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LOMWRU Laos Oxford

UNIVERSITY OF OXFORD

Lao - Oxford - Mahosot Hospital - Wellcome Trust Research Unit

Aix Marseille université IRD Institut de recherche pour le développement EHESP

SCIENTIFIC ANNUAL REPORT FOR 2014

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

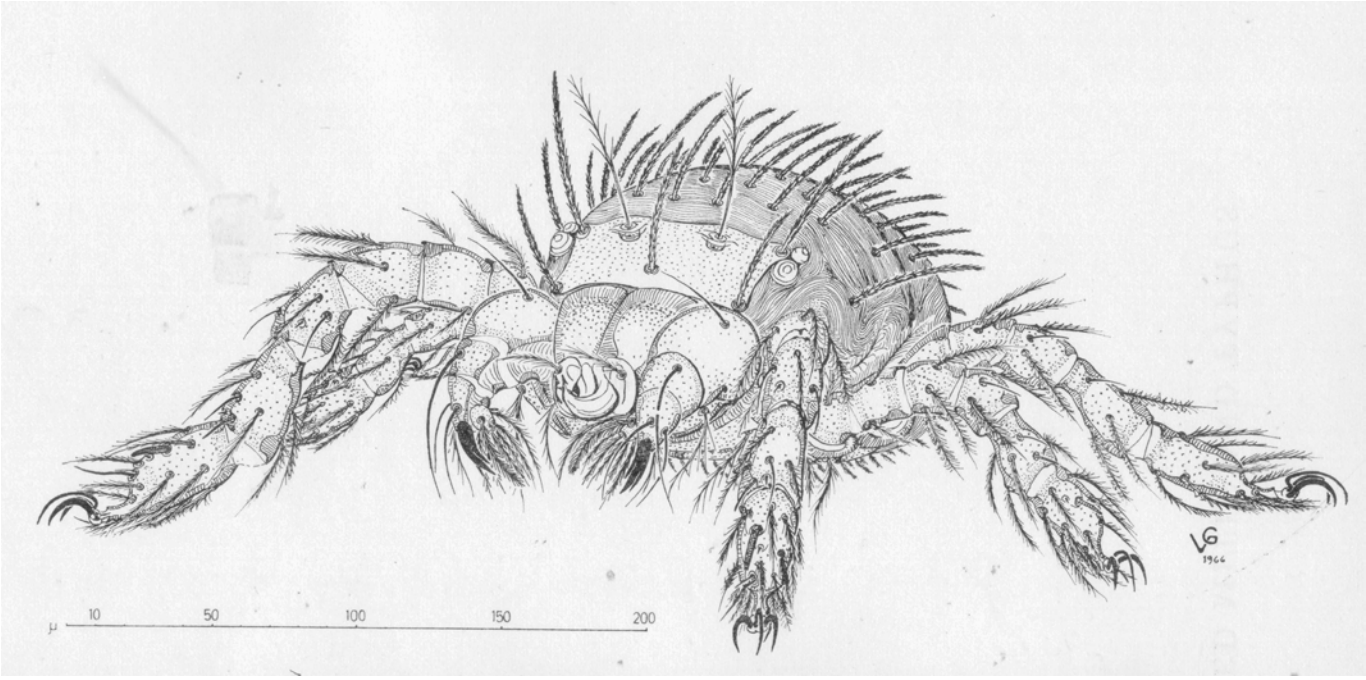
TO

MINISTRY OF HEALTH
GOVERNMENT OF THE LAO PDR




CONTENTS

A chigger mite, vector and reservoir of scrub typhus, from J Ralph Audy (1968)



ບົດສັງລວມຫຍໍ້

ກ. ໂຄງການຄົ້ນຄວ້າພະຍາດເຂດຮ້ອນລະຫວ່າງໂຮງໝໍມະໂຫສົດ-ແອວຄຳຕູ້ສ-ມະຫາວິທະຍາໄລອ໋ອກຝອດ ຫຼື The Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit (LOMWRU) ເປັນໜ່ວຍງານຄົ້ນຄວ້າທາງຄລີນິກ ເຊິ່ງນອນຢູ່ໃນພະແນກວິເຄາະຈຸລິນຊີ, ໂຮງໝໍມະໂຫສົດ. ໂຄງການນີ້ໄດ້ຮັບທຶນຊ່ວຍເຫລືອຫລັກຈາກທາງແອວຄຳຕູ້ສ ປະເທດ ອັງກິດ ແລະທຶນອີກສ່ວນໜຶ່ງແມ່ນໄດ້ຈາກ US Naval Medical Research Centre, US Centres for Disease Control (CDC), The European Union, Fondation Total/Institute Pasteur, the Foundation for Innovative New Diagnostics (FIND), the World Health Organisation (WHO), the French Government Ministry of Foreign and International Affairs and the Joint Inter-Agency Task Force of the Global Fund, PMI, and UNDP. ນອກນີ້ ທາງໂຄງການຍັງໄດ້ຮັບການຊ່ວຍເຫລືອເປັນເຄື່ອງອຸປະກອນ ຈາກສະຖາບັນຄົ້ນຄວ້າເພື່ອການພັດທະນາ/ມະຫາວິທະຍາໄລແອ໋ກຊ-ມາກໄຊ ປະເທດຝລັ່ງ ແລະ ໂຄງການຄົ້ນຄວ້າພະຍາດຣິກເກັດເຊຍ ຂອງສູນຄົ້ນຄວ້າທາງການແພດກອງທັບເຮືອ ສະຫະລັດອາເມລິກາ.

ຂ. LOMWRU ມີພະນັກງານທັງໝົດ 43 ຄົນ, ໃນນີ້ 93% ແມ່ນຄົນລາວ ແລະ 50% ເປັນເພດຍິງ. ສ່ວນພະແນກວິເຄາະຈຸລິນຊີ ມີພະນັກງານ (ພາກລັດ) ທັງໝົດ 26 ຄົນ. ພວກເຮົາມີຫ້ອງວິເຄາະທາງຄລີນິກຕ່າງໆ ເຊັ່ນ: ຫ້ອງວິເຄາະຈຸລະຊີວະວິທະຍາ, ຫ້ອງວິເຄາະທາງພັນທຸກຳສາດ, ຫ້ອງວິເຄາະເຊໂຣໂລຊີ, ແລະ ຫ້ອງວິເຄາະລະດັບ 3 (BSL3). ການປະຕິບັດງານ ໃນຫ້ອງວິເຄາະດັ່ງກ່າວ ແມ່ນເປັນໄປຕາມແນວທາງ - ລະບຽບ ການຄວາມປອດໄພ ຂອງມະຫາວິທະຍາໄລອ໋ອກຝອດ.

ຄ. LOMWRU ຊ່ວຍບໍລິການບົ່ງມະຕິພະຍາດຊຶມເຊື້ອພາຍໃນໂຮງມະໂຫສົດ ແລະ ໂຮງໝໍຕ່າງແຂວງ ເຊັ່ນ ໂຮງໝໍແຂວງ ຫລວງນ້ຳທາ, ສາລະວັນ ແລະ ຊຽງຂວາງ, ເຮັດການຄົ້ນຄວ້າທາງຄລີນິກ, ແລະ ສ້າງຄວາມເຂັ້ມແຂງໃຫ້ແກ່ພະນັກງານພາກລັດ ທາງດ້ານການບົ່ງມະຕິພະຍາດ ແລະ ການເຮັດຄົ້ນຄວ້າ ໂດຍຜ່ານການຝຶກອົບຮົມ ແລະ ການປະຕິບັດງານຕົວຈິງ.

ງ. ຈຸດສຸມສຳລັບວຽກຄົ້ນຄວ້າຂອງພວກເຮົາໄດ້ແກ່ ສາເຫດ ແລະ ລະບາດວິທະຍາຂອງໄຂ້ ລວມທັງການບົ່ງມະຕິ ແລະ ປື່ອປົວທີ່ສົມເຫດສົມຜົນ ໃນ ສປປ ລາວ, ການບົ່ງມະຕິ, ລະບາດວິທະຍາ ແລະ ການປ້ອງກັນ ພາວະຂາດວິຕະມິນເອ1 ໃນເດັກລຸ່ມ 1 ປີ, ແລະ ການສຶກສາຄົ້ນຄວ້າກ່ຽວກັບຄຸນນະພາບຂອງຢາ ໃນລະດັບສາກົນ.

ຈ. ໃນປີ 2014 ພວກເຮົາໄດ້ຕີພິມ ຫລື ກຳລັງຖືກຮັບຕີພິມເຜີຍແຜ່ຜົນຂອງການຄົ້ນຄວ້າລົງໃນວາລະສານການແພດສາກົນ ຈຳນວນ 29 ບົດ, ລົງໃນປຶ້ມຕຳລາທາງການແພດຈຳນວນ 7 ພາກ, ບົດປະກອບຄຳເຫັນ 1 ບົດ ແລະ ບົດຄວາມທາງໜັງສືພິມ 1 ບົດ. ນັບແຕ່ປີ 2000 ເປັນຕົ້ນມາ, ນັກຄົ້ນຄວ້າຂອງໂຄງການ LOMWRU ມີຜົນງານຕີພິມເຜີຍແຜ່ຜົນການຄົ້ນຄວ້າຫລາຍຫວ່າໝູ່ໃນວົງການສາທາລະນະສຸກຂອງລາວ ເຊິ່ງລວມມີ ທັງໝົດ 203 ບົດ ທີ່ຖືກຕີພິມ ຫລື ກຳລັງຖືກຮັບຕີພິມເຜີຍແຜ່ ລວມທັງປຶ້ມຕຳລາທາງການແພດອີກຈຳນວນ 13 ພາກ.

ສ. ຜົນການຄົ້ນຄວ້າຂອງ LOMWRU ທີ່ຖືກນຳໄປຜັນຂະຫຍາຍເປັນແນວທາງນະໂຍບາຍ ດ້ານສາທາລະນະສຸກພາຍໃນປະເທດ ລວມມີ: ການຈັດຕັ້ງປະຕິບັດການສັກຢາກັນພະຍາດທີ່ເກີດຈາກເຊື້ອ Pneumococcus ແລະ ການສັກຢາກັນພະຍາດອັກເສບສະໝອງຍີ່ປຸ່ນ, ການປ່ຽນແປງແນວທາງການປິ່ນປົວພະຍາດໄຂ້ຍຸງ ແລະ ພະຍາດໄຂ້ທໍລະພິດ (Typhoid). ນອກນີ້ ພວກເຮົາຍັງໄດ້ຄົ້ນພົບພະຍາດທີ່ສຳຄັນບາງຢ່າງ ເປັນຄັ້ງທຳອິດ ໃນປະເທດລາວ, ໄດ້ຊີ້ໃຫ້ເຫັນຄວາມສຳຄັນຂອງພະຍາດໄຂ້ແມງແດງ, ໄຂ້ຢ່ຽວໜູ, ໄຂ້ທໍລະພິດ, ເມລີອອຍ ໂດສຊິສ, ອັກເສບສະໝອງຍີ່ປຸ່ນ ເຊິ່ງເຮັດໃຫ້ພວກເຮົາເຂົ້າໃຈຢ່າງເລິກເຊິ່ງຕື່ມກ່ຽວກັບລັກສະນະດ້ານລະບາດວິທະຍາ ແລະ ແນວທາງການປ້ອງກັນພະຍາດດັ່ງກ່າວ.

ຊ. ສະຫລຸບຜົນຂອງການຄົ້ນຄວ້າທີ່ສຳຄັນ ເຊິ່ງໄດ້ຕີພິມເຜີຍແຜ່ ຫລື ກຳລັງຈະຖືກຕີພິມເຜີຍແຜ່ ໃນປີ 2014 ມີດັ່ງຕໍ່ໄປນີ້ (ກະລຸນາເບິ່ງລາຍລະອຽດຕື່ມໃນບົດລາຍງານ):

- ພວກເຮົາສາມາດບົ່ງມະຕິພະຍາດໄຂ້ທໍລະພິດ ຢ່າງໄວວາ, ແມ່ນຢຳ ແລະ ມີລາຄາຖືກ ໂດຍນຳໃຊ້ຊຸດການກວດວິເຄາະແບບໄວວາເພື່ອຊອກຫາແອນຕີເຈນ ຂອງເຊື້ອໄຂ້ທໍລະພິດ ໃນແກ້ວປູກເລືອດ. ນອກຈາກນີ້ ການກວດທາງພັນທຸກຳ (PCR) ຈາກຊຸດການກວດແບບໄວວາທີ່ໃຫ້ຜົນບວກ ຍັງສາມາດ

ບອກໄດ້ຢ່າງໜັ້ນການວ່າ ເຊື້ອດັ້ງກ່າວຕອບສະໜອງຕໍ່ຢາ Fluoroquinolones ຫລືບໍ່. ເຕັກນິກນີ້ ອາດຊ່ວຍໃຫ້ຫ້ອງວິເຄາະຂອງໂຮງໝໍແຂວງ ສາມາດບົ່ງມະຕິພະຍາດດັ້ງກ່າວໄດ້ ໃນອະນາຄົດ ແລະ ສາມາດສົ່ງແຜ່ນກວດໄວວາທີ່ມີຜົນບວກ ໄປກວດຫາໜັ້ນທຸກຳຢູ່ຂັ້ນສູນກາງ ເພື່ອເບິ່ງລັກສະນະການດື້ຂອງເຊື້ອຕໍ່ຢາ Fluoroquinolones.

