanello G, Mayxay M, Reed-Tsochas F, Jones COH, Kosaikanont R, Praphattong P, Manohan P, Lubell Y, Newton PN, Keomany S, Wertheim HFL, Lienert J, Xayavong T, Warapikuptanun P, Zaw YZ, U-Thong P, Benjaroon P, Sangkham N, Wibunjak K, Chai-In

P, Chailert S, Thavethanutthanawin P, Promsutt K, Thepkhamkong A, Sithongdeng N, Keovilayvanh M, Khamsoukthavong N, Phanthasomchit P, Phanthavong C, Boualaiseng S, Vongsavang S, Greer RC, Althaus T, Nedsuwan S, Intralawan D, Wangrangsimakul T, Limmathurotsakul D, Ariana P (2018a) Antibiotics and activity spaces: protocol of an exploratory study of behaviour, marginalisation and knowledge diffusion *BMJ Glob Health* 3(2): e000621.

Abstract. BACKGROUND. Antimicrobial resistance (AMR) is a global health priority. Leading UK and global strategy papers to fight AMR recognise its social and behavioural dimensions, but current policy responses to improve the popular use of antimicrobials (eg, antibiotics) are limited to education and awareness-raising campaigns. In response to conceptual, methodological and empirical weaknesses of this approach, we study people's antibiotic-related health behaviour through three research questions. RQ1: What are the manifestations and determinants of problematic antibiotic use in patients' healthcare-seeking pathways? RQ2: Will people's exposure to antibiotic awareness activities entail changed behaviours that diffuse or dissipate within a network of competing healthcare practices? RQ3: Which proxy indicators facilitate the detection of problematic antibiotic behaviours across and within communities? METHODS. We apply an interdisciplinary analytical framework that draws on the public health, medical anthropology, sociology and development economics literature. Our research involves social surveys of treatment-seeking behaviour among rural dwellers in northern Thailand (Chiang Rai) and southern Lao PDR (Salavan). We sample approximately 4800 adults to produce district-level representative and social network data. Additional 60 cognitive interviews facilitate survey instrument development and data interpretation. Our survey data analysis techniques include event sequence analysis (RQ1), multilevel regression (RQ1–3), social network analysis (RQ2) and latent class analysis (RQ3). DISCUSSION. Social research in AMR is nascent, but our unprecedentedly detailed data on microlevel treatment-seeking behaviour can contribute an understanding of behaviour beyond awareness and free choice, highlighting, for example, decision-making constraints, problems of marginalisation and lacking access to healthcare and competing ideas about desirable behaviour.

35. Haenssgen MJ, Xayavong T, Charoenboon N, Warapikuptanun P, Zaw YK (2018) The Consequences of AMR Education and Awareness Raising: Outputs, Outcomes, and Behavioural Impacts of an Antibiotic-Related Educational Activity in Lao PDR. *Antibiotics* 7, 95.

Abstract. Education and awareness raising are the primary tools of global health policy to change public behaviour and tackle antimicrobial resistance. Considering the limitations of an awareness agenda, and the lack of social research to inform alternative approaches, our objective was to generate new empirical evidence on the consequences of antibiotic-related awareness raising in a low-income country context. We implemented an educational activity in two Lao villages to share general antibiotic-related messages and also to learn about people's conceptions and health behaviours. Two rounds of census survey data enabled us to assess the activity's outputs, its knowledge outcomes, and its immediate behavioural impacts in a difference-in-difference design. Our panel data covered 1130 adults over two rounds, including 58 activity participants and 208 villagers exposed indirectly via conversations in the village. We found that activity-related communication circulated among more privileged groups, which limited its indirect effects. Among participants, the educational activity influenced the awareness and understanding of "drug resistance", whereas the effects on attitudes were minor. The evidence on the behavioural impacts was sparse and mixed, but the range of possible consequences included a disproportionate uptake of antibiotics from formal healthcare providers. Our study casts doubt on the continued dominance of awareness raising as a behavioural tool to address antibiotic resistance.

36. Henriques G, Phommasone K, Tripura R, Peto TJ, Raut S, Snethlage C, Sambo I, Sanann N, Nguon C, Adhikari B, Pongvongsa T, Imwong M, von Seidlein L, Day NPJ, White NJ, Dondorp AM, Newton PN, Ley N, Mayxay M (2018) Comparison of glucose-6 phosphate dehydrogenase status by fluorescent spot test and rapid diagnostic test in Lao PDR and Cambodia. *Malar J* 17: 243.

Abstract. Background. Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common enzymopathy worldwide. Primaquine is the only licensed drug that effectively removes *Plasmodium vivax* hypnozoites from the human host and prevents

Dr Ivo Elliott Farewell Presentation and Basci April 2018

relapse. While well tolerated by most recipients, primaquine can cause haemolysis in G6PD deficient individuals and is, therefore, underused. Rapid diagnostic tests (RDTs) could permit ascertainment of G6PD status outside of laboratory settings and hence safe treatment in remote areas. The performance of the fluorescent spot test (Trinity, Ireland; FST) and a G6PD RDT (Carestart, USA) against spectrophotometry were assessed. Methods. Participants were enrolled during cross-sectional surveys in Laos and by purposive sampling in Cambodia. FST and RDT were performed during village surveys and 3 mL of venous blood was collected for subsequent G6PD measurement by spectrophotometry. Results. A total of 757 participants were enrolled in Laos and 505 in Cambodia. FST and RDT performed best at 30% cut-off activity and performed significantly better in Laos than in Cambodia. When defining intermediate results as G6PD deficient, the FST had a sensitivity of 100% (95%CI 90–100) and specificity of 90% (95%CI 87.7–92.2) in Laos and sensitivity of 98% (94.1–99.6) and specificity of 71% (95%CI 66–76) in Cambodia ($p < 0.001$). The RDT had sensitivity and specificity of 100% (95%CI 90–100) and 99% (95%CI 97–99) in Laos and sensitivity and specificity of 91% (86–96) and 93% (90–95) in Cambodia ($p < 0.001$). The RDT performed significantly better (all $p < 0.05$) than the FST when intermediate FST results were defined as G6PD deficient. Conclusion. The interpretation of RDT results requires some training but is a good alternative to the FST.

37. Inglis R, Ayebale E, Schultz MJ (2019) Optimizing respiratory management in resource-limited settings. *Curr Opin Crit Care* 25(1):45-53.

Abstract. PURPOSE OF REVIEW. This review focuses on the emerging body of literature regarding the management of acute respiratory failure in low- and middle-income countries (LMICs). The aim is to abstract management principles that are of relevance across a variety of settings where resources are severely limited. RECENT FINDINGS. Mechanical ventilation is an expensive intervention associated with considerable mortality and a high rate of iatrogenic complications in many LMICs. Recent case series

report crude mortality rates for ventilated patients of between 36 and 72%. Measures to avert the need for invasive mechanical ventilation in LMICs are showing promise: bubble continuous positive airway pressure has been demonstrated to decrease mortality in children with acute respiratory failure and trials suggest that noninvasive ventilation can be conducted safely in settings where resources are low. SUMMARY. The management of patients with acute respiratory failure in LMICs should focus on avoiding intubation where possible, improving the safety of mechanical ventilation and expediting weaning. Future directions should involve the development and trialing of robust and context-appropriate respiratory support technology.

38. Kloprogge F, Workman L, Borrmann S, Tékété M, Lefèvre G, Hamed K, Piola P, Ursing J, Kofoed PE, Mårtensson A, Ngasala B, Björkman A, Ashton M, Friberg Hietala S, Aweeka F, Parikh S, Mwai L, Davis TME, Karunajeewa H, Salman S, Checchi F, Fogg C, Newton PN, Mayxay M, Deloron P, Faucher JF, Nosten F, Ashley EA, McGready R, van Vugt M, Proux S, Price RN, Karbwang J, Ezzet F, Bakshi R, Stepniewska K, White NJ, Guerin PJ, Barnes KI, Tarning J (2018) Artemether-lumefantrine dosing for malaria treatment in young children and pregnant women: A pharmacokinetic-pharmacodynamic meta-analysis. *PLoS Med* 12;15(6):e1002579.

Abstract. BACKGROUND. The fixed dose combination of artemether-lumefantrine (AL) is the most widely used treatment for uncomplicated *Plasmodium falciparum* malaria. Relatively lower cure rates and lumefantrine levels have been reported in young children and in pregnant women during their second and third trimester. The aim of this study was to investigate the pharmacokinetic and pharmacodynamic properties of lumefantrine and the pharmacokinetic properties of its metabolite, desbutyl-lumefantrine, in order to inform optimal dosing regimens in all patient populations. METHODS AND FINDINGS. A search in PubMed, Embase, ClinicalTrials.gov, Google Scholar, conference proceedings, and the WorldWide Antimalarial Resistance Network (WWARN) pharmacology database identified 31 relevant clinical studies published between 1 January 1990 and 31 December 2012, with 4,546 patients in whom lumefantrine concentrations were measured. Under the auspices of WWARN, relevant individual concentration-time data, clinical covariates, and outcome data from 4,122 patients were made available and pooled for the meta-analysis. The developed lumefantrine population pharmacokinetic model was used for dose optimisation through in silico simulations. Venous plasma lumefantrine concentrations 7 days after starting standard AL treatment were 24.2% and 13.4% lower in children weighing <15 kg and 15–25 kg, respectively, and 20.2% lower in pregnant women compared with non-pregnant adults. Lumefantrine exposure decreased with increasing pre-treatment parasitaemia, and the dose limitation on absorption of lumefantrine was substantial. Simulations using the lumefantrine pharmacokinetic model suggest that, in young children and pregnant women beyond the first trimester, lengthening the dose regimen (twice daily for 5 days) and, to a lesser extent, intensifying the frequency of dosing (3 times daily for 3 days) would be more efficacious than using higher individual doses in the current standard treatment regimen (twice daily for 3 days). The model was developed using venous plasma data from patients receiving intact tablets with fat, and evaluations of alternative dosing regimens were consequently only representative for venous plasma after administration of intact tablets with fat. The absence of artemether-dihydroartemisinin data limited the prediction of parasite killing rates and recrudescent infections. Thus, the suggested optimised dosing schedule was based on the pharmacokinetic endpoint of lumefantrine plasma exposure at day 7. CONCLUSIONS. Our findings suggest that revised AL dosing regimens for young children and pregnant women would improve drug exposure but would require longer or more complex schedules. These dosing regimens should be evaluated in prospective clinical studies to determine whether they would improve cure rates, demonstrate adequate safety, and thereby prolong the useful therapeutic life of this valuable antimalarial treatment.

