ກະຊວງ ສາທາລະນະສຸກ
Ministry of Health

ສະຖາບັນ ປັດສະເຕີ ລາວ
INSTITUT PASTEUR DU LAOS

Institut Pasteur du Laos

Activity Report 2019-2020

Réseau International des Instituts Pasteur

Summary

Mandate

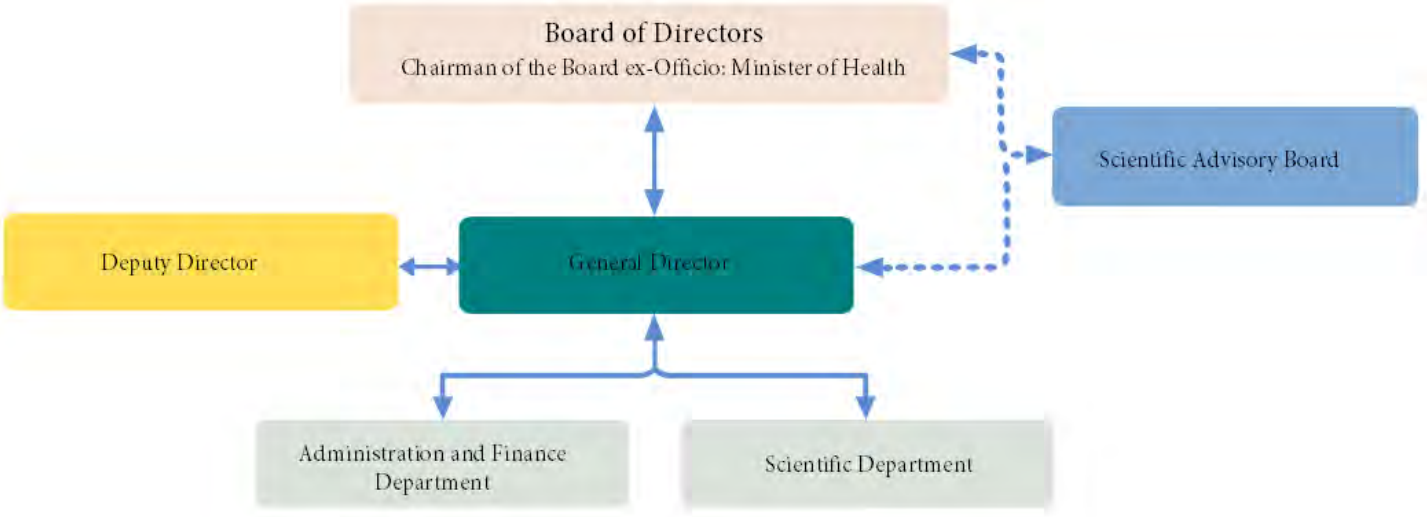
Institut Pasteur du Laos (IPL) is a Lao National Institution created by Prime Ministerial Decree in November 2007 IPL is the result of a long term and joint decision between Lao Ministry of Health and Institut Pasteur Paris which commits to stay 16 years before retroceding the full management of IPL. Sustainability will be achieved by preparing a new generation of Lao doctors and scientists to fill key positions as heads of laboratories and administration at IPL.

IPL has a mandate from Lao Ministry of Health to fulfil activities of public service :
1. Research and diagnostics on emerging infectious diseases and vector borne diseases.
2. Training, education and capacity building.
3. Technical assistance to National Center for Laboratory and Epidemiology (NCLE) for investigation of epidemics.

IPL benefits from a large degree of autonomy (legal, scientific, management, financial) and as such can be considered as a new model of Lao public institution. All the ownership belongs and remains the property of Lao PDR.

IPL has a scientific autonomy within its mandate provided by the MoH. It is able to engage freely in collaborative research and investigations with other Lao and international research and public health organisations.
Financial issues are independent from the Lao public finance system. IPL is able to receive outside funding (donations, grants, bequeaths, etc.) and to generate its own resources through its own discoveries to insure its sustainability.

Main Organigram



IPL is governed by a Board of Directors composed of 3 Lao Members appointed by the Lao Ministry of Health and 2 members appointed by IP Paris. A specificity of the Board meetings is the participation of the main contributors and stakeholders as observers in the spirit of transparency and partnership.

Actual composition of the Board of Directors:

Ass. Pr. Dr. Bounkong SYHAVONG, (Chairman), Minister of Health, Lao PDR.
Dr. Ponmek DALALOY, (Honorary Chairman of the Board), Former Minister of Health, Lao PDR.
Dr. Manivanh SOUPHANTHONG, Former Dean of University of Health Sciences, Lao PDR.
Pr. Dr. Didier SICARD, Honorary President of the National Ethic Committee of France, France.
Dr. Marc JOUAN, International Director of the Institut Pasteur, France.

Actual composition of Scientific Advisory Board:

Prof. Felix REY, Institut Pasteur, Paris.
Prof. Olivier LORTHOLARY, Necker Hospital, Paris.
Dr. Rabindra R ABEYSINGHE, WHO, Manila

Letter From

Dr. Paul BREY

Director

We have witnessed in 2019 one of the most devastating Dengue epidemics in Laos over the past decades. With indicators such as high dengue viral inoculum in the general population, low herd immunity and a very robust *Aedes aegypti / Aedes albopictus* adult and larval populations through out Vientiane Capital, IPL predicted a major Dengue epidemic for 2019 already in November of 2018. This information was shared with the Lao Ministry of Health at the National Dengue Meeting. The Ministry recognized this risk, but to conduct a major vector control campaign during the dry season when vector populations are very low is difficult to motivate and implement a vector control campaign as there is a general sentiment that the situation is not urgent enough so why carry out such a campaign when there are only a few cases of Dengue and vector mosquitoes are few. The problem is that the vector populations are too important to control once the rains have started. It is a bit like the analogy of a forest fire. If the fire is control when it is small it can be managed if you let the fire get out of control it is impossible to stop the spread. It may seem counter-intuitive to initiate control program during the dry season, but this is precisely the moment we should attack the vector when its populations are low. The dry season is the bottleneck for the next rainy season's mosquito vector population. If we can reduce the mosquito vector populations during the dry season it makes it difficult for the vector mosquitoes to expand their populations effectively once the rain season returns. Hence, effective dry season mosquito vector control could reduce the vector populations enough to slow down the spread of Dengue once the rains return. Laos is not the only country in the region that saw record-breaking number of Dengue cases; Philippines, Bangladesh, Thailand Cambodia and Vietnam also suffered a similar scourge. The World Health Organization (WHO) and expert medical entomologists need to work closely with public health authorities to push for the implementation of "dry season "vector control programs.

2019 also saw the re-emergence of Ebola virus in the Democratic Republic of Congo with some case being reported in the bordering countries. The seriousness of the re-emergence prompted the WHO to declare on 24 July 2019 a public health emergency where all countries must be on alert for imported Ebola cases. Hence, on 29 July 2019 IPL received an official mandate letter N° 916 from the Lao Ministry of Health to take on the responsibility of being the front line laboratory for Ebola diagnostics and differential diagnostics for hemorrhagic fever viruses. IPL immediately put into place its protocols for Level 4 putative hemorrhagic fever viruses sample management and diagnostics.

IPL also continued it work on vaccine preventable diseases providing the Lao Ministry of Health with evidenced based results on the Hepatitis B vaccine protection, Pentavalent vaccine efficacy and preliminary results on the Human Papilloma Virus (HPV) vaccine immunogenicity and protection in adolescent school girls in Lao PDR. Such evidence-based results are provided to the Lao Ministry of Health and WHO as policy briefs to improved the public health policy according to results.

On the Parasitology front, the SATREPS project supported by the Japanese government, which focused on Malaria, Opisthorciasis and Schistosomiasis came to an end in May 2019, but additional funds from Japanese industry have been secured to continue our work on Malaria and Malaria parasite drug resistance.

Another highlight in 2019 is the IPL's Board of Directors officially established IPL's own foundation *"Fondation Institut Pasteur du Laos"* under the auspices of the *Fondation* de France in Paris. This foundation was created as a financial tool to promote the sustainability of IPL in the future. IPL Fondation can receive donations from outside sources and the financial interest generated by the foundation will then be sent exclusively to IPL to use for IPL Lao scientist/technician salary support, scientific projects, equipment etc. An independent governance board, separate from the IPL executive, manages the IPL Fondation funds. We are now actively searching for contributes big and small to help meet our goal of 5 Million USD by 2024 (www.pasteur.la).

Finally in the educational tradition of Institut Pasteur and the IP International Network, IPL and Institut Pasteur in Paris co-organized the International Medical Entomology course 10-22 June 2019 hosted at IPL. This course included lectures from leading specialists from France, Europe and Southeast Asia, as well as hands-on laboratory practical courses in the dedicated training labs and medically important arthropod collection at IPL. The course finished with a three-day medical entomology field excursion, allowing the students to put what they learned into practice. Fourteen selected students from Laos, France, Cambodia, Myanmar, New Caledonia, Vietnam, Tunisia, Slovenia, Iran and the Kingdom of Bhutan joined the course.

Paul Brey

ສາມຈາກ
ດຣ. ໂປມ ເບຼ
ຜູ້ອຳນວຍການ

ໃນປີ 2019, ພວກເຮົາໄດ້ເຫັນວ່າ ພະຍາດໄຂ້ຍຸງລາຍ ເປັນໜຶ່ງພະຍາດ ລະບາດທີ່ຮ້າຍແຮງທີ່ສຸດ ຢູ່ປະເທດລາວ ຕະຫຼອດທົດສະວັດທີ່ຜ່ານມາ. ດ້ວຍໂຕຊີ້ວັດໃຫ້ເຫັນ ເຊັ່ນ: ເຊື້ອໄວຣັສໄຂ້ຍຸງລາຍສູງໃນຄົນທົ່ວໄປ, ເຮັດໃຫ້ພູ້ມຄຸ້ມກັນຕ່ຳ ແລະ ຄວາມແຂງແຮງຂອງຍຸງ *Aedes aegypti / Aedes albopictus* ໂຕແກ່ ແລະ ປະຊາກອນໝອນນ້ຳຍຸງລາຍ ທົ່ວນະຄອນຫຼວງວຽງຈັນ. ສະຖາບັນ ປັດສະເຕີ ລາວໄດ້ຄາດຄະເນການ ເກີດການລະບາດຂອງພະຍາດໄຂ້ຍຸງລາຍໃນປີ2019 ໄດ້ມີມາແຕ່ປີ2018. ຂໍ້ມູນນີ້ ໄດ້ຖືກເຜີຍແຜ່ຕ່ຳກະຊວງສາທາລະນະສຸກລາວ ໃນກອງປະຊຸມ ພະຍາດໄຂ້ຍຸງລາຍແຫ່ງຊາດ. ທ່ານລັດຖະມົນຕີກະຊວງສາທາລະນະສຸກ ໄດ້ຮັບຮູ້ຄວາມສ່ຽງດັ່ງກ່າວ. ແຕ່ການດຳເນີນການ ປຸກລະດົມການຄວບຄຸມ ພາຫະນຳເຊື້ອໃນລະດູແລ້ງ ໃນໄລຍະທີ່ພາຫະນຳເຊື້ອມີໜ້ອຍ ມີຄວາມ ຍຸ້ງຍາກທີ່ຈະເຮັດເນື່ອງຈາກມີຄວາມຮູ້ສຶກທົ່ວໄປວ່າສະພາບການຍັງ ບໍ່ຮີບດ່ວນ ເປັນຫຍັງຈຶ່ງຕ້ອງເຮັດ ແລະ ຄົນເຈັບກໍລະນີໄຂ້ຍຸງລາຍມີຈຳ ນວນໜ້ອຍ ແລະ ຍຸງທີ່ເປັນພາຫະນຳເຊື້ອກໍມີໜ້ອຍ. ບັນຫາກໍ່ຄືປະຊາກອນ ພາຫະນຳເຊື້ອເພີ່ມຂຶ້ນ ເກີນທີ່ຈະຄວບຄຸມໄດ້ ເມື່ອຝົນເລີ່ມຕົກເທື່ອທຳອິດ. ປຽບທຽບມັນຄືກັນກັບໄຟໄໝ້ປ່າ ຖ້າຄວບຄຸມໄຟໄດ້ ໃນໄລຍະໃດໜຶ່ງ ກໍຈະສາມາດຄວບຄຸມອັກຄີໄພໄດ້. ຖ້າຫາກທ່ານປ່ອຍໃຫ້ໄຟລາມອອກ ຈາກການຄວບຄຸມ ມັນກໍເປັນໄປບໍ່ໄດ້ທີ່ຈະຍຸດການແຜ່ລາມໄດ້. ການເລີ່ມ ຄວບຄຸມພາຫະນຳເຊື້ອໃນລະດູແລ້ງ ເຊິ່ງເປັນໄລຍະທີ່ດີ ທີ່ພວກເຮົາຄວນ ໂຈມຕີພາຫະນຳເຊື້ອໃນຕອນທີ່ມັນມີບ່ໜ້ອຍ. ໃນລະດູແລ້ງ ພາຫະນຳເຊື້ອ ພະຍາດໄຂ້ຍຸງລາຍມີຈຳກັດ ມັນຈະບໍ່ຂະຫຍາຍໃນລະດູຝົນຕໍ່ໄປ ຖ້າພວກ ເຮົາສາມາດກຳຈັດຍຸງທີ່ເປັນ ພາຫະນຳເຊື້ອໄຂ້ຍຸງລາຍໄດ້ໃນລະດູແລ້ງຈະ ເຮັດໃຫ້ຍຸງລາຍບໍ່ສາມາດຂະຫຍາຍໄດ້ ເພື່ອລຸດຜ່ອນການແຜ່ກະຈາຍຂອງ ໄຂ້ຍຸງລາຍໃນລະດູຝົນທີ່ຈະມາເຖິງ. ປະເທດລາວບໍ່ແມ່ນປະເທດດຽວທີ່ ຕິດອັນດັບສູງສຸດ ກໍລະນີພະຍາດໄຂ້ຍຸງລາຍ; ປະເທດ ຟີລິບປິນ, ບັງກາ ລາເທດ, ໄທ, ກຳປູເຈຍ ແລະ ຫວຽດນາມ ກໍ່ໄດ້ຮັບຄວາມເຈັບປວດ ແລະ ໄພອັນຍິ່ງໃຫຍ່ຈາກພະຍາດໄຂ້ຍຸງລາຍເຊັ່ນດຽວກັນ. ອົງການອະນາໄມໂລກ ແລະ ຊ່ຽວຊານດ້ານແມງໄມ້ຈຳຕ້ອງໄດ້ເຮັດວຽກ ແລະ ປະສານງານຢ່າງ ໃກ້ສິດຕິດແໜດກັບເຈົ້າໜ້າທີ ສາທາລະນະສຸກເພື່ອປະຕິບັດແຜນການ ຄວບຄຸມໃຫ້ມີຜົນສັກສິດ ແລະ ມີປະສິດທິຜົນໃນການຄວບຄຸມພາຫະນຳ ເຊື້ອໃນລະດູແລ້ງ.

ໃນປີ 2019 ພະຍາດໄວຣັສອີໂບລາໄດ້ກັບ ຄືນມາອີກເຊັ່ນດຽວກັນ ໃນສາທາລະນະລັດ ປະຊາທິປະໄຕ ກົງໂກ. ບາງກໍລະນີໄດ້ຖືກລາຍ ງານໃນ ບັນດາປະເທດທີ່ມີຊາຍແດນຕິດກັບປະເທດກົງໂກ. ຄວາມ ຮຸນແຮງຂອງການເກີດຄັ້ງໃໝ່ໄດ້ກະຕຸກຊຸກຍູ້ໃຫ້ໃຫ້ອົງການອະນາ ໄມໂລກໄດ້ປະກາດພາວະສຸກເສີນໃນວັນທີ 24 ກໍລະກົດ 2019 ເພື່ອສັນຍານລະວັງໄພກ່ຽວກັບສຸຂະພາບຂອງປະຊາຊົນໃຫ້ທຸກປະ ເທດທີ່ມີກໍລະນີ ອີໂບລາ ເຂົ້າມາ. ດັ່ງນັ້ນໃນວັນທີ 29 ກໍລະກົດ 2019 ທາງສະຖາບັນ ປັດສະເຕີ ລາວໄດ້ຮັບໜັງສືແຈ້ງ ການສະບັບເລກທີ 916 ຂອງກະຊວງສາທາລະນະສຸກ ເພື່ອຮັບຜິດ ຊອບເປັນຫ້ອງ ວິເຄາະເພື່ອບົ່ງມະຕິພະຍາດໄວຣັສອີໂບລາ ແລະ

ແລະ ປົ່ງມະຕິຄວາມແຕກຕ່າງ ຂອງໄວຣັສໄຂ້ເລືອດອອກສະຖາບັນ ປັດສະເຕີ ລາວໄດ້ກຽມພ້ອມຮັບຕົວຢ່າງ ແລະ ປົ່ງມະຕິໄວຣັສ ໄຂ້ເລືອດອອກຂັ້ນ 4.

ສະຖາບັນ ປັດສະເຕີ ລາວ ຍັງສືບຕໍ່ວຽກງານຂອງຕົນກ່ຽວກັບພະຍາດທີ່ປ້ອງກັນດ້ວຍວັກຊີນ ໄດ້ມອບໃຫ້ກະຊວງສາທາລະນະສຸກ ເຊັ່ນ: ຜົນທີ່ໄດ້ຮັບເບື້ອງຕົ້ນດ້ານການປ້ອງກັນ ດ້ວຍການສັກວັກຊີນປ້ອງກັນພະຍາດຕັບອັກເສບບີ, ປະສິດຕິພາບຂອງວັກຊີນ Pentavalent ແລະ ຜົນທີ່ໄດ້ຮັບເບື້ອງຕົ້ນກ່ຽວກັບວັກຊີນປ້ອງກັນມະເຮັງປາກມົດລູກ (HPV) ແລະ ການສັກວັກຊີນປ້ອງກັນໃນໄວໜຸ່ມຍິງໃນໂຮງຮຽນຢູ່ ສປປ ລາວ. ຜົນໄດ້ຮັບເບື້ອງຕົ້ນດັ່ງກ່າວ ໄດ້ມອບໃຫ້ກະຊວງສາທາລະນະສຸກ ແລະ ອົງການອະນາໄມໂລກ ເພື່ອປັບປຸງນະໂຍບາຍທາງດ້ານສຸຂະພາບຂອງປະຊາຊົນໃຫ້ດີຂຶ້ນ.

ກ່ຽວກັບຂະແໜງການຝ່າກວິທະຍາ, ໂຄງການ SATREPS ເຊິ່ງໄດ້ຮັບການສະໜັບສະໜູນຈາກລັດຖະບານຍີ່ປຸ່ນ ໄດ້ເນັ້ນເຈາະຈົງໃສ່ພະຍາດໄຂ້ມາລາເຣຍ, ພະຍາດໃບໄມ້ໃນຕັບ ແລະ ພະຍາດໃບໄມ້ໃນເລືອດໄດ້ສິ້ນສຸດລົງໃນເດືອນພຶດສະພາ 2019 ແຕ່ວ່າຍັງໄດ້ຮັບທຶນເພີ່ມເຕີມຈາກຍີ່ປຸ່ນເພື່ອສືບຕໍ່ວຽກງານພະຍາດມາລາເຣຍ ແລະ ການດື້ຢາຂອງພະຍາດຫາຝ່າກມາລາເຣຍ.

ອີກວຽກໜຶ່ງທີ່ພົ້ນດັ່ນໃນປີ 2019 ກໍ່ແມ່ນກອງປະຊຸມສະພາບໍລິຫານຂອງສະຖາບັນ ປັດສະເຕີ ລາວ ທີ່ໄດ້ສ້າງຕັ້ງມູນນິທິຂອງຕົນເອງ ມູນນິທິສະຖາບັນ ປັດສະເຕີ ລາວ ພາຍໃຕ້ການອຸປະຖໍາຈາກ ມູນນິທິສະຖາບັນ ປັດສະເຕີ ປາຣີ. ມູນນິທິນີ້ໄດ້ສ້າງຕັ້ງຂຶ້ນ ເພື່ອເປັນເຄື່ອງມືດ້ານການເງິນ ເພື່ອນໍາໄປສູ່ການພັດທະນາຄວາມຍືນຍົງຂອງ ສະຖາບັນ ປັດສະເຕີ ລາວ ໃນອານາຄົດ. ມູນນິທິສະຖາບັນປັດສະເຕີ ລາວ ສາມາດຮັບເງິນບໍລິຈາກ ຈາກພາຍນອກ ເງິນທີ່ໄດ້ຈາກມູນນິທິ ຈະຖືກສົ່ງມາໃຫ້ສະຖາບັນ ປັດສະເຕີ ລາວ ເພື່ອຈະນໍາໄປໃຊ້ເປັນ ເງິນເດືອນນັກວິທະຍາສາດ, ວິຊາການ ໃນສະຖາບັນ ປັດສະເຕີ ລາວ ໂຄງການວິທະຍາສາດ. ອຸປະກອນຮັບໃຊ້ ແລະ ອື່ນໆ

ຄະນະບໍລະຫານງານອິດສະລະຈາກສະຖາບັນ ປັດສະເຕີ ລາວ ເປັນ ຜູ້ຄຸ້ມຄອງບໍລິຫານເງິນທຶນຂອງມູນນິທິ ສະຖາບັນ ປັດສະເຕີ ລາວ, ພວກເຮົາກໍາລັງຄົ້ນຄວ້າຊອກຫາການຊ່ວຍເຫຼືອນ້ອຍແລະ ໃຫຍ່ໃຫ້ໄດ້ 5 ລ້ານໂດລາສະຫະລັດອາເມລິກາ ແຕ່ນີ້ຮອດປີ 2024 ເພື່ອ ເປັນການຊ່ວຍໃຫ້ພວກເຮົາໄປເຖິງຈຸດປະສົງ ແລະ ເປົ້າໝາຍ ທີ່ວາງໄວ້ (www.pasteur.la).

ສຸດທ້າຍແລ້ວ ຕາມປະເພນີຂອງການສຶກສາຢູ່ສະຖາບັນ ປັດສະເຕີ ແລະ ເຄືອຄາຍສະຖາບັນ ປັດສະເຕີ ສາກົນ ໄດ້ຮ່ວມກັນຈັດຫຼັກສູດການແພດສາກົນດ້ານແມງໄມ້ໃນວັນທີ 10-22 ມິຖຸນາ 2019 ທີ່ສະຖາບັນ ປັດສະເຕີ ລາວ ຫຼັກສູດນີ້ປະກອບມີ ການບັນຍາຍຈາກຜູ້ຊ່ຽວຊານຊັ້ນນໍາຈາກປະເທດຝຣັ່ງ, ເອີຣົບ, ແລະ ອາຊີຕາເວັນອອກສ່ຽງໃຕ້ພ້ອມທັງແນະນໍາການປະຕິບັດຕົວຈິງ ໃນຫ້ອງວິເຄາະ ແລະ ການຊະສົມແມງໄມ້ຕີນຂໍ້ (arthro-pod) ທີ່ຄວາມສໍາຄັນທາງການແພດ ຢູ່ສະຖາບັນ ປັດສະເຕີ ລາວ. ຫຼັກສູດດັ່ງກ່າວໄດ້ສໍາເລັດ ພ້ອມກັບການຝຶກອົບຮົມພາກສະໜາມ ດ້ານການແພດແມງໄມ້ ເປັນເວລາ ສາມອັນ ເພື່ອໃຫ້ນັກສຶກສານໍາໃຊ້ສິ່ງທີ່ໄດ້ຮຽນມາປະຕິບັດຕົວຈິງ. ນັກສຶກສາຈໍານວນ 14 ຄົນ ຈາກລາວ, ຝຣັ່ງ, ກໍາປູເຈຍ, ຕຸຍນີເຊຍ, ສະໂລເວເນຍ, ອີຣານ ແລະ ຣາດສະອານາຈັກມູຖານ ໄດ້ຖືກຄັດເລືອກໃຫ້ເຂົ້າຮ່ວມຊຸດຝຶກອົບຮົມນີ້.

ໂປນ ເຫຼ

IN MEMORIAM

Phengsavanh SITTIVONG

1948-2019

In 2007 when I arrived in Vientiane to establish the Institut Pasteur du Laos I needed to find a trustworthy collaborator who could speak and write fluently in French and Lao languages and who could help me with interactions with the Lao government during this initial phase of the creation of the Institut Pasteur du Laos. During a stay at the Green Park Hotel in Vientiane, I met the proprietor Mr. Chung Prarasavong who told me his "little" brother Phengsavanh was looking to find a position within an organization that was here to benefit the country.

After I met Phengsavanh, who was about 10 years my senior, I knew that he was the type of person I was looking for. An experienced jurist, trained in France with working experience within the Lao Ministry of Justice and years of experience in Canada, Phengsavanh was a remarkable person with many talents. I liked his direct approach and his remarkable ability to give me insight into the Lao culture and their way of doing things.

Shortly after hiring Phengsavanh, we were having lunch together at a local restaurant when several men approached our table and conveyed their respects in Lao, shook Phengsavanh's hand vigorously, visibly with a high degree of admiration. I teased Phengsavanh asking him if he was some kind of star? He replied frankly, "yes" he used to be the captain of the Lao National Football (Soccer) Team and was a famous player who scored 3 goals to beat the "historic rivals", the National Team of Thailand! So, I quickly realized that my co-worker Phengsavanh was the former Lao Zinedine Zidane! But Phengsavanh told me that he had given up football, but was absolutely passionate about tennis –he played the game almost everyday after work and on weekends!