- ພວກເຮົາພົບວ່າ ພະຍາດໄຂ້ແມງແດງ, ມູຣິນໄທຟັສ, ແລະ ໄຂ້ຍ່ຽວໜູ ເປັນສາເຫດທີ່ສຳຄັນຂອງ ການຊຶມເຊື້ອລະບົບປະສາດສູນກາງໃນວຽງຈັນ ແລະ ອາດພົບໃນແຫ່ງອື່ນໆຂອງຂົງເຂດອາຊີເຊັ່ນກັນ. ຂໍ້ມູນນີ້ ຊີ້ໃຫ້ເຫັນວ່າ ເຮົາຄວນພິຈາລະນານຳໃຊ້ຢາ Doxycycline ຕື່ມໃນຄົນເຈັບທີ່ເປັນອັກເສບເຍື່ອ ຫຸ້ມສະໝອງ/ສະໝອງອັກເສບ ຍ້ອນວ່າ ຢາກຸ່ມ Cephalosporins ລຸ້ນທີ 3 ທີ່ມັກໃຊ້ປິ່ນປົວພະ ຍາດໜ້ ສະໝອງ/ສະໝອງອັກເສບ ແບບເປັນປະເພນີນັ້ນ ບໍ່ສາມາດປິ່ນປົວພະຍາດໄທຟັສ
- ຜົນການຄົ້ນຄວ້າພວກເຮົາພົບວ່າ ເຊື້ອ *Staphylococcus aureus* ເປັນເຊື້ອສາເຫດຕົ້ນຕໍຂອງການ ຊຶມເຊື້ອເລືອດໃນເດັກລຸ່ມ 1 ປີ ຂອງລາວ ເຊິ່ງສາເຫດອາດເປັນຍ້ອນການນອນໄຟຂອງແມ່ຫລັງ ເກີດລູກ – ຂໍ້ມູນນີ້ຊີ້ໃຫ້ເຫັນວ່າ ເຮົາຄວນພິຈາລະນານຳໃຊ້ຢາ Cloxacillin ເພື່ອປິ່ນປົວຊຶມເຊື້ອ ເລືອດໃນເດັກເກີດໃໝ່ 3 ອາທິດແລກ ແລະ ເດັກລຸ່ມ 1 ປີ ໃນຂະນະທີ່ລໍຖ້າຜົນຢູກເລືອດ.
- ຂໍ້ມູນຈາກການສຶກສາກ່ຽວກັບສາເຫດຂອງໄຂ້ ທີ່ບໍ່ແມ່ນໄຂ້ຍູງ (Mayxay et al., 2013) ຊີ້ໃຫ້ເຫັນ ວ່າ ການກວດ C-reactive protein ສາມາດນຳໃຊ້ເປັນຕົວຊີ້ບອກວ່າ ຄົນເຈັບທີ່ເຂົ້າມາດ້ວຍ ອາ ການໄຂ້ ຈຳເປັນຕ້ອງໄດ້ຮັບຢາຕ້ານເຊື້ອ ຫລືບໍ່.
- ມາຮອດປະຈຸບັນ ພວກເຮົາພົບຄົນເຈັບທີ່ຕິດເຊື້ອ Sennetsu (*Neorickettsia sennetsu*) ໃນ ສປປ ລາວ ຈຳນວນ 5 ຄົນ ເຊິ່ງການຄົ້ນພົບນີ້ ໄດ້ນຳໄປສູ່ການພັດທະນາ ວິທີການບົ່ງມະຕິພະຍາດ *Neorickettsia sennetsu* ໂດຍກົງ.
- ເຊື້ອຈຸລິນຊີ Enterobacteriaceae ທີ່ດື້ຕໍ່ຢາຕ້ານເຊື້ອກຸ່ມເບຕາລັກຕາມິນ ບໍ່ພຽງແຕ່ເປັນສາເຫດຂອງ ການຊຶມເຊື້ອທີ່ນັບມື້ພົບຫລາຍຂຶ້ນໃນໂຮງໝໍມະໂຫສົດ ແຕ່ຍັງພົບເຫັນຫລາຍໃນອາຈົມຂອງເດັກໂຮງ ຮຽນອະນຸບານທີ່ມີສຸຂະພາບແຂງແຮງ ໃນນະຄອນຫລວງວຽງຈັນ ແລະ ແຂວງວຽງຈັນ – ການຄົ້ນພົບ ນີ້ ເຮັດໃຫ້ເຮົາກັງວົນໃນເລື່ອງການແຜ່ກະຈາຍຂອງເຊື້ອ ທີ່ດື້ຕໍ່ຢາຕ້ານເຊື້ອໃນລາວ.
- ພວກເຮົາພົບວ່າ ຄວາມຮັບຮູ້ກ່ຽວກັບລັກສະນະການຕ້ານຂອງເຊື້ອ ຕໍ່ຢາຕ້ານເຊື້ອ ຂອງທ່ານໝໍປິ່ນປົວ ຍັງຢູ່ໃນລະດັບຕ່ຳ ແລະ ການວາງຢາຕ້ານເຊື້ອໃນການປິ່ນປົວຍັງບໍ່ທັນເໝາະສົມ ເຊິ່ງອັນນີ້ຊີ້ໃຫ້ເຫັນ ເຖິງຄວາມຈຳເປັນອັນຮີບດ່ວນສຳລັບການແກ້ໄຂຊ່ອງຫວ່າງຂອງບັນຫານີ້.
- ສາເຫດຂອງອັກເສບຫົວໃຈຊັ້ນໃນ (Endocarditis) ທີ່ພົບຫລາຍກວ່າໝູ່ ແມ່ນເຊື້ອ *Streptococcus* spp ເຊິ່ງຜົນການປິ່ນປົວບໍ່ຄ່ອຍຈະດີ ແລະ ທີ່ສຳຄັນສຸດແມ່ນຕ້ອງພະຍາຍາມລະດົມໃຫ້ຄົນເຈັບ ນອນ ໂຮງໝໍດົນເພື່ອປິ່ນປົວດ້ວຍຢາຕ້ານເຊື້ອທາງເສັ້ນເລືອດ. ພວກເຮົາຍັງພົບຄົນເຈັບ ທີ່ເປັນອັກເສບຫົວ ໃຈຊັ້ນໃນຈາກເຊື້ອ *Bartonella henselae* ເປັນຄັ້ງທຳອິດໃນລາວ ສະນັ້ນ ແພດໝໍເຮົາຄວນ ຄິດຫາເຊື້ອດັ້ງກ່າວ ໃນເວລາບົ່ງມະຕິຈຳແນກພະຍາດ.
- ພະຍາດ ມື-ຕີນ-ປາກ ຖືກຄົ້ນພົບໃນລາວ ແລະ ເຊື້ອທີ່ເປັນສາເຫດໄດ້ແກ່ EV71 (ລວມທັ້ງ C4) ແລະ CVA16 (ລວມທັ້ງ B1a). ມີຄວາມຈຳເປັນຕ້ອງວາງແຜນຮັບມືກັບການລະບາດຂອງພະຍາດ ທີ່ເປັນຮຸນແຮງພາຍໃນປະເທດ.
- ພວກເຮົາຄົ້ນພົບເຊື້ອ *Tropheryma whipplei* ທີ່ກໍ່ໃຫ້ເກີດພະຍາດ Whipple ໃນອາຈົມຂອງເດັກທີ່ ມີສຸຂະພາບແຂງແຮງໃນວຽງຈັນ. ເຊື້ອຈຸລິນຊີນີ້ ກໍ່ໃຫ້ເກີດພະຍາດຖອກທ້ອງ, ການດູດຊຶມອາຫານບໍ່ດີ, ບັນຫາທາງລະບົບປະສາດສູນກາງ ແລະ ບາງຄັ້ງພາໃຫ້ເກີດອັກເສບຫົວໃຈຊັ້ນໃນ (ແຕ່ບໍ່ສູ້ພົບ) – ແຕ່ວ່າ ຄວາມສຳຄັນຂອງການກໍ່ໃຫ້ເກີດພະຍາດສຳລັບເຊື້ອພະຍາດນີ້ໃນ ລາວ ຍັງບໍ່ທັນຊັດເຈນ.
- ພວກເຮົາສາມາດຢືນຢັນ ການຊຶມເຊື້ອລະບົບຫາຍໃຈຈາກເຊື້ອ *Mycoplasma pneumoniae* ເປັນ ຄັ້ງທຳອິດໃນວຽງຈັນ ເຊິ່ງຂໍ້ມູນລະອຽດ ຈະໄດ້ກ່າວຕື່ມໃນປີ 2015. ພວກເຮົາຍັງບໍ່ທັນມີຂໍ້ມູນກ່ຽວກັບ ລັກສະນະການຕອບສະໜອງຂອງເຊື້ອນີ້ ຕໍ່ຢາຕ້ານເຊື້ອເທື່ອ, ແຕ່ພວກເຮົາຄິດວ່າ ຢາຕ້ານເຊື້ອໃນກຸ່ມ Macrolides ແລະ Fluoroquinolones ໜ້າຈະໄດ້ຜົນດີ – ແຕ່ສຳລັບຢາໃນກຸ່ມ Cephalosporins ແລະ Penicillins ຈະບໍ່ໄດ້ຜົນ.
- ການຄົ້ນຄວ້າຂອງພວກເຮົາພົບວ່າ ແຜ່ນກວດພະຍາດໄຂ້ເລືອດອອກ (Standard Diagnostics, NS1/IgM/IgG) ທີ່ເກັບຮັກສາໄວ້ໃນອຸນຫະພູມຮ້ອນຂອງບ້ານເຮົາເປັນໄລຍະເວລາດົນນານ ຍັງ ສາມາດນຳໃຊ້ໄດ້ເປັນຢ່າງດີ.
- ພວກເຮົາສາມາດຢັ້ງຢືນວ່າ ເຊື້ອ *Rickettsia felis* ພົບເຫັນໃນລາວເຮົາ ເຊິ່ງເຊື້ອນີ້ອາດເກີດຈາກ ການກັດຂອງໂຕໝັດ (Flea) ແລະ ອາດຕອບສະໜອງດີຕໍ່ຢາ Doxycycline ແຕ່ຍັງບໍ່ທັນມີຄວາມ ຊັດເຈນວ່າ ເຊື້ອນີ້ກໍ່ໃຫ້ເກີດພະຍາດໃນຄົນ ຫລືບໍ່.

- ພວກເຮົາພົບວ່າ ເຊື້ອ *Rickettsia typhi* ໃນເລືອດຄົນເຈັບທີ່ເປັນພະຍາດມູຣິນໄທຟັສ ແມ່ນມີປະລິມານຕ່ຳກວ່າ ເຊື້ອ *Orientia tsutsugamushi* ທີ່ພົບໃນຄົນເຈັບທີ່ເປັນໄຂ້ແມງແດງ. ເຕັກນິກ LAMP assay ທີ່ພວກເຮົາພັດທະນາຂຶ້ນມາ ສຳລັບບ່ງມະຕິພະຍາດມູຣິນໄທຟັສ ແມ່ນມີຄວາມແມ່ນຍຳໜ້ອຍ ຍ້ອນປະລິມານເຊື້ອທີ່ມີໃນເລືອດຄົນເຈັບມີໜ້ອຍ. ສະນັ້ນ ແນວທາງການບ່ງມະຕິທີ່ດີສຳລັບພະຍາດດັ່ງກ່າວ ຄວນຈະເປັນການສົມທົບກັນ ລະຫວ່າງ ເຕັກນິກເຊໂຣໂລຊີ ແລະ ເຕັກນິກທາງພັນທຸກຳ.
- ການຕ້ານຂອງເຊື້ອ *Burkholderia pseudomallei* ຕໍ່ຢາ Co-trimoxazole ບໍ່ຄ່ອຍພົບເຫັນໃນປະເທດລາວ ແລະ ກຳປູເຈຍ ແຕ່ພົບເຫັນເລື້ອຍໃນປະເທດໄທ. ຂໍ້ມູນນີ້ ຊີ້ໃຫ້ເຫັນວ່າ ການປິ່ນປົວດ້ວຍຢາ Co-trimoxazole ພຽງຢ່າງດຽວໃນໄລຍະ Eradication phase ຈຶ່ງເປັນທາງເລືອກທີ່ເໝາະສົມ.
- ພວກເຮົາຄົ້ນພົບເຊື້ອໄຂ້ຍຸງຟານຊີປາຣອມດື້ຕໍ່ຢາກຸ່ມ Artemisinin ທີ່ແຂວງອັດຕະປື ເຊິ່ງສະແດງອອກດ້ວຍ ການທີ່ຄົນເຈັບຫາຍຈາກອາການໄຂ້ຊ້າກວ່າປົກກະຕິ - ເຖິງວ່າ ຍັງພົບໃນຈຳນວນຄົນເຈັບໜ້ອຍໜຶ່ງກໍ່ຕາມ (6%). ສະນັ້ນ ເຮົາຈຳເປັນຕ້ອງມີການຕິດຕາມຢ່າງລະມັດລະວັງ ແລະ ຕ້ອງເພີ່ມທະວີການປ້ອງກັນການແຜ່ກະຈາຍຂອງມັນ ດ້ວຍການບ່ງມະຕິພະຍາດ ແລະ ປິ່ນປົວໃຫ້ທັນການ. ນອກນີ້ ຄວນພິຈາລະນາຫາແນວທາງກຳຈັດມັນອອກໄປ.
- ການສຶກສາຄົ້ນຄວ້າແບບທົດລອງຢູ່ທາງພາກໃຕ້ຂອງພວກເຮົາຊີ້ໃຫ້ເຫັນວ່າ ການໃຫ້ວິຕະມິນເບ1 ເສີມໃນຄົນເຈັບທີ່ເປັນໄຂ້ຍຸງຟານຊີປາຣອມ ບໍ່ໄດ້ຊ່ວຍຫລຸດຜ່ອນອາການຂອງພະຍາດໄຂ້ຍຸງລົງ ໃນໄລຍະກຳລັງປິ່ນປົວດ້ວຍຢາໄຂ້ຍຸງ.
- ພວກເຮົາໄດ້ຮ່ວມມືກັບທາງ FIND ເຮັດການຄົ້ນຄວ້າ ແລະ ພົບວ່າ ພະນັກງານສາທາລະນະສຸກຂອງລາວ ລວມທັງ ອສບ ສາມາດນຳໃຊ້ ແລະແປຜົນຊຸດກວດສອບຄຸນນະພາບແຜ່ນຈຸ່ມໄຂ້ຍຸງ (Positive control wells or PCW) ໄດ້ຢ່າງມີປະສິດທິຜົນ ພາຍຫລັງໄດ້ຮັບການອົບຮົມໄລຍະສັ້ນ.
- ຜົນການສຳຫລວດ ພາວະຂາດ G6PD ຢູ່ 6 ບ້ານ ທີ່ຊູ່ມຈາກ ແຂວງເຊກອງ ພົບວ່າ ອັດຕາການຂາດ G6PD ໃນປະຊາກອນແມ່ນມີປະມານ 4% (70/1,897).
- ຜົນຈາກການແຕ້ມແຜນທີ່ ກ່ຽວກັບ ຍຸງນຳເຊື້ອ ແລະ ແຫລ່ງເກັບເຊື້ອໄຂ້ຍຸງຊະນິດທີ 5 ຂອງຄົນ *Plasmodium knowlesi* ຊີ້ໃຫ້ເຫັນວ່າ ໄຂ້ຍຸງຊະນິດນີ້ ອາດມີໃນປະເທດລາວ.
- ພວກເຮົາພົບວ່າ ຄຸນນະພາບຂອງຢາປິ່ນປົວ ຍັງເປັນບັນຫາທີ່ໜັກໜ່ວງໃນທົ່ວໂລກ. ພວກເຮົາຄົ້ນພົບວ່າ FDA CD3 device ເປັນເຄື່ອງມືກວດຢາປອມທີ່ດີ. ນອກນີ້ ພວກເຮົາຍັງພົບວ່າ ການໜັກແບ່ງຢາເມັດ (ທີ່ເຮົາມັກເຮັດເປັນປະຈຳ) ເປັນບັນຫາທີ່ເຮົາຍັງມອງຂ້າມ ເຊິ່ງອາດມີຜົນຕໍ່ການປິ່ນປົວຄົນເຈັບ ແລະ ຍັງພົບອີກວ່າ ເຮົາຈະຕ້ອງໄດ້ເອົາໃຈໃສ່ບັນຫາ ເລື່ອງຂໍ້ຄວາມພາສາ ແລະ ຄວາມຊັດເຈນຂອງພາສາ ໃນພາຊະນະຫໍ່ຫຸ້ມຢາ.
- ຜົນຂອງການສຶກສາສົມທຽບ ລະຫວ່າງການສຳຫລວດຄຸນນະພາບຂອງຢາປົວໄຂ້ຍຸງແບບຊູ່ມ 2 ຄັ້ງ ໃນ ສປປ ລາວ ພົບວ່າ ຄຸນນະພາບຂອງຢາດັ່ງກ່າວ ແມ່ນມີການປັບປຸງດີຂຶ້ນກວ່າເກົ່າ ເມື່ອທຽບກັບ 9 ປີ ທີ່ຜ່ານມາ.
- ລະບົບການສຳຫລວດຄຸນນະພາບຂອງຢາປົວໄຂ້ຍຸງຂອງ WWARN ເພື່ອຈຸດປະສົງລາຍງານຄຸນນະພາບຂອງຢາປິ່ນປົວໄຂ້ຍຸງໃນທົ່ວໂລກ ຖືກຈັດຕັ້ງຂຶ້ນ ແລະ ມີທັງພາສາຝລັ່ງ ແລະ ອັງກິດ ແລະ ເຮົາສາມາດດາວໂຫລດເບິ່ງຂໍ້ມູນລາຍງານຂອງແຕ່ລະປະເທດໄດ້.
- ພວກເຮົາພົບວ່າ ແນວທາງສຳລັບລາຍງານຜົນການທົດລອງຢາ (CONSORT guidelines) ຄວນມີພາກທີ່ຂຽນລາຍງານກ່ຽວກັບຄຸນນະພາບຂອງຢາທີ່ໃຊ້ໃນການສຶກສາທົດລອງ ເພື່ອເປັນມາດຕະການ

SUMMARY

Mrs Phonelavanh Phoumin and Mrs Joy Silisouk and colleagues checking paperwork in the Microbiology Laboratory

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 is funded predominantly by the Wellcome Trust of Great Britain, with significant additional support from the US Naval Medical Research Centre, US Centres for Disease Control (CDC), The European Union, Fondation Total/Institute Pasteur, the Foundation for Innovative New Diagnostics (FIND), the World Health Organisation (WHO), the French Government Ministry of Foreign and International Affairs and the Joint Inter-Agency Task Force of the Global Fund, PMI, and UNDP. Considerable assistance in kind is given by the Institut de Recherche pour le Développement/Aix-Marseille University and the Rickettsial Diseases Research Program, Naval Medical Research Center, USA.