39. Limmathurotsakul D, Daily F, Bory S, Khim G, Wiersinga WJ, Torres AG, Dance DAB, Currie BJ (2019) for International Melioidosis Society and Melioidosis Research Collaborative Network. Melioidosis: the hazards of incomplete peer-review. *PLoS Neglected Tropical Diseases* 13(3): e0007123.

Abstract. As researchers working on *Burkholderia pseudomallei*, we were interested to see the review by Perumal Samy *et al.* [1], which highlights the significant clinical impact and public health threat caused by melioidosis in the tropics. However, although the

LOMWRU staff in verdant Microbiology Laboratory Garden, May 2018

general content of the abstract is valid, unfortunately the review itself contains considerable misleading, incomplete and inaccurate information. Whilst not wishing to appear unduly critical, we have become aware that the review is already causing clinicians and microbiologists confusion, which could have a dangerous impact on the care of melioidosis patients. This was reinforced during our 2nd Cambodia National Melioidosis Workshop in October 2017, with several participants noting that they had read the open access review and were following some of the (dangerously) incorrect information therein. We, therefore, felt obliged to write to highlight the issue, which we feel reflects a failure of the peer-review process and the authors' inadequate interpretation of recent advances in the melioidosis field. We have outlined some of the most important inaccuracies below.

40. Miller AK, Ghionea S, Vongsouvath M, Davong V, Mayxay M, Somoskovi A, Newton PN, Bell D, Friend M (2018) A robust incubator to improve access to microbiological culture in low resource environments. *J Medical Devices*

Abstract. To help address the limitations of operating conventional microbiological culture incubators in low resource environments, a new incubator design was developed and tested to meet the requirements of operation in laboratories without reliable power (power outages up to 12 contiguous hours) or climate control (ambient indoor temperatures from 5 °C to 45 °C). The device is designed to enable adherence to incubation temperatures recommended for growth detection, identification and drug susceptibility testing of human pathogenic bacteria. During power outages, stable temperatures are maintained in the device's internal sample compartment by employing phase change material (PCM) as a bi-directional thermal battery to maintain incubation temperature. Five prototypes were tested in a laboratory setting using environmental test chambers and programmable power supplies, and three were field tested in the Lao PDR in situations of intended use. The prototypes successfully held their temperature to within +/- 1 °C in both laboratory environmental chamber testing as well as 22 during the field test. The results indicate that

the device will maintain stable culture temperatures across periods of intermittent power supply, while enabling normal workflow This could greatly increase the availability of microbiological culture for diagnosis and antimicrobial resistance monitoring.

41. Mukhopadhyay C, Shaw T, Varghese GM, Dance DAB (2018) Melioidosis in South Asia (India, Nepal, Pakistan, Bhutan and Afghanistan). *Trop Med Infect Dis* 3(2): 51.

Abstract. Despite the fact that South Asia is predicted to have the highest number of cases worldwide, melioidosis is a little-known entity in South Asian countries. It has never been heard of by the majority of doctors and has as yet failed to gain the attention of national Ministries of Health and country offices of the World Health Organization (WHO). Although a few centers are diagnosing increasing numbers of cases, and the mortality documented from these institutions is relatively high (nearly 20%), the true burden of the disease remains unknown. In India, most cases have been reported from southwestern coastal Karnataka and northeastern Tamil Nadu, although this probably simply reflects the presence of centers of excellence and researchers with an interest in the disease. As elsewhere, the majority of cases have type 2 diabetes mellitus and occupational exposure to the environment. Most present with community-acquired pneumonia and/or bacteremia, especially during heavy rainfall. The high seropositivity rate (29%) in Karnataka and isolation of *B. pseudomallei* from the environment in Tamil Nadu and Kerala confirm India as melioidosis-endemic, although the full extent of the distribution of the organism across the country is unknown. There are limited molecular epidemiological data, but, thus far, the majority of Indian isolates have appeared distinct from those from South East Asia and Australia. Among other South Asian countries, Sri Lanka and Bangladesh are known to be melioidosis-endemic, but there are no cases that have conclusively proved to have been acquired in Nepal, Bhutan, Afghanistan or Pakistan. There are no surveillance systems in place for melioidosis in South Asian countries. However, over the past two years, researchers at the Center for Emerging and Tropical Diseases of Kasturba Medical College, University of Manipal, have established the Indian Melioidosis Research Forum (IMRF), held the first South Asian Melioidosis Congress, and have been working to connect researchers, microbiologists and physicians in India and elsewhere in South Asia to raise awareness through training initiatives, the media, workshops, and conferences, with the hope that more patients with melioidosis will be diagnosed and treated appropriately. However, much more work needs to be done before we will know the true burden and distribution of melioidosis across South Asia.

42. Newton PN, Keolouangkhot V, Lee SJ, Choumlivong K, Sisouphone S, Choumlivong K, Vongsouvath M, Mayxay M, Chansamouth V, Davong V, Phommasone K, Sirisouk J, Blacksell SD, Nawtaisong P, Moore CE, Castonguay-Vanier J, Dittrich S, Rattanavong S, Chang K, Darasavath C, Rattanavong O, Paris DH, Phetsouvanh R (2019) A prospective, open-label, randomized trial of doxycycline versus azithromycin for the treatment of uncomplicated murine typhus. *Clin Inf Dis 68(5):738-747*

Abstract. BACKGROUND. Murine typhus, or infection with *Rickettsia typhi*, is a global but neglected disease without randomized clinical trials to guide antibiotic therapy. METHODS. A prospective, open, randomized trial was conducted in nonpregnant, consenting inpatient adults with rapid diagnostic test evidence of uncomplicated murine typhus at 2 hospitals in Vientiane, Laos. Patients were randomized to 7 days (D7) or 3 days (D3) of oral doxycycline or 3 days of oral azithromycin (A3). Primary outcome measures were fever clearance time and frequencies of treatment failure and relapse. RESULTS. Between 2004 and 2009, the study enrolled 216 patients (72 per arm); 158 (73.2%) had serology/polymerase chain reaction (PCR)–confirmed murine typhus, and 52 (24.1%) were *R. typhi* PCR positive. The risk of treatment failure was greater for regimen A3 (22.5%; 16 of 71 patients) than for D3 (4.2%; 3 of 71) or D7 (1.4%; 1 of 71) (P < .001). Among *R. typhi* PCR–positive patients, the area under the time-temperature curve and the fever clearance time were significantly higher for A3 than for D3 (1.8- and 1.9-fold higher, respectively; P = .005) and D7 (1.5- and 1.6-fold higher; P = .02). No patients returned with PCR-confirmed *R. typhi* relapse. CONCLUSION. In Lao adults, azithromycin is inferior to doxycycline as oral therapy for uncomplicated murine typhus. For doxycycline, 3- and 7-day regimens have similar efficacy. Azithromycin use in murine typhus should be reconsidered. Investigation of genomic and phenotypic markers of *R. typhi* azithromycin resistance is needed.

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

[A review of medicine quality and public health]

44. Newton PN, Day NJP (in press) Scrub typhus. Hunter's Tropical Medicine and Emerging Infectious Disease 10th edition.

[A review of scrub typhus]

45. Ombelet S, Ronat J-B, Walsh T, Yansouni CP, Cox J, Vlieghe E, Martiny D, Semret M, Vandenberg O, Jacobs J, On behalf of the BACTI-LRS working group (2018) Clinical bacteriology in low resource settings – not tomorrow's but today's solutions. *Lancet Infectious Diseases* 18, 8, e248-e258.

Abstract. Low-resource settings (LRS) are disproportionately burdened by infectious diseases and antimicrobial resistance (AMR). Good quality bacteriology is a prerequisite for effective AMR control, but LRS face infrastructural, technical and behavioural challenges when implementing clinical bacteriology. This article explores the needs for successful implementation of clinical bacteriology in LRS. The majority of microbiological techniques and equipment have not been developed for the specific needs of LRS. Pending the arrival of a new generation of LRS-friendly diagnostics, we have focused on improving, adapting and implementing conventional, culture-based techniques. LRS priorities include harmonized, quality-assured and "tropicalized" equipment, consumables and techniques as well as rationalized bacterial identification and AMR testing. Diagnostics should be integrated into clinical care and patient management; moreover, clinically relevant specimens must be appropriately selected and prioritized. Open-access training materials and information management tools should be developed. We also advocate on-site validation and field-adoption of diagnostics in LRS, with considerable shortening of the time between development and implementation of diagnostics. We argue that implementing clinical bacteriology in LRS improves patient management, provides valuable surveillance for local antibiotic treatment guidelines and national policies, and augments AMR containment and hospital infection prevention and control.