Prior to the creation of the Institut Pasteur du Laos by decree of Mr. Bouason Bhoupavanh, the Lao Prime Minister, we need to establish a representation office for Institut Pasteur in Laos. Phengsavanh did all the paper work and we got our license to operate in Laos. We set up a small office in the Sihom Office building and with Phengsavanh we began to work with the Minister of Health Dr. Ponmek Dalaloy, his head of Cabinet Dr. Nao Boutta, and Professor Sithat Insisiengmay to draft a general collaboration agreement along with our counterparts at Institut Pasteur in Paris (Michèle Boccoz, International Director and Antoine des Graviers, legal council to the international division). The General Cooperation agreement would serve as a framework for the future statutes of the Institut Pasteur du Laos. The Institut Pasteur du Laos was officially established as a Lao National Institute for research and training on infectious diseases on the 16 of November 2007.

After the creation of IPL, Phengsavanh remained a crucial figure during the construction phase and during the hiring process of IPL staff. He was well liked by our Lao staff who considered him like a big brother figure. Phengsavanh's door was always open and he was always available to talk to staff and always ready to help out. He was the one the staff would call if something was not right. Phengsavanh remained once the Institut was operational continuing to provide legal advice, translate documents and participate in the daily life of IPL. Even after his retirement Phengsavanh continued working at IPL part-time in the mornings and playing tennis the afternoons.

Over the past 12 years Phengsavanh Sittivong has played an important role in the establishment of Institut Pasteur du Laos. His deep understanding of the Lao and Western cultures made him a key player in negotiations with the Lao Government, Vientiane Municipality, but also allowed the IPL administration to have a clear understanding of the Lao point of view. His legal and translational skills allowed IPL to negotiate and communicate effectively with our Lao partners. Phengsavanh's jovial good-natured personality always made our staff and guests always feel at ease and welcome. We lost our Phengsavanh on the 11th of September this year while he was playing tennis, the game he loved so much. We miss him dearly.

Paul Brey

ຄວາມຊົງຈຳ
ທ່ານ ເພັ່ງສະຫວັນ ສິດທິວົງ
1948-2019

ໃນປີ 2007 ຂ້າພະເຈົ້າ ໄດ້ມາ ນະຄອນຫຼວງວຽງຈັນ ເພື່ອຈະມາກໍ່ຕັ້ງ ສະຖາບັນ ປັດສະເຕີ ລາວ ແລະ ຂ້າພະເຈົ້າກໍ່ຕ້ອງການ ຊອກຫາຜູ້ ປະສານງານທີ່ສາມາດເວົ້າພາສາ ຝຣັ່ງ ແລະ ພາສາລາວໄດ້ເປັນຢ່າງດີ ແລະ ເຊື່ອຖືໄດ້ ເຊິ່ງຈະເປັນຜູ້ທີ່ຈະຊ່ວຍຂະພະເຈົ້າປະສານງານກັບທາງພາກ ສ່ວນລັດທະບານລາວໃນໄລຍະລິເລີ່ມການກໍ່ຕັ້ງສະຖາບັນ ປັດສະເຕີ ລາວ. ໃນລະຫວ່າງການພັກເຊົາຢູ່ທີ່ ໂຮງແຮມ ກຼີນປ່າກ ທີ່ນະຄອນຫຼວງວຽງຈັນ (Green Park Hotel) ຂ້າພະເຈົ້າກໍ່ໄດ້ພົບກັບເຈົ້າຂອງໂຮງແຮມ ລາວມີ ຊື່ວ່າ ທ່ານ ຈຸງ ພຼາລາດຊະວົງ (Mr. Chung Prarasavong) ຜູ້ທີ່ແນະນຳໃຫ້ຂະພະເຈົ້າຮູ້ຈັກກັບ ທ່ານ ເພັ່ງສະຫວັນ ເຊິ່ງເປັນນ້ອງຊາຍ ຫຼ້າຂອງລາວ ທີ່ກຳລັງຊອກຫາຕຳແໜ່ງໃດໜຶ່ງໃນອົງກອນທີ່ເປັນປະໂຫຍດ ຕໍ່ປະເທດຊາດ.

ຫຼັງຈາກຂ້າພະເຈົ້າໄດ້ພົບກັບ ທ່ານ ເພັ່ງສະຫວັນ ເຊິ່ງເປັນຜູ້ທີ່ມີອາຍຸ ຫຼາຍກວ່າຂະພະເຈົ້າປະມານ 10 ກວ່າປີ, ແລະ ຂ້າພະເຈົ້າຮູ້ໄດ້ ເລີຍວ່າລາວເປັນບຸກຄົນ ທີ່ຂ້າພະເຈົ້າກຳລັງຊອກຫາ. ເຊິ່ງມີ ປະສົບການ ທາງດ້ານກົດໝາຍ ແລະ ໄດ້ຜ່ານການຝຶກອົບຮົມຈາກ ປະເທດຝຣັ່ງ, ກະຊວງຍຸດຕິທຳ ຂອງ ສປປ ລາວ ແລະ ການາດາ ເປັນເວລາຫຼາຍປີ, ທ່ານເພັ່ງສະຫວັນ ຍັງມີຄວາມສະມາດພິເສດຫຼາຍດ້ານ. ຂ້າພະເຈົ້າມັກ ຄວາມ ກົງໄປກົງມາແບບລາວ ແລະ ຄວາມສາມາດອັນໂດດເດັ່ນ ໃນການໃຫ້ຂໍ້ມຸນ ເຊິ່ງເລີກໃນ ດ້ານວັດທະນະທຳລາວ ແລະ ການປະ ຕິບັດວຽກງານຕ່າງໆ.

ຕໍ່ມາຂ້າພະເຈົ້າກັບທ່ານເພັ່ງສະຫວັນກໍ່ໄດ້ໄປກິນເຂົ້າສວຍນຳກັນທີ່ຮ້ານ ອາຫານ ພື້ນບ້ານແຫ່ງໜຶ່ງ ໃນຂະນະນັ້ນໄດ້ມີຜູ້ຊາຍຫຼາຍຄົນ ຍ່າງເຂົ້າ ມາໂຕະຂອງ ພວກຂ້າພະເຈົ້າ ແລ້ວ ສະແດງຄວາມເຄົາລົບທຳນຽມ ແບບ ຄົນລາວ ດ້ວຍການຈັບມືທ່ານເພັ່ງສະຫວັນ, ນັ້ນສະແດງໃຫ້ເຫັນ ເຖິງການ ຊື່ນຊົມ ທ່ານເພັ່ງສະຫວັນ ເປັນຢ່າງສູງ. ຂ້າພະເຈົ້າຖາມເພື່ອນ ຂອງ ເພັ່ງສະຫວັນແບບຫຼິ້ນໆດ້ວຍການຖາມລາວວ່າ ລາວເປັນດາລາບໍ່ ແລະ ເປັນດາລາແບບໃດ. ລາວຕອບແບບກົງໄປກົງມາວ່າ ແມ່ນແລ້ວລາວເຄີຍ ເປັນກັບຕັນບານເຕະທີມຊາດລາວ ແລະ ເປັນນັກເຕະດີເດັ່ນ ທີ່ສາມາດ ຍິງປະຕູໄດ້ 3 ລຸກ ບຽດເອົາຊະນະຄູ່ແຂ່ງປະຫວັດສາດ ທີມຊາດໄທມາໄດ້! ສະນັ້ນ ຂ້າພະເຈົ້າຮູ້ໄດ້ທັນທີເລີຍວ່າ ເພື່ອນຮ່ວມງານຂອງຂ້າພະເຈົ້າ ເພັ່ງສະຫວັນ ປຽບດັ່ງອາດີດນັກເຕະບານ ສິນາດີນສິດານລາວ (Zinadine Zidane!) ແຕ່ທ່ານເພັ່ງສະຫວັນ ໄດ້ບອກຂ້າພະເຈົ້າວ່າ ລາວໄດ້ເຊົາເປັນນັກກິລາເຕະບານແລ້ວ ແຕ່ ລາວກັບຫຼົ້ງໄຫຼໃນກິລາເທນນິດ ລາວຫຼິ້ນເທນນິດເກືອບທຸກມື້ຫຼັງເລີກງານ ແລະ ວັນພັກໃນທ້າຍອາທິດ.

ກ່ອນຈະສ້າງຕັ້ງ ສະຖາບັນ ປັດສະເຕີ ລາວ ໂດຍດຳລັດຂອງ ນາຍົກລັດທະ ມົນຕີແຫ່ງ ສປປ ລາວ ທ່ານ ບົວສອນ ບຸບຜາວັນ, ພວກເຮົາມີຄວາມ ຈຳເປັນຕ້ອງໄດ້ຈັດຕັ້ງ ຫ້ອງການ ຕາງໜ້າ ສຳລັບ ສະຖາບັນ ປັດສະເຕີ ໃນລາວ ,ທ່ານ ເພັ່ງສະຫວັນແມ່ນເປັນ ຄົນແລ່ນ ເອກະສານທັ້ງໝົດ ແລະ ໄດ້ຮັບໃບອະນຸຍາດ ດຳເນີນກິດຈະກຳຢູ່ໃນ ສປປ ລາວ. ພວກເຮົາໄດ້ ຈັດຕັ້ງຫ້ອງການນ້ອຍໆ ຢູ່ອາຄານແຖວສີຫອມ ແລະ ທ່ານເພັ່ງສະຫວັນ ໄດ້ເລີ່ມເຮັດວຽກພົວພັນກັບ ລັດຖະມົນຕີກະຊວງສະທາລະນະສຸກ ກໍ່ຄື ທ່ານ ດຣ. ປອນເມັກ ດາລາລອຍ, ຫົວໜ້າຫ້ອງການ ແມ່ນ ທ່ານ ດຣ. ເນົາ ບຸດຕາ, ແລະ ອາຈານ ສີທັດ ອິນສີຊຽງໃໝ່ ເພື່ອຮ່າງຂໍ້ຕົກລົງການຮ່ວມມື ທົ່ວໄປ ຮ່ວມກັບຄູ່ຮ່ວມງານຂອງພວກເຮົາທີ່ ປັດສະເຕີ ໃນປີ8 (Mich le Boccoz, ຄືຜູ້ອຳນວຍການສາກົນ ແລະ Antoine des Graviers, ສະພາກິດໝາຍຕໍ່ພະແນກສາກົນ) ຂໍ້ຕົກລົງການຮ່ວມມືທົ່ວໄປຈະເປັນ ຮ່າງກິດລະບຽບໃນອະນາຄົດຂອງສະຖາບັນ ປັດສະເຕີ ລາວ. ສະຖາບັນ ປັດສະເຕີ ລາວ ໄດ້ຖືກສ້າງຕັ້ງຂຶ້ນຢ່າງເປັນທາງການ, ເປັນສະຖາບັນແຫ່ງ ຊາດລາວເພື່ອການຄົ້ນຄວ້າ ແລະ ຝຶກອົບຮົມກ່ຽວກັບພະຍາດຕິດຕໍ່ ໃນວັນທີ 16 ພະຈິກ 2007.

ຫຼັງຈາກການສ້າງຕັ້ງ ສະຖາບັນ ປັດສະເຕີລາວ, ທ່ານເພັ່ງສະຫວັນຍັງຄົງ ເປັນບຸກຄົນສຳຄັນໃນໄລຍະກໍ່ສ້າງ ແລະ ໃນໄລຍະການ ຮັບພະ ນັກງານຂອງ ສະຖາບັນ ປັດສະເຕີ ລາວ. ທ່ານເພັ່ງສະຫວັນໄດ້ຮັບຄວາມຮັກ ແພງຈາກພະນັກງານຄົນລາວ ເພາະພວກເຮົາຖືວ່າລາວ ເປັນອ້າຍໃຫຍ່. ປະຕູຂອງ ທ່ານເພັ່ງສະຫວັນແມ່ນ ເປີດຢູ່ສະເໝີ ແລະ ພ້ອມທີ່ ຈະສົນທະນາກັບພະນັກງານ ແລະ ພ້ອມທີ່ຈະຊ່ວຍເຫຼືອຕະຫຼອດເວລາ. ລາວເປັນທັງທີ່ປຶກສາໃຫ້ພະນັກງານເວລາມີບັນຫາບາງສິ່ງບາງຢ່າງທີ່ບໍ່ຖືກ ຕ້ອງ. ໃນເວລາ ທ່ານ ເພັ່ງສະຫວັນຍັງຢູ່ ສະຖາບັນ ປັດສະເຕີ ລາວ ແມ່ນ ໄດ້ສືບຕໍ່ໃຫ້ຄຳປຶກສາທາງດ້ານກົດໝາຍ, ແປເອກະສານ ແລະ ເຂົ້າຮ່ວມກິດ ຈະກຳຕ່າງໆ ໃນສະຖາບັນ ປັດສະເຕີ ລາວ. ເຖິງແມ່ນວ່າ ທ່ານ ເພັ່ງສະຫວັນໄດ້ລາອອກບຳນານໄປແລ້ວກໍ່ຕາມ ກໍ່ຍັງສືບຕໍ່ເຮັດວຽກຢູ່ ສະຖາບັນ ປັດສະເຕີ ລາວ ໃນເວລາຕອນເຊົ້າ ແລະ ຫຼິ້ນເທນນິດ ໃນຕອນບ່າຍ.

ຕະຫຼອດໄລຍະເວລາ 12 ປີທີ່ຜ່ານມາ ທ່ານ ເພັ່ງສະຫວັນ ສິດທິວົງ ໄດ້ມີບົດບາດສຳຄັນໃນການສ້າງຕັ້ງ ສະຖາບັນ ປັດສະເຕີ ລາວ. ຄວາມເຂົ້າ ໃຈອັນເລີກເຊິ້ງກ່ຽວກັບວັດທະນະທຳລາວ ແລະ ປະເທດຕາເວັນຕົກ ໄດ້ ເຮັດໃຫ້ລາວກາຍເປັນກຳລັງສຳຄັນໃນການພົວພັນກັບຝ່າຍລັດ ຖະບານ ລາວ, ກໍຄືຂອບເຂດນະຄອນຫຼວງວຽງຈັນ, ແລະ ຍັງຊ່ວຍໃຫ້ການບໍລິຫານ ຂອງ ສະຖາບັນ ປັດສະເຕີ ລາວ ມີຄວາມເຂົ້າໃຈຢ່າງຈະແຈ້ງກ່ຽວ ກັບຈຸດ ປະສົງຂອງຄົນລາວ. ທັກສະທາງດ້ານກົດໝາຍ ແລະ ການແປພາ ສາຂອງລາວ ຊ່ວຍໃຫ້ ສະຖາບັນ ປັດສະເຕີ ລາວ ພົວພັນ ແລະ ສື່ສານ ຢ່າງມີປະສິດທິຜົນ ກັບຄູ່ຮ່ວມງານຄົນລາວຂອງພວກເຮົາ.

ບຸກຄະລິກກະພາບທີ່ໜ້າຮັກຂອງທ່ານເພັ່ງສະຫວັນໄດ້ເຮັດໃຫ້ພະນັກງານ ແລະ ແຂກຂອງພວກເຮົາຮູ້ສຶກສະບາຍໃຈ ແລະ ຍິນດີຕ້ອນຮັບທຸກໆຄັ້ງ. ພວກເຮົາເສຍ ທ່ານ ເພັ່ງສະຫວັນຂອງພວກເຮົາໄປໃນວັນທີ 11 ເດືອນ ກັນຍາ 2019 ໃນຂະນະທີ່ລາວກຳລັງຫຼິ້ນກິລາເທັນນິສ ຄືເກມ ກິລາທີ່ລາວຮັກຫຼາຍ. ພວກເຮົາຄິດຮອດລາວ.

ໂປນ ເຢ

Scientific Activities 2019-2020

Arbovirus and Emerging viral diseases *Lao-French joint Lab 1*

Since 2012, the Arbovirus and Emerging Viral Diseases Laboratory developed combined field studies to improve knowledge of viral vector-borne diseases in the Lao PDR. Over the last three years, the team was organized around specific projects to help the young Lao scientists and technicians to acquire skills in virology with strong practical applications. In order to expand the capacities of the unit and reinforce the positioning of the laboratory in the public health arena, a new strategy has been developed. The team has now been restructured into three groups that, between them, encompass the different laboratories' areas of responsibility.

Progress in arbovirus surveillance and staff changes afforded opportunities to reorganize the team. A major part of the group is now dedicated to the global surveillance of arboviruses, providing (i) assistance to Vientiane and provincial hospitals for the confirmation of arboviral infections, (ii) updated information and alerts to national and international health authorities, and (iii) training for medical staff in hospitals. A second group focuses its activities on arbovirus ecology. Interactions with entomologists and vertebrate experts were reinforced in order to diversify the fields of investigation on arbovirus cycles and maintenance in wild environments. A third group is in charge of conducting research on more fundamental aspects of virus–host interactions and to develop new detection or diagnosis tests.

Nevertheless, the capacity of the unit to mobilize sufficient staff to face a major public health crisis in the area of the emergence of unrecorded viral pathogens in the region or re-emergence of endemic viruses on a 24/7 basis has been maintained.

Head of Laboratory: Marc GRANDADAM, PhD

Junior Scientists:
Somphavanh SOMLOR, MD
Thonglakhone XAYBOUNSOU, MD

Technicians:
Phaithong BOUNMANY
Souksakhone VIENGPHOUTHONG
Sitsana KEOSENHOM, Lab & Quality Agent
Phoutsana KHAMSUVAT
Thep aksone CHINDAVONG
Phithaya THONGSAVATH, Data clerk

Projects

- Arbovirus surveillance in Lao PDR
- Ecomore2 project
- Inputs of Research and development activities in Ecomore2 project
- Training activities
- Support to institutions
- Evaluation of new tools for arbovirus diagnosis

Executive summary

Arbovirus surveillance became over time the major tool for research and public health involvement of the Arbovirus and emerging viral diseases laboratory. The strong background of knowledge and data acquired by the laboratory over the last seven years allowed to set up capacities to anticipate dengue epidemic and improve patients' diagnostic and management. However, dengue remains a major public health concern and its transmission remains challenging for the country. Dengue surveillance was the driving activity of the laboratory through Ecomore2 project. Lao PDR experienced a continuous transmission of dengue with a global predominance of dengue 4 over the past three years. However, a sudden onset of dengue virus serotype 2 raised the question of the occurrence of a major outbreak in 2019. As of September 30th, more than 33.500 dengue suspected cases were declared at the country level within which, 66 fatal cases were attributed to dengue virus.

The development of news laboratory methods was required to fulfill some objectives of Ecomore2 project. Specific capacities were developed to improve dengue virus characterization. From a genetic point of view, a rapid screening protocol has been set up in order to determine dengue virus genotypes using partial envelop sequencing. Isolates of interest are then analyzed by extensive envelop gene sequencing. Surveillance system also aims to evaluate the risk for arbovirus importation and spreading. Experimental infection of local *Aedes* species have been performed to evaluate their competence for the transmission of different genotypes of chikungunya virus isolates from imported cases in Laos.

The expertise of the laboratory has also been requested by health authorities to reinforce the country capacities to fight against global emergencies (Ebola) or by private companies for the evaluation of new diagnostics tools.

Over these eight years of activities, the laboratory became a key partner for the national health authorities by providing recommendations for arbovirus diagnostic, control and prevention and training to reinforce health systems.

ສະຫຼຸບການປະຕິບັດວຽກງານ

ການເຝົ້າລະວັງເຊື້ອຈຸລະໂລກທີ່ມີແມງໄມ້ຕີນຂໍເປັນພາຫະກາຍເປັນເຄື່ອງມືທີ່ສຳຄັນສຳລັບ ວຽກງານທາງດ້ານການຄົ້ນຄວ້າ ແລະ ວຽກງານທາງດ້ານສາທາລະນະສຸກສາດທີ່ກ່ຽວພັນກັບຫ້ອງທົດລອງອາກໂບໄວຣັສ ແລະ ພະຍາດທີ່ເກີດຂຶ້ນໃຫ່ມ. ຄວາມເຂົ້າໃຈເລິກເຊິ່ງກ່ຽວກັບປະຫວັດຄວາມເປັນມາ ແລະ ຖານຂໍ້ມູນທີ່ໄດ້ມາຈາກຫ້ອງທົດລອງໃນຊ່ວງໄລຍະ 7 ປີ ທີ່ຜ່ານມາ ແມ່ນສ້າງຄວາມອາດສາມາດໃນການຄາດຄະເນການລະບາດ ຂອງພະຍາດໄຂ້ຍຸງລາຍ ແລະ ຊ່ວຍປັບປຸງການບົ່ງມະຕິ ແລະ ການຄຸ້ມຄອງກໍລະນີໃຫ້ແກ່ຄົນເຈັບ

ເຖິງຢ່າງໃດກໍຕາມ, ພະຍາດໄຂ້ຍຸງລາຍ ຍັງຄົງເປັນບັນຫາຫນຶ່ງທີ່ສຳຄັນທາງດ້ານສາທາລະນະສຸກສາດ ແລະ ການສົ່ງເຊື້ອກໍຍັງເປັນສິ່ງທ້າທາຍໃຫ້ແກ່ປະເທດ. ການເຝົ້າລະວັງພະຍາດໄຂ້ຍຸງລາຍແມ່ນກິດຈະກຳຫຼັກ ຂອງຫ້ອງວິເຄາະ ໂດຍພາຍໃຕ້ໂຄງການ Ecomore2. ສປປ ລາວ ໄດ້ປະສົບກັບການລະບາດຂອງພະຍາດໄຂ້ຍຸງລາຍຢ່າງຕໍ່ເນື່ອງ ພ້ອມກັນນັ້ນ ຍັງພົບເຊື້ອໄວຣັສໄຂ້ຍຸງລາຍຊະນິດທີ 4 ແມ່ນຊະນິດທີ່ພົ້ນເດັ່ນຕະຫຼອດໄລຍະ 3 ປີ ທີ່ຜ່ານມາ. ການເພີ້ມຂຶ້ນກະທັນຫັນຂອງເຊື້ອໄວຣັສໄຂ້ຍຸງລາຍຊະນິດທີ 2 ຈຶ່ງເຮັດໃຫ້ມີຄຳຖາມຂອງການເກີດການລະບາດໃຫ່ຍ ໃນປີ 2019 ນີ້.

ໃນວັນທີ 30 ກັນຍາທີ່ຜ່ານມາ, ໄດ້ມີການລາຍງານຫລາຍກວ່າ 33.500 ກໍລະນີທີ່ສົງໄສພະຍາດໄຂ້ຍຸງລາຍໃນປະເທດ, ເຊິ່ງໃນນັ້ນມີ 66 ກໍລະນີທີ່ເສຍຊີວິດ ຈາກການຕິດເຊື້ອໄວຣັສໄຂ້ຍຸງລາຍ.

ການພັດທະນາວິທີການແບບໃຫ່ມໃນຫ້ອງທົດລອງຍັງມີຄວາມຈຳເປັນຫລາຍເພື່ອໃຫ້ບັນລຸຈຸດປະສົງຂອງໂຄງການ Ecomore2. ຄວາມສາມາດສະເພາະຈຶ່ງໄດ້ພັດທະນາຂຶ້ນເພື່ອປັບປຸງການຈຳແນກລັກສະນະຂອງເຊື້ອໄວຣັສໄຂ້ຍຸງລາຍ. ພ້ອມກັນນັ້ນຍັງມີການສຶກສາທາງດ້ານ ພັນທຸກຳ ເຊິ່ງຂັ້ນຕອນການປະຕິບັດງານແມ່ນໄດ້ມີການສ້າງ protocol ການຄັດກອງແບບໄວ ເພື່ອຊອກຫາ genotype ຂອງເຊື້ອໄວຣັສໄຂ້ຍຸງລາຍ ໂດຍນຳໃຊ້ບາງສ່ວນ envelop ມາກວດດ້ວຍເຕັກນິກ sequencing. ຫລັງຈາກນັ້ນຈຶ່ງໄດ້ມີການວິເຄາະສາຍພັນທີ່ໜ້າສົນໃຈດ້ວຍການກວດຫາ envelop ທີ່ຍາວຂຶ້ນດ້ວຍ ເຕັກນິກ sequencing ລະບົບການເຝົ້າລະວັງນີ້ ຍັງມີຈຸດປະສົງເພື່ອຢາກປະເມີນຄວາມສ່ຽງການນຳເຊື້ອເຂົ້າມາ ແລະ ການກະຈາຍອອກຂອງເຊື້ອພະຍາດທີ່ມີແມງໄມ້ຕີນຂໍເປັນພາຫະ. ການທົດລອງການຕິດເຊື້ອໃນຍຸງປະຈຳຖິ່ນຊະນິດ *Aedes* ແມ່ນ ໄດ້ປະຕິບັດຂຶ້ນ ເພື່ອປະເມີນຄວາມສາມາດໃນການຖ່າຍທອດພັນທຸກຳຕ່າງໆ ຂອງໄວຣັສ chikungunya ຈາກກໍລະນີຄົນເຈັບທີ່ນຳເຊື້ອເຂົ້າມາລາວ.