B. The Microbiology Laboratory is composed of 26 Lao Government staff and 43 project-funded staff; 93% are Lao and 50% are female. The Microbiology Laboratory has clinical microbiology, molecular, serology and BSL3 laboratories. It follows University of Oxford biosafety policies and guidelines.

C. LOMWRU supports the infectious disease diagnostic service of Mahosot Hospital, assists provincial hospitals in Luang Nam Tha, Salavan and Xieng Khouang, performs clinical research and builds diagnostic and research human capacity through training and active participation. The main objective of the work is to inform health policy globally, in Asia and in Laos and improve public health.

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

E. In 2014 we published or have in press 29 peer-reviewed papers and 7 book chapters, one report for the World Health Organisation, one commentary and one magazine article. LOMWRU authors are the most cited in the Lao public health literature since 2000, with 204 papers published or in press, including 13 book chapters.

F. Previous LOMWRU research translated into policy in Laos includes the implementation of vaccination against the pneumococcus and the Japanese encephalitis virus (JEV) and the change in national antimalarial and typhoid treatment policies. It also demonstrated the presence of numerous important pathogens for the first time in Laos, and highlighted the global importance of scrub typhus, leptospirosis, typhoid, melioidosis and JEV, providing evidence on their epidemiology and prompting interventions.

G. The main findings, in brief, from work published or in press or in preparation in 2014 (please see caveats in text!), are:

- Typhoid bacteria can be accurately, quickly and relatively inexpensively identified in blood culture fluid using typhoid antigen detecting rapid diagnostic tests (RDTs). Furthermore, PCR assays of the pad of positive RDTs can quickly determine whether the bacteria are likely to be susceptible to fluoroquinolones. These techniques may enable provincial hospital laboratories to diagnose this important disease with a centralised system of PCR testing for markers of fluoroquinolone resistance.
- Scrub typhus, murine typhus and leptospirosis are important causes of central nervous system infections in Vientiane and probably elsewhere in Asia. These data suggest a low threshold for use of doxycycline in patients with meningitis/encephalitis as the conventional empirical therapy, third generation cephalosporins, will not treat typhus.
- *Staphylococcus aureus* is the leading cause of bacteraemia in Lao infants, probably because of maternal hot bed use, suggesting that cloxacillin use should be especially considered as empirical treatment for late onset sepsis in infants in their first three weeks of life.
- Data from the non-malarial fever study (Mayxay *et al.* 2013) suggest that C-reactive protein assays are potentially useful markers of the need for antibiotic therapy amongst those presenting with fever.
- We have now documented five patients with sennetsu (*Neorickettsia sennetsu*) infection in Laos and have established comprehensive direct diagnostic methods for *Neorickettsia sennetsu*.
- Extended spectrum beta-lactamase producing Enterobacteriaceae are not only increasingly important causes of infection in Mahosot Hospital but are also common in stools of healthy kindergarten children in Vientiane City and Province. This is of great concern for the spread of drug resistance in Laos and greater emphasis on antibiotic stewardship and regulation is needed.
- Knowledge about antibiotic resistance patterns and appropriate prescribing amongst doctors was relatively low – interventions targeting these gaps are urgently needed.
- The most frequent causes of endocarditis in Laos are *Streptococcus* spp. Outcome is poor and efforts to try to ensure that patients remain in hospital for the recommended duration of intravenous antibiotic therapy are vital. *Bartonella henselae* endocarditis also occurs in Laos and should be considered in the differential diagnosis.
- Hand, foot and mouth disease (HFMD) in Laos is caused by both EV71 (including C4) and CVA16 (including B1a). Planning is needed for interventions for when the inevitable outbreak of severe disease occurs in Laos.

An arboreal rocket launch during the Rocket Festival in Laos

- The agent of Whipple's disease, *Tropheryma whipplei,* occurs in the stools of healthy children in Vientiane. This bacterium can cause diarrhoea, malabsorption, CNS problems and, rarely, endocarditis – its importance in causing disease in Laos is uncertain.
- That *Mycoplasma pneumoniae* respiratory infection occurs in Vientiane has been confirmed – more data will be available in 2015. We have no data yet on antibiotic susceptibility but macrolides and fluoroquinolones are likely to be efficacious, but cephalosporins and penicillins will not be.
- At least one brand of dengue RDT (Standard Diagnostics, NS1/IgM/IgG) retains diagnostic accuracy when exposed to Lao hot season temperatures long term
- That *Rickettsia felis* occurs in Laos has been confirmed. This bacterium is probably flea-borne and would be

Fieldwork in the rain !

Luang Nam Tha Provincial Hospital

expected to respond to doxycycline but its significance as a pathogen is unclear.

- The *Rickettsia typhi* bacterial load in blood is low in patients with murine typhus, usually less than the loads of *Orientia tsutsugamushi* in scrub typhus. The loop-mediated isothermal amplification (LAMP) assay for murine typhus that LOMWRU developed had low sensitivity because of these low bacterial loads. It is likely that the optimum diagnosis of these typhus diseases will involve combined serological and molecular detection
- Co-trimoxazole resistance in *Burkholderia pseudomallei* is much rarer in Laos (and Cambodia) than the Thai literature would suggest, and co-trimoxazole alone is the treatment of choice for the majority of patients with melioidosis during the eradication phase
- *Plasmodium falciparum* artemisinin resistance, as manifested by prolonged parasite clearance, is present in Attapeu, southern Laos, albeit in a minority of patients (~6%). This needs to be monitored carefully and prevention, diagnosis and treatment of malaria enhanced. Targeted malaria elimination should be considered.
- A clinical trial suggested that thiamin supplementation does not reduce the frequency of adverse events after anti-malarial therapy among patients with falciparum malaria in southern Laos
- In collaboration with FIND, we have shown that Lao health workers, including village health volunteers, are able to correctly perform and interpret prototype positive control wells (PCW) developed for malaria RDT quality control, after brief training.
- A G6PD deficiency survey conducted in six randomly selected villages of Sekong province demonstrated that the frequency of people with phenotypic G6PD deficiency was ~4% (70/1,897).
- Mapping of the vectors and reservoirs of *Plasmodium knowlesi* suggests that this monkey and human malaria is likely to occur in Laos
- There remain severe, at least focal, problems with the quality of diverse medicines globally. As shown in Laos the FDA CD3 device is a promising tool for detection of falsified packaging. Tablet splitting is a common practice that may have a neglected deleterious impact on patient outcomes and more attention needs to be paid to packaging language and readability.
- Comparison of results from two random surveys of antimalarial quality in Laos suggest that the quality has improved over the last nine years.
- The WWARN Antimalarial Quality Surveyor system, for mapping reports of antimalarial quality globally is now available in French as well as English and allows downloadable country reports.
- We argue that the CONSORT guidelines on trial reports should include a requirement to determine and describe the quality of medicines used, as a measure to correct this under-recognized and neglected critical weak link in clinical trials.

INTRODUCTION

Mrs Somsavanth Sihalath and Mrs Latsaniphone Boutthasavong working with Petri dishes in the Microbiology Laboratory

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

Oxford University headquarters are at the Centre for Tropical Medicine & Global Health, in the Nuffield Department of Medicine on the Churchill Hospital site. We are greatly assisted by the supplies, logistic and accounting staff of Mahidol Oxford Research Unit (MORU) in the Faculty of Tropical Medicine, Mahidol University, Bangkok, and have many shared projects with MORU.

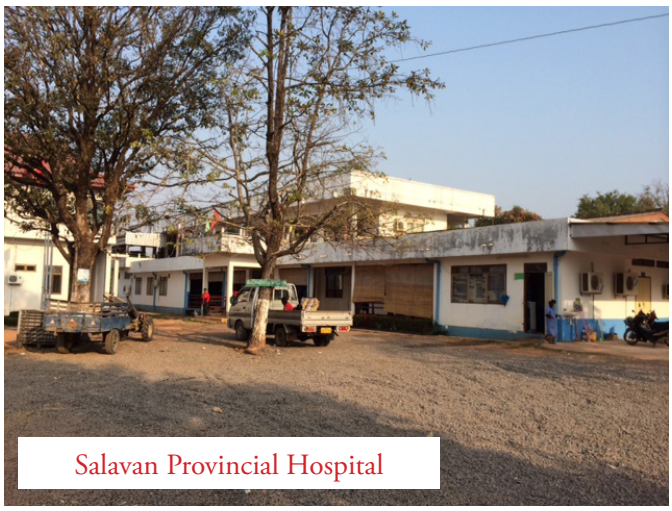
MORU, the Shoklo Malaria Research Unit (SMRU), in Mae Sot, Thailand, the Cambodia-Oxford Medical Research Unit (COMRU) and LOMWRU are integrated into the Thailand/Lao Major Overseas Programme of the Wellcome Trust and Oxford University. We are also linked to the Oxford University Clinical Research Unit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

The Microbiology Laboratory is composed of 26 Lao Government staff and 43 project-funded staff; 93% are Lao and 50% are female. In addition, we have four goats, resident in the Laboratory garden, which assist with the preparation of blood agar. LOMWRU has received significant recent support, in addition to that from the Wellcome Trust, from the US Naval Medical Research Centre, the US Centres for Disease Control (CDC), the Foundation for Innovative New Diagnostics (FIND), the World Health Organisation (WHO), the Institut de Recherche sur l'Asie du Sud-Est Contemporaine (IRASEC) and the Joint Inter-Agency Task Force of the Global Fund, PMI and UNDP. Considerable assistance in kind is given by the Institut de Recherche pour le Développement (IRD)/Aix-Marseille University and the University Rickettsial Diseases Research Program, Naval Medical Research Center, USA

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

Khouang), the far south (Salavan) and other hospitals and institutions on request, performs clinical research and builds diagnostic and research human capacity. The main objective of the work is to inform health policy globally, in Asia and in Laos and improve public health. In 2014 the Laboratory processed 4,970 blood culture pairs, 310 cerebrospinal fluid samples, 1,267 urine samples, 458 stool samples, 844 pus samples, 3,062 genital swabs and 1,112 throat swabs. Dengue IgG, IgM and NS1 ELISAs were performed for 2,026 patients and scrub typhus and murine typhus rapid diagnostic tests for 501 patients.

Since 2000 we have published or have in press 204 papers, including 13 book chapters. LOMWRU authors are the most cited in the Lao public health literature since 2000 and are the most cited authors on medicine quality & public health. In 2014 we published or have in press 29 peer-reviewed papers and 7 book chapters, one report for the World Health Organisation, one commentary and one magazine article. Here we describe this work and briefly summarize diverse activities over the past year.



Salavan Provincial Hospital

Microbiology Laboratory Team at the That Luang Festival

RESEARCH RESULTS AND THEIR PUBLIC HEALTH IMPLICATIONS

Dr Onanong Sengvilaypaseuth and Ms Oyanong Phonemixay preparing for ELISAs in the Infectious Disease Centre

Infectious Disease Epidemiology and Treatment

A. Fever in rural Laos. The data published in Mayxay *et al.* (2013) in Lancet Global Health (see 2013 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 expanded this work to include Xieng Khouang Provincial Hospital, along with those in Salavan and Luang Nam Tha and now have a physician and technician running the study in each of the three hospitals. The National Centre for Laboratory & Epidemiology (NCLE) is analyzing throat and nasal swabs from these hospitals for national influenza surveillance.

Further analysis of the non-malarial fever data from Laos suggests that serum C-Reactive Protein (CRP) is a potentially accurate predictor for the need for antibiotics amongst patients with fever. We are about to start examining this prospectively. Further programmatic and cost-effectiveness analysis of different algorithms is being planned.

We are also looking in the blood samples of patients without a diagnosis for diverse other pathogens such as *Bartonella* spp., *Neorickettsia sennetsu*, *Anaplasma* and *Ehrlichia* species at the three provincial sites and at Mahosot (see B). We have identified a further four patients with sennetsu.

We have completed the analysis of data collected on the causes of fever amongst patients with suspected malaria presenting at Phalanxay District Hospital. Consistent with the data presented in Mayxay *et al.* (2013), this suggests that dengue, leptospirosis, Japanese encephalitis virus infection and scrub typhus were the predominant causes (Mayxay *et al.* submitted).

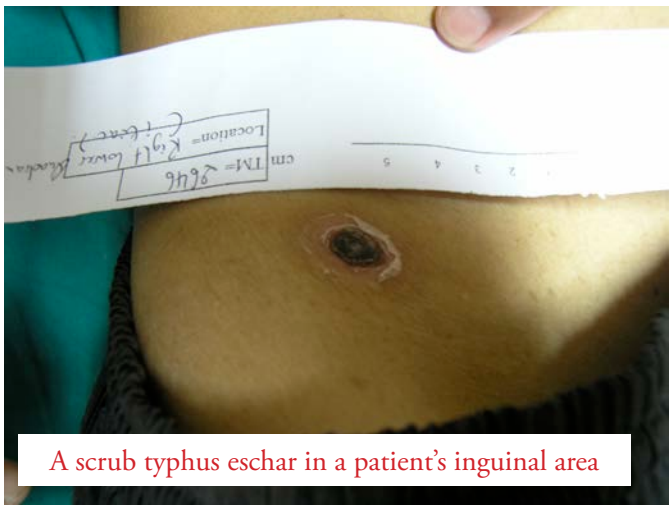
B. Causes of fever at Mahosot. We are working on amalgamating all the data on the causes of sepsis (conventional bacteraemia, rickettsia, leptospira, dengue and JEV) over four recent years so that we can estimate the frequency of hospital admission of diverse aetiologies for a large series of patients and describe their comparative clinical features. For the conventional bacteria we are also analyzing how antimicrobial resistance patterns have changed since 2000.

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

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

This work has been expanded as the SEAe project in collaboration with the Institut Pasteur, Paris, funded by the Total Foundation to investigate the aetiology and impact of encephalitis and meningoencephalitis in Vietnam (at National Institute of Hygiene and Epidemiology, Hanoi), Cambodia (at Kantha Bopha Hospital, Phnom Penh) and Laos (at Mahosot Hospital) using common study protocols.

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 co-authored a review discussing Japanese encephalitis virus infection (Tarantola *et al.* 2014) pointing out the large gaps in our understanding of this infection and other causes of encephalitis in SE Asia. There are few data globally on whether the disability associated with JEV encephalitis improves in the longer term and we are investigating this using the Liverpool Outcome Score for patients with long-term follow up.



A scrub typhus eschar in a patient's inguinal area

E. Aetiology and impact of fever in pregnancy. The large cohort study of the causes and impact of fevers in pregnancy in Pak Gnum District, Vientiane, is nearly completed. Maternal mortality in Laos is reported as the highest in SE Asia and data from Mahosot Hospital (Chansamouth *et al.* in prep.) suggests that common infectious diseases, such as dengue and scrub typhus may be important contributors. This study is linked to the National Centre of Laboratory and Epidemiology for surveillance of respiratory infections in pregnant women, supported by US CDC in Laos. To date 856 pregnant women have been recruited, of whom 86 have had fevers, whose aetiology is being determined. When the follow up of 1,000 pregnant women has been completed the data will be analysed and further plans discussed.

F. Cryptodex Trial. This clinical trial of dexamethasone *versus* placebo in HIV-positive patients receiving amphotericin B for cryptococcal meningitis, coordinated by OUCRU (Day *et al.* 2014) and including Mahosot Hospital, has been stopped on the recommendation of the Trial Steering Committee and the Data Monitoring and Ethics Committee. Further details are awaited.

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

Clinical Bacteriology

A. Accelerated, inexpensive detection of typhoid in blood cultures. Typhoid (*Salmonella enterica* serovar Typhi) remains an important pathogen in Laos but there are very few health facilities with accessible blood culture and antimicrobial susceptibility testing facilities. We

Prof Valy Keoluangkhot and Ward Round in Adult Infectious Disease Ward

demonstrated that brief blood culture bacterial incubation followed by testing of blood culture fluid for *S.* Typhi antigen using rapid diagnostic tests (RDT) is an accurate and inexpensive tool for the accelerated diagnosis of patients with acute typhoid in Laos (Castonguay-Vanier *et al.* 2013, See 2013 Annual Report).

In 2014 we expanded this work and demonstrated that molecular markers of *S.* Typhi fluoroquinolone resistance could by detected by PCR assays on extracts from the RDTs (Nic Fhogartaigh *et al.* submitted). This work suggests that *S.* Typhi antigen detecting RDTs could be used in rural Asia for diagnosing typhoid after brief blood culture incubation. Positive RDTs could be sent to a central facility for the rapid determination of fluoroquinolone resistance by PCR.