46. Pastorino B, Sengvilaipaseuth O, Chanthongthip A, Vongsouvath M, Souksakhone M, Mayxay M, Thirion L, Newton PN, de Lamballerie X, Dubot-Pérès A (in press) Low Zika Virus Seroprevalence in Vientiane, Laos, 2003-2015. *Am J Trop Med Hyg*

Abstract. *Zika virus* (ZIKV) has been presumed to be endemic in Southeast-Asia (SEA), with a low rate of human infections. Although the first ZIKV evidence was obtained in the 1950s through serosurveys the first laboratory confirmed case was only detected in 2010 in Cambodia. The epidemiology of ZIKV in SEA remains uncertain due to the scarcity of available data. From 2016, subsequent to the large outbreaks in the Pacific and Latin America, several Asian countries started reporting increasing numbers of confirmed ZIKV patients, but no global epidemiological assessment is available to date. Here, with the aim of providing information on ZIKV circulation and population immunity, we conducted a seroprevalence study amongst blood donors in Vientiane, Laos. Sera from 359 asymptomatic consenting adult donors in 2003-2004 and 687 in 2015 were screened for anti-ZIKV IgG using NS1 ELISA assay (Euroimmun). Positive and equivocal samples were confirmed for anti-ZIKV neutralizing antibodies by Virus Neutralisation Tests.

Our findings suggest that ZIKV has been circulating in Vientiane over at least the last decade. ZIKV seroprevalence observed in the studied blood donors was low, 4.5% in 2003-2004 with an increase in 2015 to 9.9% ($p = 0.002$), possibly reflecting the increase of ZIKV incident cases reported over this period. We did not observe any significant difference in seroprevalence according to gender. With a low herd immunity in the Vientiane population, ZIKV represents a risk for future large scale outbreaks. Implementation of a nation-wide ZIKV surveillance network as well as epidemiological studies throughout the country are needed.

47. Phetsouvanh R, Habe S, Newton P, Vongsouvaht M, Horii H, Doanh PN, Nawa Y (2018) Spontaneous Emergence Of A *Gnathostoma spinigerum* Adult Worm From The Abdominal Skin Of A Laotian Woman: A Case Report. *Southeast Asian J Trop Med Public Health* 49 (1) 1-5.

Abstract. Gnathostomiasis caused by infection with the Spirurine nematode, *Gnathostoma* species, is a common fish-borne parasitic zoonosis in Asia. We present here the case of the spontaneous emergence

of an adult *Gnathostoma spinigerum* worm from the abdominal skin of a Laotian woman. We review the literature on gnathostomiasis and discover that infective *G. spinigerum* larvae can grow into immature and mature worms in humans more commonly than expected.

48. Phouangsouvanh S, Mayxay M, Keoluangkhot V, Vongsouvath M, Davong V, Dance DAB (2018) Antimicrobial susceptibility of *Neisseria gonorrhoeae* isolates in Vientiane, Lao PDR. *J Glob Antimicrob Resist* 13: 91–93.

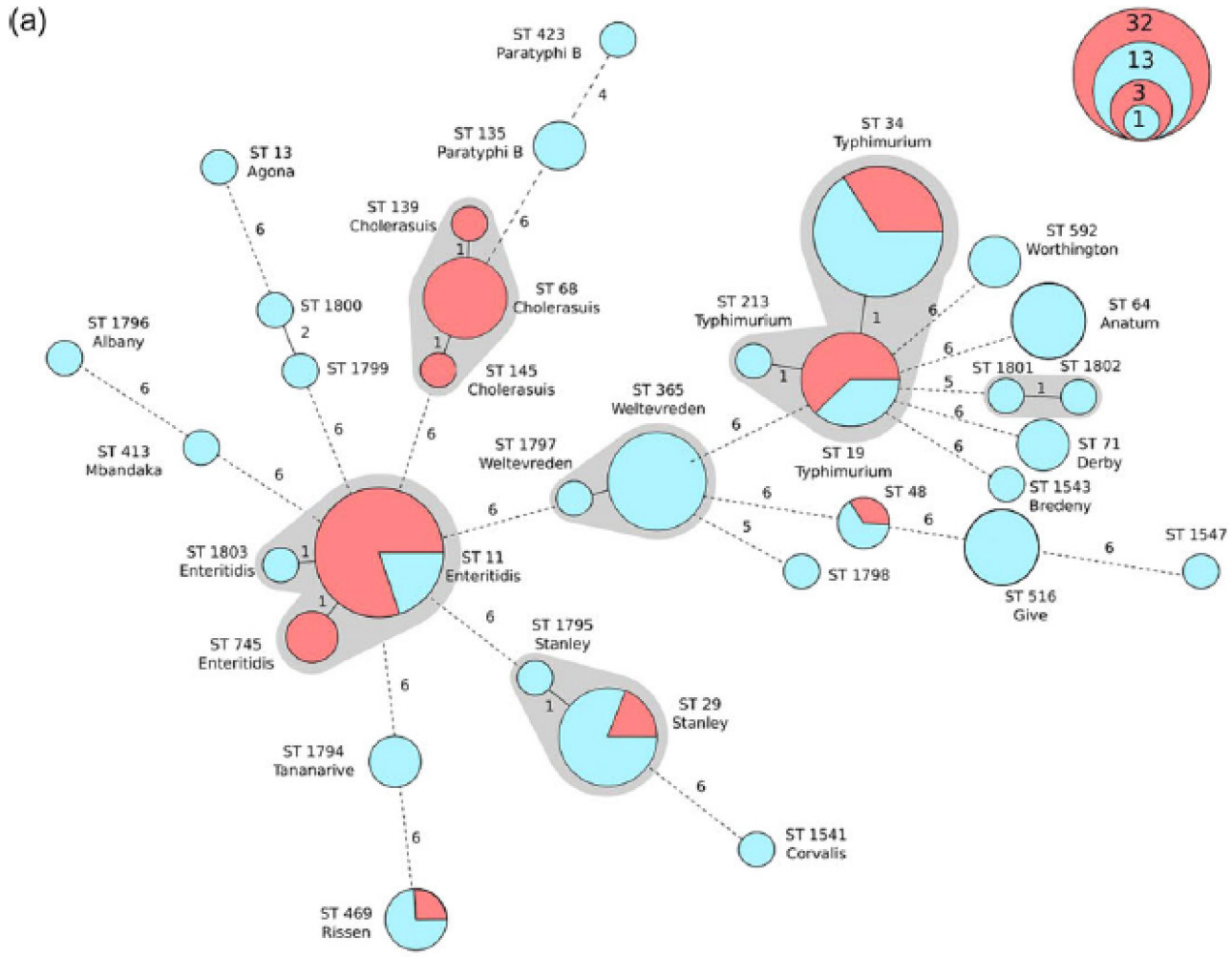
Abstract. OBJECTIVES. The aim of this study was to determine the antimicrobial susceptibility of *Neisseria gonorrhoeae* isolates in the Lao People's Democratic Republic (Laos). METHODS. A total of 165 gonococcal isolates (1.3%) were obtained from 12 281 genital samples routinely submitted to a diagnostic laboratory in Vientiane, Laos, between 2011 and 2015. Susceptibility to five antibiotics was determined by the standard disk diffusion method for 158 of the isolates. RESULTS. Rates of resistance to penicillin (by β-lactamase production), tetracycline and ciprofloxacin were 89.9%, 99.4% and 84.8%, respectively. All isolates were susceptible to ceftriaxone and spectinomycin. CONCLUSIONS. The situation in Laos is similar to that in neighbouring countries; this fortunately means that the latest Lao national guidelines for treating gonorrhoea should still be effective.

49. Phuong TLT, Rattanavong S, Vongsouvath M, Davong V, Lan NPH, Campbell JI, Darton TC, Thwaites GE, Newton PN, Dance DAB, Baker S (2018) Non-typhoidal Salmonella serovars associated with invasive and non-invasive disease in the Lao People's Democratic Republic *Trans R Soc Trop Med Hyg* 111(9): 418–424.

Abstract. BACKGROUND. Invasive non-typhoidal *Salmonella* (iNTS) disease is a well-described cause of mortality in children and human immunodeficiency virus (HIV)-infected adults in sub-Saharan Africa. Additionally, there is an ill-defined burden of iNTS disease in Southeast Asia. METHODS. Aiming to investigate the causative serovars of non-invasive and iNTS disease and their associated antimicrobial susceptibility profiles in the Lao People's Democratic Republic, we performed multilocus sequence typing and antimicrobial susceptibility profiling on 168 NTS (63 blood and 105 faecal) organisms isolated in Lao between 2000 and 2012. RESULTS. Six different serovars were isolated from blood; *Salmonella enterica* serovar Enteritidis (n=28), *S. enterica* serovar Typhimurium (n=19) and *S. enterica* serovar Choleraesuis (n=11) accounted for >90% (58/63) of the iNTS disease cases. In contrast, the isolates from diarrhoeal faeces were comprised of 18 different serovars, the mostly commonly identified being *S. enterica* Typhimurium (n=28), *S. enterica* Weltevreden (n=14) and *S. enterica* Stanley (n=15). *S. enterica* Enteritidis and *S. enterica* Choleraesuis were significantly more associated with systemic disease than diarrhoeal disease in this patient group (p<0.001). CONCLUSIONS. We find a differing distribution of *Salmonella* sequence types/serovars between those causing iNTS disease and non-invasive disease in Lao. We conclude that there is a small but not insignificant burden of iNTS disease in Lao. Further clinical and epidemiological investigations are required to assess mortality and the role of comorbidities such as HIV.

50. Phuklia W, Panyanivong P, Sengdetka D, Sonthayanon P, Newton PN, Paris DH, Day NPJ, Dittrich S (2018) Novel high-throughput screening method using quantitative PCR to determine the antimicrobial susceptibility of *Orientia tsutsugamushi* clinical isolates. *J Antimicrob Chemother* 74, 1, 1, 74–81.