ຄວາມຊຳນິຊຳນານຂອງຫ້ອງທົດລອງຈຶ່ງເຮັດໃຫ້ໄດ້ຮັບການຮຽກຮ້ອງຈາກຫ່ວຍງານດ້ານສຸຂະພາບໃຫ້ເພີ້ມຄວາມອາດຄວາມສາມາດຂອງປະເທດໃນການຮັບມືກັບພາວະສຸກເສີນທົ່ວໂລກ (Ebola) ຫລືຈາກ ບໍລິສັດ ເອກະຊົນຕ່າງໆ ໃນການປະເມີນເຄື່ອງມືໃນການບົ່ງມະຕິແບບໃຫ່ມ.

ກິດຈະກຳຕະຫຼອດໄລຍະ 8 ປີທີ່ຜ່ານມາ ໄດ້ເຮັດໃຫ້ຫ້ອງທົດລອງໄດ້ກາຍເປັນເພື່ອນຮ່ວມງານທີ່ສຳຄັນໃຫ້ແກ່ຫ່ວຍງານດ້ານສາທາລະນະສຸກແຫ່ງຊາດ ເຊິ່ງຫ້ອງທົດລອງໄດ້ ໃຫ້ຄຳແນະນຳໃນການບົ່ງມະຕິ, ການຄວບຄຸມ ແລະ ການປ້ອງກັນເຊື້ອຈຸລະໂລກທີ່ມີແມງໄມ້ຕີນຂໍເປັນພາຫະ ແລະ ການຝຶກອົບຮົມ ເພື່ອເສີມສ້າງລະບົບສາທາລະນະສຸກໃຫ້ດີຂຶ້ນ.

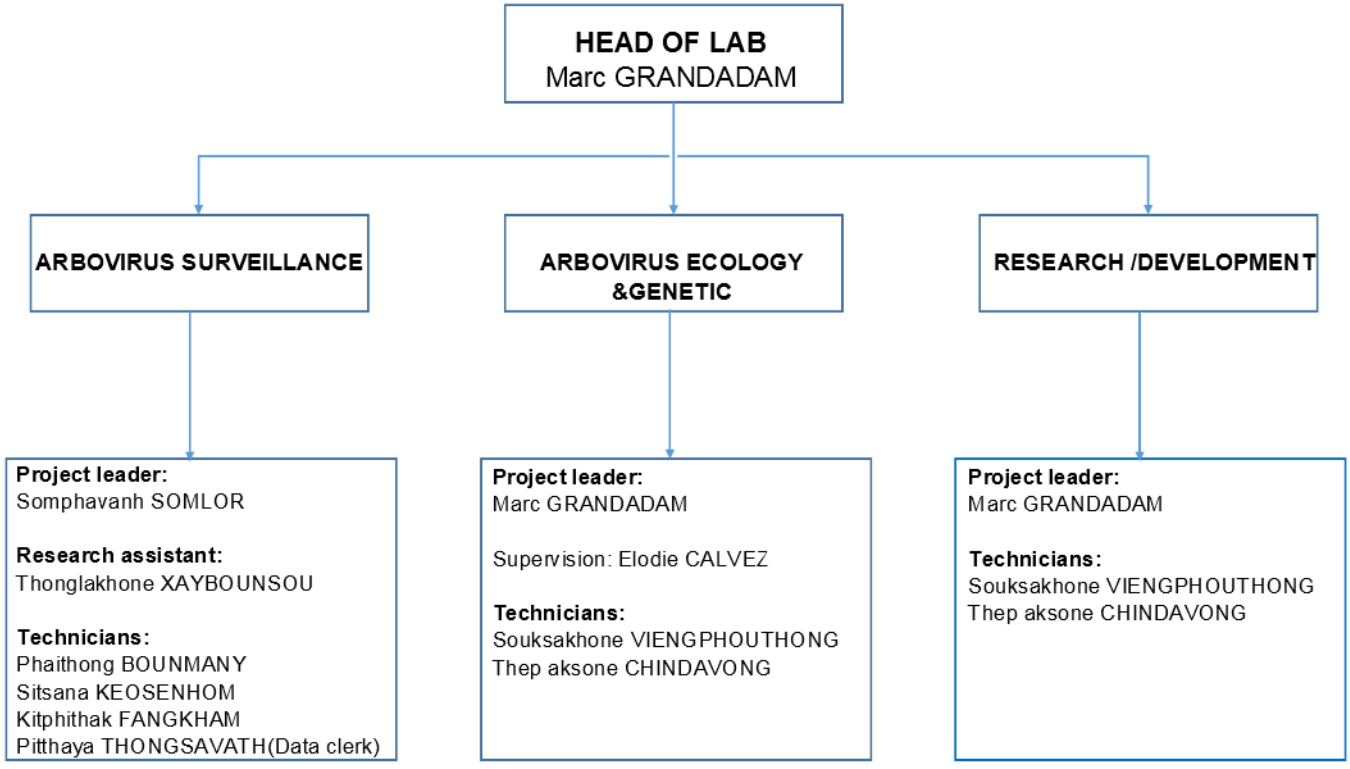


Figure 1: Arbovirus and Emerging Viral Diseases Laboratory organigram

Figure 2: Arbovirus and emerging viral diseases laboratory regional partnerships

Arbovirus surveillance in Lao PDR

Project Ecomore2 funded by Agence Française du développement (AFD)

Project coordinators: Marc GRANDADAM ; Somphavanh SOMLOR
Staff members: Thonglakhone XAYBOUNSOU, Phaihong BOUNMANY, Sitsana KEOSENHOM

Dengue surveillance in Vientiane Capital

Arboviruses surveillance

For now eight consecutive years, the AVED laboratory provides real time information on dengue transmission to the Lao Ministry thanks to its surveillance network in Vientiane Capital. Since 2015, the surveillance network includes several Lao provinces after specific a request of the MOH (Saravan; Attapeu) and as an outcome of a large training program for the Lao Army health service (Arboshield project; Champassack; Luang Prabang….). The sum of the data allows to establish, with a certain degree of certainty, predictions regarding the level of transmission of dengue in Vientiane Capital city (Table 1). In November 2018, the indicators suggested that, if environmental conditions were met, a major dengue epidemic could be expected in Laos. This trend was confirmed in the first months of 2019, when a significant level of dengue cases were confirmed with a predominance of serotype 2 (Figure n°3).

Table 1: Indicators used for dengue prediction for 2019

INTER-SEASON 2012-13 / 2017-2018

		2012 / 2013	2017 / 2018
FACTORS	Characteristics	Status	Status
Dengue virus	Serotype	- DENNv-3; 2 genotypes - (Re)emergent - Predominant - > 6 months	- DENv-4, DENv-2 - (Re)emergent - Predominant - > 6 months
Human population	Immunity Age class Confirmed cases (weekly dry season) Density	Low All 5<X<15 (+ asymptomatic) To be determined	Low All 5<X<10 (+ asymptomatic) To be determined ↑
Vector	*Aedes* species Stage Activity Density Insecticide resistance	*Ae aegypti; albopictus* Larvae; adults Permanent ? ?	*Ae aegypti; albopictus* Larvae; adults Permanent Preliminary Data I/R
Climat (December)	Temperature Rainfalls Humidity	26.5°C 2.79 mm 65.4%	24.3°C 0 65.9%
Environment	Construction sites	To evaluate	To evaluate ↑

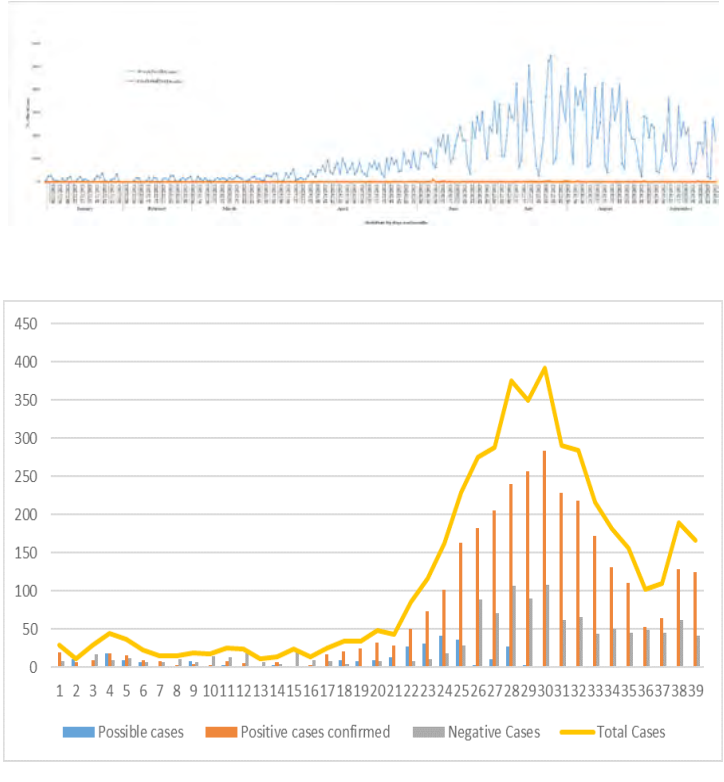


Figure 3: Comparison of the national syndromic and Vientiane Capital laboratory based surveillance data. (A) Weekly of dengue suspected cases recorded at the national level (source NCLE); (B) Laboratory surveillance data from IPL.

As of September 30, 2019, 33.200 cases of dengue were reported at the national level, including 66 fatal cases attributed to dengue. Of them, 21 were recorded in Vientiane Capital city. investigated. A dengue virus infection could be confirmed for 11 of the 13 cases investigated by the AEVD laboratory. All the three circulating serotypes (i.e. DENV-1; DENV-2 and DENV-4) were found at the origin of these fatal cases. AEVD laboratory investigated a large part of the suspected cases. From January to September 2019, 6211 dengue suspected cases from 9 provinces (Vientiane Capital, Vientiane Province, Saravan, Attapeu LuangPrabang, Xayabouly, XiengKhouang, Champassack and Savannakhet) were tested giving a global confirmation rate of 65%. At the acme of the outbreak, between weeks 27 to 33, the confirmation rate reached 80%. At this stage the number samples received by the laboratory was not manageable anymore (Picture 1). Specific clinical criteria were determined and discussed with the Ministry of Health and WHO in order to save laboratory capabilities and prioritize the investigation of critical cases. The number of samples tested for dengue virus serotyping has also been reduced. The drop in the number of cases observed in the surveillance in Vientiane Capital is mostly a result of the recommendations for a pre-screening selection of the suspected cases (Figure n°3). As shown by the syndromic surveillance system, the number of cases recorded began to decrease from week 34. However, confirmation rate remained beyond 70%, suggesting the epidemic may continue until the end of the year. Among the RT-PCR positive cases, 10 to 30% were randomly selected for serotyping. This procedure, experienced during the 2012-2013 DENV-3 epidemic, demonstrated to give an accurate vision of the distribution of the dengue virus serotypes. The predominance of DENV-2, anticipated in October 2018, was confirmed and was associated with a co-circulation of DENV-1 and DENV-4 (Figure n°4).

Picture 1: Reception and bio-banking of dengue suspected cases' samples

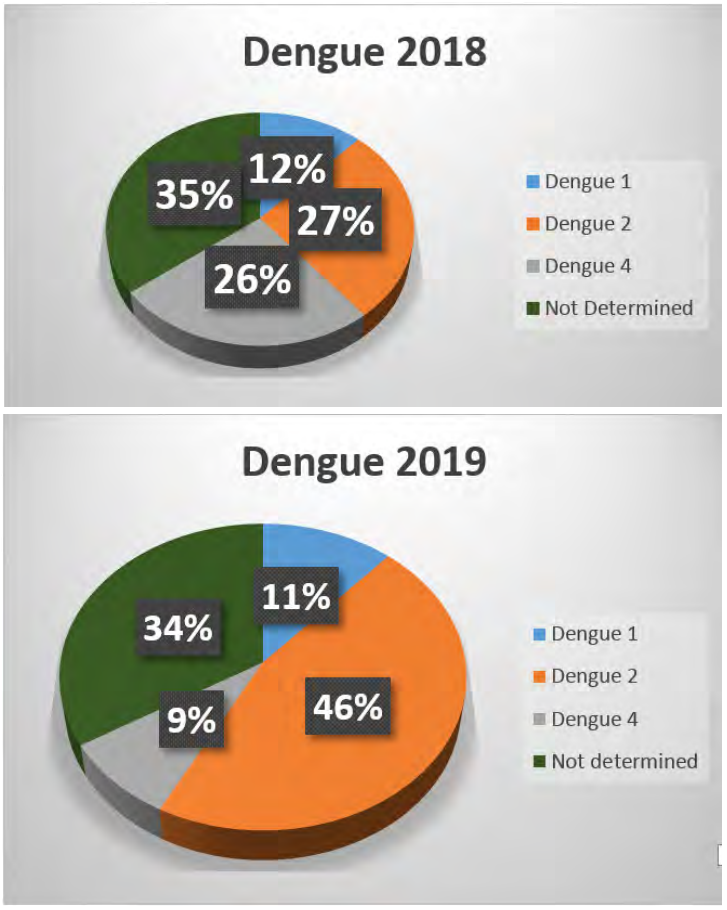


Figure n°4: Dengue serotypes distribution in Vientiane Capital

Differential diagnosis

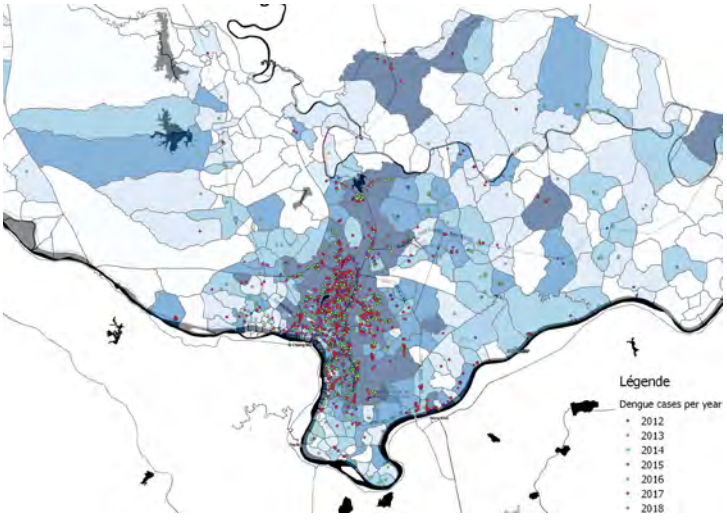
The algorithm used for the investigation of dengue suspected cases includes a differential diagnosis approach. At this stage, chikungunya and zika viruses have been identified as possible threats with potential high impact in the general Lao population. As diagnostic capacities and capabilities remain limited, the passive surveillance of these viruses is the keystone for the early detection of the importation and/or circulation in the country.

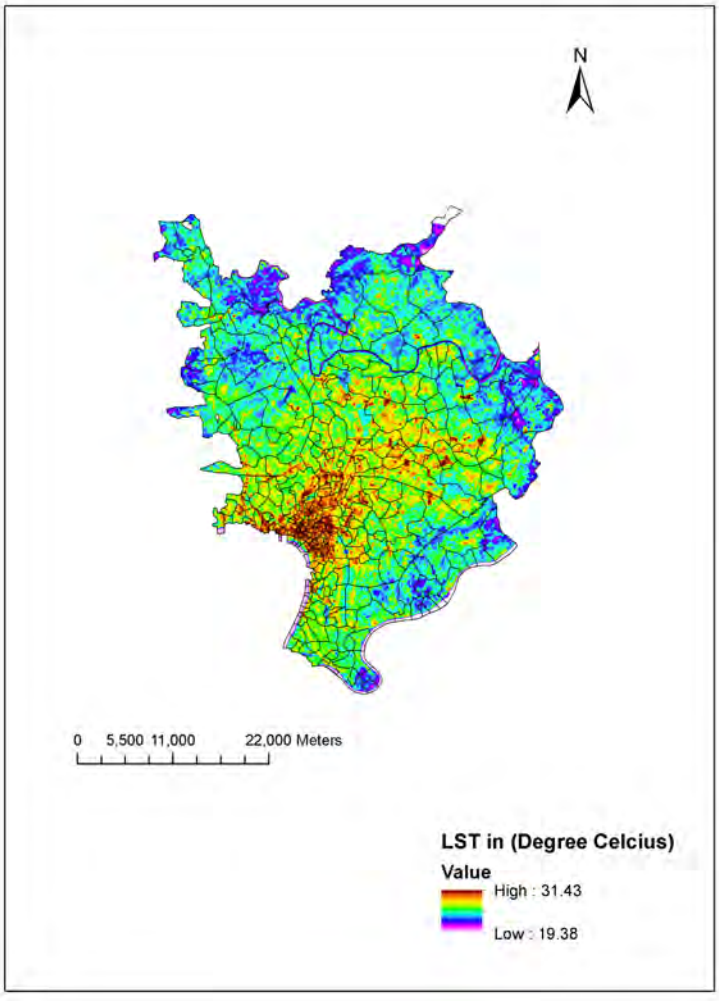
Urine collection has been promoted to the clinicians for now four years to improve zika virus direct diagnosis by RT-PCR. When available, the suspected cases negative for dengue were tested for the presence of zika virus genome in urine by RT-PCR. No case of zika infection could be identify in 2018-2019.

Two cases of chikungunya virus infection were recorded in foreigners returning respectively from Indonesia and Myanmar. Genetic and vector competence on Lao populations of *Aedes* mosquitoes are now under investigation.

Ecomore2 project

The main objective of ECOMRE2 is to gather different sources of information (disease surveillance; entomologic indicators; socio-demographic data; meteo/climatic data…) to reinforce the capacity to predict dengue epidemic by developing a multi data sources modelling system. The dengue surveillance system provides the core source of information of the future outbreak simulator including GPS coordinates' of dengue confirmed cases for real time mapping in Vientiane Capital. Socio-demographic data from the last census and satellite pictures were used to characterize the city (Figure n°5).

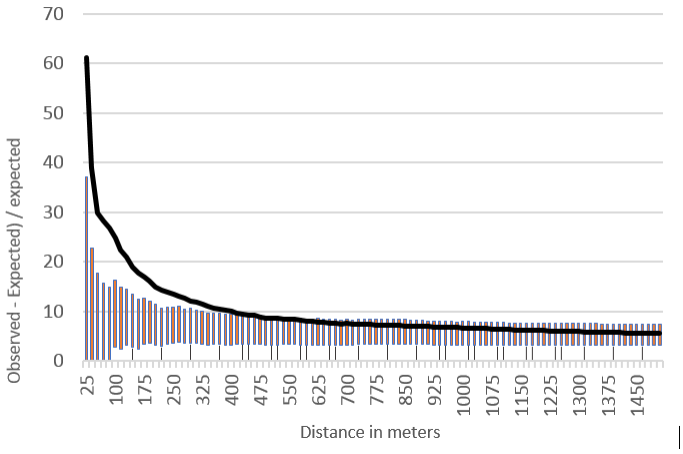


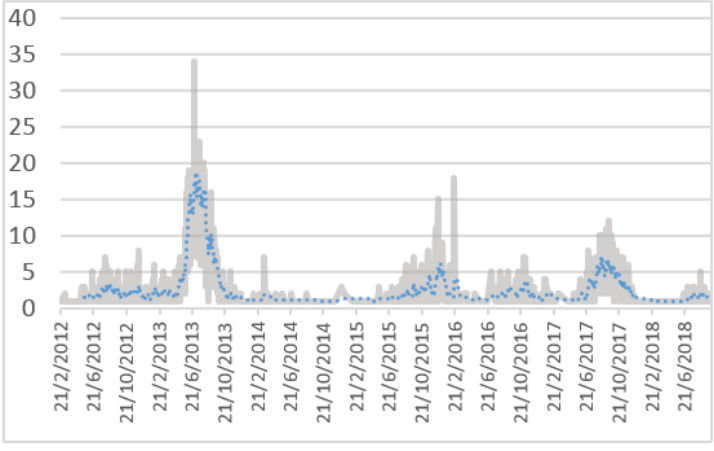


B

Figure 5: Different layers used for dengue transmission analysis in Vientiane Capital province. (A) Distribution of dengue confirmed cases based on households GPS coordinates. (B) Ground temperature

The superposition of the different layers of data used for the mapping will help to determine the driving factors of dengue transmission specific to Vientiane Capital city. Additional approaches based on clustering analysis of the confirmed cases will help to analyse the dynamic of the outbreak (Figure n°6). The study of the different epidemic cycles observed over the last past eight years evidenced different patterns of confirmed cases clustering.





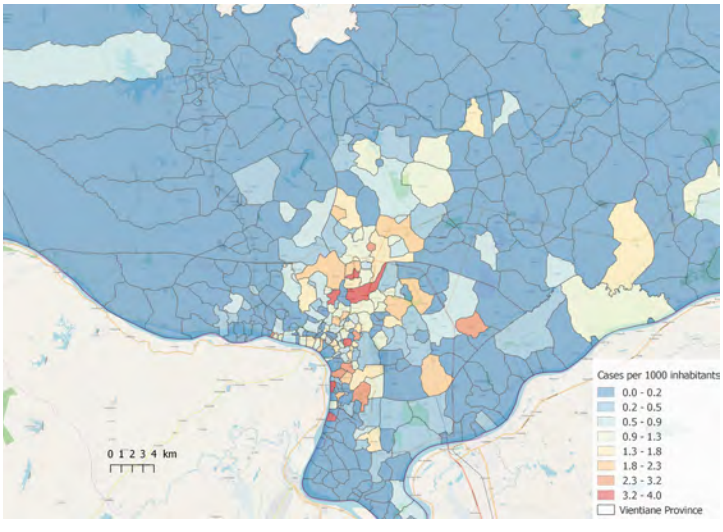


Figure n°6: Clustering of dengue confirmed cases based on the combination of laboratory surveillance

Inputs of Research and development activities in Ecomore2 project

Ecomore2 project in Lao PDR aims to gather multiple sources of data for the development of an outbreak simulator. Dengue virus genetic information and the level of herd immunity of the Lao population against dengue were considered as key issue for the accuracy of the future simulator. Specific tools were designed for the rapid analysis of large series of virus or human samples.

Rapid genotyping protocol for dengue virus mobility studies

From a genetic point of view, the four dengue virus serotypes are subdivided in closely related patterns of isolates also known as "genotypes". The full envelop gene sequencing is used as the gold standard to determine dengue virus genotypes. However, the cost and the workload are limiting this type of analysis to a limited number of isolates. Rapid genotyping protocols were developed in order to determine the genotype(s) of large series of isolates with the aim to facilitate virus mobility studies. Preliminary results were obtained were already obtained for the two main serotypes of dengue recorded in 2018-2019.

Dengue virus serotype 2 phylogeny

Since 2012, dengue virus serotype 2 (DENV-2) has been regularly detected in Lao People's Democratic Republic at a low level but the epidemiologic profile changed in 2018. Indeed, in October 2018, DENV-2 burden progressively increases and DENV-2 became predominant Vientiane Capital in 2019. In this study, we updated DENV-2 polymorphism data observed in Vientiane Capital and at the country level. To determine DENV-2 isolates origins and diversity, a Genotype Screening Protocol (GSP) based on partial envelope gene sequencing has been set up. A panel of 49 DENV-2 isolates, gathering both autochthonous and imported cases collected in six Lao provinces between 2015 to 2019, was analyzed.

The molecular epidemiology revealed the co-circulation of two DENV-2 genotypes (Cosmopolitan and Asian genotypes) and lineages within each genotype over the last five years. Sequence comparison suggested multiple events of introductions of DENV-2 strains in the country from neighboring countries.

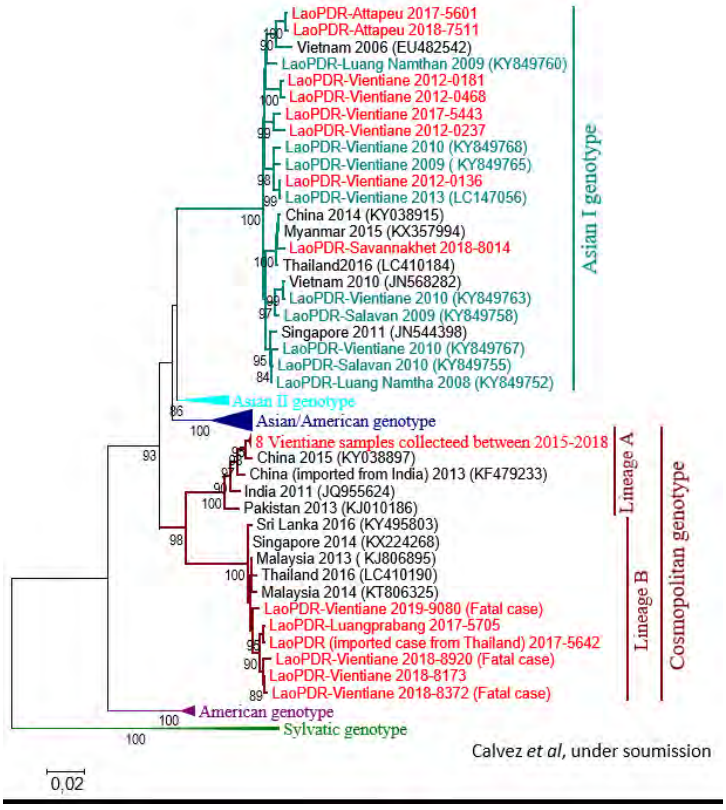


Figure n°7: Phylo-geography of Lao DENV-2 isolates determined by the genotype screening protocol

Dengue virus serotype 4 phylogeny

In 2013, the arbovirus surveillance network detected the emergence of DENV-4 in Vientiane Capital. In 2014-2015 the virus spread at the country level and DENV-4 became predominant in 2017-2018. In this study, we determined DENV-4 polymorphism observed in Vientiane Capital and at the country level.