B. Bacteraemia in Lao infants. We reviewed the aetiology and antibiotic susceptibilities of bacteraemia in young infants admitted at Mahosot Hospital (Anderson *et al.* 2014). As *Staphylococcus aureus* is the leading cause of bacteraemia in Lao infants, we also examined risk factors for this infection, in particular the local practice of warming mothers during the first weeks postpartum with hot coals under their beds (hot beds). The most common isolates were *S. aureus, Escherichia coli* and *Klebsiella pneumoniae*. Whereas no methicillin-resistant *S. aureus* was found, only 18% of *E. coli* isolates were susceptible to ampicillin. A history of sleeping on a hot bed with mother was significantly associated with *S. aureus* bacteraemia. The study therefore suggests that *S. aureus* is a surprisingly common pathogen in young infants and that hot beds are a major risk factor for infant hospital admission with *S. aureus* sepsis. We are planning further studies to investigate this, to measure the temperatures infants are exposed to on hot beds, and to perform prospective investigations to test this relationship and to increase our understanding of the knowledge, attitude and practice of maternal and infant hot bed use to inform interventions.

C. Diagnosis of infectious diseases using filter paper as a substrate for blood and CSF. Prompted by the demonstration that dried cerebrospinal fluid (CSF) conserved on filter paper could be used as a substrate for accurate PCR diagnosis of important causes of bacterial meningitis, we reviewed the literature on this subject. In collaboration with the London School of Hygiene and Tropical Medicine (LSHTM) the literature, including for diverse types of body fluid and for veterinary medicine and especially for neglected tropical diseases, was overviewed (Smit *et al.* 2014). This suggests that, at least for some pathogens, filter papers are appropriate substrates, but there remains much confusion over terminology, analysis and reporting that needs to be improved.

D. *Burkholderia pseudomallei* and the environment. We are completing two field studies with the Institut de Recherche pour le Développement (IRD) to examine the distribution of *B. pseudomallei* in soil and water in Laos

Ms Amphayvanh Suepsanith labelling samples

in relation to physicochemical variables. We have also optimised molecular methods for the detection of *B. pseudomallei* in soil and developed methodologies to be used with water samples (Knappik *et al*. submitted). We established an artificial soil system, which can hopefully be extended as an *in vitro* system to study *B. pseudomallei* ecology in the BSL-3 laboratory.

E. *Burkholderia pseudomallei* and clinical microbiology. We reviewed *B. pseudomallei* Etest sulphamethoxazole-trimethoprim (SXT) MIC data from Vientiane and Siem Reap, Cambodia, confirming that primary resistance of *B. pseudomallei* to SXT is extremely uncommon and should rarely be a contraindication to SXT monotherapy. This is especially important as after the MERTH clinical trial in Thailand international guidelines for the eradication phase of meliodosis have changed from SXT plus doxycycline to SXT alone (Dance *et al.* 2014a, Dance *et al.* 2014b). We are analyzing the data from the first ~800 patients with culture positive melioidosis diagnosed at Mahosot Hospital since 1999. We are also evaluating a new RDT for *B. pseudomallei* antigen detection in blood cultures and other body fluids using the same principle as the work on the detection of typhoid (see A).

F. Extended spectrum beta-lactamase (ESBL) carriage. We have completed a pilot study with Institut de la Francophonie pour la Médecine Tropicale (IFMT) on childhood faecal carriage of Extended spectrum beta-lactamase producing Enterobacteriaceae (ESBLE) in Vientiane kindergartens, finding that 23% were colonised with ESBLE, mainly *Escherichia coli* carrying bla_{CTX-M} and *Klebsiella pneumoniae* carrying bla_{SHV} or bla_{CTX-M}, which were frequently resistant to multiple unrelated antibiotics (Stoesser *et al.* in press). Faecal carriage of ESBLE was more common in Vientiane Capital (30%) than the more rural Vientiane Province (16%). Only antibiotic use in the last three months was found to be an independent risk factor for ESBLE carriage on multivariable analysis.

G. *Clostridium difficile*. We recently, not unexpectedly, found evidence for *Clostridium difficile* in the stools of patients at Mahosot Hospital. This along with the finding of ESBL (see F) suggests that strategies to reduce inappropriate antibiotic consumption both within health services and in the community are urgently needed.

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

genomics of *Burkholderia pseudomallei*, and with Public Health England on the genomics of *Staphylococcus aureus*.

I. Endocarditis. We are investigating the aetiology of endocarditis in collaboration with Paris Descartes University and the University Aix-Marseille and have recently described the first two patients with *Bartonella henselae* endocarditis. We argue that it is likely that *Bartonella* endocarditis is neglected and more widespread than appreciated, as there are few laboratories in Asia able to make the diagnosis (Rattanavong *et al.* 2014). This is important, as it is likely that proven *Bartonella* endocarditis can be treated with simpler and less expensive regimens than endocarditis caused by "conventional" organisms.

We also reviewed the 36 patients admitted with definite or possible IE, by modified Duke criteria, between 2006-2012 to Mahosot Hospital. Underlying heart diseases included: rheumatic valve disease in 33%, congenital heart disease in 19%, degenerative valve disease in 8%, and of unknown origin in 39% patients. The most frequent pathogens were *Streptococcus* spp. in 19% and blood cultures were negative in 61% patients. Fourteen (39%) had died by follow-up after a median of 2.1 years. These data, some of the few from tropical Asia, suggest that centralised services for acute endocarditis surgery should be considered as an intervention to reduce this mortality (Mirabel *et al.* 2014).

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

J. Respiratory infections. We have started 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 and its serotypes to examine how their frequencies change with the introduction of 13 valent *S. pneumoniae* vaccination in Laos.

K. Leptospirosis. We have been determining the optimal fraction of blood for the molecular diagnosis of leptospirosis and conducting a large prospective evaluation of different

The outer case of a cluster bomb being used to grow spring onions in Savannakhet Province

RDTs and PCR assays for diagnosing leptospirosis, with the aim that this will inform the optimal diagnostic techniques for Laos. We have completed a review of the literature to estimate the untreated mortality of leptospirosis.

N. *Nocardia aobensis.* We have described a 30 year old farmer with actinomycetoma caused by *Nocardia aobensis* in Savannakhet Province (Vongphoumy *et al.* in preparation). This is the first record of this pathogen in actinomycetoma. We described the first patient with actinomycetoma in Laos in 2012 (Rattanavong *et al.* 2012), a patient with *Actinomadura madurae* infection from Xieng Khouang province.

Virology

The virology work of LOMWRU is strongly supported by the Institut de Recherche pour le Développement (IRD)/ Aix-Marseille University. A key part of the work in 2014 is the design and provision of freeze-dried PCR reagents for the diagnosis of pathogens causing central nervous system disease and respiratory disease, as part of the SEAe and ARIVI studies (see above).

A. Dengue epidemiology. Thankfully 2014 had low incidence of dengue in Laos, unlike 2013. We are analyzing the distribution of dengue serotypes in Vientiane 2006-2010 and in Luang Nam Tha and Salavan in 2008 to 2010.

B. Dengue RDTs. We are evaluating the diagnostic accuracy of dengue rapid diagnostic tests (RDTs) and determining whether dengue PCR can be performed, for determination of dengue serotype, on extracts of the pad of the dry NS1 positive RDT. If this technique is accurate it could facilitate the Lao national monitoring of dengue serotypes by the shipping of RDTs from the provinces to Vientiane for dengue PCR of NS1 positive RDTs. There are problems with the thermal stability of malaria RDTs and we therefore examined long term stability of the diagnostic accuracy of dengue RDTs in the laboratory and in the field. The data

The foot of a 30 year old rice farmer from Xieng Khuang Province with actinomycetoma of her left foot caused by *Actinomadura madurae*. She recovered after prolonged antibiotic therapy

suggest that at least one brand of dengue NS1/IgM/IgG RDT maintains diagnostic accuracy long term at hot Lao temperatures. The RDTs had 100% consistency over the two-year study, despite high temperatures, including in a hut in which temperatures exceeded the manufacturer's recommendations for 29% of time points (Phommasone *et al.* submitted).

C. Hand, Foot and Mouth disease. We support enteroviral PCR for surveillance of Hand, Foot and Mouth disease (HFMD) as it is likely that there will be a large outbreak in Laos, as has happened in adjoining countries in the last decade. We described, for the first time, a SYBR Green real-time RT-PCR system validated to detect all 8 EV-A71 genogroups (Dubot-Pérès *et al.* 2014). So far we have only found EV-A71 in children with uncomplicated disease. We performed the first genomic characterization of EV-A71 and coxsackievirus A16 strains isolated in 2011 from Lao patients. Isolates were related to EV-A71 genotype C4 and CV-A16 genotype B1a that circulated in neighbouring countries during the same period (Nguyen *et al.* 2014). We are liaising with OUCRU in Ho Chi Minh City over a multicentre placebo-controlled trial of the efficacy of immunoglobulin in severe EV71 disease.

Rickettsiology

A. Scrub typhus genotypes. The collaboration with Rickettsial Diseases Research Program, Naval Medical Research Center, USA, is progressing with the whole genome sequencing (WGS) of multiple *Orientia tsutsugamushi* genotypes to examine whether different genotypes are associated with disease severity. Twelve isolates have had successful WGS performed and more genotypes are expected soon. In addition, we are working on the genetic diversity of the pathogen across Laos using a multilocus sequence typing (MLST) scheme.

B. *Rickettsia felis* in Laos. We described three Lao patients with PCR evidence for *Rickettsia felis* infection (from Vientiane, Luang Nam Tha and Salavan) but all had co-morbidities and the pathogenicity of the bacterium and the vectors responsible for transmission in Asia are questioned (Dittrich *et al.* 2014a).

C. Murine typhus diagnosis using LAMP assays. Although treatment of murine typhus (*Rickettsia typhi*) with tetracycline antibiotics is effective, treatment is often misguided or delayed due to diagnostic difficulties. As the gold standard immunofluorescence assay is imperfect, we developed and evaluated a loop-mediated isothermal amplification assay (LAMP) (Dittrich *et al.* 2014). In the prospective evaluation amongst 266 consecutive patients with suspected scrub typhus or murine typhus, the clinical sensitivity was low at 33% (95% CI: 9.2 - 56.8) (specificity: 98.5% (95% CI: 97.0% - 100%)). This low diagnostic accuracy was attributed to low patient *R. typhi* bacterial loads and suggests that LAMP assays for *R. typhi* are unlikely to be diagnostically useful.

D. Antibiotic susceptibility of rickettsial species. We have started a project examining the antibiotic susceptibility of *O. tsutsugamushi* and *Rickettsia typhi*.

E. Ticks. We are working with Institut Pasteur-Laos on the detection of *Rickettsia, Bartonella, Orientia, Anaplasma* and *Ehrlichia* species in a large collection of ticks from Kammouane Province. We have found a significant proportion contain *Rickettsia* spp. DNA and are determining the species present. In addition *Ehrlichia chaffeensis*, *Coxiella burnetii, Anaplasma phagocytophilum* and *Borellia* spp. were identified. This should help us to narrow down what tick-borne bacterial pathogens we may find in patients in Laos.

F. Mapping of scrub typhus. We are working with VectorMap (http://www.vectormap.org/), the Spatial Ecology and Epidemiology Group of Oxford University, Liverpool University and many partners on the global mapping of chigger vectors/reservoirs and infected rodents and humans.

G. Revisiting the natural history of scrub typhus. Dr Ivo Elliott has been awarded a Wellcome Trust Fellowship to work in LOMWRU from 2015, revisiting the research on the natural history of scrub typhus in the 1930s/1950s using modern techniques such as whole genome sequencing and geographical information systems.

H. Untreated mortality of scrub typhus. We have completed a review of the literature to estimate the untreated mortality of scrub typhus.

Plus, We are working on determining the optimal fraction of blood for the molecular diagnosis of rickettsial diseases, disease severity scores for scrub typhus, and analyzing

the scrub typhus and murine typhus clinical trials of doxycycline and azithromycin and the first PK-PD work on typhus and doxycycline and azithromycin therapy with the Pharmacology Department of MORU. We continue to work on the epidemiology of *Neorickettsia sennetsu*, with the University of North Dakota, looking for this pathogen in a variety of invertebrate and vertebrate taxa to try to elucidate the natural history of this intriguing pathogen.

Malaria

A. Artemisinin resistance. We participated, with the Centre for Malariology, Parasitology and Entomology and coordinated by MORU, in the multicentre TRAC study 2011/12 that provided the first evidence that artemisinin resistant *Plasmodium falciparum* parasites are present in southern Laos, as has been documented on the Cambodia/Thailand, Thailand/Burma borders and southern Vietnam (Ashley *et al.* 2014). Of 120 patients with uncomplicated *P. falciparum* malaria recruited in Phouvong District, Attapeu, 6% had parasitaemia on day three and the slopes of the parasite clearance curves were reduced. These data suggest that the situation should be closely monitored in Attapeu and elsewhere in southern Laos. We plan to continue this monitoring with CMPE as part of the TRAC-2 project in 2015.

B. Molecular markers of antimalarial resistance. The recent description of a molecular marker of artemisinin resistance ('K-13 propeller') has facilitated the mapping the extent of these parasites. We are working with MORU to examine the distribution of different K-13 mutations across southern Laos. With independent emergence of mutations in different geographic areas such monitoring will be very important (Takala-Harrison *et al.* 2014).

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

C. Thiamin supplementation and malaria. We found evidence (Mayxay *et al.* 2007) that one third of Lao patients presenting with uncomplicated *Plasmodium falciparum* malaria had biochemical evidence of thiamin deficiency, which was associated with a higher incidence of adverse events. We therefore conducted a double-blind, parallel group, placebo-controlled, superiority trial of thiamin supplementation in patients with uncomplicated and severe falciparum malaria in Xepon District, Savannakhet Province. The trial suggested that thiamin supplementation does not reduce the frequency of adverse events after anti-malarial therapy among patients with falciparum malaria in southern Laos

D. The cost of treating malaria. Parenteral artesunate is the recommended treatment for patients with severe *Plasmodium falciparum* malaria but uptake in national policy has been slow (but not in Laos) – one potential reason is that parenteral artesunate is costlier than quinine. We documented the resources used in treating falciparum malaria by either parenteral artesunate or quinine in a hospital on the Thai-Myanmar border. This analysis found no evidence for a difference in total costs for malaria inpatients treated with artesunate as compared with quinine.

E. Positive control wells for malaria RDTs. We have been working with the Foundation for Innovative New Diagnostics (FIND) to evaluate positive control wells (PCWs) in the quality control of malaria rapid diagnostic tests (RDTs). We have shown that Lao health workers, including village health volunteers, are able to correctly perform and interpret prototype positive control wells (PCW) developed for malaria RDT quality control, after a half-day training and to maintain this standard over 6 months of routine use. Also, PCWs appear to improve health workers' confidence in malaria RDT validity.

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

G. *Plasmodium knowlesi* malaria. We have looked for *Plasmodium knowlesi* in filter paper blood spots from malaria patients but have not found evidence for this pathogen in Laos. However, work with the Spatial Ecology & Epidemiology Group in Oxford University to investigate the potential geographical range of this pathogen suggests that it should occur in Laos (Moyes *et al.* 2014).

H. 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 use of primaquine. We have therefore conducted surveys of the prevalence of phenotypic and genotypic markers of G6PD deficiency in Sekong and Salavan Provinces in collaboration with CMPE, SMRU and Institut de Recherche pour le Développement. A G6PD deficiency survey conducted in six randomly selected villages of two districts of Sekong province demonstrated that, using Trinity fluorescence spot test, the frequency of people with phenotypic G6PD deficiency was ~ 4% (70/1,897).

I. Targeted malaria elimination. We are planning with CMPE and MORU a large study of the implementation of Targeted Malaria Elimination (TME) in Savannakhet Province in 2015 funded by the Bill and Melinda Gates Foundation.