Abstract. OBJECTIVES. To develop a method to enable the large-scale antimicrobial susceptibility screening of *Orientia tsutsugamushi* clinical isolates, using one timepoint and one concentration of antibiotics to considerably speed up the time to result. METHODS. Growth, harvesting, multiplicity of infection (moi) and the day to determine the MICs were optimized using five *O. tsutsugamushi* reference strains [susceptible (Karp, Kato and Gilliam) and putatively resistant (AFC-3 and AFSC-4)], one clinical isolate (UT76) and one rodent isolate (TA763). Subsequently, the MICs of azithromycin, chloramphenicol and doxycycline for these strains and 51 clinical isolates including AFSC-7 were determined. An optimal concentration was calculated using the epidemiological cut-off value. RESULTS. The conditions for *O. tsutsugamushi* infection, growth and harvesting were determined to be an moi of 100:1 and trypsinization with the peak growth on day 10. The resulting MICs were in line with previously published susceptibility data for all reference strains, except for



Identified Non-typhoidal Salmonella serovars causing invasive and non-invasive disease in Laos. Minimum spanning tree of 168 Lao NTS isolates created using seven-allele MLST profiling; the sources of the organisms are colour coded (red, blood; blue, faeces). The sequence type (ST) of each allele profile is shown along with the inferred serovar.

Karp and AFSC-4, which showed azithromycin MICs of 0.0156 and 0.0313 mg/L, compared with 0.0078 and 0.0156 mg/L, respectively, in previous reports. The MIC of doxycycline for AFC-3 was 0.125 mg/L compared with >4 mg/L in earlier reports. The final single screening concentrations were identified as: azithromycin, 0.125 mg/L; chloramphenicol, 8 mg/L; and doxycycline, 1 mg/L. CONCLUSIONS. This simplified procedure facilitates the simultaneous screening of 48 isolates for actively monitoring potential resistance of this important fever pathogen, with an 8-fold throughput improvement over early methods. The data do not support the existence of doxycycline- and chloramphenicol-resistant scrub typhus.

51. Pongvongsa T, Phommasone K, Adhikari B, Henriques G, Chotivanich K, Hanboonkunupakarn B, Mukaka M, Peerawaranun P, von Seidlein L, Day NPJ, White NJ, Dondorp AM, Imwong M, Newton PN, Singhasivanon P, Mayxay M, Pukrittayakamee S (2018) The dynamic of asymptomatic *Plasmodium falciparum* infections following mass drug administrations with dihydroarteminisin-piperaquine plus a single low dose of primaquine in Savannakhet Province, Laos. *Malar J* 17(1):405.

Abstract. BACKGROUND. The increase in multidrug resistant *Plasmodium falciparum* infections threatens the malaria elimination goals in countries within the Greater Mekong Sub-region. A multi-pronged approach assuring access to basic malaria control measures, including insecticide-treated bed nets and early diagnosis and treatment was followed by mass drug administrations (MDA) in southern Savannakhet Province, Laos. The main objective of this study was

Boat Race Party at Paul's House, October 2018

to evaluate the effectiveness and safety of mass drug administrations as well as their effects on the dynamic of asymptomatic *P. falciparum* infections in 4 malaria endemic villages. METHODS. Two villages were randomized to early MDA consisting of 3 rounds of a 3-day course of dihydroartemisinin–piperaquine with a single low dose of primaquine. In the other 2 villages MDA was deferred by 1 year. A total of 1036 residents were enrolled in early MDA villages and 883 in control villages (deferred-MDA). Tri-monthly parasitaemia surveys using uPCR were conducted for a year in the 4 villages. RESULTS. Eighty-four percent (872/1036) of the residents participated in the MDAs, of whom 90% (781/872) completed 3 rounds of MDA (9 doses). In intervention villages, the prevalence of asymptomatic *P. falciparum* infections decreased by 85% after MDA from 4.8% (95% CI 3.4–6.4) at baseline (month 0 or M0) to 0.7% (95% CI 0.3–1.6) at month 12. In control villages there was a decrease of 33% in *P. falciparum* prevalence between M0: 17.5% (95% CI 15.9–20.3) and M12: 11.6% (95% CI 9.3–14.2). In bivariate and multivariate analyses *P. falciparum* infections were significantly reduced with early MDA (adjusted incidence rate ratios (AIRR): 0.08, CI 0.01–0.091) and completion of 3 MDA rounds (AIRR: 0.06; CI 0.01–0.66). A quarter of participants (226/872) reported adverse events of which 99% were mild. CONCLUSION. The study found a significant reduction in *P. falciparum* prevalence and incidence following MDA. MDA was safe, well tolerated, feasible, and achieved high population coverage and adherence. MDAs must be integrated in multi-pronged approaches such as vector control and preventive measures with a focus on specific risk groups such as mobile, migrant population and forest goers for a sustained period to eliminate the remaining parasite reservoirs.

52. Robinson MT, Vongphayloth K, Hertz JC, Brey P, Newton PN (2018) Tick-transmitted human infections in Asia. *Microbiology Australia.*

[A review of the epidemiology, biology and clinical significance of tick-transmitted pathogens in Asia]

53. Rolim DB, Lima RXR, Ribeiro AKC, Colares RM, Lima LDQ, Rodríguez-Morales AJ, Montúfar

FE, David A. B. Dance (2018) Melioidosis in South America. *Trop Med Infect Dis* 3(2): 60.

Abstract. Melioidosis is an emerging disease in the Americas. This paper reviews confirmed cases, the presence of *Burkholderia pseudomallei* and the organization of national surveillance policies for melioidosis in South America. Confirmed cases in humans have been reported from Ecuador, Venezuela, Colombia, Brazil, and Peru. The bacterium has been isolated from the environment in Brazil and Peru. The state of Ceará, northeastern region of Brazil, is the only place where specific public strategies and policies for melioidosis have been developed. We also discuss the urgent need for health authorities in South America to pay greater attention to this disease, which has the potential to have a high impact on public health, and the importance of developing coordinated strategies amongst countries in this region.

54. San Martin PFM, Chua JC, Bautista RLP, Nailes JM, Panaligan MM, Dance DAB (2018) Melioidosis in the Philippines. *Trop Med Infect Dis* 3(3): 99.

Abstract. The first documented case of melioidosis in the Philippines occurred in 1948. Since then, there have been sporadic reports in the literature about travelers diagnosed with melioidosis after returning from the Philippines. Indigenous cases, however, have been documented rarely, and under-reporting is highly likely. This review collated all Philippine cases of melioidosis published internationally and locally, as well as unpublished case series and reports from different tertiary hospitals in the Philippines. In total, 25 papers and 41 cases were identified. Among these, 23 were indigenous cases (of which 20 have not been previously reported in the literature). The most common co-morbidity present was diabetes mellitus, and the most common presentations were pulmonary and soft tissue infections. Most of the cases received ceftazidime during the intensive phase, while trimethoprim-sulfamethoxazole was given during the eradication phase. The known mortality rate was 14.6%, while 4.9% of all cases were reported to have had recurrence. The true burden of melioidosis in the country is not well defined. A lack of awareness among clinicians, a dearth of adequate laboratories, and the absence of a surveillance system for the disease are major challenges in determining the magnitude of the problem.

Wild bananas showing the large seeds that cause bezoars

55. Satzke C, Dunne EM, Choummanivong M, Ortika BD, Neal EFG, Pell CL, Nation ML, Fox KK, Nguyen CD, Gould KA, Hinds J, Chanthongthip A, Xeuatvongsa A, Mulholland EK, Sychareun V, Russell FM (2019) Pneumococcal carriage in vaccine-eligible children and unvaccinated infants in Lao PDR two years following the introduction of the 13-valent pneumococcal conjugate vaccine. *Vaccine.* 37(2):296-305.

Abstract. Pneumococcal carriage is a prerequisite for disease, and underpins herd protection provided by pneumococcal conjugate vaccines (PCVs). There are few data on the impact of PCVs in lower income settings, particularly in Asia. In 2013, the Lao People's Democratic Republic (Lao PDR) introduced 13-valent PCV (PCV13) as a 3 + 0 schedule (doses at 6, 10 and 14 weeks of age) with limited catch-up vaccination. We conducted two cross-sectional carriage surveys (pre- and two years post-PCV) to assess the impact of PCV13 on nasopharyngeal pneumococcal carriage in 5-8 week old infants (n = 1000) and 12-23 month old children (n = 1010). Pneumococci were detected by quantitative real-time PCR, and molecular serotyping was performed using DNA microarray. Post PCV13, there was a 23% relative reduction in PCV13-type carriage in children aged 12-23 months (adjusted

prevalence ratio [aPR] 0.77 [0.61-0.96]), and no significant change in non-PCV13 serotype carriage (aPR 1.11 [0.89-1.38]). In infants too young to be vaccinated, there was no significant change in carriage of PCV13 serotypes (aPR 0.74 [0.43-1.27]) or non-PCV13 serotypes (aPR 1.29 [0.85-1.96]), although trends were suggestive of indirect effects. Over 70% of pneumococcal-positive samples contained at least one antimicrobial resistance gene, which were more common in PCV13 serotypes ($p < 0.001$). In 12-23 month old children, pneumococcal density of both PCV13 serotypes and non-PCV13 serotypes was higher in PCV13-vaccinated compared with undervaccinated children ($p = 0.004$ and $p < 0.001$, respectively). This study provides evidence of PCV13 impact on carriage in a population without prior PCV7 utilisation, and provides important data from a lower-middle income setting in Asia. The reductions in PCV13 serotype carriage in vaccine-eligible children are likely to result in reductions in pneumococcal transmission and disease in Lao PDR.