For this purpose, we conducted a phylogenetic study on DENV-4 samples, isolated by the arbovirus surveillance system, during the major DENV-4 outbreak in Laos PDR between 2014 and 2018. The preliminary results demonstrated an introduction of the genotype II in 2014. However, since 2015, only genotype I was detected in Lao

Discriminant ELISA for typing Arbovirus antiobodies (DELTAa)

Seroprevalence studies were implemented to determine the level of exposure of different groups of the Lao population to arboviral diseases (cf BiolaoPlus project in Epidemiology section; Ecomore2). A major limitation in the interpretation of the data are the cross-reactions between and within antigenic complex. The gold standard to discriminate antibody cross reactivity is the plaque reduction neutralization test (PRNT). This technics is labour intensive and sometimes requires a significant volume of sample as it is required to test different virus in parallel to establish the interpretation thresholds. Only limited series of samples can be analyse at the same time. A new approach based on a modified indirect ELISA, based on antibody affinity, has been set up to overcome these obstacles. Preliminary comparison with PRNT showed that the DELTAa assay allow the discrimination of antibody reactivity at the antigenic complex level. Tests are now ongoing to evaluate how this approach may help to determine virus specific antibodies and could be apply for dengue virus serotyping. The DELTAa assay has been used for the screening of the samples collected to determine dengue virus seroprevalence in Vientiane Capital city. The level of herd immunity of the population in Vientiane against dengue and the burden of each serotypes are part of the information that will be used for the development of the outbreak simulator.

Blood samples were collected in 9 sentinel hospitals in Vientiane Capital between February to April 2019 (Figure n°8; Picture 2). A total of 1317 volunteers were included defining a cohort with a mean age of 30.1 years (min-max 3-88 years) and a sex ratio of 1.4 (F/M). Among them, 66.3 % (95% CI 63.4-68.5) displayed reactive IgG against dengue antigen using ELISA. The DENV seroprevalence increased progressively from 14.1% (95% CI 8.6-20.0) in children aged 3 to 6 years, to 64.7% (95% CI 59.7-69.8) among participants aged 19 to 30 years and finally 92.8% (CI 95% 82.6-96.1) among adults over 50 years.

When measuring the antibody affinity using the DELTAa assay with a threshold of 60%, the overall DENV seroprevalence rate felt to 21.3% (95% CI 29.4-34.5). Seroprevalence rates increased with age 2% (95% CI 0.6-6.3) among children aged 3 to 6 years to 20.2% (95% CI 25.8-35.5) in young adults (19 - 30 years) and was only 36.6% (CI 95% 43.6-56.4) among adults over 50 years. Dengue seroprevalence rate based on high antibody affinity was found to be limited in Vientiane Capital with a slight increase with age. This may explain at least in part, the permanent impact of dengue in Vientiane Capital city. The same type of approach is now ongoing to establish the seroprevalence rates for each serotypes.

Picture 2: Inclusion of outpatients by students of the Lao Tropical and Public Health Institute

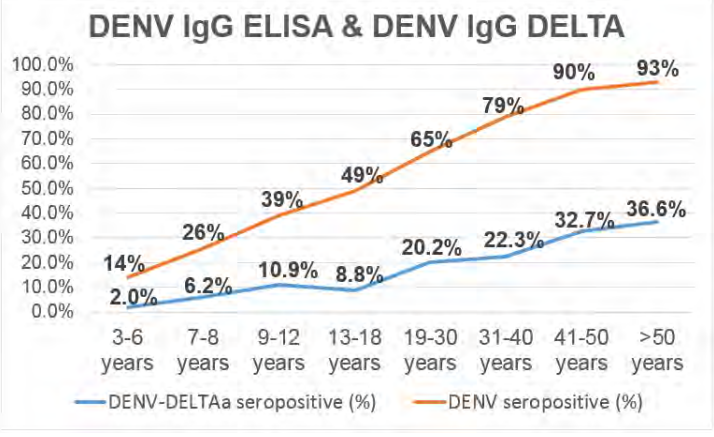


Figure n°8: Comparison of dengue seroprevalence measured by both conventional indirect ELISA and DELTAa assay

Training activities

- 2 days training on GIS in the frame of Ecomore 2 for IPL, NCLE and Xaysettha district hospital (Institut Pasteur du Laos, Marc Choisy; Olivier Telle)
- Arboshield project, year 3: 21 days of training for military staffs on health surveillance system (module 1), biosafety/biosecurity (module 2) and diagnostic of viral vector borne diseases (module 3).
- Co-supervision of a Lao master student (Palamy Changleuxai; Flinders University, Adelaide, Australia)
- Supervision of two master student of the Lao Tropical and Public Health Institute.

Education activities (Elodie Calvez)

- Arboshield program: 8 participants divided into 2 group (1 group per year) from Lao Army Institute of Preventive Medicine. In this course, six topics were described: Biosafety and biosecurity, Concentration and dilution, General biology, Molecular biology, Immunology, Pathogen vector transmission. This training is organized 1 hour per week since 2018.
- IPL staff training: Every week, IPL staff divided into two group participated to a course and different topic were presented like Concentration and dilution, General biology, Molecular biology, and Sequence analysis.
- Journal club organization: Every two weeks a journal club is organized with the IPL junior scientists. The aims of the journal club are to develop the ability of the junior scientist to research and analyse a publication and also to be able to present and explain the results.
- Implementation of the Biosafety e-learning (Kaptitude program): This new program was organized to increase the knowledge of the IPL laboratory but also maintenance staff in Biological and Chemical security.

Support to institutions

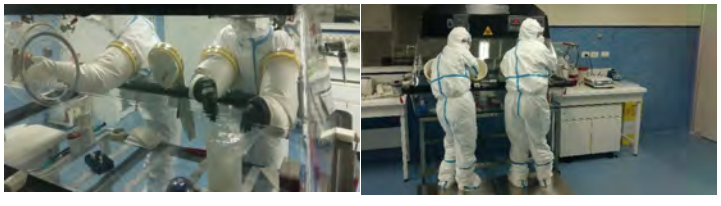
Table II: Biological material transfer activity in 2019

Consignee	Biological material
Institut Pasteur de Bangui	Antigens: Dengue; Chikungunya for IgM and IgG detection
Institut Pasteur de Côte d'Ivoire	Antigens: Dengue; Chikungunya for IgM and IgG detection

• A financial support has been provided to colleagues of the dengue surveillance network from Vientiane Capital, Salavan and Attapeu provinces to attend to the National dengue meeting in Champassack (10-11 December 2018).

• Ecomore2 project supported the development of alert and prevention application by staffs of Xaysettha district hospital by providing a smartphone and a computer. These staff were also invited to GIS training organized at the Institut Pasteur du Laos and Institut Pasteur du Cambodge.

• Following the international alert on Ebola launched by WHO in July 2019, the AEVD laboratory has been mandated by the Lao Ministry of health to reactivate laboratory capabilities for the first line investigation of Ebola suspected cases. Staffs of AEVD laboratory followed a refreshing training on management of biological samples suspected to contain BSL-4 pathogens (Picture 2). Several exercises were performed to check the reagents and the performances of the team.



Picture 2: Ebola training session in IPL BSL-3

Evaluation of new tools for arbovirus diagnosis

Surveillance activities performed by the AEVD laboratory over the last past years have generated a collection of nearly 15.000 human samples. The ethical agreements and the patients' inform consent allowed the AEVD laboratory to use the leftovers for research and to improve diagnosis on arboviral infections. The laboratory has been solicited by private companies and the Foundation for Innovative New Diagnostics (FIND) to perform evaluation studies of automated serological tests and lateral flow immunochromatographic assays (Picture 3). These requests concretize the level of expertise of the laboratory and its staff and offers a new source of funding to support IPL activities.

Picture 3: Evaluation a new dengue diagnostic tools

Scientific communications

• Somphavanh SOMLOR *et al.* "Institut Pasteur dengue surveillance network". Annual dengue surveillance meeting. December 11th 2018. (Oral communication).
• Somphavanh SOMLOR *et al.* Annual dengue surveillance meeting. "Dengue diagnostic and laboratory surveillance". December 11th 2018. Champasack, Lao PDR. (Oral communication).

- Somphavanh SOMLOR *et al.* "Chikungunya" Symposium - "Outbreak Preparedness and Readiness in the Greater Mekong Subregion" on May 28th and 29th 2019 in KunMing, Yunnan, China.(Oral communication).

- Elodie Calvez *et al,* "Genetic landscape of dengue virus serotype 2 in Vientiane Capital, Lao PDR". National Research Forum, Vientiane, October 17th, 2019 (Oral communication).

- Thonglakhone XAYBOUNSOU *et al.* "Kinetic of dengue virus cellular viremia in Lao patients". National Research Forum, Vientiane, October 17th, 2019 (Oral communication).

- Somphavanh SOMLOR *et al.* Dengue seroprevalence in Vientiane Capital, Lao PDR. National Research Forum, Vientiane, October 17th, 2019 (Oral communication).

- Somphavanh SOMLOR *et al.* Incidence of dengue infection in Lao teenagers in Vientiane Capital. Adolescent health research day Vientiane, October 15th, 2019 (poster).

Publications

- Elliott F. Miot, Elodie Calvez, Fabien Aubry, Stéphanie Dabo, Marc Grandadam, Sébastien Marcombe, Catherine Oke, James G. Logan, Paul T. Brey, Louis Lambrechts. Potential of the sylvatic mosquito *Aedes malayensis* to act as an arbovirus bridge vector in a forested area of the Nakai district, Laos". Parasites & Vectors. (submitted)

- Temmam S, Vongphayloth K, Hertz J, Sutherland I, Douangboubpha B, Grandadam M, Thomas Bigot T, Brey P, Eloit M. Six nearly-complete genome segments of a novel reovirus identified in Laotian batflies. 2019. Microbiology Resource Announcements (in press)

- Nouanthong P, Hübschen JM, Billamay S, Mongkhoune S, Vilivong K, Khounvisith V, Sinner R, Grandadam M, Phonekeo D, Black AP, Muller CP. Varicella zoster and fever rash surveillance in Lao People's Democratic Republic. BMC Infect Dis. 2019 May 8;19(1):392. doi: 10.1186/s12879-019-3990-7.

- Tangena JA, Marcombe S, Thammavong P, Chonephetsarath S, Somphong B, Sayteng K, Grandadam M, Sutherland IW, Lindsay SW, Brey PT. Bionomics and insecticide resistance of the arboviral vector *Aedes albopictus* in northern Lao PDR. PLoS One. 2018 Oct 25;13(10):e0206387. doi: 10.1371

Medical Entomology & Biology of Disease Vectors Laboratory *Lao-French joint Lab 2*

The main objective of our lab is to study the biology and ecology of arthropod vectors (mosquitoes, sandflies, ticks, etc.), as well as the transmission cycles of the viruses, parasites and other microbial pathogens they transmit. Furthermore, we are working on ways to mitigate vector borne disease transmission in Lao PDR via vector control training programs.

Head of Laboratory: Dr. Paul BREY, PhD

Scientists:
Khamsing VONGPHAILOTH, MD
Sébastien MARCOMBE, PhD

Junior Scientist:
Phoutmany THAMMAVONG, MD

PhD Student:
Elliott MIOT, MSc

Technicians:
Somsanith CHONEPHESARATH
Nothasine PHOMMAVANH
Kaithong LAKEOMANY
Somphat NILAXAY
Phonesavanh LUANGAMATH
Vaekey VUNGKYLY
Bouapha NGONETHACHACK
Inthava KATIYALATH
Kongphaeng SAIYALATH
Khanmany OUDOMSOUK

Projects

- ECOnomic Development, ECOsystem Modifications, and Emerging Infectious Diseases Risk Evaluation. Entomology work package.
- ARBOVEC-PLUS
- Inventory of cave-dwelling hematophagous insects in Laos
- PhD thesis: Potential of the mosquito *Aedes malayensis* as an arbovirus vector in South East Asia

Executive summary

As previous years the Medical Entomology and Vector-Borne disease laboratory has been very active. This year we have published eight articles in reputable international journals.

In 2019 the IPL entomology team hosted the International Medical Entomology Course of the Pasteur International Network for 15 high level students from: Laos, Cambodia, Vietnam Myanmar, France, New Caledonia, Slovenia, Iran, Tunisia and the Kingdom of Bhutan.

This year's annual report relates our scientific results on the ECOMORE 2 (Agence Français du Développement - AFD funded) This project explored *Aedes aegypti* and *Aedes albopictus* larval and adult populations throughout Vientiane Capital and analysed the usefulness of novel Pyriproxyphen-treated In2Care traps as a means of targeted *Aedes* vector control.

In addition, in the Arbovec Plus project we continued our collaboration with the US Naval Medical Research Center Asia (NMRC-A) in Singapore on insecticide resistance in *Aedes aegypti* and *Aedes albopictus* vectors from six provinces of Lao PDR, to validate the usefulness of novel molecular markers of insecticide resistance and finally to validate high-throughput PCR diagnostic tests to identify insecticide resistance in *Aedes aegypti.*

In the context of the National Geographic Project we carried out an inventory of Culicideae, Phlebotominae and Ceratapogonidae as well as their diversity density and population dynamics inside and around caves. This study was carried out to determine the dipteran vectors in caves where bats are living. With the increase in cave visits for tourists in Lao PDR it is requisite to have a better understanding of infectious emerging disease risk that these vectors represent.

Finally we carried out the Spillback project to assess the risk of Dengue Virus to migrate from urban and peri-urban environments to simian populations in forested areas of Lao PDR (75% of the country) via bridge vectors, such as *Aedes albopictus* and *Aedes malayensis*. The "spillback" of Dengue virus into Simian populations lead to the establishment of a forest cycle of Dengue in Lao PDR, as is already the case in Malaysia. We explore and test this hypothesis in our studies both in Laos Singapore and France.

ສະຫຼຸບການປະຕິບັດວຽກງານ

ຄືດັ່ງຫລາຍປີທີ່ຜ່ານມາ, ຫ້ອງວິເຄາະແມງໄມ້ວິທະຍາທາງການແພດ ແລະ ຊີ ວະວິທະຍາຂອງພະຍາດທີ່ມີແມງໄມ້ເປັນພາຫະ ແມ່ນຂະແໜງ ການຄົ້ນຄ້ວາ ທີ່ຫ້າວຫັນ. ໃນປີນີ້ພວກເຮົາໄດ້ມີການຕີພິມບົດຄົ້ນຄ້ວາແປດສະບັບ ໃນວາລະ ສານໃນລະດັບສາກົນ.

ໃນປີ 2019 ທີ່ຜ່ານມານີ້ ຂະແໜງແມງໄມ້ຂອງ ສະຖາບັນ ປັດສະເຕີ ລາວ ໄດ້ເປັນເຈົ້າພາບໃນການຈັດການຝຶກອົບຮົມກ່ຽວກັບແມງໄມ້ວິທະຍາທາງ ການແພດໃນລະດັບສາກົນ (the International Medical Entomology Course) ຂອງເຄືອຄ່າຍສະຖາບັນປັດສະເຕີນາໆຊາດ (the Pasteur International Network) ສຳລັບນັກຮຽນ 15 ຄົນ ທີ່ມາຈາກປະເທດ ລາວ, ກຳປູເຈຍ, ຫວຽດນາມ, ພະມ້າ, ຝລັ່ງ, ນິວກາເລໂດເນຍ, ສະໂລເວເນຍ, ອີລານ, ຕູນີເຊຍ ແລະ ບູຕານ.

ລາຍງານປະຈຳປີຂອງປີນີ້ແມ່ນກ່ຽວກັບຜົນທາງວິທະຍາສາດຂອງ ໂຄງການ ECOMORE 2 (ໃຫ້ທຶນໂດຍ Agence Fran ais du D veloppement - AFD). ໂຄງການນີ້ໄດ້ຄົ້ນຄ້ວາກ່ຽວກັບປະຊາກອນ ຫນອນນ້ຳ ແລະ ຍຸງລາຍ *Aedes aegypti* and *Aedes albopictus* ໃນນະຄອນຫລວງວຽງຈັນ ແລະ ສຶກສາວິເຄາະຜົນປະໂຫຍດການນຳໃຊ້ກັບ ດັກຍ້ອມຢາຊະນິດໃຫ່ມທີ່ເອີ້ນວ່າ Periproxyphen-treated In2Care traps ເປັນເຄື່ອງມືແນໃສ່ການຄວບຄຸມພະຫະຍຸງລາຍ.

ນອກນັ້ນ ໃນໂຄງການ Arbovec Plus project ຂອງພວກເຮົາຍັງໄດ້ສືບ ຕໍ່ຮ່ວມມືກັບສູນຄົ້ນຄ້ວາທາງການແພດກອງທັບເຮືອສະຫະລັດປະຈຳເອເຊຍ (the US Naval Medical Research Center Asia [NMRC-A]) ຢູ່ສິງກາໂປ ເພື່ອຕິດຕາມການຕ້ານຕ່ຳຢາຂ້າແມງໄມ້ຂອງພາຫະໄຂ້ເລືອດອອກ ໃນຫົກແຂວງຂອງລາວ, ເພື່ອສຶກສາຜົນປະໂຫຍດການນຳໃຊ້ເຄື່ອງໝາຍທາງ ພັນທຸກຳທີ່ກ່ຽວຂ້ອງກັບການຕ້ານຕ່ຳຢາຂ້າແມງໄມ້ ແລະ ສຸດທ້າຍແມ່ນເພື່ອ ແນ່ໃສ່ການນຳໃຊ້ເຕັກນິກ PCR ເພື່ອບົ່ງມະຕິການຕ້ານຕ່ຳຢາຂ້າແມງໄມ້ຂອງ ຍຸງລາຍ *Aedes aegypti.*

ກ່ຽວກັບເນື້ອໃນຂອງໂຄງການ National Geographic Project ແມ່ນພວກເຮົາໄດ້ສຳຫລວດຊະນິດພັນຂອງຍຸງ, ຮິ້ນ ແລະ ຮິ້ນຝອຍຊາຍ ລວມເຖິງຄວາມຫລາກຫລາຍ ແລະ ຈຳນວນປະຊາກອນຂອງພວກມັນຢູ່ໃນຖ້ຳ ແລະ ບໍລິເວນຖ້ຳ. ການສຶກສານີ້ແມ່ນເພື່ອຊອກຫາແມງໄມ້ພາຫະທີ່ຢູ່ໃນຖ້ຳທີ່ ມີເຈຍອາໄສຢູ່. ເນື່ອງຈາກວ່າຢູ່ລາວເຮົານັກທອງທ່ຽວເຂົ້າມາທອງທ່ຽວຖ້ຳມີ ຈຳນວນເພີ່ມຂຶ້ນມັນຈຶ່ງສຳຄັນທີ່ຈະຕ້ອງເຂົ້າໃຈຄວາມສ່ຽງກ່ຽວກັບພະຍາດທີ່ ອາດຈະເກີດຂຶ້ນໃຫ່ມທີ່ມີແມງໄມ້ເປັນພາຫະ.

ສຸດທ້າຍນີ້, ພວກເຮົາໄດ້ຈັດຕັ້ງປະຕິບັດໂຄງການທີ່ເອີ້ນວ່າ: ການກະຈາຍ ກັບຄືນສູ່ປ່າ (Spillback project) ເພື່ອປະເມີນຄວາມສ່ຽງຂອງໄຂ້ ເລືອດ ອອກໃນການກະຈາຍຈາກຕົວເມືອງໄປສູ່ເຂດຊົນນະບົດ ແລະ ໄປສູ່ປະຊາ ກອນລີງທີ່ຢູ່ໃນປ່າ (ປ່າກວມເອົາ 75% ຂອງປະເທດ) ຍ້ອນພາຫະທີ່ເປັນຂົວຕໍ່ເຊັ່ນ: ຍຸງລາຍ *Aedes albopictus* ແລະ *Aedes malayensis.* ການກະຈາຍກັບຄືນສູ່ປ່າ spillback ຂອງໄຂ້ເລືອດອອກ ໄປສູ່ປະຊາກອນລີງແມ່ນອາດສາມາດນຳໄປສູ່ວົງຈອນຊີວິດຂອງໄຂ້ເລືອດ ອອກໃນປ່າຂອງ ລາວ, ເຊິ່ງເຄີຍມີກໍລະນີມາແລ້ວຢູ່ປະເທດມາເລເຊຍ. ພວກ ເຮົາໄດ້ຄົ້ນຄ້ວາແລະທົດສອບສົມມຸດຕິຖານດັ່ງກ່າວຢູ່ທັງລາວ, ສິງກາໂປ ແລະ ຝຣັ່ງ.

ECOMORE II

ECOnomic Development, ECOsystem Modifications, and Emerging Infectious Diseases Risk Evaluation. Entomology work package.

Project coordinator:
Dr. Sebastien Marcombe
Dr. Paul Brey

Entomology field collection, identification, and insecticide resistance monitoring:
Dr. Sebastien Marcombe
Dr. Phoutmany Thammavong
Khaithong Lakeomany
Nothasine Phommavanh
Phonesavanh Luangamath
Numpherng Xayyavong
Somphat Nilaxay

Finance and administration
Dr. Antoine des Graviers
Phouvanhnamalee Vilaysouk

Description of the entomological work package

Mosquito surveillance, insecticide resistance, and innovative vector control method

Mosquito surveillance

The recent economic development of Laos, characterized by one of the highest rates of urbanization in the ASEAN community and the development of outer-urban zones where the density of population is combined with rural activities, offers ideal ecosystems for the proliferation of *Aedes* mosquitoes, specifically in Vientiane. An epidemiological study carried out in Vientiane since 2012 has shown the existence of dengue transmission hotspots in several areas of the city (Figure 1). It is important to know the dynamics of the mosquito populations during the dry and rainy seasons in these various localities. Indeed, it is crucial to understand the geography of *Aedes* mosquitoes regarding their environment in order to understand if risk factors are related to the *Aedes* index or—as suggested by other researches—to other factors. To do so, mosquito larval measurement (Breteau index and container index) will be carried out in different types of areas (created by GIS). The result of the surveillance will allow us to identify times and locations where

mosquito control would have the maximum impact on disease diffusion in the city (in central or rural spaces, during transmission or low transmission season, etc.). If other mosquito species are present during the *Aedes* surveillance, they will be collected and screened for arboviruses.

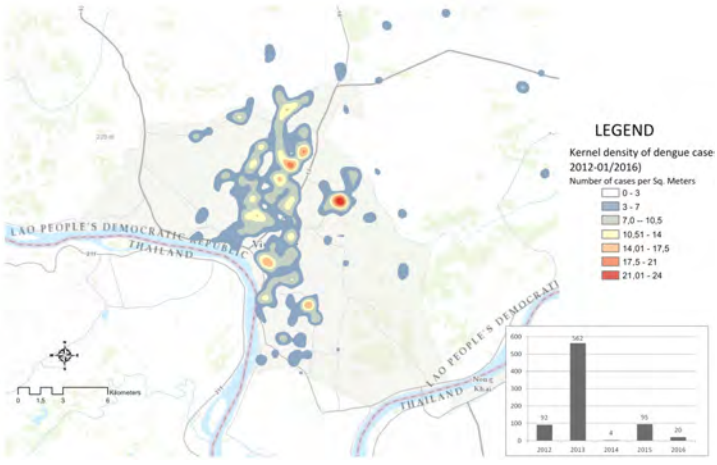


Figure 1: Kernel density of dengue cases in Vientiane, Laos (2012–01/2016).

Insecticide resistance

During inter-epidemic periods or when the elimination of mosquito breeding habitats is not easily achievable, insecticide application in larval habitats is routinely conducted by public health services in many countries, including the Lao PDR (Rawlins 1998, Rodriguez et al., 2002, Thavara et al., 2004). Space-spraying applications are conducted during epidemics or when the entomological indices of mosquitoes are high. For both larviciding and adulticiding, organophosphates and pyrethroids are the insecticide families of choice, worldwide and in the Lao PDR. Unfortunately, many dengue vector control programs are threatened by the development of insecticide resistance in *Aedes* populations across the world (Marcombe et al. 2009). Strong levels of resistance to organophosphates and pyrethroids have been detected in *Aedes aegypti* populations in Southeast Asia (Marcombe et al., 2009, 2014 ; Jirakanjanakit et al., 2007; Ranson, 2010; Kamgang et al., 2010 ; Vontas et al., 2012). The organophosphate temephos (larvicide) and insecticides from the pyrethroid family (permethrin, deltamethrin; adulticides) have been used in the Lao PDR PDR for decades and specifically during the last outbreak in 2013. Compared to its neighboring countries (Vietnam and Thailand), there was no information available on the resistance status of *Aedes* populations and the possible impact of the resistance on vector control operations in the country. A preliminary study led in 2015 by the Institut Pasteur du Laos showed insecticide resistance in *Aedes* populations from several provinces as well as in Vientiane (Marcombe et al. *in prep*). In view of these results, it is important now to know more precisely the distribution and resistance levels of the populations of vectors in Vientiane in order to propose alternative strategies. Resistance to conventional insecticides (temephos, deltamethrin, permethrin, and malathion) were tested on *Aedes aegypti* and *Ae. albopictus* larvae and adults collected in Vientiane. Distribution maps of the resistance by species and at larval/adult stages will be drawn up and shared with stakeholders to improve the efficiency of the vector control campaign.