First aid training

Community perceptions and engagement

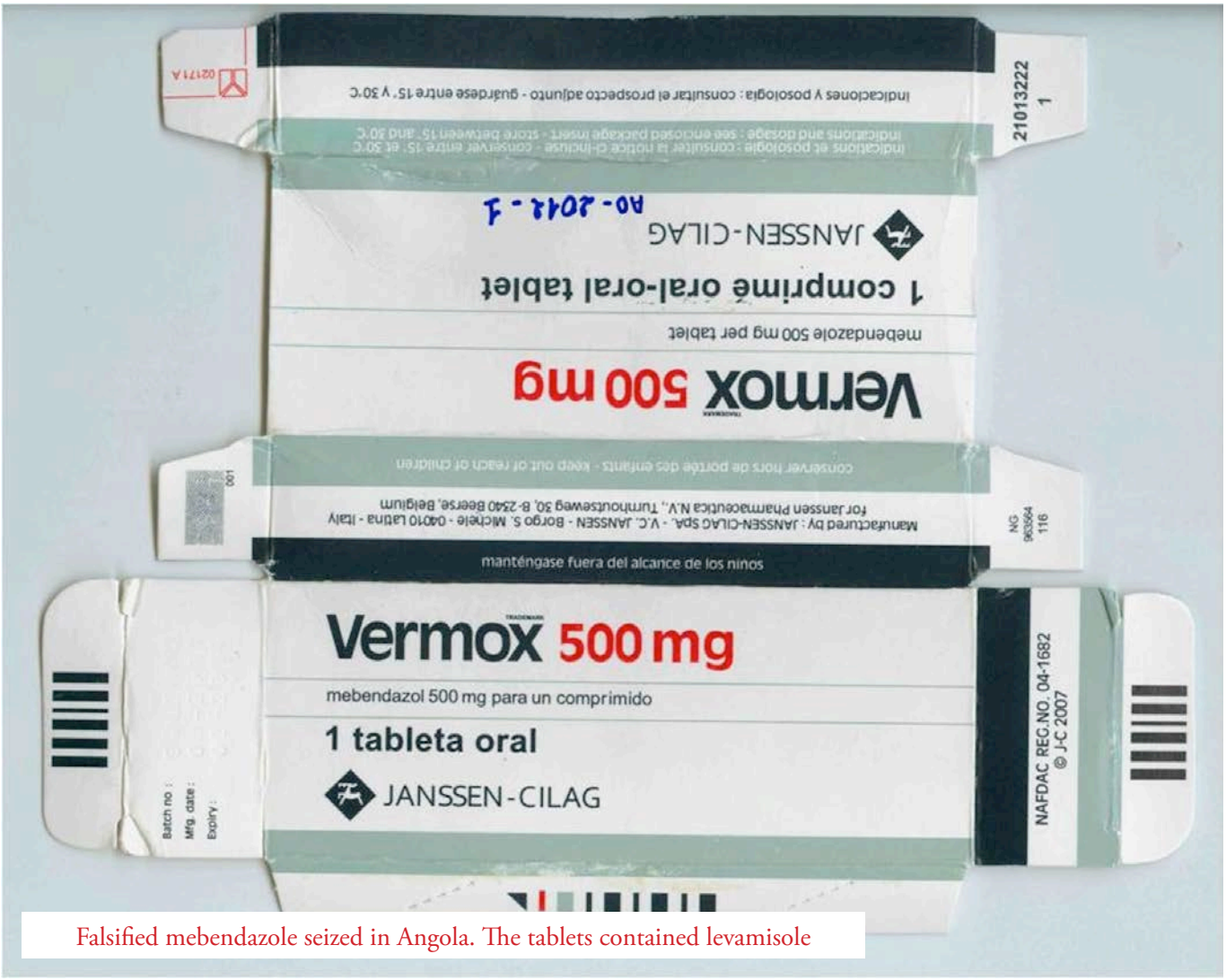
Now that there are more data on infectious disease epidemiology in Laos we are planning both public engagement research and implementation. This will be a key component of the TME project (above).

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

Medicine quality

A. Worldwide Antimalarial Resistance Network (WWARN). The WWARN Antimalarial Quality Scientific Group continues to tabulate and map reports of the quality of antimalarials (see http://www.wwarn.org/resistance/surveyors/antimalarial-quality). It has been improved in 2014 with a French language version and a system for the automatic creation of pdf country reports. We hope to be able to extend this to other classes of essential medicines in 2015. This is supported by the Institut de Recherche sur l'Asie du Sud-Est Contemporaine (IRASEC) at the French Embassy, Bangkok and the Bill & Melinda Gates Foundation.

We analysed the WWARN Antimalarial Quality Surveyor database of anti-malarial quality reports in English, French and Spanish since 1946 (Tabernero *et al.* 2014). No publicly available reports on antimalarial quality for 61% of the 104 malaria-endemic countries were found. Out of 9,348 antimalarials sampled, 30% failed chemical/packaging quality tests with 39% classified as falsified, 2% as substandard and 58% as poor quality without evidence available to categorize them as either substandard or falsified. Only 32% of the reports explicitly described the definitions of medicine quality used and just 9% of



Falsified mebendazole seized in Angola. The tablets contained levamisole

the samples collected in six surveys were conducted using random sampling techniques. Twenty different wrong active ingredients were found in falsified anti-malarials. The available evidence demonstrates severe public health problems with antimalarial quality, many gaps and the need for more investment to improve medicine regulatory authority capacity and both sampling and analytical methodology and to achieve consensus in defining different types of poor quality medicines.

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

C. Angola seizure. We described the seizure in Luanda, Angola, of falsified medicines labelled as the antimalarial 'Coartem' 'artemether-lumefantrine' bearing the Affordable

Medicines Facility–malaria (AMFm) logo and others labelled as the antihelminthic 'mebendazole' 'Vermox (Newton *et al.* 2014). No mebendazole was detected in the 'mebendazole' formulation, but levamisole (270mg/tablet) was present. Both 'products' showed marked differences in packaging characteristics from genuine products. The discovery of falsified artemether-lumefantrine and without any detectable antimalarial is of considerable concern for malaria control. Presence of levamisole in falsified 'mebendazole' is also of great concern as it has significant adverse effects. Enhanced collaboration between African MRAs/police and the authorities in China to stop criminal transcontinental trade in falsified essential medicines is urgently needed. Delays in reporting and action must be reduced by mandatory notification systems and independent public health risk assessments. Despite multiple reports, public health research has failed to stimulate actions required to improve the quality of global drug supply

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

E. Falsified contraceptives. We investigated falsified 'morning after pills' discovered in Peru that contained no detectable levonorgestrel, but did contain the antibiotic sulfamethoxazole (Monge *et al.* 2014).

F. Reports for the Joint Inter-Agency Task Force (JIATF). We write reports every two months on medicine quality problems for the Joint Inter-Agency Task Force (JIATF) of The Global Fund's Office of Inspector General, the Presidents Malaria Initiative, and UNDPs Office of Audit and Investigations.

G. Forensics. We are collaborating with specialist laboratories 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.

H. Guidelines. We are revising the MEDQUARD guidelines (Newton *et al.* 2009; *PLoS Medicine* 6, e1000052) on conducting and reporting surveys for the quality of medicines for the WHO. These have been reviewed by a WHO committee and are being revised as a WHO report for 2015. See: http://www.who.int/medicines/areas/quality_safety/quality_assurance/WHO_SamplingProcedures_GeneralDocument_QAS14_590_10062014.pdf?ua=1

I. Tablet splitting. Tablet splitting is commonly practiced globally but there has been relatively little discussion on their clinical consequences for essential medicines in low- and middle-income countries. We therefore examined the accuracy of splitting and found severe problems, especially with coated and unscored tablets. Wider ranges of dosage units, particularly for narrow therapeutic index and critical dosage medicines, are needed so that splitting is not required (Elliott *et al.* 2014).

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

K. Medicine Stability. There are very few 'field' data as to what actually happens to the composition of medicines in the tropics if they are not stored according to the manufacturer's recommendations. We are therefore performing long-term stability studies on antimalarials both in hot rural Laos and in the laboratory to examine the consequences of heat and time and determine the degradation products in collaboration with the CDC and the Georgia Institute of Technology in the USA.

L. Counterfeit Detection Device. We conducted a blinded evaluation of the diagnostic accuracy of the Counterfeit Detection Device 3 (CD-3), developed by the US Food and Drug Administration Forensic Chemistry Center, with the Bureau of Food and Drug Inspection (BFDI), Food and Drug Quality Control Centre (FDQCC), Government of the Lao PDR, NIH and CDC (Ranieri *et al.* 2014). This innovative, portable and relatively inexpensive instrument facilitates the examination of packaging to detect falsified medicines. Two hundred three samples of the oral antimalarial artesunate were compared with authentic products using the CD-3 by a trainer and two trainees. The specificity (95% confidence interval [95% CI]), sensitivity (95% CI), positive predictive value (95% CI), and negative predictive value (95% CI) of the CD-3 for detecting counterfeit (falsified) artesunate were 100% (93.8-100%), 98.4% (93.8-99.7%), 100% (96.2-100%),

and 97.4% (90.2-99.6%), respectively. Inter-observer agreement for 203 samples of artesunate was 100%. The CD-3 holds promise as a relatively inexpensive and easy to use instrument for field evaluation of medicines, potentially empowering drug inspectors, customs agents, and pharmacists. This paper was reported on in Minerva of the BMJ (http://www.bmj.com/content/349/bmj.g6260).

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

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

The Lao PDR with the main research sites. Yellow are fever study sites and green are malaria and G6PD study sites

Visit of Professor Mike Turner and Dr Michael Chew, from the Wellcome Trust, to Professor Dr Bounthapany Bounxouei, Director of Mahosot Hospital

KEY COLLABORATIONS

The plaque on the front of the Infectious Disease Centre

Within Lao PDR

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

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

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

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

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

Professor Sharon Peacock, University of Cambridge, UK

Dr Mike Green, CDC, Atlanta, Georgia, USA

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

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

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

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

Dr Dallas Mildenhall, GNS Science, New Zealand

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

Dr Céline Caillet and Prof Anne Roussin, Faculté de Pharmacie, UMR1027 Inserm-Universite Toulouse III, France

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

Drs Nadine Fievet, Valérie Briand and Jean-Yves Le Hesran, Institut de Recherche pour le Développement, UMR 216, Université Paris Descartes, Paris

Drs Olivier Ribolzi and Alain Pierret, Institut de Recherche pour le Développement, Laos and Université de Toulouse, France

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

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

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

Professor Adrian Linacre, Flinders University, Australia

Ms Aline Plançon, INTERPOL, Lyon, France

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

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

Drs David Schellenberg, Shunmay Yeung and Harparkash Kaur, ACT Consortium, London School of Hygiene and Tropical Medicine, London, UK

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

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

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

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

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

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

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

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

Dr Damien Chaussabel, Sidra Medical and Research Center, Qatar

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

Dr Souly Phanouvong and Dr Patrick Lukulay, United States Pharmacopeia, Rockville, Virginia, USA

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

Professor Xavier Nicolas de Lamballerie, UMR190_ Emergence des Pathologies Virales (Institut de Recherche pour le Développement, Aix-Marseille University, EHESP French School of Public Health), Marseille, France.

Professor Didier Raoult and Professor Pierre-Edouard Fournier, Rickettsial Reference Laboratory, Aix-Marseille University, France

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

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

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

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

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

Dr Philippe Guerin and WWARN, WWARN, Centre for Tropical Medicine & Global Health, University of Oxford, UK

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

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

Professor Albert Ko, Yale School of Public Health, USA

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

STAFF AND HUMAN CAPACITY BUILDING

Dr Rattanaphone Phetsouvanh inviting everyone for international food exchange lunch !

Seventy-seven students and doctors in diverse health disciplines studied in the Microbiology Laboratory in 2014 and three residents are writing their theses related to the work of the Laboratory. The Laboratory staff assisted with the post-graduate internal medicine and paediatric training programme teaching. Three Lao postgraduate fellows in infectious disease are conducting their research theses with us.

Dr Rattanaphone Phetsouvanh is completing her PhD from Mahidol University, Bangkok, on scrub typhus, and Dr Manivanh Vongvousath is reading for her MSc in Clinical Tropical Medicine also at Mahidol University. Ms Kristin Mullins of the Uniformed Services University, USA, is doing her PhD with us on scrub typhus disease severity and *O. tsutsugamushi* genotypes. We hosted three London School of Hygiene and Tropical Medicine MSc students for three months in 2014, working on chiggers and the molecular diagnostic of scrub typhus and murine typhus and the untreated mortality of leptospirosis.

Ms Bountoy Sibounheuang spent a further two months in the laboratory of Professor Xavier Nicolas de Lamballerie at Marseille working with Dr Audrey Dubot-Pérès, funded by the Institut de Recherche pour le Développement. She will spend two months a year there for the next two years, working on Lao projects to replicate the procedures used in Marseille in Vientiane. Dr Audrey Dubot-Pérès, who leads the LOMWRU virology, is based in Marseille but returned for three months of intensive virology work in LOMWRU in 2014.

We are also fortunate to have strong links with Public Health England (PHE) who support a microbiology/ infectious disease registrar to spend a year of training with us. Dr Caoimhe Nic Fhogartaigh and then Dr Kate Woods have conducted much laboratory, clinical and research work during 2014. This has been a very useful synergistic programme. In addition, Dr Andrew Taylor from the UK is working in LOMWRU for a year, contributing much to the clinical and research work.

Regular classes have been held in English. We are planning Good Clinical Practice and statistics training in 2015.

In 2014, we supported nine Lao staff to attend conferences and meetings outside of Laos.

We have continued to build capacity within the Unit with hands-on training in microbiology, ELISA, molecular diagnostic and BSL3 Laboratory work. In addition we have weekly teaching sessions for the doctors working within the

Unit (both at Mahosot and those visiting from the Provinces) covering clinical and laboratory aspects of infectious diseases and microbiology directly relevant to both their clinical and research activities. We have a Lao Deputy Safety Officer, a Lao Head of Field Research, a Lao deputy head of Virology, a Lao deputy WWARN Antimalarial Quality Coordinator, a Lao Laboratory Manager and a Lao BSL3 Manager. Following a WHO-led workshop in late 2013, we have appointed a Laboratory Management Adviser who is co-ordinating a programme of work towards ISO15189 accreditation for the Microbiology Laboratory, and we are working closely with other laboratories in Laos that are also planning to work towards accreditation.

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

Entrance to the BSL3 Laboratory antechamber


Teachers and attendees of the Good Clinical Practice course run at Mahosot Hospital

OTHER ACTIVITIES

Lunch in the garden

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

B. E-Library. We have been working with the University of Health Sciences (UHS) to build a page on their website as an e-library – as a repository of published and grey literature information about Lao public health. This will be completed in early 2015.

C. 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 will be freely downloadable on the e-library at UHS.

D. LOMWRU website. We are revising the LOMWRU components of the www.tropmedres.ac site.

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

F. Talks etc. The Laboratory runs monthly lunchtime journal clubs, monthly scientific seminars and has frequent talks by academic visitors.

G. Wellcome Trust visit. We were visited by Professor Michael Turner and Dr Michael Chew from the Wellcome Trust in October.

Microbiology Laboratory staff cleaning in preparation for National Day

TITLES AND ABSTRACTS OF PAPERS PUBLISHED OR IN PRESS 2014

1. Tarantola A, Goutard F, Newton PN, de Lamballerie X, Lortholary O, Cappelle J, Buchy (2014) Estimating the burden of Japanese encephalitis virus and other encephalitides in countries of the Mekong Region. *PLoS NTD* 8: e2533.

Abstract. Diverse aetiologies of viral and bacterial encephalitis are widely recognized as significant yet neglected public health issues in the Mekong region. A robust analysis of the corresponding health burden is lacking. We retrieved 75 articles on encephalitis in the region published in English or in French from 1965 through 2011. Review of available data demonstrated that they are sparse and often derived from hospital-based studies with significant recruitment bias. Almost half (35 of 75) of articles were on Japanese encephalitis virus (JEV) alone or associated with dengue. In the Western Pacific region the WHO reported 30,000-50,000 annual JEV cases (15,000 deaths) between 1966 and 1996 and 4,633 cases (200 deaths) in 2008, a decline likely related to the introduction of JEV vaccination in China, Vietnam, or Thailand since the 1980s. Data on dengue, scrub typhus and rabies encephalitis, among other aetiologies, are also reviewed and discussed. Countries of the Mekong region are undergoing profound demographic, economic and ecological change. As the epidemiological aspects of Japanese encephalitis (JE) are transformed by vaccination in some countries, highly integrated expert collaborative research and objective data are needed to identify and prioritize the human health, animal health and economic burden due to JE and other pathogens associated with encephalitides.