56. Saralamba N, Mayxay M, Newton PN, Smithuis F, Nosten F, Archasuksan L, Pukrittayakamee S, White NJ, Day NPJ, Dondorp AM, Imwong M (2018) Genetic polymorphisms in the circumsporozoite protein of *Plasmodium malariae* show a geographical bias. *Malar J.* 17: 269.

Abstract. BACKGROUND. *Plasmodium malariae* is characterized by its long asymptomatic persistence in the human host. The epidemiology of *P. malariae* is incompletely understood and is hampered by the limited knowledge of genetic polymorphisms. Previous reports from Africa have shown heterogeneity within the *P. malariae circumsporozoite protein* (*pmcsp*) gene. However, comparative studies from Asian countries are lacking. Here, the genetic polymorphisms in *pmcsp* of Asian isolates have been characterized. METHODS. Blood samples from 89 symptomatic *P. malariae*-infected patients were collected, from Thailand ($n = 43$), Myanmar ($n = 40$), Lao PDR ($n = 5$), and Bangladesh ($n = 1$). *pmcsp* was amplified using semi-nested PCR before sequencing. The resulting 89 *pmcsp* sequences were analysed together with 58 previously published *pmcsp* sequences representing African countries using BioEdit, MEGA6, and DnaSP. RESULTS. Polymorphisms identified in *pmcsp* were grouped into 3 populations: Thailand, Myanmar, and Kenya. The nucleotide diversity and the ratio of nonsynonymous to synonymous substitutions (dN/dS) in Thailand and Myanmar were higher compared with that in Kenya. Phylogenetic analysis showed clustering of *pmcsp* sequences according to the origin of isolates (Asia vs. Africa). High genetic differentiation ($Fst = 0.404$) was observed between *P. malariae* isolates from Asian and African countries. Sequence analysis of *pmcsp* showed the presence of tetrapeptide repeat units of NAAG, NDAG, and NAPG in the central repeat region of the gene. *Plasmodium malariae* isolates from Asian countries carried fewer copies of NAAG compared with that from African countries. The NAPG repeat was only observed in Asian isolates. Additional analysis of 2 T-cell epitopes, Th2R and Th3R, showed limited heterogeneity in *P. malariae* populations. CONCLUSIONS. This study provides valuable information on the genetic polymorphisms in *pmcsp* isolates from Asia and advances our understanding of *P. malariae* population in Asia and Africa. Polymorphisms in the central repeat region of *pmcsp* showed association with the geographical origin of *P. malariae* isolates and can be potentially used as a marker for genetic epidemiology of *P. malariae* population.

57. Shrestha P, Roberts T, Homsana A, Myat TO, Crump JA, Lubell Y, Newton PN (2018) Febrile illness in Asia: gaps in epidemiology, diagnosis, management for informing health policy. *Clinical Microbiology and Infection* 24, 8, 815–826.

Abstract. BACKGROUND. Increasing evidence is becoming available on the aetiology and management of fevers in Asia; the importance of these fevers has increased with the decline in the incidence of malaria. Aims: To conduct a narrative review of the epidemiology and management of fevers in South and South- East Asia and to highlight gaps in our knowledge that impair evidence-based health policy decisions. SOURCES. A narrative review of papers published since 2012 on developments in fever epidemiology, diagnosis and treatment in South and South-East Asia. The papers that the authors felt were pivotal, from their personal perspectives, are discussed. CONTENT. We identified 100 studies. Among the 30 studies (30%) including both children and adults that investigated three or more pathogens, the most frequently reported fever aetiology was dengue (reported by 15, 50%), followed by leptospirosis (eight, 27%), scrub typhus (seven, 23%) and *Salmonella* serovar Typhi (six, 20%). Among four studies investigating three or more pathogens in children, dengue and *Staphylococcus aureus* were the most frequent, followed by non-typhoidal *Salmonella* spp, *Streptococcus pneumoniae*,

Salmonella serovar Typhi, and *Orientia tsutsugamushi*. Increased awareness is needed that rickettsial pathogens are common but do not respond to cephalosporins, and that alternative therapies, such as tetracyclines, are required. Implications: Many key gaps remain, and consensus guidelines for study design are needed to aid comparative understanding of the epidemiology of fevers. More investment in developing accurate and affordable diagnostic tests for rural Asia and independent evaluation of those already on the market are needed. Treatment algorithms, including simple biomarker assays, appropriate for empirical therapy of fevers in different areas of rural Asia should be a major aim of fever research. Enhanced antimicrobial resistance (AMR) surveillance and openly accessible databases of geography-specific AMR data would inform policy on empirical and specific therapy. More investment in innovative strategies facilitating infectious disease surveillance in remote rural communities would be an important component of poverty reduction and improving public health.

58. Sonthayanon S, Jaresitthikunchai J, Mangmee S, Thiangtrongjit T, Wuthiekanun V, Amornchai P, Newton PN, Phetsouvanh R, Day NPJ, Roytrakul S (in press) Whole cell matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) for identification of *Leptospira* spp. in Thailand and Lao PDR. *PloS NTD*

Abstract. Leptospirosis is a zoonosis with a worldwide distribution, caused by pathogenic spirochetes of the genus *Leptospira*. The classification and identification of leptospires can be conducted by both genotyping and serotyping which are time-consuming and established in few reference laboratories. This study used matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) as rapid and accurate tool for the identification of leptospires. The whole cell protein spectra of 116 *Leptospira* isolates including 15 references *Leptospira* spp. (pathogenic, n=8; intermediate, n=2; non-pathogenic, n=5) and 101 *Leptospira* spp. clinical isolates was created as an in-house MALDI-TOF MS database. Ninety-seven clinical isolates from Thailand and Laos was validated with these protein spectra and revealed 98.9% correct identification when compared with 16S rRNA gene sequences method. Moreover, MALDI-TOF MS could identify spiked leptospires whole cell in urine. Biomarkers for differentiation of leptospires phylogeny and specific protein spectra for most found *Leptospira* spp. in this area (*L. interrogans, L. kirschneri, L. borgpetersenii)* based on MALDI-MS algorithm were demonstrated.

59. Srisutham S, Saralamba N, Sriprawat K, Mayxay M, Smithuis F, Nosten F, Pukrittayakamee S, Day NPJ, Dondorp AM, Imwong M (2018) Genetic diversity of three surface protein genes in *Plasmodium malariae* from three Asian countries. *Malar J* 17: 24.

Abstract. BACKGROUND. Genetic diversity of the three important antigenic proteins, namely thrombospondin-related anonymous protein (TRAP), apical membrane antigen 1 (AMA1), and 6-cysteine protein (P48/45), all of which are found in various developmental stages of *Plasmodium* parasites is crucial for targeted vaccine development. While studies related to the genetic diversity of these proteins are available for *Plasmodium falciparum* and *Plasmodium vivax*, barely enough information exists regarding *Plasmodium malariae*. The present study aims to demonstrate the genetic variations existing among these three genes in *P. malariae* by analysing their diversity at nucleotide and protein levels. METHODS. Three surface protein genes were isolated from 45 samples collected in Thailand (N = 33), Myanmar (N = 8), and Lao PDR (N = 4), using conventional polymerase chain reaction (PCR) assay. Then, the PCR products were sequenced and analysed using BioEdit, MEGA6, and DnaSP programs. RESULTS. The average pairwise nucleotide diversities (π) of *P. malariae trap*, *ama1*, and *p48/45* were 0.00169, 0.00413, and 0.00029, respectively. The haplotype diversities (Hd) of *P. malariae trap*, *ama1*, and *p48/45* were 0.919, 0.946, and 0.130, respectively. Most of the nucleotide substitutions were non-synonymous, which indicated that the genetic variations of these genes were maintained by positive diversifying selection, thus, suggesting their role as a potential target of protective immune response. Amino acid substitutions of *P. malariae* TRAP, AMA1, and P48/45 could be categorized to 17, 20, and 2 unique amino-acid variants, respectively. For further vaccine development, carboxyl terminal of P48/45 would be a good candidate according to conserved amino acid at low genetic diversity (π = 0.2–0.3). CONCLUSIONS. High mutational diversity was observed in *P. malariae trap* and *ama1* as compared to *p48/45* in *P. malariae* samples isolated from Thailand, Myanmar, and Lao PDR. Taken together, these results suggest that P48/45 might be a good vaccine candidate against *P. malariae* infection because of its sufficiently low genetic diversity and highly conserved amino acids especially on the carboxyl end.

60. Tabernero P, Swamidoss I, Mayxay M, Khanthavong M, Phonlavong C, Vilayhong C, Yeuchaixiong S, Sichanh C, Sengaloundeth S, Green MD, Newton PN (in press) A random survey of the prevalence of falsified and substandard antibiotics in the Lao PDR. Journal of Antimicrobial Chemotherapy

Abstract. In 2012 a stratified random survey, using mystery shoppers, was conducted to investigate the availability and quality of antibiotics sold to patients in the private sector in five southern provinces of the Lao People's Democratic Republic (Laos). A total of 147 outlets were sampled in 10 districts. The active pharmaceutical ingredient (API) content measurements for 909 samples, including nine APIs (amoxicillin, ampicillin, ceftriaxone, ciprofloxacin, doxycycline, ofloxacin, sulfamethoxazole, tetracycline and trimethoprim), were determined using High-Performance Liquid Chromatography (HPLC).