Innovative vector control method

In recent years, a number of new tools to combat vectors have been developed and present an interesting potential to replace or strengthen the current expensive and laborious methods of larvicide treatments and fumigation. Furthermore, the actual efficiency of these methods is often discussed (Marcombe et al. 2012). Among these tools, the method described as "auto-dissemination" presents an innovative technique for the treatment of breeding sites. It consists of using female mosquitoes themselves as carriers of an insecticide, the pyriproxyfen (insect growth regulator), and to make them transfer the insecticide to the larval breeding sites in urban areas (Figure 2). The potential of this technique and this insecticide was proved in small-scale trials in Peru, Italy, and Madeira (Guess et al., 2009; Caputo et al., 2012) and is in development on other sites. We suggest in this project studying the efficacy of this method to control vectors in the specific urban environment of the city of Vientiane.

Figure 2. In2Care® mosquito trap.

Objectives

- Define the dynamics of vectors
- Organize an active entomological surveillance in representative districts to document the annual dynamics of the populations of vectors
- Set up a systematic entomological balance sheet of the housing environment of the confirmed cases and possibly the other possible sites of contamination to identify active zones of transmission
- Measure the levels of insecticide resistance of the *Aedes* populations against conventional insecticides used in Vientiane to recommend the appropriate methods of vector control to the authorities
- Estimate innovative strategies of vector control by measuring their efficacy on the populations of mosquitoes to propose alternatives to the conventional methods
- Investigate the possibility of alternative vectors.

Methodology

Entomological surveillance

After selection of 4 sentinel sites (Figure 3), vector density is surveyed during the dry and rainy season. Adults and larvae were trapped by 2 Ovitraps and 2 BG sentinel trap inside and outside the houses. Traps are checked weekly and shipped to IPL entomology unit where larvae are reared to adults for taxonomy and resistance testing and adult mosquitoes are identified and thus kept at -80oC for arboviruses screening.

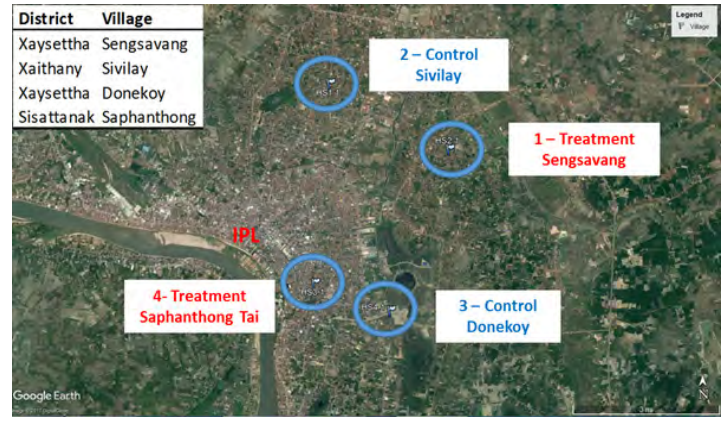


Figure 3. Location of sites for entomological surveillance

Insecticide resistance

Larval bioassays on the *Aedes* sp. populations were run with diagnostic dose (WHO recommended) of deltamethrin (0.00132 mg/L), permethrin (0.0032 mg/L), temephos (0.0132 mg/L) and DDT (0.04 mg/L). Mortality was recorded after 24h. Following WHO criteria a population is considered resistant if the mortality after 24 h is under 90%, resistance is suspected with mortality between 90 and 98% and a population is susceptible with mortality over 98%.

Adult bioassays were run using filter papers treated with diagnostic doses of deltamethrin (0.05%), permethrin (0.25%), DDT (4%), and malathion (0.8%) following a WHO protocol. Mortality resulting from tarsal contact with treated filter papers was measured using WHO test kits on adult mosquitoes of the different populations. Four batches of 25 non-blood-fed females (2–5 days of age) were introduced into holding tubes and maintained for 60 minutes at 27 ± 2°C and a relative humidity of 80 ± 10%. Insects were then transferred into the exposure tubes and placed vertically for 60 minutes under subdued light. Mortality was recorded 24 hours after exposure. Mortality was recorded after 24h. Following WHO criteria a population is considered resistant if the mortality after 24 h is under 90%, resistance is suspected with mortality between 90 and 98% and a population is susceptible with mortality over 98%.

Innovative vector control method

The new tool is the In2Care® Mosquito Trap that attracts and kills *Aedes* females with ingredients that target both mosquito larvae and adults. It is the first to exploit the concept of 'auto-dissemination', resulting in an effective kill of mosquito larvae in breeding sites surrounding the trap. In2Care® Mosquito Traps can be placed both indoors and outdoors at a recommended density of 1/400m2 (10 traps per acre) and be maintained every 4-6 weeks using refill sachets. The Trap uses a combination of a US-EPA approved entomopathogenic fungus that kills the adult mosquitoes, coupled with a WHO-recommended juvenile growth hormone mimic (Pyriproxyfen (PPF)) that is specific to diptera and kills larvae. The In2Care® mosquito trap has been validated in field studies (Snetselaar et al. 2014). The female mosquitoes are attracted to the trap to lay eggs (oviposition). Once in the trap they become contaminated with fungal spores and PPF. She will then fly off with both spores and PPF to find oviposition sites and upon laying her eggs will contaminate the water with PPF. The PPF will then kill the developing larvae. The female herself will eventually die from the fungal infection (Figure 2).

In2Care® Mosquito Trap field study in Vientiane

The field trial study started in July 2018 and two villages were selected as treatment sites (Sensavang and Saphanthong tai) and two other villages were selected as control (Donekoy and Sivilay, Figure 5). We coordinated the vector control campaigns with the vector control units of the different district and villages of Vientiane in order to avoid any interferences in our study sites. Dengue transmission rates (confirmed cases/dominant serotype) and mosquito abundance (larval/adult traps/indexes) was measured before and, during the interventions to estimate the efficacy of the new vector control method. The traps were maintained every 6 weeks (water and insecticide refill) and until august 2019. The water from 5 traps in each villages is sampled and brought back to the laboratory and *Ae. aegypti* larvae (3rd instar) are exposed to this water and the mortality was evaluated.

Results

Vector surveillance

Figure 4 shows the abundance of *Aedes* larvae in in the 4 villages and Figure 4 shows the number of adult collected in the BG-Sentinel traps in Vientiane. For both, adult and larval abundance, the results showed that the abundance pattern over time follow the dry/rainy season model (i.e. high abundance during season and vice-versa) and show that is these locations the mosquito vectors are present throughout the year. Data anylasis are currently being implemented to show the efficacy of the new technology on the number of mosquito collected.

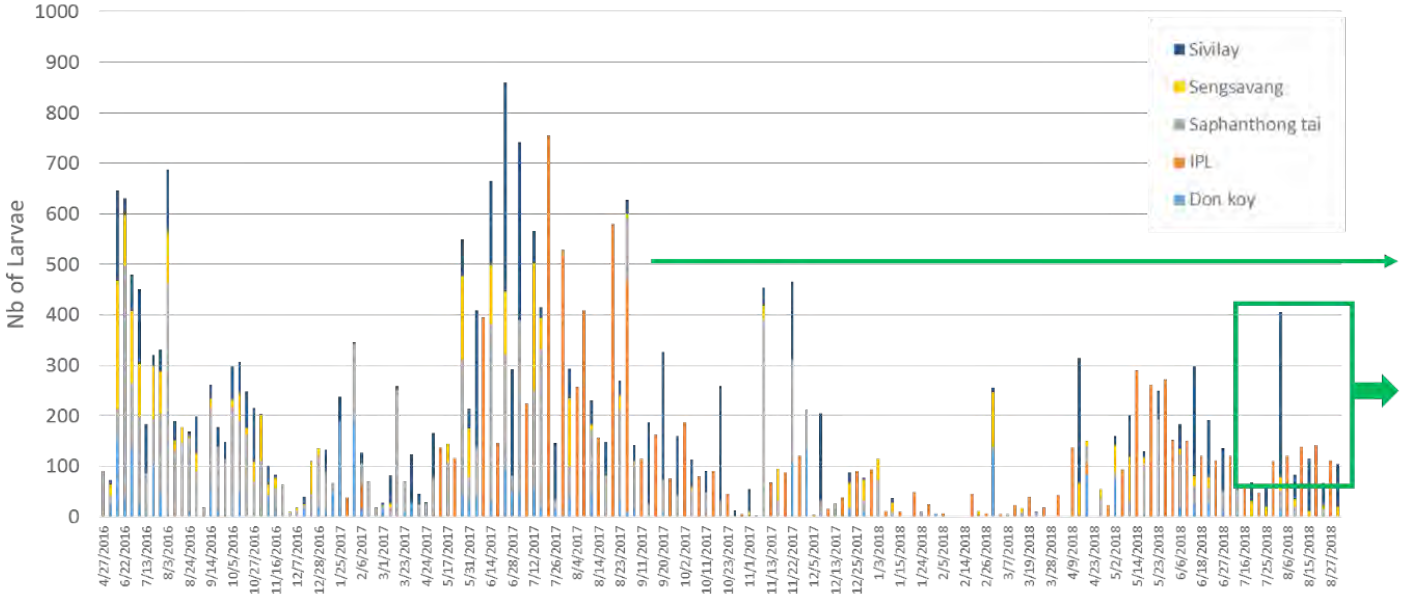


Figure 4. Abundance of *Aedes* sp. larvae in Vientiane, Laos

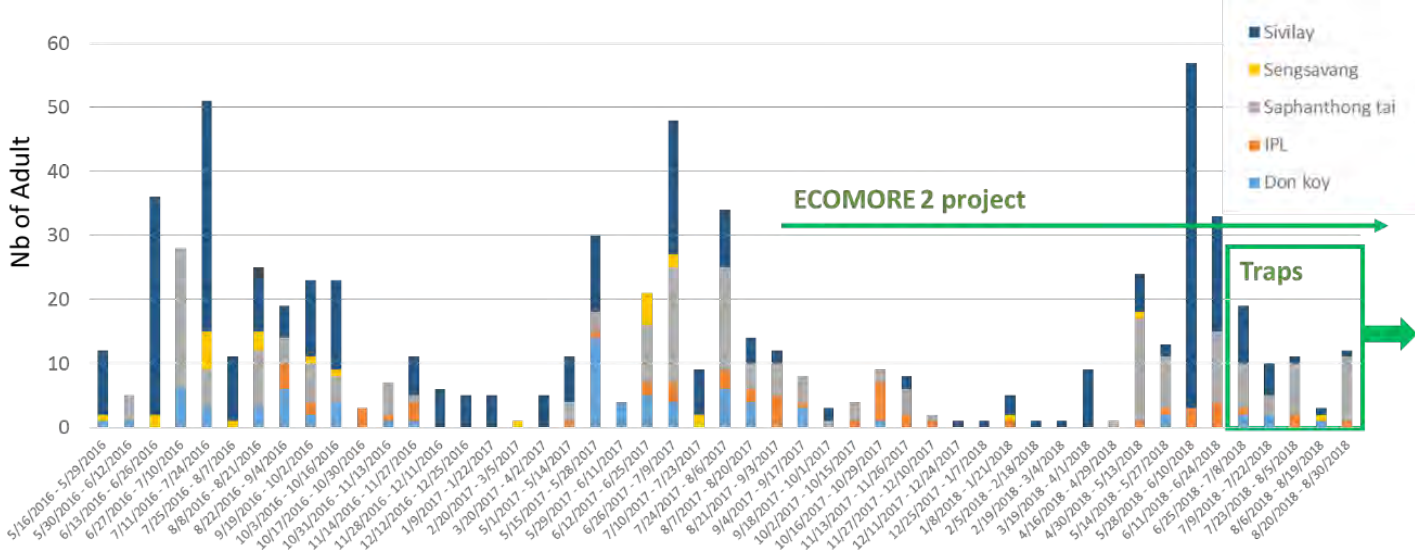


Figure 5. Abundance of *Aedes* sp. adult in Vientiane, Laos

Insecticide resistance

The results of the larval bioassays are presented in Figure 7. Larval *Ae. aegypti* and *Ae. albopictus* mosquito populations from 4 villages in Vientiane Capital were tested. Two larval *Ae. aegypti* populations tested were highly resistant to deltamethrin, permethrin and DDT. All larval populations were resistant to temephos except the population from Sivilay village (100% mortality). *Aedes aegypti* adult populations from 4 villages were tested. All the populations tested were highly resistant to all the insecticides tested. The results showed that larval *Aedes albopictus* mosquito populations from Sengsavang and IPL were resistant and suspected resistant, respectively. Both populations were resistant to DDT. The populations from Sengsavang was susceptible to deltamethrin and suspected resistant to permethrin. The IPL population was susceptible to permethrin and suspected resistant to deltamethrin. The results of the adult bioassays showed that the 2 *Aedes albopictus* mosquito populations were highly resistant to the organochlorine DDT and the organophosphate malathion. Both populations were susceptible to deltamethrin and were susceptible and /or suspected resistant to permethrin.

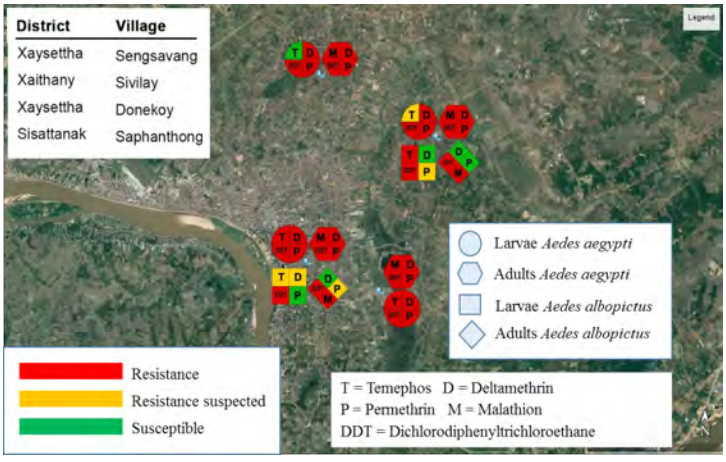
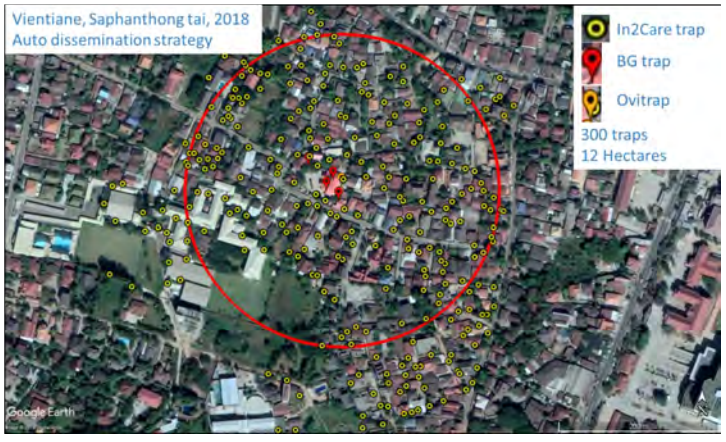
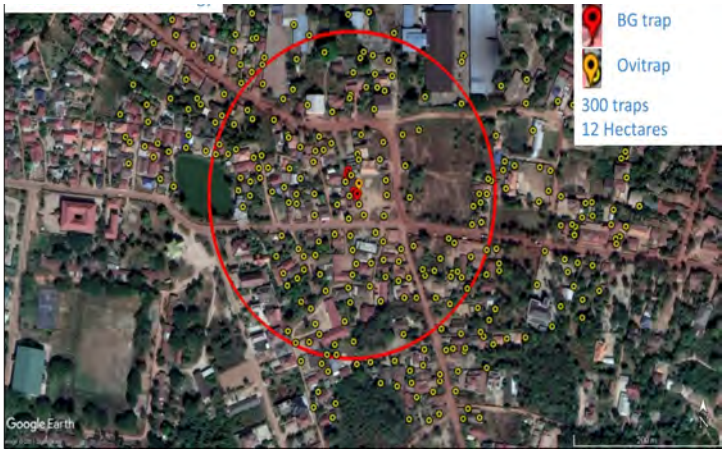


Figure 7. Insecticide resistance distribution in dengue vectors in Vientiane, Laos.

In2Care® Mosquito Trap field study in Vientiane

Figures show the distribution of the auto-dissemination traps in Saphanthong tai and Sensavang villages, respectively. The large scale field trial was finished in August 2019 and results will be presented next year.





The pictures show the team (IPL staff, Vientiane districts health officers, village volunteers and Lao military staff)

Financial support

This work was supported by the Agence Française de Développement.

ສະຖາບັນ ປັດສະເຕີ ລາວ
INSTITUT PASTEUR DU LAOS

CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE

ກະຊວງ ສາທາລະນະສຸກ
Ministry of Health

ARBOVEC-PLUS

Project coordinator:
Dr. Sebastien Marcombe
Dr. Paul Brey

Entomology field collection, identification, and insecticide resistance monitoring:
Dr. Sebastien Marcombe
Dr. Phoutmany Thammavong
Khaithong Lakeomany
Nothasine Phommavanh
Phonesavanh Luangamath
Numpherng Xayyavong
Vaekey Vungkyly
Somphat Nilaxay
Somsanith Chonephetsarath

Finance and administration
Dr. Antoine des Graviers
PhouvanhnamaleeVilaysouk

Background

This 2-year project stems from analysis of data related to the ARBOVEC project implemented in Laos from April 2014 to August 2015. Insecticide resistance levels in 10 *Ae. aegypti* populations from 5 provinces were measured following WHO protocols. Several larval populations showed moderate resistance to the organophosphate insecticide temephos and high resistance to the pyrethroid deltamethrin. Bioassays performed on adult mosquitoes confirmed the presence of pyrethroid and organophosphate resistance with some populations being resistant to permethrin and malathion but also pointed out high resistance to the organochlorine DDT.

In 2013, Lao PDR has faced one its most severe dengue outbreak in decades (>50,000 cases and 100 death; WHO 2013). The most recent outbreak was in 2017 with 11,000 cases reported, with a predominance of mostly DENV serotype 4. The mosquitoes *Ae. aegypti* and *Ae. albopictus* are the main dengue virus vector in Laos. Because there is still no vaccine or specific treatment available against this virus, vector control remains the only strategy for reducing dengue transmission. Effective vector control measures rely on active community participation, health education programs, and environmental management (Erlanger et al., 2008). During inter-epidemic periods or when the elimination of breeding habitats of the mosquito is not easily achievable, insecticide application in larval habitats is routinely conducted by public health services in many countries including Laos (Rawlins 1998, Rodriguez et al., 2002). Space spraying applications are conducted during epidemics or when the entomological indices of mosquitoes are high. For both larviciding and adulticiding, organophosphates and pyrethroids are the insecticide families of choice against dengue vectors worldwide and in Laos.

Unfortunately, many dengue vector control programs are now threatened by the development of insecticide resistance in *Aedes sp.* populations worldwide. Insecticide resistance is associated with mutations in the sequence of the target proteins (target-site resistance, kdr mutation for pyrethroids and DDT), and/or an increase activity of

particular detoxification enzymes leading to an increase biodegradation of insecticides by resistant mosquitoes (metabolic-based resistance). Metabolic-based resistance usually involves several large enzyme families called "detoxification enzymes" including cytochrome P450 monooxygenases (P450s), glutathione S-transferases (GSTs) and carboxy/cholinesterases (CCEs). A recent study performed by Faucon et al. (2015; http://genome.cshlp.org/content/25/9/1347) used a cutting-edge next generation sequencing approach for identifying novel molecular markers of metabolic resistance in *Ae. aegypti.* This study identified several gene Copy Number Variations (CNVs), Single Nucleotide Polymorphisms (SNPs) and differentially expressed genes (DE genes) strongly associated with resistance in populations from south-east Asia and showed that these novel resistance markers will well complement current target-site markers (*kdr* mutations) for monitoring the different resistance mechanism in natural populations and improving vector control strategies.

Indeed, during the 2013 dengue outbreak in Lao PDR, more than $300,000 of temephos (Abate® formulation) were bought by the government without knowing the insecticide resistance status of *Aedes* sp. populations. Until the recent ARBOVEC project (NMRHC-A funding, 1 year project at IPL, April 2014-August 2015) and compared to its neighboring countries there was barely no information available on the resistance status of dengue vectors populations in Laos. We now know that resistance levels to organophosphate and pyrethroid insecticides are high in several provinces and that metabolic based resistance and kdr mutation are likely involved (Dr. Marcombe, ARBOVEC project) and investigations on the specific metabolic mechanisms involved in the resistance at the gene level are now needed.

In this frame, the screening and validation of novel resistance markers will be implemented in resistant vector populations of several regions of Lao PDR representing various environmental characteristics which could influence the development of particular mechanisms. Covering a large part of Laos will allow us to develop tools able to detect a large variety of resistance markers in various settings.

The validation and implementation of novel resistance markers will reduce the cost and the charge of the resistance monitoring compared to the usual techniques and will allow monitoring the spatial and temporal dynamic of resistant alleles/genes in the field in order to manage resistance locally.

Objectives

- Determine the resistance levels of *Aedes aegypti* mosquitoes from six provinces of Laos against conventional insecticides.
- Validate the usefulness of novel molecular markers of insecticide resistance, recently identified in *Ae. aegypti,* in Laos.
- Validate high-throughput PCR-based diagnostic tests to identify insecticide resistance in *Ae. aegypti.*

Methods

Mosquito collections

Mosquito collections were implemented in 12 provinces in 2017-2019 (Figure1).

Figure 1. Map showing selected provinces for insecticide resistance monitoring in Laos.

Morphological mosquito identification

For all the mosquito populations collected, larvae were reared until adults (F1 generation; Figure 1). After adult identification, mosquitoes obtained were separated by species and location. Only *Aedes aegypti* and *Ae. albopictus* were kept for breeding. Females mosquitoes were then blood fed using quail and the eggs obtained were kept for the larval and adult bioassays.

Insecticide resistance status

We tested the susceptibility of *Aedes aegypti* mosquitoes to a range of insecticides representative of those historically and currently used for mosquito control in Lao PDR (ie DDT, temephos, malathion, deltamethrin and permethrin). Larval and adult bioassays were be performed following WHO guidelines (WHO 2005, 2006).

Larval bioassays on *Ae. aegypti* were performed using late third- and early fourth-instar larvae of the field strains. For each bioassay, larvae of each strain were transferred to cups containing 99 mL of distilled water and 1 mL of the insecticide tested at the desired concentration. Larval bioassays on the *Ae. aegypti* and *Ae. albopictus* populations were run with diagnostic doses (WHO recommended) of deltamethrin (0.00132 mg/L), permethrin (0.014 mg/L), temephos (0.02 mg/L) and DDT (0.04 mg/L). Mortality was recorded after 24h. Following WHO criteria, a population is considered resistant if the mortality after 24 h is under 90%, resistance is suspected with mortality between 90 and 98% and a population is susceptible with mortality over 98%.

Adult bioassays were run using filter papers treated with diagnostic doses of deltamethrin (0.05%), permethrin (0.25%), DDT (4%), and malathion (0.8%) on *Ae. aegypti* and *Ae. albopictus* populations. Mortality resulting from tarsal contact with treated filter papers was measured using WHO test kits on adult mosquitoes of the different populations. Four batches of 25 non-blood-fed females (2–5 days of age) were introduced into holding tubes and maintained for 60 minutes at 27 ± 2°C and a relative humidity of 80 ± 10%. Insects were then transferred into the exposure tubes and placed vertically for 60 minutes under subdued light. Mortality was recorded 24 hours after exposure.

Characterization of genetic markers for insecticide resistance in *Ae. aegypti* from Laos

To validate the usefulness of novel resistance markers (CNVs, SNPs and DE genes) identified in Faucon et al (2015) a composite population being representative of Lao PDR was built by pooling F1 eggs obtained from previously colonized resistant populations (10 *Ae. aegypti* populations from the ARBOVEC project). This composite population (named Metalao) was sent to the LECA-CNRS laboratory and was colonized for future resistance mechanisms studies. A composite population of *Ae. albopictus* from 5 provinces was built at IPL for further insecticide resistance and molecular analysis. This composite population (named MetalaoAlbo) was sent to the LECA-CNRS laboratory (February 2018)

and is being characterized for insecticide resistance phenotypes and mechanisms.

Experimental set-up

The *Aedes aegypti* composite population was established from various populations collected in Laos by the Institute Pasteur du Laos. Two generations (G1 and G2, without selection) were done in the laboratory to allow the mosquitoes to acclimatize to the conditions of the insectarium. Four generations of selection were done (from G3 to G6) with three different insecticides: deltamethrin and permethrin (pyrethroid family), and malathion (organophosphate), to segregate the resistant alleles. For every generations, 32 lots of 30 mosquitoes were exposed to lethal doses of these insecticides (60 to 80 % mortality maximum of the individuals). The first lineage (not selected) was raised in parallel without selection pressure. Four pools of 15 females were exposed to insecticides from G2 to estimate mortality rates (48 hours after exposure) during the selection.