2. Dittrich S, Castonguay-Vanier J, Moore CE, Thongyoo N, Newton PN, Paris DH (2014) Loop-mediated isothermal amplification for *Rickettsia typhi* (murine typhus) - problems with diagnosis at the limit of detection. *J Clin Micro* 52, 832-838.

Abstract. Murine typhus is a flea-borne disease of worldwide distribution caused by *Rickettsia typhi*. Although treatment with tetracycline antibiotics is effective, treatment is often misguided or delayed due to diagnostic difficulties. As the gold standard immunofluorescence assay is imperfect, we aimed to develop and evaluate a loop-mediated isothermal amplification (LAMP) assay. LAMP assays have the potential to fulfill the WHO ASSURED criteria (affordable, sensitive, specific, user friendly, robust and rapid, equipment free, deliverable to those who need them) for diagnostic methodologies, as they can detect pathogen-derived nucleic acid with low technical expenditure. The LAMP assay was developed using samples of bacterial isolates (n=41), buffy coat specimens from *R. typhi* PCR-positive Lao patients (n=42), and diverse negative controls (n=47). The method was then evaluated prospectively using consecutive patients with suspected scrub typhus or murine typhus (n=266). The limit of detection was 40 DNA copies/LAMP reaction, with an analytical sensitivity of <10 DNA copies/reaction based on isolate dilutions. Despite these low cutoffs, the clinical sensitivity was disappointing, with 48% (95% confidence interval [95% CI], 32.5 to 62.7%) (specificity, 100% [95% CI, 100 to 100%]) in the developmental phase and 33% (95% CI, 9.2 to 56.8%) (specificity, 98.5% [95% CI, 97.0% to 100%]) in the prospective study. This low diagnostic accuracy was attributed to low patient *R. typhi* bacterial loads (median, 210 DNA copies/ml blood; interquartile range, 130 to 500). PCR-positive but LAMP-negative samples demonstrated significantly lower bacterial loads than LAMP-positive samples. Our findings highlight the diagnostic challenges for diseases with low pathogen burdens and emphasize the need to integrate pathogen biology with improved template production for assay development strategies.

Xieng Khouang Provincial Hospital

3. Smit PW, Elliott I, Peeling RW, Mabey D, Newton PN (2014) An overview of the clinical use of filter paper in the diagnosis of tropical diseases. *Am J Trop Med Hyg* 90, 195-210.

Abstract. Tropical infectious diseases diagnosis and surveillance are often hampered by difficulties of sample collection and transportation. Filter paper potentially provides a useful medium to help overcome such problems. We reviewed the literature on the use of filter paper, focusing on the evaluation of nucleic acid and serological assays for diagnosis of infectious diseases using dried blood spots (DBS) compared with recognized gold standards. We reviewed 296 eligible studies and included 101 studies evaluating DBS and 192 studies on other aspects of filter paper use. We also discuss the use of filter paper with

other body fluids and for tropical veterinary medicine. In general, DBS perform with sensitivities and specificities similar or only slightly inferior to gold standard sample types. However, important problems were revealed with the uncritical use of DBS, inappropriate statistical analysis, and lack of standardized methodology. DBS have great potential to empower healthcare workers by making laboratory-based diagnostic tests more readily accessible, but additional and more rigorous research is needed.

4. Mayxay M, Soukaloun D, Newton PN (2014) A two-month-old Lao girl with dysnoea, irritability, poor breastfeeding and grunting. In: Clinical Cases in Tropical Medicine, Ed. Camilla Rothe, Elsevier-Saunders, pp. 185-187.

[A clinical description of a patient with infantile beriberi and discussion of epidemiology and treatment]

5. Slesak G, Inthalad S, Newton PN (2014) Extensive skin lesions on the lower leg in a 72-year-old farmer from Laos. In: Clinical Cases in Tropical Medicine, Ed. Camilla Rothe, Elsevier-Saunders, pp. 123-125.

[A clinical description of a patient with chromoblastomycosis and discussion of diagnosis and management]

6. Rattanavong S, Douangnoulak V, Norindr B, Newton PN, Nic Fhogartaigh C (2014) A 3 year old boy with Right Suppurative Parotitis. In: Clinical Cases in Tropical Medicine, Ed. Camilla Rothe, Elsevier-Saunders, pp. 92-94.

[A clinical description of a patient with *Burkholderia pseudomallei* parotitis and discussion of management]

7. Rattanavong S, Keoluangkhot V, Sisouphnh S, Lattaphasavang V, Dance D, Fhogartaigh CN (2014) A 44 year old male farmer from Laos with diabetes and a back abscess. In: Clinical Cases in Tropical Medicine, Ed. Camilla Rothe, Elsevier-Saunders, pp. 145-148.

[A clinical description of a diabetic patient with *Burkholderia pseudomallei* septicaemia and abscess and discussion of management]

8. Newton PN, Keoluangkhot V, Mayxay M, Michael D Green, Facundo M Fernández (2014) A 30-year-old male Chinese trader with fever. In: Clinical Cases in Tropical Medicine, Ed. Camilla Rothe, Elsevier-Saunders, pp. 168-170.

[A clinical description of a patient with a recent history of falciparum malaria but failure of 'cure' with discussion of poor medicine quality]

Village in Savannakhet Province

9. Dance DAB, Davong V, Soeng S, Phetsouvanh R, Newton PN, Turner P (2014a) Co-trimoxazole resistance in *Burkholderia pseudomallei*. *Int J Antimicrob Ag* 44, 368–369.

[The MERTH study suggests that trimethoprim/sulfamethoxazole (SXT) monotherapy may be used in the treatment of melioidosis during the eradication phase. It is therefore important to know the prevalence of SXT resistance in *Burkholderia pseudomallei* - but this is difficult to test in vitro, with disc diffusion testing overestimating resistance. Even using the Etest method to estimate the minimum inhibitory concentration (MIC), SXT resistance rates as high as 24% have occasionally been reported from Thailand. This paper reviews *B. pseudomallei* Etest SXT MIC data from Vientiane and Siem Reap, Cambodia, and confirms that primary resistance of *B. pseudomallei* to SXT is extremely uncommon and should rarely be a contraindication to SXT monotherapy.]

10. Anderson M, Luangxay K, Sisouk K, Vorlasan L, Soumphonphakdy B, Sengmouang V, Chansamouth V, Phommasone K, Van Dyke R, Chong E, Dance DAB, Phetsouvanh R, Newton PN (2014) Epidemiology of bacteremia in young hospitalized infants in Vientiane, Laos 2000-2011. *J Trop Pediatrics* 60, 10-16.

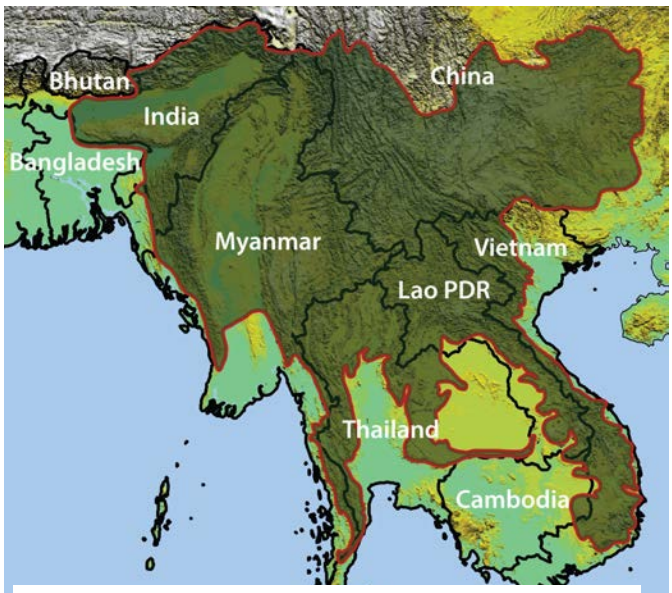
As data about the causes of neonatal sepsis in low-income countries are inadequate, we reviewed the etiology and antibiotic susceptibilities of bacteremia in young infants

The Board Round

in Laos. As *Staphylococcus aureus* is the leading cause of bacteremia in Lao infants, we also examined risk factors for this infection, in particular the local practice of warming mothers during the first weeks postpartum with hot coals under their beds (hot beds). Clinical and laboratory data regarding infants aged 0-60 days evaluated for sepsis within 72 h of admission to Mahosot Hospital in Vientiane, Laos, were reviewed, and 85 of 1438 (5.9%) infants' blood cultures grew a clinically significant organism. Most common were *S. aureus, Escherichia coli* and *Klebsiella pneumoniae*. Whereas no methicillin-resistant *S. aureus* was found, only 18% of E. coli isolates were susceptible to ampicillin. A history of sleeping on a hot bed with mother was associated with *S. aureus* bacteremia (odds ratio 4.8; 95% confidence interval 1.2-19.0).

11. Tabernero P, Fernández FM, Green MD, Guerin PJ, Newton PN (2014) Mind the gaps - the epidemiology of poor-quality anti-malarials in the malarious world - analysis of the WorldWide Antimalarial Resistance Network database. *Malaria Journal* 13, 139.

BACKGROUND: Poor quality medicines threaten the lives of millions of patients and are alarmingly common in many parts of the world. Nevertheless, the global extent of the problem remains unknown. Accurate estimates of the epidemiology of poor quality medicines are sparse and are influenced by sampling methodology and diverse chemical analysis techniques. In order to understand the existing data, the Antimalarial Quality Scientific Group at WWARN built a comprehensive, open-access, global database and linked Antimalarial Quality Surveyor, an online visualization tool. Analysis of the database is described here, the limitations of the studies and data reported, and their public health implications discussed. METHODS: The database collates customized summaries of 251 published anti-malarial quality reports in English, French and Spanish by time and location since 1946. It also includes information on assays to determine quality, sampling and medicine regulation. RESULTS: No publicly available reports for 60.6% (63) of the 104 malaria-endemic countries were found. Out of 9,348 anti-malarials sampled, 30.1% (2,813) failed chemical/packaging quality tests with 39.3% classified as falsified, 2.3% as substandard and 58.3% as poor quality without evidence available to categorize them as either substandard or falsified. Only 32.3% of the reports explicitly described their definitions of medicine quality and just 9.1% (855) of the samples collected in 4.6% (six) surveys were conducted using random sampling techniques. Packaging analysis was only described in 21.5% of publications and up to twenty wrong active ingredients were found in falsified anti-malarials. CONCLUSIONS: There are severe neglected problems with anti-malarial quality but there are important caveats to accurately estimate the prevalence and distribution of poor quality anti-malarials. The lack of reports in many malaria-endemic areas, inadequate sampling techniques and inadequate chemical analytical methods and instrumental procedures emphasizes the



Zomia - the dark green area illustrates the vast SE Asian massif or Zomia. This large population, including Laos, has sparse public health information

need to interpret medicine quality results with caution. The available evidence demonstrates the need for more investment to improve both sampling and analytical methodology and to achieve consensus in defining different types of poor quality medicines.

12. Dubot-Pérès A, Tan CYQ, de Chesse R, Sibounhoueng B, Vongsouvath M, Phommasone K, Bessaud M, Gazin C, Thirion L, Phetsouvanh R, Newton PN, de Lamballerie X (2014) SYBR Green Real-time PCR for the detection of all Enterovirus-A71 genogroups. *PloS One* 9, e89963.

Enterovirus A71 (EV-A71) has recently become an important public health threat, especially in South-East Asia, where it has caused massive outbreaks of Hand, Foot and Mouth disease every year, resulting in significant mortality. Rapid detection of EV-A71 early in outbreaks would facilitate implementation of prevention and control measures to limit spread. Real-time RT-PCR is the technique of choice for the rapid diagnosis of EV-A71 infection and several systems have been developed to detect circulating strains. Although eight genogroups have been described globally, none of these PCR techniques detect all eight. We describe, for the first time, a SYBR Green real-time RT-PCR system validated to detect all 8 EV-A71 genogroups. This tool could permit the early detection and shift in genogroup circulation and the standardization of HFMD virological diagnosis, facilitating networking of laboratories working on EV-A71 in different regions.

13. Moyes CL, Henry AJ, Golding N, Huang Z, Singh B, Baird JK, Newton PN, Huffman M, Duda KA, Drakeley CJ, Anstey NM, Elyazar IRF, Chen Q, Zommers Z, Bhatt S, Gething PW, Hay AI (2014) Defining the geographical range of the *Plasmodium knowlesi* reservoir. *PloS NTD* 8, e2780.

BACKGROUND: The simian malaria parasite, *Plasmodium knowlesi*, can cause severe and fatal disease in humans yet it is rarely included in routine public health reporting systems for malaria and its geographical range is largely unknown. Because malaria caused by *P. knowlesi* is a truly neglected tropical disease, there are substantial obstacles to defining the geographical extent and risk of this disease. Information is required on the occurrence of human cases in different locations, on which non-human primates host this parasite and on which vectors are able to transmit it to humans. We undertook a systematic review and ranked the existing evidence, at a subnational spatial scale, to investigate the potential geographical range of the parasite reservoir capable of infecting humans. METHODOLOGY/ PRINCIPAL FINDINGS: After reviewing the published literature we identified potential host and vector species and ranked these based on how informative they are for the presence of an infectious parasite reservoir, based on current evidence. We collated spatial data on parasite occurrence and the ranges of the identified host and vector species. The ranked spatial data allowed us to assign an evidence score to 475 subnational areas in 19 countries and we present the results on a map of the Southeast and South Asia region. CONCLUSIONS/SIGNIFICANCE: We have ranked subnational areas within the potential disease range according to evidence for presence of a disease risk to humans, providing geographical evidence to support decisions on prevention, management and prophylaxis. This work also highlights the unknown risk status of large parts of the region. Within this unknown category, our map identifies which areas have most evidence for the potential to support an infectious reservoir and are therefore a priority for further investigation. Furthermore we identify geographical areas where further investigation of putative host and vector species would be highly informative for the region-wide assessment.

14. Elliott I, Mayxay M, Yeuichaixong S, Lee SJ, Newton PN (2014) The practice and clinical implications of tablet splitting in international health. *Trop Med Int Hlth* 19, 754-60.

OBJECTIVE: Tablet splitting is frequently performed to facilitate correct dosing, but the practice and implications in low-income settings have rarely been discussed. METHODS: We selected eight drugs, with narrow therapeutic indices or critical dosages, frequently divided in the Lao PDR (Laos). These were split, by common techniques used in Laos, by four nurses and four laypersons. The mean percentage deviation from the theoretical expected weight and weight loss of divided tablets/capsules were recorded. RESULTS: Five of eight study drugs failed, on splitting, to

meet European Pharmacopoeia recommendations for tablet weight deviation from the expected weight of tablet/capsule halves with 10% deviating by more than 25%. There was a significant difference in splitting accuracy between nurses and laypersons (P = 0.027). Coated and unscored tablets were less accurately split than uncoated (P = 0.03 and 0.0019 for each half) and scored (0.0001 for both halves) tablets. CONCLUSION: These findings have potential clinical implications on treatment outcome and the development of antimicrobial resistance. Investment by drug companies in a wider range of dosage units, particularly for narrow therapeutic index and critical dosage medicines, is strongly recommended.

15. Monge ME, Dwivedi P, Zhou M, Payne M, Harris C, House B, Juggins Y, Cizmarik P, Newton PN, Fernández FM, Jenkins D (2014) A Tiered Analytical Approach for Investigating Poor Quality Emergency Contraceptives. *Plos One* 9, e95353.