All the analysed samples contained the stated API and we found no evidence for falsification. All except one sample had all the units %API between 75-125% of the content stated on the label. However, we identified the presence of substandard antibiotics; 19.6% (201/1025) of samples had their units outside the 90-110% content of the label claim and 60.2% (617/1025) of the samples had units outside of the International Pharmacopoeia uniformity of content limit range. Amoxicillin had a high number of samples (151/225, 67.1%) with units above its International Pharmacopoeia limit range, followed by ciprofloxacin (10/17, 58.8%) and ofloxacin (39/68, 57.4%). Trimethoprim, sulfamethoxazole and ceftriaxone had a high number of samples with API content below the Pharmacopoeia limit range (64/124, 51.6%; 43/124, 34.7%; and 4/7 57.1% of samples, respectively). Significant differences in %API were found between stated countries of manufacture and stated manufacturers.

Substandard antibiotics will have reduced therapeutic efficacy, impacting public health and control of bacterial infections. Furthermore, although the contribution made by poor quality medicines to the development of antimicrobial resistance (AMR) remains poorly understood, substandard antibiotics are likely to engender AMR.

61. Tauran PM, Wahyunie S, Saad F, Dahesihdewi A, Graciella M, Muhammad M, Lestari DC, Aryati A, Parwati I, Loho T, Pratiwi DIV, Mutiawati VK, Loesnihari R, Anggraini D, Rahayu SI, Wulan WN, Antonjaya U, Dance DAB, Currie BJ, Limmathuthurotsakul D, Arif M, Aman AT, Budayanti NNS, Iskandriati D (2018) Emergence of Melioidosis in Indonesia and Today's Challenges. *Trop Med Infect Dis* 3(1): 32.

Abstract. A recent modeling study estimated that there could be as many as 20,000 human melioidosis cases per year in Indonesia, with around 10,000 potential deaths annually. Nonetheless, the true burden of melioidosis in Indonesia is still unknown. The Indonesia Melioidosis Network was formed during the first melioidosis workshop in 2017. Here, we reviewed 101 melioidosis cases (99 human and two animal cases) previously reported and described an additional 45 human melioidosis cases. All 146 culture-confirmed cases were found in Sumatra (n = 15), Java (n = 104), Kalimantan (n = 15), Sulawesi (n = 11) and Nusa Tenggara (n = 1). Misidentification of *Burkholderia pseudomallei* was not uncommon, and most cases were only recently identified. We also evaluated clinical manifestations and outcome of recent culture-confirmed cases between 2012 and 2017 (n = 42). Overall, 15 (36%) cases were children (age <15 years) and 27 (64%) were adults (age ≥15 years). The overall mortality was 43% (18/42). We conducted a survey and found that 57% (327/548) of healthcare

After Jana Li's Leaving Basci December 2017

workers had never heard of melioidosis. In conclusion, melioidosis is endemic throughout Indonesia and associated with high mortality. We propose that top priorities are increasing awareness of melioidosis amongst all healthcare workers, increasing the use of bacterial culture, and ensuring accurate identification of *B. pseudomallei* and diagnosis of melioidosis.

62. Thanh LT, Phan TH, Rattanavong S, Nguyen TM, Duong AV, Dacon C, Hoang TN, Nguyen LPH, Tran CTH, Davong V, Nguyen CVV, Thwaites GE, Boni MF, Dance D, Ashton PM, Day JN (2018) Multilocus sequence typing of *Cryptococcus neoformans* var. grubii from Laos in a regional and global context. *Med Mycol* Oct 19th

Abstract. Cryptococcosis causes approximately 180 000 deaths each year in patients with human immunodeficiency virus (HIV). Patients with other forms of immunosuppression are also at risk, and disease is increasingly recognized in apparently immunocompetent individuals. *Cryptococcus neoformans* var. grubii, responsible for the majority of cases, is distributed globally. We used the consensus ISHAM Multilocus sequence typing (MLST) scheme to define the population structure of clinical *C. neoformans* var. grubii isolates from Laos (n = 81), which we placed into the global context using published MLST data from other countries (total N = 1047), including a reanalysis of 136 Vietnamese isolates previously reported. We observed a phylogeographical relationship in which the Laotian population was similar to its neighbor Thailand, being dominated (83%) by Sequence Types (ST) 4 and 6. This phylogeographical structure changed moving eastwards, with Vietnam's population consisting of an admixture of isolates dominated by the ST4/ST6 (35%) and ST5 (48%) lineages. The ST5 lineage is the predominant ST reported from China and East Asia, where it accounts for >90% of isolates. Analysis of genetic distance (Fst) between different populations of *C. neoformans* var. grubii supports this intermediate structure of the Vietnamese population. The pathogen and host diversity reported from Vietnam provide the strongest epidemiological evidence of the association between ST5 and HIV-uninfected patients. Regional anthropological genetic distances suggest diversity in the *C. neoformans* var. grubii population across Southeast Asia is driven by ecological rather than human host factors. Where the ST5 lineage is present, disease in HIV-uninfected patients is to be expected.

63. Vickers S, Bernier M, Zambrzycki S, Fernández FM, Newton PN, Caillet C (2018) Field detection devices for medicines quality screening: a systematic review. *BMJ Global Health* 3:e000725.

Abstract. BACKGROUND. Poor quality medicines have devastating consequences. A plethora of innovative portable devices to screen for poor quality medicines has become available, leading to hope that they could empower medicine inspectors and enhance surveillance. However, information comparing these new technologies is woefully scarce. METHODS. We undertook a systematic review of Embase, PubMed, Web of Science and SciFinder databases up to 30 April 2018. Scientific studies evaluating the performances/abilities of portable devices to assess any aspect of the quality of pharmaceutical products were included. RESULTS. Forty-one devices, from small benchtop spectrometers to 'lab-on-a-chip' single-use devices, with prices ranging from <US$10 to >US$20 000, were included. Only six devices had been field-tested (GPHF-Minilab, CD3/CD3+, TruScan RM, lateral flow dipstick immunoassay, CBEx and Speedy Breedy). The median (range) number of active pharmaceutical ingredients (APIs) assessed per device was only 2 (1–20). The majority of devices showed promise to distinguish genuine from falsified medicines. Devices with the potential to assay API (semi)-quantitatively required consumables and were destructive (GPHF-Minilab, PharmaChk, aPADs, lateral flow immunoassay dipsticks, paper-based microfluidic strip and capillary electrophoresis), except for spectroscopic devices. However, the 10 spectroscopic devices tested for their abilities to quantitate APIs required processing complex API-specific calibration models. Scientific evidence of the ability of the devices to accurately test liquid, capsule or topical formulations, or to distinguish between chiral molecules, was limited. There was no comment on cost-effectiveness and little information on where in the pharmaceutical supply chain these devices could be best deployed. CONCLUSION. Although a diverse range of portable field detection devices for medicines quality screening is available, there is a vitally important lack of independent evaluation of the majority of devices, particularly in field settings. Intensive research is needed in order to inform national medicines regulatory authorities of the optimal choice of device(s) to combat poor quality medicines.

64. von Seidlein L, Peto TJ, Landier J, Nguyen TN, Tripura R, Phommasone K, Pongvongsa T, Lwin KM, Keereecharoen L, Kajeechiwa L, Thwin MM, Parker DM, Wiladphaingern J, Nosten S, Proux S, Corbel V, Tuong-Vy N, Phuc-Nhi TL, Son DH, Huong-Thu PN, Tuyen NTK, Tien NT, Dong LT, Hue DV, Quang HH, Nguon C, Davoeung C, Rekol H, Adhikari B, Henriques G, Phongmany P, Suangkanarat P, Jeeyapant A, Vihokhern B, Watson J, van der Pluijm R, Lubell Y, White LJ, Aguas R, Promnarate C, Sirithiranont P, Malleret B, Rénia L, Onsjö C, Chan XH, Chalk J, Miotto O, Patumrat K, Chotivanich K, Hanboonkunupakarn B, Jittmala P, Kaehler N, Cheah PY, Pell C, Dhorda M, Imwong M, Snounou G, Mukaka M, Peerawaranun P, Lee SJ, Simpson JA, Pukrittayakamee S, Singhasivanon P, Grobusch MP, Cobelens F, Smithuis F, Newton PN, Thwaites GE, Day NPJ, Mayxay M, Hien TT, Nosten FH, Dondorp AM, White NJ (2019) The impact of targeted malaria elimination with mass drug administrations on falciparum malaria in South-East Asia: a cluster randomised trial. *PloS Medicine* 16(2): e1002745.

Abstract. BACKGROUND. The emergence and spread of multidrug resistant *P.falciparum* in the Greater Mekong Subregion (GMS) threatens global malaria elimination efforts. Mass drug administration (MDA), the presumptive treatment of an entire population to clear the subclinical parasite reservoir, is a strategy to accelerate malaria elimination. We report a cluster-randomized trial to assess the duration of effectiveness of MDA on falciparum malaria incidence and prevalence in 16 remote village populations in four GMS countries where artemisinin resistance was prevalent. METHODS. 16 villages were targeted for malaria elimination. After establishing vector control, community-based case management and following intensive community engagement, 8 villages were selected by restricted random methods to receive early MDA and the other 8 villages served as controls for 12 months after which they received deferred MDA. The MDA comprised three monthly rounds of three doses dihydroartemisinin-piperaquine and a single low-dose primaquine (except Cambodia). Exhaustive, cross-sectional surveys of the entire village populations were conducted at quarterly intervals using ultrasensitive qPCR to detect *Plasmodium* infections. RESULTS. 8,445 residents (52%) living in 16 villages were randomised. Of 4,135 (52%) residents living in eight villages randomised to early MDA, 3,790 (86%) participated in at least one MDA round and 2,520 (57%) participated in all three rounds. By three months (M3) the *P.falciparum* prevalence had fallen by 92% (from 5.1% to 0.4%) in MDA villages and by 29% (from 7.2% to 5.1%) in control villages. Over the following 9 months the *P.falciparum* prevalence increased in early MDA villages to 3.3% and in control villages to 6.1% (adjusted incidence rate ratio [IRR] 0.41; [95%CI 0.20 to 0.84]). Individual protection was proportional to the number of completed MDA rounds. Of 221 participants with subclinical *P.falciparum* infections who participated in MDA and could be followed up, 207 (94%) cleared their infections including 9 of 10 infections with artemisinin and piperaquine resistance. The MDAs were well tolerated; there were no severe drug attributable adverse events. INTERPRETATION. Added to community-based basic malaria control measures, three rounds of MDA with dihydroartemisinin-piperaquine reduced the incidence and prevalence of falciparum malaria over a one-year period in areas affected by artemisinin resistance. Malaria was reintroduced, presumably from surrounding areas. MDA deployed in contiguous areas of higher transmission could be a useful additional tool for accelerating malaria elimination in the GMS.