Ten pools of 5 females and 30 individual females were sampled before the selections (G2-NS for not-selected) and among the survivors of the 4th generation of selection (G6-deltamethrin, G6-permethrin, G6-malathion). Females of the first lineage maintained without selection in G6 (G6-NS) were sampled for the study. Besides, mosquitoes from the reference insecticide susecptible strain (Bora) were used as reference compared to the composite population.

DNA extraction

DNA extraction was performed using the CTAB method (cetyltrimethylammonium bromure). Mosquitoes were crushed in 250 µL CTAB 2%, then heated up to 65°C during 10 minutes before adding 250 µL of chloroform. After 5 minutes of centrifugation at 12000 RPM, 200 µL of the superior aqueous form containing the DNA were sampled and mixed with isopropanol. After centrifugation, isopropanol was removed and 200 µL of ethanol 70% was added. Ultrapure water was then added to the DNA (60 µL for pools and 30 µL for single specimen. DNA was dosed with a fluorimeter (Qubit®) and dilutions were done to obtain the same DNA quantity for each samples (0,1 ng/µL).

Kdr mutations genotyping

Kdr mutations were genotyped by qPCR with specific primers to compare the allelic frequencies of the non-selected specimen compared to the insecticide selected ones after few generations. The following codons were amplified: F1534C (Phenylalanine → Cysteine) and V1016G (Valine → Glycine) (Brengues *et al.*, 2003).

The qPCR method used was described by Saavedra-Rodriguez et al. (2007). The mosquito genotype was determined by using meltcurves temperatures (resistant homozygote RR at 85,5°C, or homozygote sensible SS at 81°C for 1534 / 78,5°C for 1016), and 2 pics for these two temperatures for heterozygote RS.

CNV detection

Faucon *et al.* (2015) showed that several gene cluster duplications were present in Ae. aegypti genome. The following genes, belonging to different clusters, were chosen for the detection of CNVs: CYP9J28, CYP6Z-like, CYP6Z8, CYP6BB2, GSTE2 and, CCEAE3A. Detection of the CNVs was done by qPCR with specific primers.

The gene copy numbers was made with the $\Delta\Delta C_t$ relative method: the quantification of each interest genes was normalized by using the quantification of domestic genes identified in the genome by Faucon *et al.* (2015). These data were then normalized with the average of the CNVs obtained in the reference strain (Bora) to obtain DNA relative quantity between susceptible and resistant mosquito pools.

Results

Insecticide resistance bioassays

The results of the adult and larval bioassays are presented in Figure 2. Larval *Aedes aegypti* and *Ae. albopictus* mosquito populations from Xayaboury, Bokeo,

Luang Namtha, Borlikhamxay, Khammouane and, Champasak
provinces were tested. All larval *Ae. aegypti* populations tested were moderately resistant to temephos. Most of the *Ae. albopictus* were resistant to temephos.

All the adult populations tested were highly resistant to the organochlorine DDT. All the *Ae. aegypti* populations tested against permethrin (pyrethroid family) presented high levels of resistance. Most of the *Ae. aegypti* mosquito populations were resistant or suspected resistant to deltamethrin, which is also an insecticide from the pyrethroid family. Only three populations, from Xayaboury, Vientiane Capital and Luang Prabang provinces showed full susceptibility to this insecticide (100% mortality). All the populations tested against malathion, an insecticide from the organophosphate family, were resistant to this insecticide (mortality<90%). Only one population, from Xayaboury province showed full susceptibility to this insecticide (100% mortality).

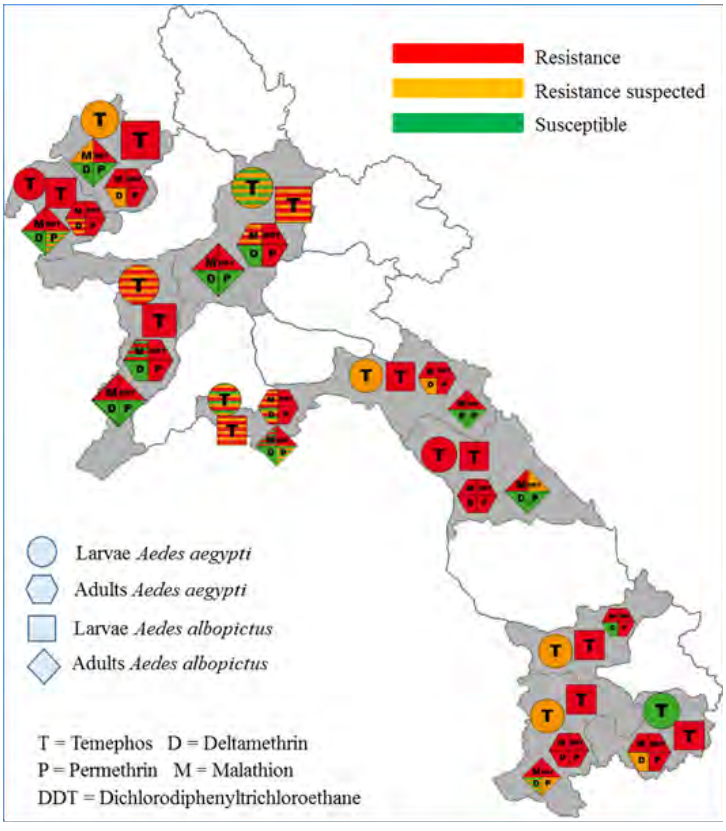


Figure 2. Insecticide resistance distribution of dengue vectors in Laos. (This map also shows result from the ARBOVEC project, see previous IPL annual reports for

Characterization of genetic markers for insecticide resistance in *Ae. aegypti* from Laos (LECA - CNRS)

A composite population of *Ae. aegypti* from 10 provinces was built at IPL for insecticide resistance and molecular analysis. This composite population (named MetaLao) was sent to the LECA laboratory and was characterized for insecticide resistance. The composite population was resistant to temephos to deltamethrin and DDT and resistance was suspected to permethrin (Table 1).

Table 1. Resistance status of *Aedes aegypti* composite population (MetaLao) on larvae to temephos, deltamethrin, permethrin and, DDT.

Insecticide	Concentration	n	% Mortality	Status
temephos	0.0132mg/l	100	86	Resistant
deltamethrin	0.00132mg/l	100	89	Resistant
permethrin	0.0032mg/l	100	97	Suspected
DDT	0.04mg/l	100	11	Resistant

Table 2. Resistance status of *Aedes aegypti* composite population (MetaLao) on adults to deltamethrin, permethrin, malathion and, DDT.

Insecticide	n	% Mortality	Status
deltamethrin	103	96.1	Suspected
malathion	98	93.9	Suspected
permethrin	100	90	Suspected
DDT	100	40	Resistant

Diagnostic doses of deltamethrin (0.05%), permethrin (0.25%), DDT (4%), and malathion (0.8%) were used.

At LECA laboratory, further to the reception of the *Ae. aegypti* metapopulation from Laos, a generation without insecticide selection was made to obtain sufficient number of adult for the future selections. In parallel, preliminary tests were realized on the same generation to define times of exposures for each of the insecticides tested here: deltamethrin (0.05 %), malathion (5 %) and permethrin (0.75 %). The objective was to define a time of exposure corresponding to a lethal dose between 60 and 80min for females only. Up to now, six generations of selection were realized (F2 to F6). The objective was to realize at least 4 generations of selection.

For each generation, 32 pools of 30 mosquitoes (male and female) were exposed to 20 min for deltamethrin, 10 min for malathion and 25 min for permethrin. For each of the selections, the mortality rates were measured independently of the selection by doing 4 pools of 20 individuals (female only).

Mortality rates during selection

For every generation of selection, the mortality rates were measured for all the insecticides. For F2, the percentages of dead mosquitoes were calculated according to the insecticides to which they were exposed: 90 % for malathion, 85 % for permethrin and 70 % for deltamethrin. A significant reduction in the mortality, showing an effect of the selection, was observed for all the lineages from G5 (P<0,05). For G6, the mortality rates were 52 % for malathion, 40 % for deltamethrin and 20 % for the permethrin.

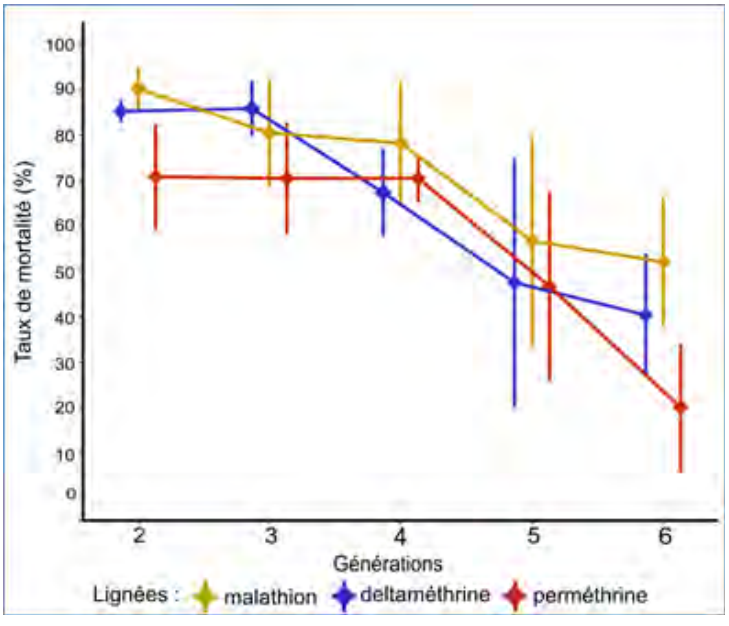


Figure 3: Mortality rates of mosquitoes to the different insecticide over the generations (Fisher test: P<0,05 * compared with G2).

Cross resistance data from insecticide-selected lines

We previously showed that selecting a composite *Aedes aegypti* population from Lao PDR with different insecticides (deltamethrin, permethrin and malathion) lead to a significant increase of resistance to each insecticide in only 4 generations, suggesting that resistance alleles to these insecticides are circulating in Lao PDR.

To assess the specificity of the resistance phenotypes observed in each selected-line, we then performed comparative bioassays on each selected line with all insecticides. Bioassays consisted in exposing adults females from each line (not selected, deltamethrin-selected, permethrin-selected, malathion-selected) to a diagnostic dose of each insecticide and recording mortality rate 24h later.

These comparative bioassays revealed that the resistance observed for both deltamethrin-selected and malathion-selected lines are highly specific while permethrin-selected line also displays some cross-resistance with other lines (Figure 4).

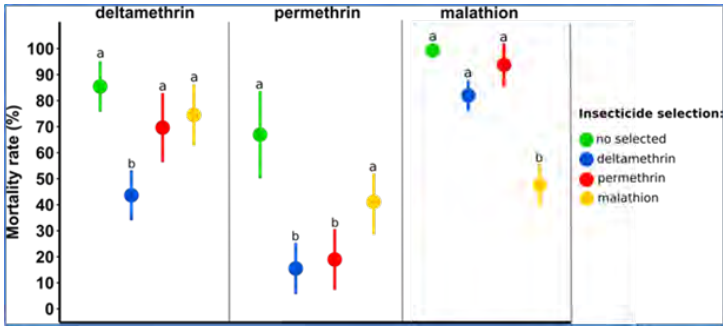


Figure 4. Cross resistance test. A GLMer (binomial family) was performed to analyze these data. Each selected-line is identified by a different color. Different letters indicate significant mortality differences between lines for each insecticide tested.

Genotype frequency of *kdr* mutations during election

The evolution of the resistant allelic frequencies of the two kdr mutations was measured. In G2, the 1016 resistant mutation allele frequency was 0.13 % (Figure 5). The frequency increased significantly (0.4%) in G6 when mosquitoes were selected with deltamethrin (P<0.001) and permethrin (P<0.01). In the same time, the frequency of the resistant allele slightly increased for the lineage selected with malathion, and decreased significantly for the non-selected lineage (P<0.05).

For G2, the resistance allelic frequency for the codon 1534 was 0,6. This frequency then remain stable: no significant difference with the composite population of departure was measured.

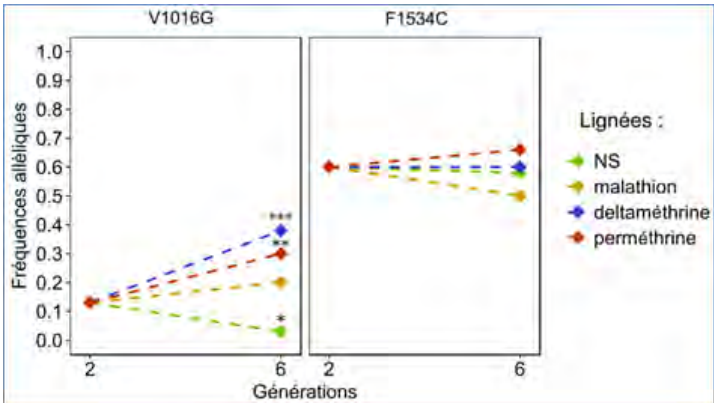


Figure 5: Allelic frequency of *kdr* mutations (V1016G and F1534C) for the different lineages (Fisher test: P<0.001 ***; P<0.01 **; P<0.05 *). NS: Not Selected.

These results were also studied in the haplotypes form (Figure 6). A disappearance of the susceptible homozygotes (SS / SS) was observed in G6 with deltamethrin exposure and with permethrin. In the same time, a strong increase of the number of resistant allele individuals for every mutation (RS / RS) was measured: 29 % compared with G2-NS for permethrin, and 38 % for deltamethrin.

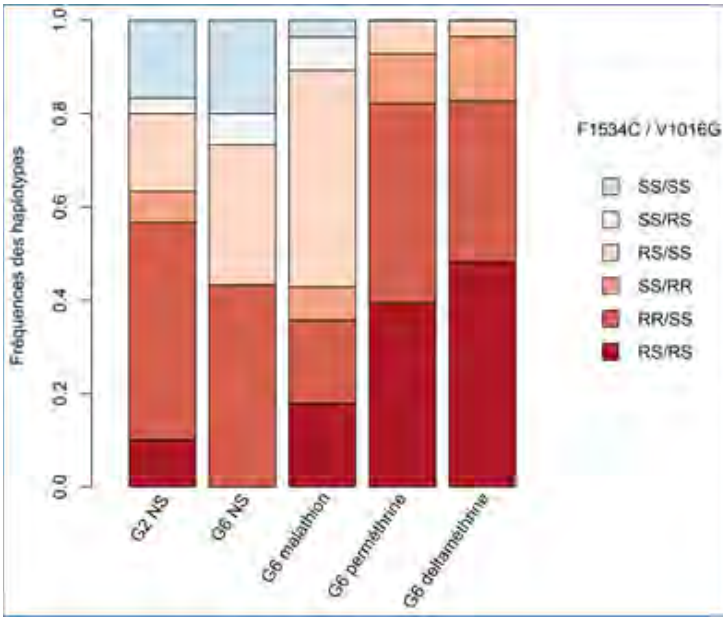


Figure 6: Cumulated frequencies of *kdr* mutations haplotypes (F1534C and V1016G).

Detection of Copy number variations (CNV) associated with resistance

To know if the Lao composite population have already showed genomic increases for the chosen markers, the quantity of DNAg was compared between G2-NS and the susceptible Bora strain. It was significantly superior in G2-NS for the genes CCEAE3A (P<0.05), CYP6BB2 (P<0.01), and GSTE2 (P<0.01), which showed the presence of duplications (Figure 7).

The quantities of DNAg of G6 of each of the lineages were then compared with those of G2-NS. A single difference was significant, the G6-malathion for the gene CCEAE3A, in which the quantity of DNAg four times superior to that measured in the G1 population (P<0.01). No other significant increase was noted after selection, even by including the genes where there was no effect in G2-NS.

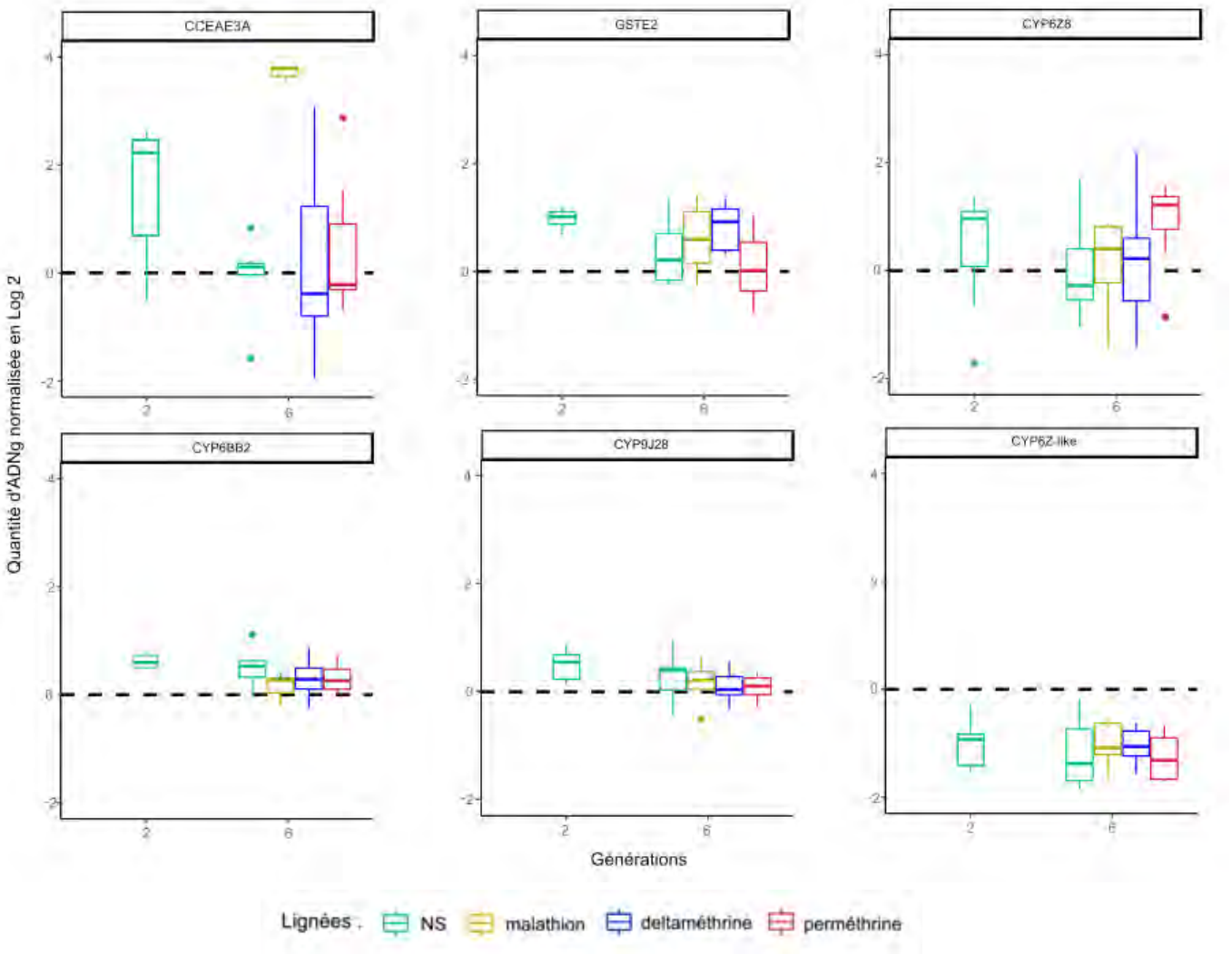


Figure 7: DNAg relative quantity variation for each gene. The black line represent the average CNV for the susceptible Bora strain.

Previous attempts to identify CNV associated with resistance in each selected line by qPCR was pushed further in order to confirm prior results and to enlarge the panel of markers by targeting other potential genes of interest.

These experiments allowed to confirm the strong association between the genomic amplification of the carboxylesterase CCEAE3A and malathion resistance. By designing novel primers to target other neighboring esterase genes we showed that the esterase AAEL019678 is also specifically amplified in the malathion line (Fig 8A). A potential CNV signal was also detected for the esterase CCEAE1A (also in the same cluster) but with a strong polymorphism, suggesting that the amplification of this esterase cluster is probably not extending to this gene in most individuals of the malathion-selected line (Fig 8A).

We also targeted two glutathione-S transferase genes, GSTE4 and GSTE6 but no signal of CNV were identified for any selected-line (Fig 8B).

Finally, in attempt to identify CNV markers of pyrethroid resistance in Lao PDR, we targeted additional P450 genes (CYP genes) belonging to two CYP6 and CYP9 clusters previously associated with resistance in other geographic regions. This included the genes CYP6CC1 and CYP6P12 in the CYP6 cluster (Fig8C) and genes AAEL014614 and CYP9J15 in the CYP9 cluster of CYP9J (Fig 8D). However, qPCR data confirmed the absence of CNV affecting these genes in any selected-line, suggesting that Kdr mutations remain the best DNA markers of pyrethroid resistance in Lao PDR.

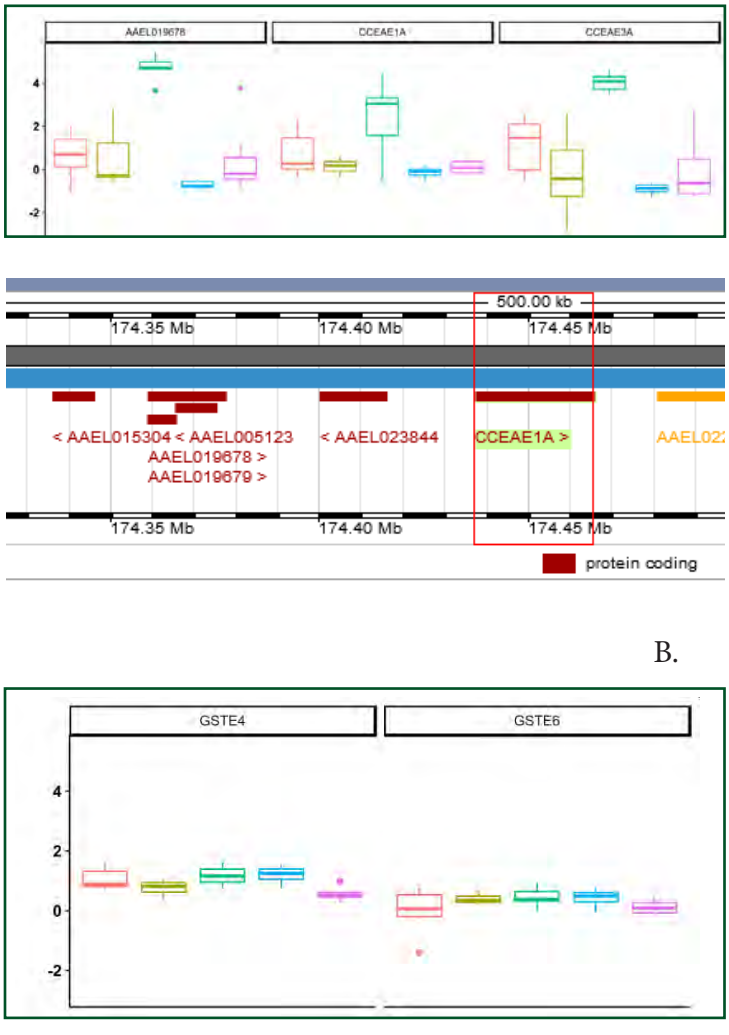


Figure 8. Normalized gDNA quantity for each gene between each line. G2 NS: Lao composite population before insecticide selection; G6 NS: Lao composite population not selected with any insecticide for 5 generation, G6 delta/Mal/Perm: Selected-lines originating from G2 NS but having selected with a given insecticide (deltamethrin, malathion and permethrin respectively) for 4 generations.

Developing a high-throughput molecular assay for detecting esterase amplification in natural populations (LECA - CNRS)

Besides the identification of novel DNA markers of resistance, the project also aimed at developing new resistance diagnostic tools to track insecticide resistance in the field. In this frame, we are currently trying to develop a PCR-based assay allowing to genotype the "presence/absence" of the esterase amplification mentioned above in individual mosquitoes. If our primer design strategy is successful, such PCR assay should be positive in presence of the genomic amplification and negative in absence of amplification (see figure 8).

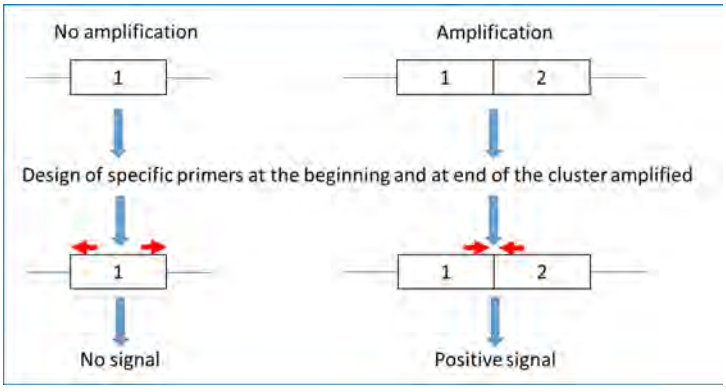


Figure 8. Primer design strategy adopted for developing a new PCR assay to genotype the presence/absence of a genomic amplification associated with insecticide resistance.