Reproductive health has been deleteriously affected by poor quality medicines. Emergency contraceptive pills (ECPs) are an important birth control method that women can use after unprotected coitus for reducing the risk of pregnancy. In response to the detection of poor quality ECPs commercially available in the Peruvian market we developed a tiered multi-platform analytical strategy. In a survey to assess ECP medicine quality in Peru, 7 out of 25 different batches showed inadequate release of levonorgestrel by dissolution testing or improper amounts of active ingredient. One batch was found to contain a wrong active ingredient, with no detectable levonorgestrel. By combining ultrahigh performance liquid chromatography-ion mobility spectrometry-mass spectrometry (UHPLC-IMS-MS) and direct analysis in real time MS (DART-MS) the unknown compound was identified as the antibiotic sulfamethoxazole. Quantitation by UHPLC-triple quadrupole tandem MS (QqQ-MS/MS) indicated that the wrong ingredient was present in the ECP sample at levels which could have significant physiological effects. Further chemical characterization of the poor quality ECP samples included the identification of the excipients by 2D Diffusion-Ordered Nuclear Magnetic Resonance Spectroscopy (DOSY 1H NMR) indicating the presence of lactose and magnesium stearate.

16. Dittrich S, Phommasone K, Anantatat T, Panyanivong P, Slesak G, Blacksell SD, Dubot-Pérès A, Castonguay-Vanier J, Stenos J, Newton PN, Paris DH (2014a) *Rickettsia felis* infections and co-morbidities in Laos. *Emerg Inf Disease* 20, 1402-1403.

[Three Lao patients with PCR evidence for *Rickettsia felis* infection are described (from Vientiane, Luang Nam Tha and Salavan) but all had co-morbidities and the pathogenicity of the bacterium and the vectors responsible for transmission in Asia are questioned]

Lunch !

17. Mayxay M, Khanthavong M, Cox L, Chanthongthip O, Imwong M, Pongvongsa T, Hongvanthong B, Phompida S, Vanisaveth V, White NJ, Newton PN (2014) Thiamin supplementation does not reduce the frequency of adverse events after anti-malarial therapy among patients with falciparum malaria in southern Laos. *Malaria Journal* 13, 275.

BACKGROUND: In a recent study one third of Lao patients presenting with uncomplicated *Plasmodium falciparum* malaria had biochemical evidence of thiamin deficiency, which was associated with a higher incidence of adverse events. Thiamin supplementation might, therefore, reduce adverse events in this population. METHODS: An exploratory, double-blind, parallel group, placebo-controlled, superiority trial of thiamin supplementation in patients of all ages with uncomplicated and severe falciparum malaria was conducted in Xepon District, Savannakhet Province, southern Laos. Patients were randomly assigned to either oral thiamin 10 mg/day for 7 days immediately after standard anti-malarial treatment then 5 mg daily until day 42, or identical oral placebo. RESULTS: After interim analyses when 630 patients (314 in thiamin and 316 in placebo groups) had been recruited, the trial was discontinued on the grounds of futility. On admission biochemical thiamin deficiency (alpha ≥ 25%) was present in 27% of patients and 9% had severe deficiency (alpha > 31%). After 42 days of treatment, the frequency of thiamin deficiency was lower in the thiamin (2%, 1%

severe) compared to the placebo (11%, 3% severe) groups ($p < 0.001$ and $p = 0.05$), respectively. Except for diarrhoea, 7% in the placebo compared to 3% in the thiamin group ($p = 0.04$), and dizziness on day 1 (33% vs 25%, $p = 0.045$), all adverse events were not significantly different between the groups ($p > 0.05$). Clinical, haematological, and parasitological responses to treatment did not differ significantly between the two groups. CONCLUSION: Thiamin supplementation reduced biochemical thiamin deficiency among Lao malaria patients following anti-malarial drug treatment, but it did not reduce the frequency of adverse events after anti-malarial therapy or have any detected clinical or parasitological impact.

18. Newton PN, Tabernero P, Dwivedi, Culzoni MJ, Monge ME, Swamidoss I, Mildenhall D, Green MD, Jähnke R, Santos de Oliveira M, Simao J, White NJ, Fernández FM (2014) Falsified medicines in Africa and public health - 'No Action-Talk Only'. *Lancet Global Health* 2, e509-e510.

[The paper describes the seizure in Luanda, Angola, in June 2012 of falsified medicines labelled as the antimalarial 'Coartem' 'artemetherlumefantrine' bearing the Affordable Medicines Facility–malaria (AMFm) logo and others labelled as the antihelminthic 'mebendazole' 'Vermox®500 mg'. The tablets were analysed by an array of analytical platforms including high performance liquid chromatography, ambient ionization mass spectrometry, Raman spectroscopy, X-ray powder diffraction analysis, nuclear magnetic resonance spectroscopy, isotope-ratio mass spectrometry, botanical assays and packaging analysis, using the portable counterfeit detection device CD-3. No artemether or lumefantrine or other active pharmaceutical ingredients were detected in the 'artemether-lumefantrine' tablets. Brushite and three different yellow dyes and few pollen grains were found. No mebendazole was detected in the 'mebendazole' formulation, but calcite and levamisole (270mg/tablet) were present. Both 'products' showed marked differences in packaging characteristics from genuine products. The discovery of falsified artemether-lumefantrine, labelled as an AMFm product and without any detectable antimalarial, is of considerable concern for malaria control. Presence of levamisole in falsified 'mebendazole' is also of great concern as it has been banned for human use. Enhanced collaboration between African MRAs/police and the authorities in China to stop criminal transcontinental trade in falsified essential medicines is urgently needed. Delays in reporting and action must be reduced by mandatory notification systems and independent public health risk assessments. Despite multiple reports, public health research has failed to stimulate actions required to improve the quality of global drug supply.]

19. Dance D (2014b) Treatment and prophylaxis of melioidosis. *Int J Antimicrob Agents*. 43, 310-8.

Melioidosis, infection with *Burkholderia pseudomallei*, is being recognised with increasing frequency and is probably more common than currently appreciated. Treatment recommendations are based on a series of clinical trials conducted in Thailand over the past 25 years. Treatment is usually divided into two phases: in the first, or acute phase, parenteral drugs are given for ≥10 days with the aim of preventing death from overwhelming sepsis; in the second, or eradication phase, oral drugs are given, usually to complete a total of 20 weeks, with the aim of preventing relapse. Specific treatment for individual patients needs to be tailored according to clinical manifestations and response, and there remain many unanswered questions. Some patients with very mild infections can probably be cured by oral agents alone. Ceftazidime is the mainstay of acute-phase treatment, with carbapenems reserved for severe infections or treatment failures and amoxicillin/clavulanic acid (co-amoxiclav) as second-line therapy. Trimethoprim/sulfamethoxazole (co-trimoxazole) is preferred for the eradication phase, with the alternative of co-amoxiclav. In addition, the best available supportive care is needed, along with drainage of abscesses whenever possible. Treatment for melioidosis is unaffordable for many in endemic areas of the developing world, but the relative costs have reduced over the past decade. Unfortunately there is no likelihood of any new or cheaper options becoming available in the immediate future. Recommendations for prophylaxis following exposure to *B. pseudomallei* have been made, but the evidence suggests that they would probably only delay rather than prevent the development of infection.

20. Ashley EA, Dhorda M, Fairhurst RM, Amaratunga C, Lim P, Suon S, Sreng S, Anderson JM, Mao S, Sam B, Sopha C, Chuor CM, Nguon C, Sovannaroth S, Pukrittayakame S, Jittamala P, Chotivanich K, Chutasmit K, Suchatsoonthorn C, Runcharoen R, Hien TT, Thuy-Nhien NT, Thanh NV, Phu NH, Htut Y, Han KT, Aye

Microbiology Laboratory Football Team - The Chiggers

Planting paddy

KH, Mokuolu OA, Olaosebikan RR, Folaranmi OO, Mayxay M, Khanthavong M, Hongvanthong B, Newton PN, Onyamboko MA, Fanello CI, Tshefu AK, Mishra N, Valecha N, Phyo AP, Nosten F, Yi P, Tripura R, Borrmann S, Bashraheil M, Peshu J, Faiz MA, Ghose A, Hossain MA, Samad R, Rahman MR, Hasan MM, Islam A, Miotto O, Amato R, MacInnis B, Stalker J, Kwiatkowski DP, Bozdech Z, Jeeyapant A, Cheah PY, Sakulthaew T, Chalk J, Intharabut B, Silamut K, Lee SJ, Vihokhern B, Kunasol C, Imwong M, Tarning J, Taylor WJ, Yeung S, Woodrow CJ, Flegg JA, Das D, Smith J, Venkatesan M, Plowe CV, Stepniewska K, Guerin PJ, Dondorp AM, Day NP, White NJ; Tracking Resistance to Artemisinin Collaboration (TRAC) (2014) Spread of artemisinin resistance in *Plasmodium falciparum* malaria. *N Engl J Med*. 371: 411-23.

BACKGROUND: Artemisinin resistance in *Plasmodium falciparum* has emerged in Southeast Asia and now poses a threat to the control and elimination of malaria. Mapping the geographic extent of resistance is essential for planning containment and elimination strategies. METHODS: Between May 2011 and April 2013, we enrolled 1241 adults and children with acute, uncomplicated falciparum malaria in an open-label trial at 15 sites in 10 countries (7 in Asia and 3 in Africa). Patients received artesunate, administered orally at a daily dose of either 2 mg per kilogram of body weight per day or 4 mg per kilogram, for 3 days, followed by a standard 3-day course of artemisinin-based combination therapy. Parasite counts in peripheral-blood samples were measured every 6 hours, and the parasite clearance half-lives were determined. RESULTS: The median parasite clearance half-lives ranged from 1.9 hours in the Democratic Republic of Congo to 7.0 hours at the Thailand-Cambodia border. Slowly clearing infections (parasite clearance half-life >5 hours), strongly associated with single point mutations in the "propeller" region of the *P. falciparum* kelch protein gene on chromosome 13 (kelch13), were detected throughout mainland Southeast Asia from southern Vietnam to central Myanmar. The incidence of pretreatment and post-treatment gametocytemia was higher among patients with slow parasite clearance, suggesting greater potential for transmission. In western Cambodia, where artemisinin-based combination therapies are failing, the 6-day course of antimalarial therapy was associated with a cure rate of 97.7% (95% confidence interval, 90.9 to 99.4) at 42 days. CONCLUSIONS: Artemisinin resistance to *P. falciparum*, which is now prevalent across mainland Southeast Asia, is associated with mutations in kelch13. Prolonged courses of artemisinin-based combination therapies are currently efficacious in areas where standard 3-day treatments are failing.

21. Nguyen VH, Sibounheuang B, Phommasone K, Vongsouvath M, Newton PN, Piorkowski G, Baronti C, de Lamballerie X, Dubot-Pérès A (2014) First Isolation and Genomic Characterisation of Enterovirus A71 and Coxsachievirus A16 from Hand Foot and Mouth Disease

Patients in the Lao PDR. *New Microb New Infect* 2, 63.

Enterovirus A71 (EV-A71) and coxsackievirus A16 (CV-A16) are major aetiological agents of hand, foot and mouth disease in Asia. We established the first genomic characterization of strains isolated in 2011 from Lao patients. Isolates were related to EV-A71 genotype C4 and CV-A16 genotype B1a that circulated in neighbouring countries during the same period. This confirms the regional character of hand, foot and mouth disease epidemiology and makes plausible the occurrence of severe disease in the Lao population.

22. Newton PN (2014) Unregulated fake medicines are threatening the fight against diseases like malaria. *New Statesman* 29th August 2014.

[A report summarizing the policy implications of Newton PN et al. (2014) Falsified medicines in Africa and public health - 'No Action-Talk Only'. *Lancet Global Health* 2, e509-e510]

23. Takala-Harrison S, Jacob CG, Arze C, Cummings MP, Silva JC, Dondorp AM, Fukuda MM, Hien TT, Mayxay M, Noedl H, Nosten F, Kyaw MP, Nhien NTT, Imwong M, Bethell D, Se Y, Lon C, Tyner SD, Saunders DL, Ariey F, Mercereau-Puijalon O, Menard D, Newton PN, Khanthavong M, Hongvanthong B, Starzengruber P, Fuehrer H-P, Swoboda P, Khan WF, Phyo AP, Nyunt MM, Nyunt M, Brown TS, Adams M, Pepin CS, Bailey J, Tan JC, Ferdig MT, Clark TG, Miotto O, MacInnis B, Kwiatkowski DP, White NJ, Ringwald P, Plowe CV (2014) Independent emergence of *Plasmodium falciparum* artemisinin resistance mutations in Southeast Asia. *J Infect Dis* DOI: 10.1093/infdis/jiu491.

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

A road to the house of a patient in the Fever in Pregnancy Cohort Study

revealed both population-specific emergence of mutations and independent emergence of the same mutation in different geographic areas. CONCLUSIONS: K13 appears to be a major determinant of artemisinin resistance throughout Southeast Asia. While we found some evidence of spreading resistance, there was no evidence of resistance moving westward from Cambodia into Myanmar.

24. Day J, Imran D, Ganiem AR, Tjahjani N, Wahyuningsih R, Adawiyah R, Dance D, Mayxay M, Newton PN, Phetsouvanh R, Rattanavong S, Chan AK, Heyderman R, van Oosterhout JJ, Chierakul W, Day N, Kamali A, Kibengo F, Ruzagira E, Gray A, Lalloo DG, Beardsley J, Binh TQ, Chau TT, Chau NV, Cuc NT, Farrar J, Hien TT, Van Kinh N, Merson L, Phuong L, Tho LT, Thuy PT, Thwaites G, Wertheim H, Wolbers M (2014) CryptoDex: A randomised, double-blind, placebo-controlled phase III trial of adjunctive dexamethasone in HIV-infected adults with cryptococcal meningitis: study protocol for a randomised control trial. *Trials* 15(1):441.

BACKGROUND: Cryptococcal meningitis (CM) is a severe AIDS-defining illness with 90-day case mortality as high as 70% in sub-Saharan Africa, despite treatment. It is the leading cause of death in HIV patients in Asia and Africa. No major advance has been made in the treatment of CM since the 1970s. The mainstays of induction therapy are amphotericin B and flucytosine, but these are often poorly available where the disease burden is highest. Adjunctive

The charring of a Ta Oy traditional mask

treatments, such as dexamethasone, have had dramatic effects on mortality in other neurologic infections, but are untested in CM. Given the high death rates in patients receiving current optimal treatment, and the lack of new agents on the horizon, adjuvant treatments, which offer the potential to reduce mortality in CM, should be tested. The principal research question posed by this study is as follows: does adding dexamethasone to standard antifungal therapy for CM reduce mortality? Dexamethasone is a cheap, readily available, and practicable intervention. METHOD: A double-blind placebo-controlled trial with parallel arms in which patients are randomised to receive either dexamethasone or placebo, in addition to local standard of care. The study recruits patients in both Asia and Africa to ensure the relevance of its results to the populations in which the disease burden is highest. The 10-week mortality risk in the control group is expected to be between 30% and 50%, depending on location, and the target hazard ratio of 0.7 corresponds to absolute risk reductions in mortality from 30% to 22%, or from 50% to 38%. Assuming an overall 10-week mortality of at least 30% in our study population, recruitment of 824 patients will be sufficient to observe the expected number of deaths. Allowing for some loss to follow-up, the total sample size for this study is 880 patients. To generate robust evidence across both continents, we aim to recruit roughly similar numbers of patients from each continent. The primary end point is 10-week mortality. Ethical approval has been obtained from Oxford University's Tropical Research Ethics Committee (OxTREC), and as locally mandated at each site.

25. Kyaw SS, Drake T, Ruangveerayuth R, Chierakul W, White NJ, Newton PN, Lubell Y (2014) Cost of treating inpatient falciparum malaria on the Thai-Myanmar border. *Malar J,* 13(1):416.