65. Wiersinga W, Virk H, Limmathurotsakul D, Dance D, Peacock S, Torres A, Currie B (2018) Melioidosis. *Nature Reviews Disease Primers* 4:17107.

Abstract. *Burkholderia pseudomallei* is a Gram-negative environmental bacterium and the aetiological agent of melioidosis, a life-threatening infection that is estimated to account for 89,000 deaths per year worldwide. Diabetes mellitus is a major risk factor for melioidosis, and the global diabetes pandemic could increase the number of fatalities caused by melioidosis. Melioidosis is endemic across tropical areas, especially in Southeast Asia and northern Australia. Disease manifestations can range from acute septicaemia to chronic infection, as the facultative intracellular lifestyle and virulence factors of *B. pseudomallei* promote survival and persistence of the pathogen within a broad range of cells, and the bacteria can manipulate the host's immune responses and signalling pathways to escape surveillance. The majority of patients present with sepsis, but specific clinical presentations and their severity vary depending on the route of bacterial entry (skin penetration, inhalation or ingestion), host immune function and bacterial strain and load. Diagnosis is based on clinical and epidemiological features as well as bacterial culture. Treatment requires long-term intravenous and oral antibiotic courses.

The departure of the old International Clinic, Mahosot Hospital, December 2018

Delays in treatment due to difficulties in clinical recognition and laboratory diagnosis often lead to poor outcomes and mortality can exceed 40% in some regions. Research into *B. pseudomallei* is increasing, owing to the biothreat potential of this pathogen and increasing awareness of the disease and its burden; however, better diagnostic tests are needed to improve early confirmation of diagnosis, which would enable better therapeutic efficacy and survival.

66. Win MM, Ashley EA, Zin KN, Aung MT, Swe MMM, Ling CL, Nosten F, Thein WM, Zaw NN, Aung MY, Tun KM, Dance DAB, Smithuis FM (2018) Melioidosis in Myanmar. *Trop Med Infect Dis* 3(1): 28.

Abstract. Sporadic cases of melioidosis have been diagnosed in Myanmar since the disease was first described in Yangon in 1911. Published and unpublished cases are summarized here, along with results from environmental and serosurveys. A total of 298 cases have been reported from seven states or regions between 1911 and 2018, with the majority of these occurring before 1949. Findings from soil surveys confirm the presence of *Burkholderia pseudomallei* in the environment in all three regions examined. The true epidemiology of the disease in Myanmar is unknown. Important factors contributing to the current gaps in knowledge are lack of awareness among clinicians and insufficient laboratory diagnostic capacity in many parts of the country. This is likely to have led to substantial under-reporting.

67. Win MM, Hla T, Phyu KP, Aung WW, Win KKN, Aye SN, Wah TT, Aye KM, Htwe TT, Htay MT, San KK, Dance DAB (2019a) A study of *Burkholderia pseudomallei* in the environment of farms in Thanlyin and Hmawbi townships, Myanmar. *American Journal of Tropical Medicine and Hygiene*

Abstract. Melioidosis is a tropical infection, first described in Myanmar but now rarely diagnosed there, which is widespread in South East Asia. The infection is predominantly acquired by people and animals through contact with soil or water. This study aimed to detect the causative organism, *Burkholderia pseudomallei,* in environmental samples from farms

in Thanlyin and Hmawbi townships near Yangon, Myanmar. One hundred and twenty soil samples and 12 water samples were collected and processed using standard microbiological methods. *Burkholderia* species were isolated from 50 of 120 (42%) soil samples but none of the water samples. Arabinose assimilation was tested to differentiate between *B. pseudomallei* and the non-pathogenic *Burkholderia thailandensis*, and seven of 50 isolates (14%) were negative. These were all confirmed as *B. pseudomallei* by a species-specific multiplex PCR. This is the first study to detect environmental *B. pseudomallei* in Myanmar and confirms that melioidosis is still endemic in the Yangon area.

68. Win TT, Su KK, Than AM, Htut ZM, Pyar KP, Ashley EA, Dance DAB, Tun KM (2019b). Presence of *Burkholderia pseudomallei* in the 'Granary of Myanmar'. *Tropical Medicine and Infectious Diseases* 4, 8; doi:10.3390/tropicalmed4010008.

Abstract. Melioidosis is a frequently fatal infectious disease caused by the Gram negative bacillus *Burkholderia pseudomallei*. Although it was originally discovered in Myanmar, the disease disappeared from sight for many decades. This study focuses on detection of *B. pseudomallei* in soil in selected sampling sites in an attempt to start to fill the gaps in the current status of our knowledge of the geographical distribution of *B. pseudomallei* in soil in Myanmar. This cross-sectional study consists of 400 soil samples from 10 selected study townships from two major paddy growing regions. Bacterial isolation was done using a simplified method for the isolation of *Burkholderia pseudomallei* from soil. In this study, only 1% (4/400) of soil samples were found to be positive; two of four were found at 90 cm depth and another two positive samples were found at 30 cm and 60 cm. This survey has confirmed the presence of environmental *B. pseudomallei* in Myanmar indicating that the conditions are in place for melioidosis acquisition.

69. Woods K, Nic-Fhogartaigh C, Arnold C, Boutthasavong L, Phuklia W, Lim C, Chanthongthip A, Tulsiani SM, Craig SB, Burns M-A, Weier SL, Davong V, Sihalath S, Limmathurotsakul D, Dance DAB, Shetty N, Zambon M, P.N. Newton, S. Dittrich (2018) A comparison of two molecular methods for diagnosing leptospirosis from three different sample types in patients presenting with fever in Laos. *Clin Microbiol Infect* 24(9): 1017.e1–1017.e7.

Abstract. OBJECTIVES. To compare two molecular assays (*rrs* quantitative PCR (qPCR) versus a combined 16SrRNA and *LipL32* qPCR) on different sample types for diagnosing leptospirosis in febrile patients presenting to Mahosot Hospital, Vientiane, Laos. METHODS. Serum, buffy coat and urine samples were collected on admission, and follow-up serum ~10 days later. *Leptospira* spp. culture and microscopic agglutination tests (MAT) were performed as reference standards. Bayesian latent class modelling was performed to estimate sensitivity and specificity of each diagnostic test. RESULTS. In all, 787 patients were included in the analysis: 4/787 (0.5%) were *Leptospira* culture positive, 30/787 (3.8%) were MAT positive, 76/787 (9.7%) were *rrs* qPCR positive and 20/787 (2.5%) were 16SrRNA/*LipL32* qPCR positive for pathogenic *Leptospira* spp. in at least one sample. Estimated sensitivity and specificity (with 95% CI) of 16SrRNA/*LipL32* qPCR on serum (53.9% (33.3%–81.8%); 99.6% (99.2%–100%)), buffy coat (58.8% (34.4%–90.9%); 99.9% (99.6%–100%)) and urine samples (45.0% (27.0%–66.7%); 99.6% (99.3%–100%)) were comparable with those of *rrs* qPCR, except specificity of 16SrRNA/*LipL32* qPCR on urine samples was significantly higher (99.6% (99.3%–100%) vs. 92.5% (92.3%–92.8%), p <0.001). Sensitivities of MAT (16% (95% CI 6.3%–29.4%)) and culture (25% (95% CI 13.3%–44.4%)) were low. Mean positive Cq values showed that buffy coat samples were more frequently inhibitory to qPCR than either serum or urine (p <0.001). CONCLUSIONS. Serum and urine are better samples for qPCR than buffy coat, and 16SrRNA/*LipL32* qPCR performs better than *rrs* qPCR on urine. Quantitative PCR on admission is a reliable rapid diagnostic tool, performing better than MAT or culture, with significant implications for clinical and epidemiological investigations of this global neglected disease.

70. Woods K, Boutthasavong L, NicFhogartaigh C, Lee SL, Davong V, AuCoin D, Dance DAB (2018) Evaluation of a Rapid Diagnostic Test for Detection of *Burkholderia pseudomallei* in the Lao People's Democratic Republic. *J Clin Microbiol* 56(7): e02002-17.