Currently, we are analyzing genome sequencing data previously obtained from an Ae. aegypti population from Thailand also carrying this esterase gene amplification at high frequency in order to identify the best primer design for this PCR assay. More precisely, we are using both read coverage and reads status to precisely identify the breakpoints (boundaries) of this large genomic amplification. Based on these NGS data, a first set of PCR primers have been ordered and will be tested on mosquitoes from Lao and Thailand in October-November 2018. If our PCR assay is validated, we will then use it to genotype the presence of this esterase gene amplification in individual mosquitoes from various field populations previously collected by IPL in Lao PDR.

Partner

Laboratoire d'Ecologie Alpine, LECA,UMR UGA-USMB-CNRS 5553, Université Grenoble Alpes.

Financial support

This work is supported by the NMRC-Asia.

Publications related to ARBOVEC and ARBOVECPLUS projects

MarcombeS,TangenaJAA,,ThammavongP,Chonephetsarath, Sompong B, Xayteng K, Grandadam M, Sutherland IW, Lindsay SW, Brey PT. Bionomics and insecticide resistance of the arboviral vector *Aedes albopictus* in northern Lao PDR. 2018. PLoS ONE 13(10): e0206387.

Marcombe S., Khounsombat K., Hertz J, Sutherland I, and Brey PT. Alternative insecticides for larval control of the dengue vector *Aedes aegypti* in Lao PDR: insecticide resistance and semi-field trial study. 2018. Parasites & Vectors 2018, 11:616.

Marcombe S, Fustec B, Cattel J, Chonephetsarath S, Thammavong P, et al. Distribution of insecticide resistance and mechanisms involved in the arbovirus vector *Aedes aegypti* in Laos and implication for vector control. December 2019. Accepted in Plos Neglected Tropical Diseases.

M. Motoki, D. Fonseca, Ian W. Sutherland, J. Hertz, PT Brey and S. Marcombe. Population genetic structure of *Aedes albopictus* from east and South-East Asia. December 2019. Accepted in Parasites & Vectors.

Marcombe S, …, Brey PT and Jones A. Malaria and Dengue mosquito vectors from Lao PDR show a lack of the rdl mutant allele responsible for cyclodiene insecticide resistance. 2019. Accepted in Journal of Medical Entomology.

Draft. Marcombe S and Brey PT. Insecticide resistance status of the dengue vector *Ae. albopictus* of Lao PDR and mechanisms involved (2020).

Draft. M. Motoki, J. Hertz, PT Brey and S. Marcombe. Population genetic structure of *Aedes aegypti* from Lao PDR (2020).

Draft. J. Cattel, S Marcombe, PT Brey and JP David. Characterization of genetic markers for insecticide resistance in *Ae. aegypti* from Laos (2020).

Inventory of cave-dwelling hematophagous insects in Laos

Project coordinator:
Dr. Khamsing Vongphayloth; under supervisor of Dr. Paul Brey, Director, Institut Pasteur du Laos, Vientiane, Lao PDR

Staff members:
Khaithong Lakeomany
Nothasine Phommavanh
Phonesavanh Luangamath

Funding support: National Geographic Society: Exploration Grant

Duration: 1 year (2019)

Background and Relevance

Vector-borne diseases (VBDs) were the second cause of emerging infectious disease (EID) events after zoonotic pathogens (1). Over the past decades, there has been significant emergence and reemergence of several vector-borne diseases such as malaria, leishmaniasis, dengue, Yellow fever, Zika, Chikungunya and plague (2-4). The emergence of new and resurgence of old known vector-borne pathogens can be associated with several factors including adaptation and change of microorganism, habitat changes, globalization, tourism and travel etc. (5, 6).

Cave ecosystems are specific environment conditions that provide suitable place for insects and harbor many different opportunist pathogens, such as viruses, bacteria and fungi (7, 8). Many of these pathogens infect cave-dwelling vertebrates, especially bats (9-13). Some of them could be transmitted from one vertebrate host to another by hematophagous arthropod vectors (e.g. mosquitoes, sandflies, batflies and biting midges) (14-16). Human and animals are risk to exposure to the arthropod vectors in caves and in the surrounding areas.

In South East Asia (SE-Asia), cave visiting is more and more popular for many reasons, such as for resource gathering by the local populations or economic purposes, such as ecotourism or spiritual purposes (cave dwelling monks). Therefore, such growing human incursion into caves may increase the risk of exposure and spillover of emerging pathogens that circulate among cave-dwelling vertebrates. The recent discovery in humans of the monkey parasite *Plasmodium knowlesi* stands out as an excellent example of the potential transfers of animal parasites to humans (17, 18). SE-Asia is a known hotspot of biodiversity. However, very little is known about cave-dwelling hematophagous insects that are medically important, such as mosquitoes (Culicidae), sandflies (Phlebotominae) and biting midges (Ceratopogonidae).

To date, eight mosquito species are known to be true troglodytes, two species are known to enter caves, and two others have been found at the entrances of caves (19). Little is known about mosquito species in caves in Laos.

Before 1990s, SE-Asia, was considered as a region without autochthonous transmission of leishmaniasis hence there is a paucity of knowledge on phlebotomine sandflies. A total of 27 species of sandflies have been identified in Thailand with widespread distribution, high density and diversity inside caves (20-23). *Se. gemmea* and *Se. barraudi*, were suspected as the vector of *Leishmania siamensis* in southern part of Thailand (24). In Laos, only one cavernicolous species of sandfly, Chinius genus, was reported by N. Léger et al (25), while two species of the *Phlebotomus* genus and five species of the *Sergentomyia* genus were reported by L. Quate (26). From our preliminary results of SandMap project of the Institut Pasteur du Laos (IP-Laos) in 2015 (Khammouane Province),

From our preliminary results of SandMap project of the Institut Pasteur du Laos (IP-Laos) in 2015 (Khammouane Province), showed that at least 20 different groups of sandfly females were classified using their morphological characters (unpublished data). Conversely, nothing is known about biting midges in caves in Laos. Sixty-six species of Culicoides were reported in Laos (27). To our knowledge, data on the diversity of cavernicolous insects of medical or veterinary interest are very limited. Moreover, almost nothing is known about their biology, population dynamics and community structure. Thus, to fulfill this gap, we would like to investigate mosquitoes, sandflies and biting midges in caves in Laos. Our hypothesis is that density and species richness of cave-dwelling insects in Laos are high, similar in each cave from different provinces, and related to environmental factors, especially inside humidity, temperature and outside rainfall.

Goals and Objectives

Inventory of Culicidae, Phlebotominae and Ceratopogonidae will help to address the knowledge gap in their diversity, density and population dynamic in caves and areas near caves in Laos in relation with relevant environmental parameters, particularly cave humidity, temperature and external rainfall. The specific objectives are as following: 1) to describe density and species richness of cave-dwelling insects in Laos, 2) to investigate the cave similarity in terms of species composition and density, and 3) to investigate the relationships between inside humidity, temperature, monthly rainfall (chosen as environmental variables) and the variations of insect density and diversity indices during the study period.

Methodology

Field collections

Mosquitoes, sand flies and biting midges were collected inside caves and outside caves alongside karstic limestone mountains (peri-karstic caves) that are located in the Vientiane Province. Three districts of karstic limestone mountains were selected: Fueng District (18°30'N, 101°59'E), Hin Heup District (18°46'N, 102°16'E), and Vang Vieng District (18°58'N, 102°20'E). Among three districts, Vang Vieng is well known for cave exploration with increasing numbers of tourists visiting each year. It is one of the top tourism destinations in the central part of Laos. To obtain baseline data on the diversity and population dynamics of hematophagous insects in karstic caves and peri-karstic cave areas, two or three caves from each district were selected. One-week surveys were conducted at the selected field sites (Table 1 for more detail).

Table 1: Field collection sites and dates

District	Month of collection	Latitude	Longitude	Season
Feung	May 2019	18°30'N	101°59'E	Dry
	Oct 2019			Rainy
Hin Heup	Feb 2019	18°46'N	102°16'E	Dry
	Sep 2019			Rainy
Vang Vieng	Jan 2019	18°58'N	102°20'E	Dry
	Jul 2019			Rainy

Collection procedure

Collections were conducted two times in each site, during the dry season (approximately November to April) and rainy season (May to October), to maximize collection yield and observe potential temporal patterns. In each selected location, standard collection methods using CDC light traps were used for hematophagous insect collection inside caves, and near cave areas between 4–6 p.m. and 8–9 a.m for 6-7 nights. Climate data were also recorded by Easy data logger.

All insects were feezed at −20 °C for 20–30 minutes. Specimens were stored at 70% ethanol, and then transported to the IP-Laos laboratory in Vientiane capital for morphological identification.

Specimen preparation and identification

Mosquitoes were identified under stereo-microscope using related identification keys (28-33). Mosquito specimens were pinned and deposited at the Insect Arthropod Collection Room of the IP-Laos. For the sandfly and biting midge specimens, head, wings, and abdomen genitalia of both sexes were cut under stereomicroscope using sterile needles. Head, wings, and genitalia were mounted on slides using PVA mediums and morphological identification under compound-microscope using related morphological identification keys. Sandflies were morphologically identified using dichotomous keys of Lewis and other related references (26, 34, 35). Biting midges were tentative identified using the keys and illustrations of Ratanaworabhan (36) and Howarth (37). Both sandfly and biting midge sample slides will be provided to Dr. Jerome Depaquit at the Université de Reims Champagne-Ardenne for quality assurance and deposited the Arthropod Collection Room of the IP-Laos.

Preliminary results

Density of Diptera assemblages

A total of 21,518 arthropod hematophagous insects were collected from both inside and outside caves, of which 20,518 were sandflies, 1,216 were mosquitoes and 95 were possibly biting midges. . In all sampled districts, sandflies were predominant (Table1) with the apparent density (number of specimens collected per trap and per day) of 118.35 in Fueng, 52.24 in Hin Heup and 22.79 in Vang Vieng. The density of sandflies was slightly higher in dry season, except in Vang Vieng district. Overall the density of mosquitoes was low in all districts, with a value of 3.91 and was lower in dry season than rainy season (Table 2).

Table 2: Total number of samples collected by district

District	Culicoides		Mosquitoes		Sandflies		Total	
	n	%	n	%	n	%	n	%
Feung	57	0.26	417	1.94	12,427	57.75	12,901	59.95
Hinheup	14	0.07	469	2.18	5,433	25.25	5,916	27.49
Vangvieng	24	0.11	330	1.53	2,347	10.91	2,701	12.55
Total	**95**	**0.44**	**1,216**	**5.65**	**20,207**	**93.91**	**21,518**	**100**

Table 3: Density of arthropod hematophagous insects (number of specimens collected per trap and per day) collected by season

Site/Season	Ceratopogonidae (Biting midges)	Culicidae (Mosquitoes)	Phlebotominae (Sandflies)	Total
Feung				
Dry season	1.16	2.69	160.39	164.24
Rainy season	0.00	5.09	81.57	86.66
Total	**0.54**	**3.97**	**118.35**	**122.87**
Hin Heup				
Dry season	0.08	2.98	59.06	62.13
Rainy season	0.18	5.86	46.39	52.43
Total	**0.13**	**4.53**	**52.24**	**56.90**
Vang Vieng				
Dry season	0.00	2.66	5.64	8.30
Rainy season	0.45	3.74	38.96	43.15
Total	**0.23**	**3.21**	**22.79**	**26.23**
Total	**0.30**	**3.91**	**64.77**	**68.98**

Inventory and diversity of Diptera assemblages

A total of 567 mosquitoes was quickly screen to species so far, around 40 species from 12 genera was suspected identified, many species reported here still need to confirm identification (Table 4). Uranotaenia was the most abundant genus followed by Culex spp.

A total of 3,183 sandflies were cut and morphological identification, at least 17 species were suspected identified from 4 genera: Chinius, Idiophlebotomus, Phlebotomus and Sergentomyia. Sergentomyia was the most abundant genus (1,862/3,183).

Table 4: Diversity of mosquitoes collected by district

Species	District			
	Fueng	Hin Huep	Vang Vieng	Total
Aedes	8		18	26
Ae. albopictus	5		17	22
Ae. gardnerii	2			2
Ae. sp			1	1
Ae. malikuli?	1			1
Anopheles	1	2	41	44
An. aconitus			1	1
An. barbirostris			2	2
An. gardnerri			1	1
An. indefinitus			4	4
An. jamesis			3	3
An. kochi			11	11
An. minimus		2	5	7
An. sp	1		6	7
An. vagus			8	8
Armiegres	3	5	18	26
Ar. subalbatus	2		3	5
Ar. inchoatus		1	2	3
Ar. Kesseli		4	10	14
Ar. kuchingensis	1		3	4
Bothaella			4	4
Bo. eldridgei			2	2
Bo. helenae			2	2
Culex	65	8	72	145
Cx. baiyi			2	2
Cx. nigropunctatus	4	1	42	47
Cx. phangngae			4	4
Cx. quinquefasciatus		4	3	7
Cx. vishnui	56	1	14	71
Cx. scanioni?		1		1
Cx. sp		1	5	6
Cx. gelidus	5			5
Cx. bitaeniorliynetus			2	2
Downsiomyia			2	2
Do. novonivae			1	1
Do. sp			1	1
Heizmannia			3	3
Hz. Macdonaldi?			2	2
Hz. Sp			1	1
Malaya		5	8	13
Malaya sp		5	8	13
Mansonia			2	2
Mansonia			2	2
Phagomyia			2	2
Ph. lophoventralis			2	2
Tripteroides			2	2
Tr. caeruleocephalus			2	2
Uranotaenia	43	131	124	298
Ur. macfarlanei			1	1
Ur. rampae	1		1	2
Ur. sombooni	18		1	19
Ur. metatarsata	5	126	119	250
Ur. obscura?			1	1
Ur. macfarlonei	17	4		21
Ur. sp		1	1	2
Ur. lateralis	1			1
Ur. hebes	1			1
Total	120	151	296	567

Table 5: Diversity of sandflies collected during our study

Genus/Species	Sex		
	Female	Male	Total
Sergentomyia			
Se. anodontis	166		166
Se. bailyi	48		48
Se. barraudi	257		257
Se. hivernus	368		368
Se. indica	60		60
Se. iyengari	146		146
Se. khawi	164		164
Se. sp.	69	531	600
Se. sp. near hivernus	1		1
Se. sp. near tambori ?	1		1
Se. sp. near to barraudi	51		51
Sergentomyia Total	1,331	531	1,862
Phlebotomus			
Ph. argentipes	1		1
Ph. baurguesae	1		1
Ph. betisi	24		24
Ph. mascomai	67		67
Ph. sp.	41	200	241
Ph. sp. near argentipes	1		1
Ph. sp. near kiangsusis	33		33
Ph. sp. near macomia	1		1
Ph. sp. near major mojor	100		100
Ph. stantoni	155	14	169
Phlebotomus Total	424	214	638
Chinius			
C. eunicegalatiae	452	117	569
Chinius Total	452	117	569
Idiophlebotomus			
I. sp	49	65	114
Idiophlebotomus Total	49	65	114
Grand Total	2,256	927	3,183

Discussion

This is the first study of cave-dwelling blood sucking insect in Laos, where the data on the diversity of cavernicolous insects of medical or veterinary interests are very limited. Overall, 21,518 arthropod hematophagous insects were collected from both inside and outside caves, of which 20,518 were sandflies, 1,216 were mosquitoes and 95 were possibly biting midges.

So far, around 40 species from 12 mosquito genera and at least 17 species of 4 sandfly genera: Chinius, Idiophlebotomus, Phlebotomus and Sergentomyia suspected identified. More than 8 species of mosquitoes and sandflies are suspected to be new records for Lao PDR. We will continue to work on identification confirmation. The identification of biting midges is now ongoing. More detail analysis on density and species richness; cave similarity in term of species composition and density; and the relationships between inside humidity, temperature, and monthly rainfall (chosen as environmental variables) and the variations of insect density and diversity indices will be performed and submitted to an international journal (Entomology).

Works Cited

1. Jones KE, Patel NG, et al. Global trends in emerging infectious diseases. Nature. 2008;451(7181):990-3.
2. Chan JF, Choi GK, et al. Zika fever and congenital Zika syndrome: An unexpected emerging arboviral disease. The Journal of infection. 2016;72(5):507-24.
3. Gratz NG. Emerging and resurging vector-borne diseases. Annual review of entomology. 1999;44:51-75.
4. Vasconcelos PF, Calisher CH. Emergence of Human Arboviral Diseases in the Americas, 2000-2016. Vector borne and zoonotic diseases. 2016;16(5):295-301.
5. Harrus S, Baneth G. Drivers for the emergence and re-emergence of vector-borne protozoal and bacterial diseases. International journal for parasitology. 2005;35(11-12):1309-18.
6. Dash AP, Bhatia R, et al. Emerging and re-emerging arboviral diseases in Southeast Asia. Journal of vector borne diseases. 2013;50(2):77-84.
7. Obame-Nkoghe J, Leroy EM, et al. Diversity and role of cave-dwelling hematophagous insects in pathogen transmission in the Afrotropical region. Emerging microbes & infections. 2017;6(4):e20.
8. Jurado V, Laiz L, et al. Pathogenic and opportunistic microorganisms in caves. Int J Speleol. 2010;39(1):15–24.
9. Konstantinov OK, Diallo SM, et al. [The mammals of Guinea as reservoirs and carriers of arboviruses]. Meditsinskaia parazitologiia i parazitarnye bolezni. 2006(1):34-9.
10. Gomez-Hernandez C, Bento EC, et al. Leishmania infection in bats from a non-endemic region of Leishmaniasis in Brazil. Parasitology. 2017;144(14):1980-6.
11. Maganga GD, Bourgarel M, et al. Bat distribution size or shape as determinant of viral richness in african bats. PloS one. 2014;9(6):e100172.
12. McKee CD, Kosoy MY, et al. Diversity and phylogenetic relationships among Bartonella strains from Thai bats. PloS one. 2017;12(7):e0181696.
13. Anh PH, Van Cuong N, et al. Diversity of Bartonella spp. in Bats, Southern Vietnam. Emerging infectious diseases. 2015;21(7):1266-7.
14. Kamani J, Baneth G, et al. Bartonella species in bats (Chiroptera) and bat flies (Nycteribiidae) from Nigeria, West Africa. Vector borne and zoonotic diseases. 2014;14(9):625-32.
15. Adam JP. [Transmission of Hemosporidia by Anopheles Cavernicolus in the Caves of the Congo (Brazzaville)]. Bulletin of the World Health Organization. 1965;32:598-602.
16. Grard G, Fair JN, et al. A novel rhabdovirus associated with acute hemorrhagic fever in central Africa. PLoS pathogens. 2012;8(9):e1002924.
17. Maeno Y, Culleton R, et al. Plasmodium knowlesi and human malaria parasites in Khan Phu, Vietnam: Gametocyte production in humans and frequent co-infection of mosquitoes. Parasitology. 2017;144(4):527-35.
18. Singh B, Daneshvar C. Human infections and detection of Plasmodium knowlesi. Clinical microbiology reviews. 2013;26(2):165-84.
19. Harbach RE, Taai K. Nyctomyia biunguiculata, a new cavernicolous species of tribe Aedini (Diptera: Culicidae) from southern Thailand. Zootaxa. 2014;3895(3):427-32.
20. Apiwathnasorn C, Samung Y, et al. Cavernicolous species of phlebotomine sand flies from Kanchanaburi Province, with an updated species list for Thailand. The Southeast Asian journal of tropical medicine and public health. 2011;42(6):1405-9.
21. Apiwathnasorn C, Sucharit S, et al. A brief survey of phlebotomine sandflies in Thailand. The Southeast Asian journal of tropical medicine and public health. 1989;20(3):429-32.

22. Polseela R, Apiwathnasorn C, et al. Seasonal variation of cave-dwelling phlebotomine sandflies (Diptera:Psychodidae) in Phra Phothisat Cave, Saraburi Province, Thailand. The Southeast Asian journal of tropical medicine and public health. 2007;38(6):1011-5.
23. Polseela R, Depaquit J, et al. Description of Sergentomyia phadangensis n. sp. (Diptera, Psychodidae) of Thailand. Parasites & vectors. 2016;9:21.
24. Chusri S, Thammapalo S, et al. Animal reservoirs and potential vectors of Leishmania siamensis in southern Thailand. The Southeast Asian journal of tropical medicine and public health. 2014;45(1):13-9.
25. Leger N, Depaquit J, et al. Chinius eunicegalatiae n. sp. (Diptera; Psychodidae), a cavernicolous sandfly from Laos. Annals of tropical medicine and parasitology. 2010;104(7):595-600.
26. Quate LW. A REVIEW OF THE INDO-CHINESE PHLEBOTOMINAE. PACIFIC INSECTS. 1962;4(2):251-67.
27. Howarth FG. Biosystematics of the Culicoides of Laos (Diptera: Ceratopogonidae). International Journal of Entomology. 1985;27:1–96.
28. Rattanarithikul R, Harbach RE, et al. Illustrated keys to the mosquitoes of Thailand. VI. Tribe Aedini. The Southeast Asian journal of tropical medicine and public health. 2010;41 Suppl 1:1-225.
29. Rattanarithikul R, Harbach RE, et al. Illustrated keys to the mosquitoes of Thailand V. Genera Orthopodomyia, Kimia, Malaya, Topomyia, Tripteroides, and Toxorhynchites. The Southeast Asian journal of tropical medicine and public health. 2007;38 Suppl 2:1-65.
30. Rattanarithikul R, Harrison BA, et al. Illustrated keys to the mosquitoes of Thailand. IV. Anopheles. The Southeast Asian journal of tropical medicine and public health. 2006;37 Suppl 2:1-128.
31. Rattanarithikul R, Harrison BA, et al. Illustrated keys to the mosquitoes of Thailand III. Genera Aedeomyia, Ficalbia, Mimomyia, Hodgesia, Coquillettidia, Mansonia, and Uranotaenia. The Southeast Asian journal of tropical medicine and public health. 2006;37 Suppl 1:1-85.
32. Rattanarithikul R, Harbach RE, et al. Illustrated keys to the mosquitoes of Thailand. II. Genera Culex and Lutzia. The Southeast Asian journal of tropical medicine and public health. 2005;36 Suppl 2:1-97.
33. Rattanarithikul R, Harrison BA, et al. Illustrated keys to the mosquitoes of Thailand I. Background; geographic distribution; lists of genera, subgenera, and species; and a key to the genera. The Southeast Asian journal of tropical medicine and public health. 2005;36 Suppl 1:1-80.
34. Lewis DJ. A Taxonomic Review of the Genus Phlebotomus (Diptera, Psychodidae): British Museum (Natural History); 1982.
35. Lewis DJ. The phlebotomine sandflies Diptera: Psychodidae of the Oriental Region1978.
36. Ratanaworabhan NC. An illustrated key for the genera of Ceratopogonidae (Diptera) of the world 1969.
37. Howarth FG. Biosystematics of the Culicoides of Laos (Diptera: Ceratopogonidae). International Journal of Entomology. 1985;27:1-96.
38. Shannon CE. The mathematical theory of communication. 1963. MD computing : computers in medical practice. 1997;14(4):306-17.
39. Chao A. Estimating the population size for capture-recapture data with unequal catchability. Biometrics. 1987;43(4):783-91.
40. Magurran AE. Ecological diversity and its measurement. Princeton, N.J.: Princeton University Press; 1988.
41. Magurran AE. Measuring Biological Diversity New York, NY: John Wiley & Sons; 2013. Available from: http://nbn-resolving.de/urn:nbn:de:101:1-2014122012826.

PhD thesis: Potential of the mosquito *Aedes malayensis* as an arbovirus vector in South East Asia

Survey of the sylvatic mosquito fauna in a forested area of the Nakai Nam Theun National Protected Area

Supervisors
Dr. Paul Brey (IPL)
Dr. Louis Lambrechts (Institut Pasteur / CNRS 2000)

PhD Student
Elliott Miot

Background

The Lao mosquito fauna is still not well characterized, especially in remote forested areas such as our study area. The work of Rueda et al. [1] began to establish a comprehensive checklist of mosquito species in the country, but it was mainly based on a literature review and only few field collections.

Methods

In order to update the checklist of mosquitoes in our study area, we performed larval and adult mosquito collections in the NNT NPA along several rivers: Nam Theun (January 2012), Nam Mon (March 2012), Nam Noy (December 2011, March-April 2012, February, March, May, August, November, December 2017, and February-March 2018), and Nam On (May 2017) (Figure 1).

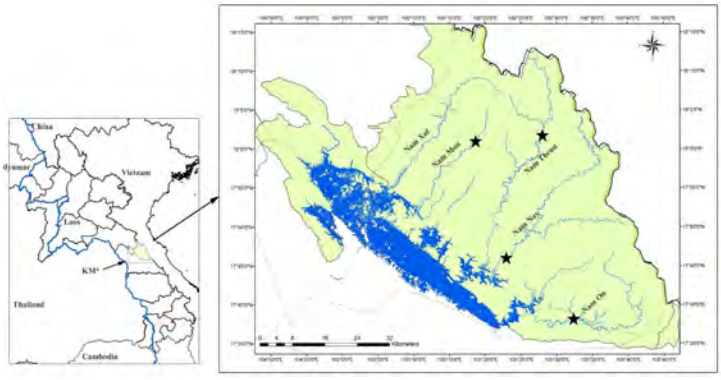


Figure 1. Map showing sampling locations of mosquitoes in Lao PDR. KM = Khammuane Province; Black stars = sampling locations; Blue line = Mekong River and its tributaries.