BACKGROUND: Despite demonstrated benefits and World Health Organization (WHO) endorsement, parenteral artesunate is the recommended treatment for patients with severe *Plasmodium falciparum* malaria in only one fifth of endemic countries. One possible reason for this slow uptake is that a treatment course of parenteral artesunate is costlier than quinine and might, therefore, pose a substantial economic burden to health care systems. This analysis presents a detailed account of the resources used in treating falciparum malaria by either parenteral artesunate or quinine in a hospital on the Thai-Myanmar border. METHODS: The analysis used data from four studies, with random allocation of inpatients with falciparum malaria to treatment with parenteral artesunate or quinine, conducted in Mae Sot Hospital, Thailand from 1995 to 2001. Detailed resource use data were collected during admission and unit costs from the 2008 hospital price list were applied to these. Total admission costs were broken down into five categories: 1) medication; 2) intravenous fluids; 3) disposables; 4) laboratory tests; and 5) services. RESULTS: While the medication costs were higher for patients treated with artesunate, total admission costs were similar in those treated with quinine, US$ 243 (95% CI: 167.5-349.7) and in those treated with artesunate US$ 190 (95% CI: 131.0-263.2) (P=0.375). For cases classified as severe malaria (59%), the total cost of admission was US$ 298 (95% CI: 203.6-438.7) in the quinine group as compared with US$ 284 (95% CI: 181.3-407) in the artesunate group (P=0.869). CONCLUSION: This analysis finds no evidence for a difference in total admission costs for malaria inpatients treated with artesunate as compared with quinine. Assuming this is generalizable to other settings, the higher cost of a course of artesunate should not be considered a barrier for its implementation in the treatment of malaria.

26. Mirabel M, Rattanavong S, Frichitthavong K, Chu V, Kesone P, Thongsith P, Jouven X, Fournier P-E, Dance DAB, Newton PN (2014) Infective endocarditis in the Lao PDR.: clinical characteristics and outcomes in a developing country. *International Journal of Cardiology* 180, 270-273.

INTRODUCTION: Data on infective endocarditis (IE) in Southeast Asia are scarce. OBJECTIVES: To describe the clinical epidemiology of IE in Lao PDR, a lower middle-income country. METHODS: A single centre retrospective study at Mahosot Hospital, Vientiane. Patients aged over 1 year of age admitted 2006-2012 to Mahosot Hospital with definite or possible IE by modified Duke criteria were included. RESULTS: Thirty-six patients fulfilled the inclusion criteria; 33 (91.7%) had left-sided IE. Eleven (30.6%) had definite IE and 25 (69.4%) possible left-sided IE. Median age was 25 years old [IQR 18-42]. Fifteen patients (41.7%) were males. Underlying heart diseases included: rheumatic valve disease in 12 (33.3%), congenital heart disease in 7 (19.4%), degenerative valve disease in 3 (8.3%), and of unknown origin in 14 (38.9%) patients. Native valve IE was present in 30 patients (83.3%), and prosthetic valve IE in 6 patients (16.7%). The most frequent pathogens were *Streptococcus* spp. in 7 (19.4%).

Blood cultures were negative in 22 patients (61.1%). Complications included: heart failure in 11 (30.6%), severe valve regurgitation in 7 (19.4%); neurological event in 7 (19.4%); septic shock or severe sepsis in 5 (13.9%); and cardiogenic shock in 3 patients (8.3%). No patient underwent heart surgery. Fourteen (38.9%) had died by follow-up after a median of 2.1 years [IQR 1-3.2]; and 3 (8.3%) were lost to follow-up. CONCLUSIONS: Infective endocarditis, a disease especially of young adults and mainly caused by *Streptococcus* spp., was associated with rheumatic heart disease and had high mortality in Laos.

27. Ranieri N, Tabernero P, Green MD, Verbois L, Herrington J, Sampson E, Satzger R, Phonlavong C, Thao K Newton PN, Witkowski M (2014) Evaluation of a new hand held instrument for the detection of counterfeit artesunate by visual fluorescence comparison. *American Journal of Tropical Medicine & Hygiene* 91, 920-4.

There is an urgent need for accurate and inexpensive handheld instruments for the evaluation of medicine quality in the field. A blinded evaluation of the diagnostic accuracy of the Counterfeit Detection Device 3 (CD-3), developed by the US Food and Drug Administration Forensic Chemistry Center, was conducted in the Lao People's Democratic Republic. Two hundred three samples of the oral antimalarial artesunate were compared with authentic products using the CD-3 by a trainer and two trainees. The specificity (95% confidence interval [95% CI]), sensitivity (95% CI), positive predictive value (95% CI), and negative predictive value (95% CI) of the CD-3 for detecting counterfeit (falsified) artesunate were 100% (93.8-100%), 98.4% (93.8-99.7%), 100% (96.2-100%), and 97.4% (90.2-99.6%), respectively. Interobserver agreement for 203 samples of artesunate was 100%. The CD-3 holds promise as a relatively inexpensive and easy to use instrument for field evaluation of medicines, potentially empowering drug inspectors, customs agents, and pharmacists.

28. Rattanavong S, Fournier P-E, Chu V, Frichitthavong K, Kesone P, Mayxay M, Mirabel M, Newton PN (2014) *Bartonella henselae* endocarditis in Laos - 'the unsought will go undetected. *PloS NTD* 8, e3385.

BACKGROUND: Both endocarditis and *Bartonella* infections are neglected public health problems, especially in rural Asia. *Bartonella* endocarditis has been described from wealthier countries in Asia, Japan, Korea, Thailand and India but there are no reports from poorer countries, such as the Lao PDR (Laos), probably because people have neglected to look. METHODOLOGY/PRINCIPAL FINDINGS: We conducted a retrospective (2006-2012), and subsequent prospective study (2012-2013), at Mahosot Hospital, Vientiane, Laos, through liaison between the microbiology laboratory and the wards. Patients aged >1 year

The bleeding of a goat

admitted with definite or possible endocarditis according to modified Duke criteria were included. In view of the strong suspicion of infective endocarditis, acute and convalescent sera from 30 patients with culture negative endocarditis were tested for antibodies to *Brucella melitensis, Mycoplasma pneumoniae, Bartonella quintana, B. henselae, Coxiella burnetii* and *Legionella pneumophila*. Western blot analysis using *Bartonella* species antigens enabled us to describe the first two Lao patients with known *Bartonella henselae* endocarditis. CONCLUSIONS/SIGNIFICANCE: We argue that it is likely that *Bartonella* endocarditis is neglected and more widespread than appreciated, as there are few laboratories in Asia able to make the diagnosis. Considering the high prevalence of rheumatic heart disease in Asia, there is remarkably little evidence on the bacterial etiology of endocarditis. Most evidence is derived from wealthy countries and investigation of the aetiology and optimal management of endocarditis in low income countries has been neglected. Interest in *Bartonella* as neglected pathogens is emerging, and improved methods for the rapid diagnosis of *Bartonella* endocarditis are needed, as it is likely that proven *Bartonella* endocarditis can be treated with simpler and less expensive regimens than "conventional" endocarditis and multicenter trials to optimize treatment are required. More understanding is needed on the risk factors for *Bartonella* endocarditis and the importance of vectors and vector control.

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

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

Data Entry - Mr Lianexay Saisomsaard, Dr Phouthasone Kouaykesone, Dr Phonevilay Thongsith, Mr Chanthachone Khamsy & Mr Somphanh Sidavong

30. Dance DA (2014) Melioidosis in Puerto Rico: The Iceberg Slowly Emerges. Clin Infect Dis. Sep 30.

[A commentary on a paper describing meliodiosis in Puerto Rico]

31. Dance DAB. Melioidosis. In: Manson's Tropical Diseases, Twenty third edition. Chapter 36, pp. Elsevier 2014.

[A review of the epidemiological, clinical and microbiological aspects of melioidosis]

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

Objectives: To assess antibiotic (AB) prescribing of medical doctors in Lao PDR through a knowledge, attitude and practice survey. Methods: Questionnaires were distributed among 463 medical doctors in 25 public hospitals in four provinces in Lao PDR. Findings: Response rate was 83.4% (n=386); 64.7% participants prescribed AB at least once weekly. Participants considered AB resistance (ABR) less of a problem in their own practice as compared to world- and nationwide (61.9% versus 75.2% and 83.7%, $p < 0.001$). A total of 59.8%(n =270) participants declared not to have enough information about AB; information sources included national guidelines, pharmaceutical companies and internet (86.5%, 76.9% and 73.9%, respectively). Knowledge about AB prescribing was poor (mean score of 5.9 ± 1.3, range 2 to 10); only 14.0% recognized cephalosporin cross-resistance in methicillin-resistant-*Staphylococcus aureus*. Most participants had no information about local ABR for Salmonella Typhi (54.8%) and hospital-acquired pneumonia (65.9%). Unnecessary AB prescriptions were considered as harmless by 29.8% participants and 38.3% considered locally available AB to be of poor quality. Generic ABs were perceived by 34.4% and 5.6% of participants as substandard and counterfeit respectively. ABs were prescribed by International Nonproprietary Name as frequently as by brand name (55.2% versus 44.8%, of participants respectively). Nearly three-quarts (72.5%) of participants agreed that it was difficult to select the correct AB. The majority (96.6%) welcomed educational programs on AB prescribing and 65.0% preferred local over international AB guidelines. Conclusion: The present KAP-survey highlights preparedness of medical doctors in Lao PDR for AB prescribing interventions.

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

Background. Scrub typhus, murine typhus and leptospirosis are common causes of febrile illness in Asia, and meningitis and meningoencephalitis are recognised severe complications. However, limited data exist on the burden of these pathogens in patients with central nervous system disease in endemic countries. Methods. Between 2003-2011, this prospective study enrolled 1,112 consecutive patients admitted with CNS symptoms/signs requiring a lumbar puncture, to a single hospital in Vientiane, the capital of Laos. Microbiological examinations (culture/PCR/serology) targeted 'conventional' bacterial infections (*Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae*, *S. suis*) and *Orientia tsutsugamushi*, *Rickettsia typhi*/*Rickettsia* spp. and *Leptospira* spp. infections in blood and/or cerebrospinal fluid (CSF). Aetiologies, clinical and CSF characteristics were analysed and compared between patient groups. **Findings**. Among 1,051 (95%) patients with available CSF, 254 (24·2%) had a CNS infection attributable to a bacterial or fungal pathogen and 90/254 (35·4%) of those were caused by *O. tsutsugamushi*, *R. typhi*/*Rickettsia* spp. or *Leptospira* spp. These pathogens were found significantly more frequently than 'conventional' bacterial infections (90/1,051, 9% versus 42/1,051, 4%; p<0·0001) using conservative diagnostic definitions. CNS infections had a high mortality (236/876, 27%), with 18% (13/71) for *R. typhi*/*Rickettsia* spp., *O. tsutsugamushi* and *Leptospira* spp. combined, and 33% (13/39) for 'conventional' bacterial infections (P=0·076).
Interpretation. These data suggest that *R. typhi*/*Rickettsia* spp., *O. tsutsugamushi* and *Leptospira* spp. infections are important causes of CNS infections in Laos. Antibiotics, such as tetracyclines, required for the treatment of murine typhus and scrub typhus, are not routinely advised for empirical treatment of CNS infections. These severely neglected infections require more attention, as they represent a potentially large proportion of treatable CNS disease burden across vast endemic areas.

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

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

35. Newton PN, Schellenberg D, Ashley EA, Ravinetto R, Green MD, ter Kuile FO, Tabernero P, White NJ, Guerin PJ (in press) The quality assurance of medicines used in clinical trials: Proposal for adaptation of the CONSORT guidelines. *BMJ*

[The CONSORT guidelines have been widely adopted to guide the design, conduct and reporting of clinical trials and have been very influential in improving practice and ensuring that trial results appropriately guide public policy. Although substandard and falsified medicines are an enormous but neglected public health problem, particularly in the developing world, clinical trials have been considered immune from the problem. This is not so. We give examples from clinical research, including the degradation of vitamin A capsules, poor quality sulfadoxine-pyrimethamine for a study of malaria in pregnancy, falsified clopidogrel shipped for use as a comparator in a clinical trial and important differences in bioequivalence between products. We propose that the CONSORT guidelines on trial reports should include a requirement to determine and state the quality of medicines, as a measure to correct this under-recognized and neglected critical weak link in trials. This proposed change will require development of infrastructure and accessible analytical capacity but enormous investment in trials will be wasted and their interpretation into public policy incorrect if the quality of medicines and medical devices used is not assured.]

36. Yong YL, Plançon A, Lau YH, Hostetler DM, Fernández FM, Green MD, Sounvoravong S, Nara S, Boravann M, Dumrong T, Bangsawan N, Tuc VV, Low MY, Lim C-C, Choo RLA, Newton PN (in press) Collaborative health and enforcement operations on the quality of antimalarials and antibiotics in SE Asia. *Am J Trop Med Hyg*

Counterfeit (or falsified) and substandard medicines pose a major public health risk. We describe the findings of Operation Storm I & II conducted in 2008/2009 to combat counterfeit medicines through partnership between national customs, Drug Regulatory Agencies (DRAs) and police in Cambodia, Indonesia, Laos, Myanmar, Singapore, Thailand and Vietnam. Samples were obtained from seizures and market surveillance by national DRAs. Laboratory analysis using spectroscopic and chromatographic techniques and examination of packaging were performed.

Ninety-three antibiotic and 95 antimalarial suspect samples were collected. Of the antibiotics, twenty-nine (31%) had % active pharmaceutical ingredient content (%API) <85% or >115% (including one counterfeit). Of the 95 antimalarials, 30 (32%) had %API <85 >115% API (including one counterfeit). A significant minority of samples, antimalarials (13%) and antibiotics (15%), were collected in plastic bags with minimal or no labeling. Of 20 ampicillin samples, 13 (65%) contained <85%API (with one counterfeit containing additional amoxicillin). Of 34 oral artesunate samples, 7 (21%) contained %API out of the

The Microbiology Laboratory Sil-la-pa Team

85-115% range. Coordinated and synergistic partnership adopted by the participating countries, INTERPOL, WHO and laboratories facilitated a platform for discussions and intelligence sharing, helping to improve each participating country's capacity to combat poor quality medicines.

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

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

38. Keita AK, Dubot-Pérès A, Phommasone K, Sibounheuang B, Vongsouvath M, Mayxay M, Raoult D, Newton PN, Fenollar F (in press) High prevalence of *Tropheryma whipplei* carriage in Lao kindergarten children. *PLoS NTD*

Tropheryma whipplei is a bacterium commonly carried in feces of young children in Africa but with no data from Asia. Using specific PCR, we estimated the prevalence of *T. whipplei* carriage in 113 feces from 106 children in Vientiane, the Lao PDR (Laos). *T. whipplei* was detected in 48% (51/106) of children. Those aged ≤4 years were significantly less frequently positive (17/52, 33%) than older children (34/54, 63%; $p< 0.001$). Positive samples were genotyped. Eight genotypes were detected including 7 specific to Laos. Genotype 2, previously detected in Europe, was circulating (21% of positive children) in 2 kindergartens (Chomphet and Akad). Genotypes 136 and 138 were specific to Chomphet (21% and 15.8%, respectively) whereas genotype 139 was specific to Akad (10.55%). *T. whipplei* is a widely distributed bacterium, highly prevalent in feces of healthy children in Vientiane.

Further research is needed to identify the public health significance of this finding.

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

Synopsis. Objectives: Intestinal carriage constitutes an important reservoir of antimicrobial-resistant bacteria, with some of the highest rates reported from Asia. Antibiotic resistance has been little studied in Laos, where some antibiotics are available without restriction but others, such as carbapenems, are not available. Patients and methods: We collected stools from 397 healthy children in 12 randomly selected pre-school childcare facilities in and around Vientiane. Colonisation with ESBL-producing Enterobacteriaceae (ESBLE) and carbapenemase-producing Enterobacteriaceae (CPE) were detected using a disk diffusion screening test and ESBLE were characterised using whole genome sequencing (WGS). Risk factor data were collected by questionnaire. Results: Ninety-two children (23%) were colonised with ESBLE, mainly *Escherichia coli* carrying $bla_{\text{CTX-M}}$ and *Klebsiella pneumoniae* carrying bla_{SHV} or $bla_{\text{CTX-M}}$, which were frequently resistant to multiple antibiotic classes. Although residence in Vientiane Capital, foreign travel, maternal level of education, antibiotic use in the preceding three months and attending a childcare facility with a "good" level of hygiene were all associated with ESBLE colonisation on univariable analysis, only antibiotic use in the previous three months was an independent predictor of ESBL colonisation in a random effects model stratified by childcare facility. WGS analysis suggested transmission in both childcare facilities and community settings. Conclusions: The high prevalence of paediatric colonisation with ESBLE in Laos, one of the highest reported in Asia, is probably the result of inappropriate antibiotic use. Paediatric colonisation with CPE was not identified in this study, but it is important to continue to monitor the spread of antibiotic resistant Enterobacteriaceae in Laos.