Abstract. *Burkholderia pseudomallei* causes significant global morbidity and mortality, with the highest disease burden in parts of Asia where culture-based diagnosis is often not available. We prospectively evaluated the Active Melioidosis Detect (AMD; InBios

International, USA) lateral flow immunoassay (LFI) for rapid detection of *B. pseudomallei* in turbid blood cultures, pus, sputum, sterile fluid, urine, and sera. The performance of this test was compared to that of *B. pseudomallei* detection using monoclonal antibody latex agglutination (LA) and immunofluorescence assays (IFA), with culture as the gold standard. AMD was 99% (99/100; 95% confidence interval, 94.6 to 100%) sensitive and 100% (308/308; 98.8 to 100%) specific on turbid blood culture bottles, with no difference from LA or IFA. AMD specificity was 100% on pus (122/122; 97.0 to 100%), sputum (20/20; 83.2 to 100%), and sterile fluid (44/44; 92 to 100%). Sensitivity on these samples was as follows: pus, 47.1% (8/17; 23.0 to 72.2%); sputum, 33.3% (1/3; 0.84 to 90.6%); and sterile fluid, 0% (0/2; 0 to 84.2%). For urine samples, AMD had a positive predictive value of 94% (32/34; 79.7 to 98.5%) for diagnosing melioidosis in our cohort. AMD sensitivity on stored sera, collected prospectively from melioidosis cases during this study, was 13.9% (5/36; 4.7% to 29.5%) compared to blood culture samples taken on the same day. In conclusion, AMD is an excellent tool for rapid diagnosis of melioidosis from turbid blood cultures and maintains specificity across all sample types. It is a promising tool for urinary antigen detection, which could revolutionize diagnosis of melioidosis in resource-limited settings. Further work is required to improve sensitivity on nonblood culture samples.

71. Wootton CI, Bell S, Philavanh A, Phommachack K, Soukavong M, Kidoikhammouan S , Walker SL, Mayxay M (2018) Assessing skin disease and associated health-related quality of life in a rural Lao community. *BMC Dermatol.* 18: 11.

Abstract. BACKGROUND. Skin diseases are common and often have an impact on an individual's health-related quality of life. In rural communities where access to healthcare may be limited and individuals rely on farming for food and income, the impact of skin diseases may be greater. The objectives for this study were to perform an assessment of skin disease prevalence in a rural village in Laos and assess the associated impact of any skin disease found using the Dermatology Life Quality Index (DLQI). METHODS. A rural village was purposively selected and 340 participants examined by dermatologists over a four day period. Brief questionnaires were performed, followed by full body skin examinations and DLQI questionnaires completed were relevant. The data were analysed using chi square and Wilcoxon signed rank tests. RESULTS. One hundred and eighty-one participants were found to have a skin disease (53%). The six most common skin diseases were: eczema (22%), dermatophyte infections (19%), acne (10%), scabies infestation (9%), melasma (8%) and pityriasis versicolor (4%). Just over half of those with skin disease (51%) completed the DLQI, with scores ranging from 0 to 24. Those with skin problems on examination were significantly more likely to be farmers, have had a previous skin problem, be older or live in a smaller family. CONCLUSIONS. This study represents the first formal documentation of skin disease prevalence in Laos and establishes the high rate of skin disease in the rural community and the associated impact these diseases have on health-related quality of life.

Sunset across the Mekong River

72. Zhu, L., Tripathi, J., Rocamora, F. M., Miotto, O., van der Pluijm, R., Voss, T. S., Mok, S., Kwiatkowski, D. P., Nosten, F., Day, N., White, N. J., Dondorp, A. M., Bozdech, Z., Tracking Resistance to Artemisinin Collaboration I (2018) The origins of malaria artemisinin resistance defined by a genetic and transcriptomic background. *Nature Communications* 9(1), 5158. doi:10.1038/s41467-018-07588-x.

Abstract. The predisposition of parasites acquiring artemisinin resistance still remains unclear beyond the mutations in *Pfk13* gene and modulation of the unfolded protein response pathway. To explore the chain of casualty underlying artemisinin resistance, we reanalyze 773 *P. falciparum* isolates from TRACI-study integrating TWAS, GWAS, and eQTL analyses. We find the majority of *P. falciparum* parasites are transcriptomically converged within each geographic site with two broader physiological profiles across the Greater Mekong Subregion (GMS). We report 8720

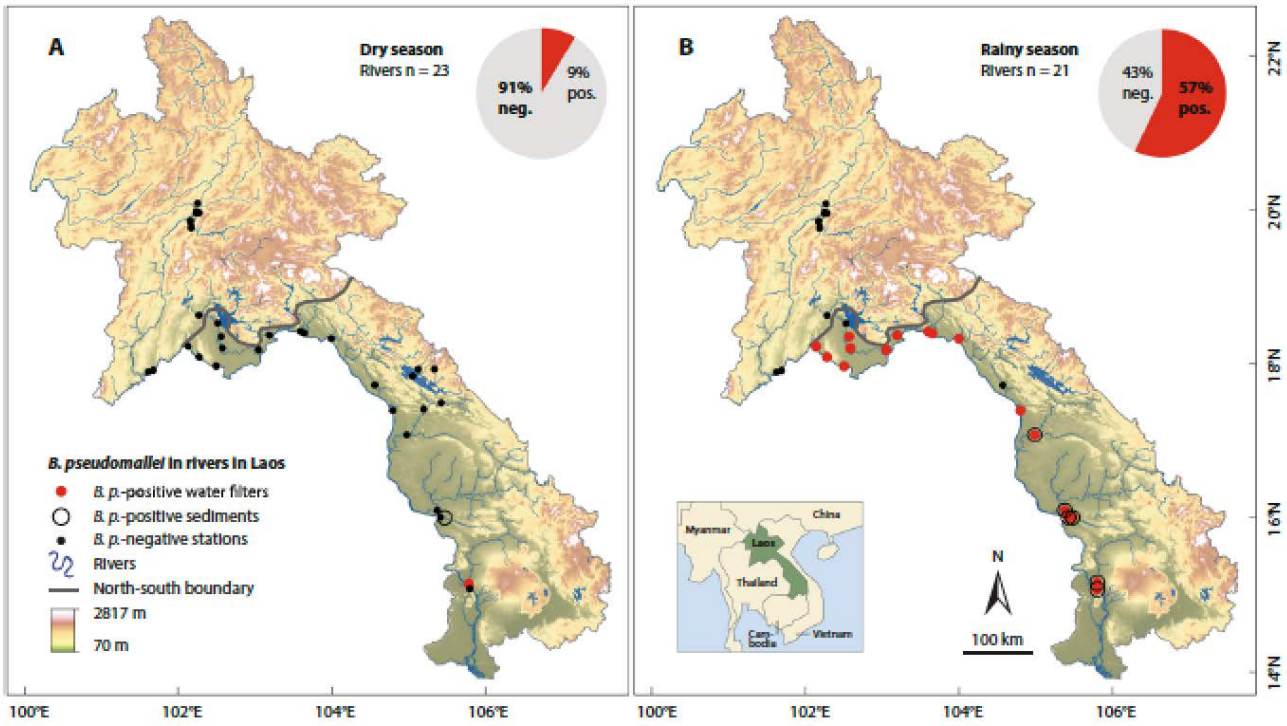


Figure 1. *B. pseudomallei* (*B.p.*)-positive and -negative stations and rivers in the dry season (**A**) and rainy season. (**B**) North-south boundary based on[38,39], map background based on elevation data (U.S. Geological Survey, https://earthexplorer.usgs.gov; Central Intelligence Agency, https://www.cia.gov/library/publications/the-world-factbook/index.html) and rivers/lakes/country shapefiles provided by the Centre for Development and Environment (CDE), CDE Lao Country Office, Laos. Geographic coordination system: WGS 1984, latitude and longitude in degrees; altitude of highest and lowest point in meters above mean sea level.

SNP-expression linkages in the eastern GMS parasites and 4537 in the western. The minimal overlap between them suggests differential gene regulatory networks facilitating parasite adaptations to their unique host environments. Finally, we identify two genetic and physiological backgrounds associating with artemisinin resistance in the GMS, together with a farnesyltransferase protein and a thioredoxin-like protein which may act as vital intermediators linking the *Pfk13* C580Y mutation to the prolonged parasite clearance time.

73. Zimmermann RE, Ribolzi O, Pierret A, Rattanavong S, Robinson MT, Newton PN, Davong V, Auda Y, Zopfi J, Dance DAB (2018) Rivers as carriers and potential sentinels for *Burkholderia pseudomallei* in Laos. *Sci Rep* 8: 8674.

Abstract. *Burkholderia pseudomallei*, causative agent of the often fatal disease melioidosis, dwells in tropical soils and has been found in freshwater bodies. To investigate whether rivers are potential habitats or carriers for *B. pseudomallei* and to assess its geographical distribution in Laos, we studied 23 rivers including the Mekong, applying culture-based detection methods and PCR to water filters and streambed sediments. *B. pseudomallei* was present in 9% of the rivers in the dry season and in 57% in the rainy season. We found the pathogen exclusively in Southern and Central Laos, and mainly in turbid river water, while sediments were positive in 35% of the *B. pseudomallei*-positive sites. Our results provide evidence for a heterogeneous temporal and spatial distribution of *B. pseudomallei* in rivers in Laos with a clear north-south contrast. The seasonal dynamics and predominant occurrence of *B. pseudomallei* in particle-rich water suggest that this pathogen is washed out with eroded soil during periods of heavy rainfall and transported by rivers, while river sediments do not seem to be permanent habitats for *B. pseudomallei*. Rivers may thus be useful to assess the distribution and aquatic dispersal of *B. pseudomallei* and other environmental pathogens in their catchment area and beyond.

Dr Liz Ashley, who will start as LOMWRU Director late April 2019

Dr Andy Simpson, who will start as LOMWRU Clinical Microbiologist April 2019

Mr Duangchan Chanthadala
(Joy)
Tuk -Tuk Driver

Mr Phonesavath Minolath
(Phone)
Tuk-Tuk Driver

Bushmeat at a Lao market, including Pallas's squirrels, bamboo rat, rat species, barbets and a hill myna with chilli and parsley – the mammals are links in zoonotic disease ecology in Laos [photograph courtesy of Khongsy Khammavong and the Wildlife Conservation Society].