Mosquito larvae were collected in various types of breeding sites such as rock pools along the riverbanks, tree holes, temporary pools of water, fruit husks, and bamboo shoots. We also constructed bamboo traps, i.e. large bamboos cut in small pieces and filled with fresh water, that we deployed across the Nam Noy field site to create semi-natural breeding sites. Adult mosquitoes were captured using a combination of commercial traps (e.g. CDC light traps, BG sentinel traps) and active collections using vacuum-powered and mouth aspirators, and butterfly nets. Larvae were reared until adulthood and sorted morphologically back in the laboratory.

Results

During our field surveys, we identified 54 mosquito taxa belonging to 11 genera, of which 15 species were new records in Laos. We also described *Ae. malayensis,* a species found throughout South East Asia [2-4], for the first time in Laos based on morphological characters and molecular analyses. We produced photos of the morphological characters allowing proper identification of *Ae. malayensis* and distinction from closely related species that are found in sympatry, such as *Ae. albopictus* (Figure 2 & 3). We also provided information on the bionomics of *Ae. malayensis.*

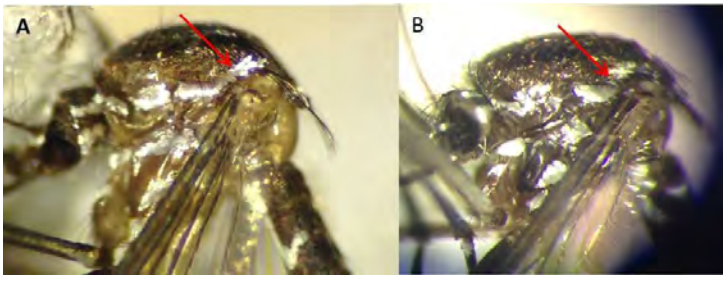


Figure 2: Morphological comparison of the thorax. (A) *Ae. malayensis* - a supraalar area of thorax with a patch of pale scales extended upward toward the scutellum. (B) *Ae. albopictus* - a supraalar area of thorax with spot of pale scales not extended toward the scutellum.

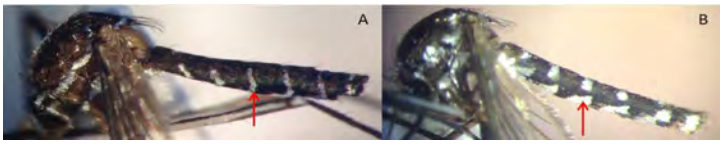


Figure 3: Morphological comparison of the abdominal terga IV-VI. (A) *Ae. malayensis* - dorsal white bands connected to lateral pale patches. (B) *Ae. albopictus* - dorsal white bands separated from lateral spots.

Successful colonies of *Ae. albopictus* and *Ae. malayensis* were derived from the collections, which allowed subsequent laboratory studies on these sylvatic mosquito populations.

Publications

Motoki MT, Vongphayloth K, Rueda LM, Miot EF, Hiscox A, Hertz JC, and Brey PT. New records and updated checklist of mosquitoes (Diptera: Culicidae) from Lao People's Democratic Republic, with special emphasis on adult and larval surveillance in Khammuane Province. J Vector Ecol. 2019;44: 76–88. doi:10.1111/jvec.12331

Motoki MT, Miot EF, Rueda LM, Vongphayloth K, Phommavanh N, Lakeomany K, Debboun M, Hertz JC, and Brey PT. First Record of *Aedes (Stegomyia) malayensis* Colless (Diptera: Culicidae) in the Lao People's Democratic Republic, Based on Morphological Diagnosis and Molecular Analysis. US Army Med Dep J. 2018;: 1–7.

Identification of sylvatic mosquito species attracted to humans in a forested area of the Nakai Nam Theun National Protected Area

Background

Little is known about the vector status, ecology, and behavior of mosquito species in Laos. In addition to vector competence, vectorial capacity strongly depends on mosquito ecology and feeding behavior.

Host preference, host availability, vector abundance, feeding frequency, encounter rate, and the temporal pattern of blood-feeding activity are some of the main ecological parameters that determine the effective contact between vectors and hosts [5]. Thus, characterizing the abundance, richness, diversity, and biting behaviors of mosquito species is an essential step in order to assess the risk of spillover of zoonotic, vector-borne pathogens to the human population [6]. The same is true for assessing the risk of spillback of human vector-borne pathogens establishing novel sylvatic transmission cycles in regions where they were previously absent [7,8].

Methods

We conducted a total of four 1-week missions in August (rainy season), November-December (dry season) 2017 and twice in March 2018 (intermediate season) in the NNT NPA. Mosquito species attracted to humans were specifically targeted using human-baited double-net traps [9] (Figure 4) deployed in three habitats (riverside, low-cover forest, high-cover forest) along a trail going from the Nam Noy riverbank to deeper in the forest (Figure 5). Mosquitoes were captured around the clock, sacrificed, grouped per 1-hour interval, and identified morphologically back in the laboratory. Based on the captures, we evaluated the abundance, richness, diversity, and daily activity pattern of human-attracted mosquito species and compared these indices between habitats and seasons.

Figure 4: Human-baited double-net traps *in situ.*

Figure 5: Map of the Nam Noy field site with the location of the collection sites. Generated using ©Google Maps. Purple, red and yellow marks show the collection locations in the riverside habitat, low-cover forest habitat, and high-cover forest habitat, respectively.

Results

We collected a total of 1,018 females that were morphologically assigned to 9 genera and 26 mosquito taxa, of which 87.6% represented only 8 mosquito species (Figure 6). Some of them are known or putative arbovirus vectors such as *Ae. albopictus*, *Cx. vishnui, Ar. subalbatus,* and *Ae. malayensis*. Others have been poorly studied and little is known about their potential as pathogen vectors such as Heizmannia species, *Ae. desmotes,* and *Ar. jugraensis.*

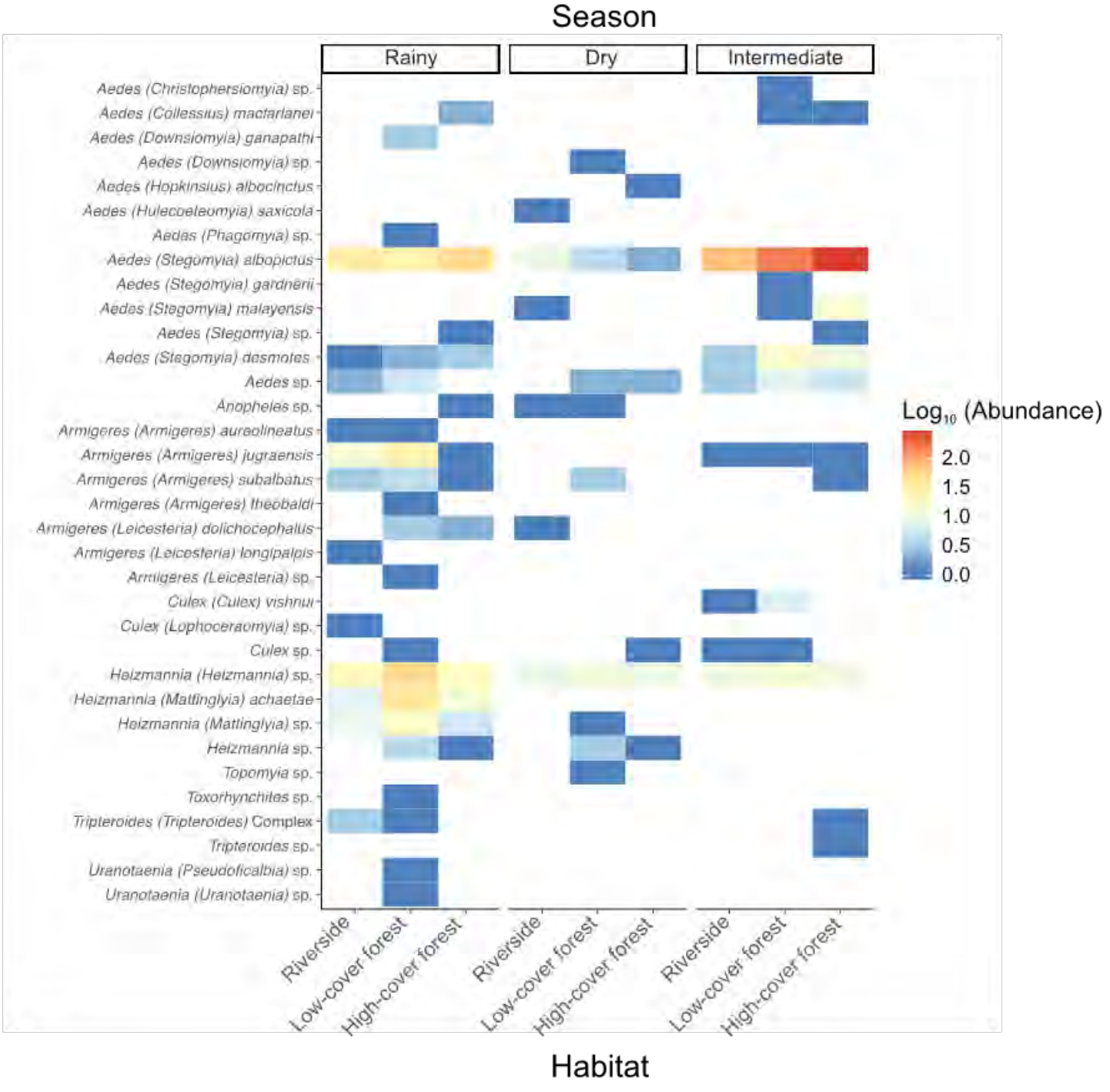


Figure 6: Species abundance of human-attracted mosquitoes stratified by season and habitat. Heat map of the log10-transformed total number of mosquitoes caught in the human- baited traps shown by season (rainy, dry, intermediate) and habitat (riverside, low-cover forest, high-cover forest) for each species. Abundance ranges from dark blue (low) to red (high).

Analyses of species richness and diversity revealed that they were significantly higher during the rainy season, especially in the low-cover forest habitat (Figure 7 & 8).

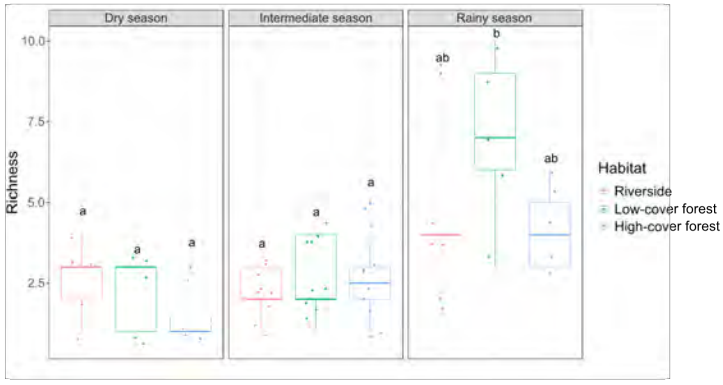


Figure 7: Species richness of human-attracted mosquitoes stratified by season and habitat. Boxplots of the daily number of mosquitoes caught in the human-baited traps shown by season (rainy, dry, intermediate) and habitat (riverside, low-cover forest, high-cover forest). Each point in the boxplots represents the species richness of a 24-hour mosquito collection period. Letters above the boxplots indicate statistical significance of the differences. Conditions sharing a letter are not significantly different.

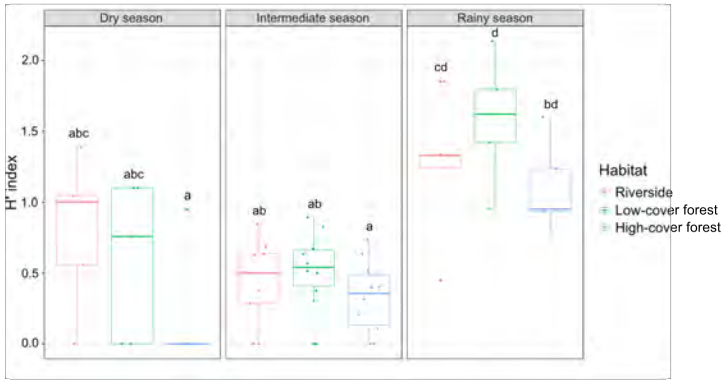


Figure 8: Diversity of human-attracted mosquitoes stratified by season and habitat. Boxplots of the daily Shannon diversity (H') index of mosquitoes caught in the human-baited traps by season (rainy, dry, intermediate) and habitat (riverside, low-cover forest, high-cover forest). Each point in the boxplots represents the H' index of a 24-hour mosquito collection period. Letters above the boxplots indicate statistical significance of the differences. Conditions sharing a letter are not significantly different.

Daily activity patterns showed that all human-attracted mosquito species collected were only active during daytime regardless of seasons or habitats (Figure 9). Host-seeking activity differed between the low-cover forest habitat, where mosquitoes were mostly active during the afternoon, and the high-cover forest habitat, where they were more active in the morning, regardless of the season.

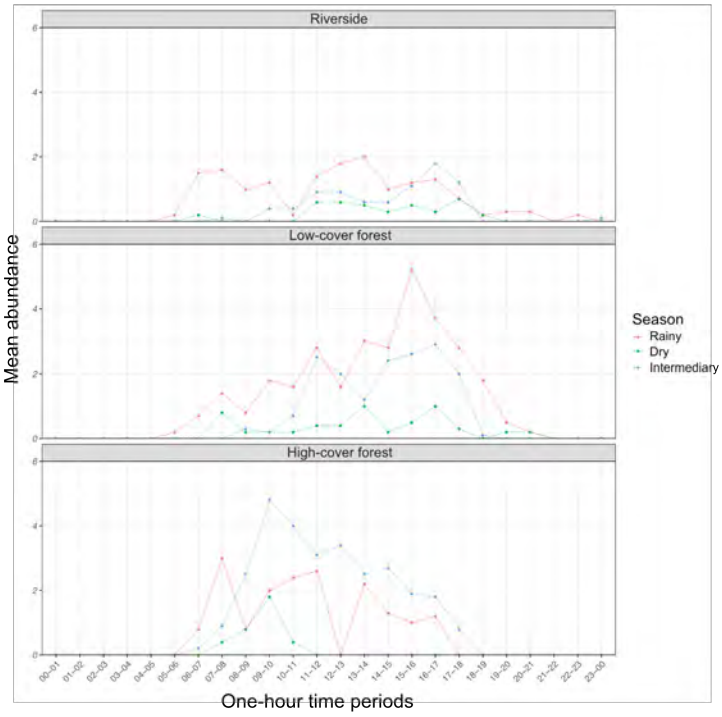


Figure 9: Daily pattern of human-seeking mosquito activity stratified by habitat and season. The mean hourly number of mosquitoes caught in the human-baited traps is plotted over time by habitat (riverside, low-cover forest, high-cover forest) and season (rainy, dry, intermediate). Hourly data were collected for 24-hour periods (rainy season: 6 consecutive days and nights; dry season: 6 consecutive days and nights; intermediate season: 2 x 6 consecutive days and nights).

The results of this study improve our understanding of mosquito-human interactions in the NNT NPA by providing new information on the species abundance, richness, diversity, and daily activity pattern of mosquitoes caught in human-baited traps. We characterized several human-attracted mosquito species, including

Ae. malayensis, in a primary forest of Laos, which paves to way for identification of potential bridge vectors in this area.

Publication

Miot EF, Motoki MT, Phommavanh N, Lakeomany K, Vongphayloth K, Hertz JC, Lambrechts L, and Brey PT. Diversity, abundance and daily activity pattern of human-seeking mosquitoes in a forested area of the Nakai district, Laos. Manuscript in preparation.

Potential of the sylvatic mosquito *Aedes malayensis* to act as an arbovirus bridge vector in forested area of the Nakai Nam Theun National Protected Area

Background

Following our initial field surveys, we chose to focus on the mosquito *Ae. malayensis* to further characterize its potential to act as an arbovirus bridge vector in the NNT NPA. This was done by evaluating its vector competence and its attraction to human scent in laboratory conditions.

Methods

We measured the vector competence of our laboratory colony of *Ae. malayensis* recently derived from a sylvatic population (see Chapter 1) for DENV and YFV, using an urban *Ae. aegypti* population from Laos as a positive control. Mosquito were orally challenged with a low-passage DENV-1 isolate from Laos and a low-passage YFV isolate belonging to the West African lineage. Additionally, we performed behavioral experiments using a dual-port olfactometer to assess the specific attraction of this *Ae. malayensis* population to human odor in the presence of CO_2 (Figure 12A).

Results

We found that our sylvatic *Ae. malayensis* population was competent for DENV-1, but to a lesser extent than our *Ae. aegypti* control (Figure 10). Due to low YFV infection rates overall, we were not able to conclusively demonstrate that this *Ae. malayensis* population was competent for YFV, but it was likely less competent than our *Ae. aegypti* control (Figure 11).

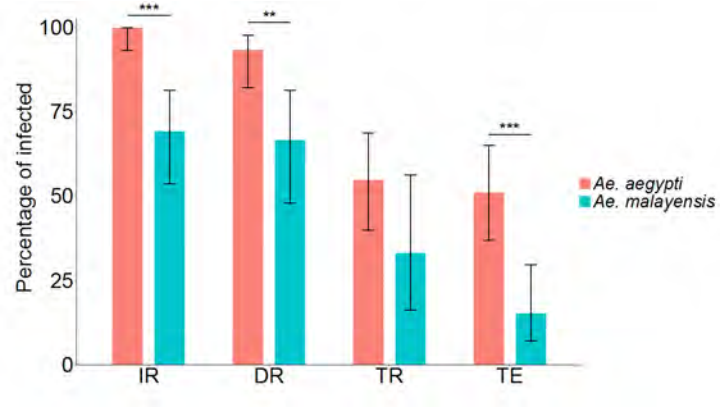


Figure 10: Vector competence of sylvatic *Ae. malayensis* and *Ae. aegypti* controls after exposure to 1.38 x 107 FFUs/ml of DENV-1. Bars represent the percentage of virus-positive mosquitoes 14 days post infectious blood meal and the error bars are the 95% confidence intervals of the percentages. Infection rate (IR) is the proportion of blood-fed females with an infected body. Dissemination rate (DR) is the proportion of infected females with virus disseminated to the head tissues. Transmission rate (TR) is the proportion of females with a disseminated infection that shed virus in their saliva. Transmission efficiency (TE) is the overall proportion of blood-fed females that shed virus in their saliva. The *Ae. aegypti* population was included as a positive control. The figure compiles data from two independent experiments that did not differ significantly. $^{**}p < 0.01$; $^{***}p < 0.001$.

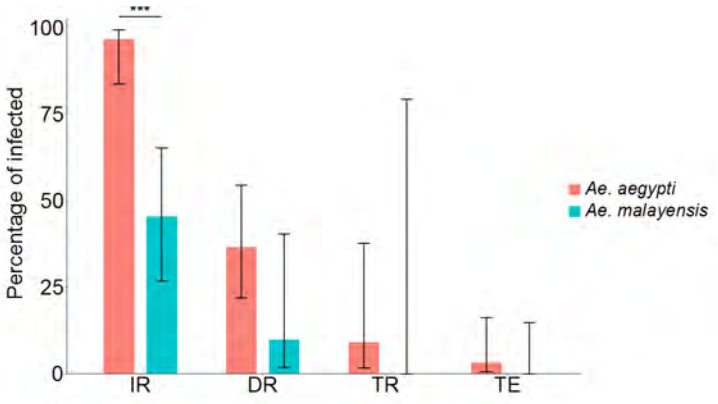


Figure 11: Vector competence of sylvatic *Ae. malayensis* and *Ae. aegypti* controls after exposure to 1.84 x 106 FFUs/ml of YFV. Bars represent the percentage of virus-positive mosquitoes 14 days post infectious blood meal and the error bars are the 95% confidence intervals of the percentages. Infection rate (IR) is the proportion of blood-fed females with an infected body. Dissemination rate (DR) is the proportion of infected females with virus disseminated to the head tissues. Transmission rate (TR) is the proportion of females with a disseminated infection that shed virus in their saliva. Transmission efficiency (TE) is the overall proportion of blood-fed females that shed virus in their saliva. The *Ae. aegypti* population was included as a positive control. ***p < 0.001.

The olfactometer bioassays allowed us to measure both flight activity (i.e. percentage of females that spontaneously left the release chamber) and specific attraction to human odor (i.e. percentage of trapped mosquitoes that chose the trap with human odor). We observed that in the presence of human odor combined with CO_2, a higher proportion of *Ae. malayensis* females were activated and started flying compared to CO_2 alone (Figure 12B). However, there was no specific attraction to human odor, with equal proportions of mosquitoes caught in the trap with human odor combined with CO_2 and in the trap with CO2 alone (Figure 12C).

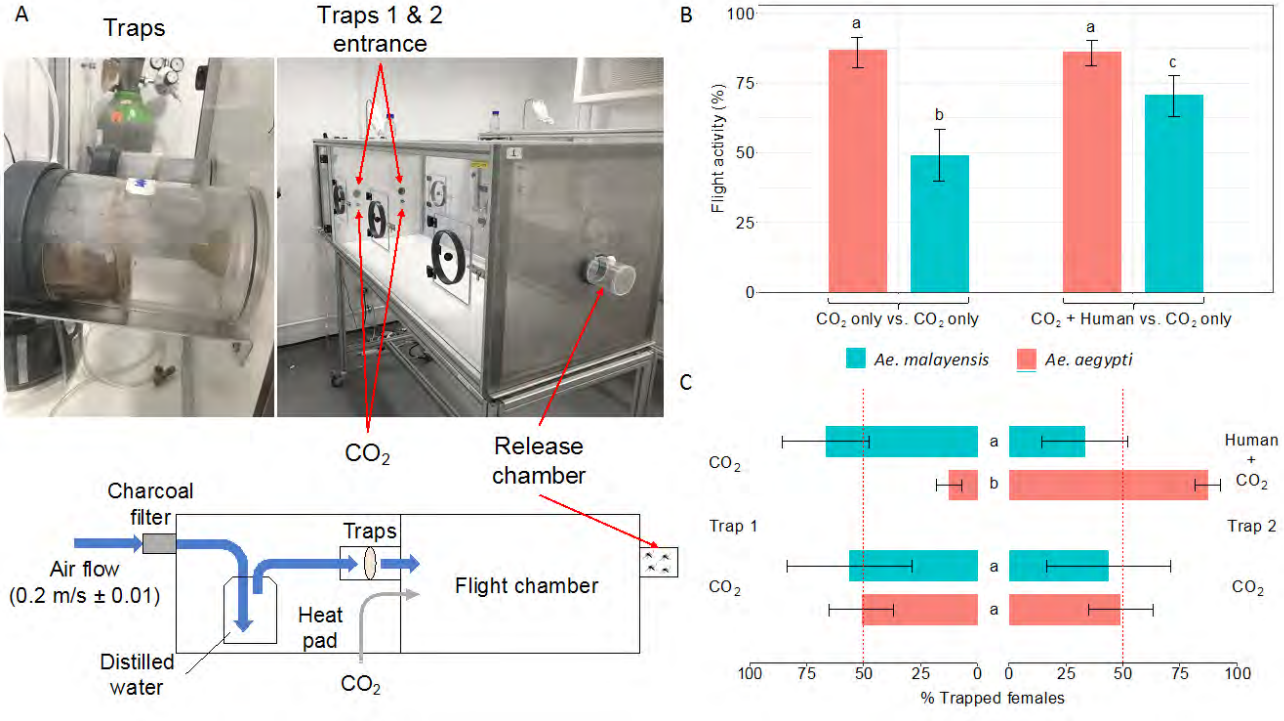


Figure 12: Lack of laboratory evidence for *Ae. malayensis* attraction to human odor. (A) Schematic and pictures of the experimental setup to measure attraction to human odor in a dual-port olfactometer. (B) Flight activity is the percentage of female mosquitoes that exited the release chamber after 20 min. (C) Attraction to human odor was estimated as the percentage of trapped mosquitoes that chose the trap with human odor, which ranges from 0% (full attraction to CO_2 without human odor) to 100% (full attraction to CO_2 with human odor). The red, vertical dashed lines indicate the expected percentage of trapped mosquitoes when there is no preference for either trap (50%). Error bars represent 95% confidence intervals of the percentages. Letters next to or above the bars indicate statistical significance. Conditions with a letter in common are not significantly different from each other. The data shown in (B) and (C) are pooled from 6 and 9 separate replicate trials for CO_2 only vs. CO_2 only and CO_2 + Human odor vs. CO_2 only designs, respectively, which did not differ significantly. The *Ae. aegypti* population was included as a positive control.

The relatively modest vector competence for DENV and YFV, combined with a lack of detectable attraction to human odor in laboratory conditions, indicate a low potential for this sylvatic population of *Ae. malayensis* to act as an arbovirus bridge vector in our study area. Nevertheless, we caution that opportunistic feeding on humans (see Chapter 2) may occasionally contribute to bridge sylvatic and human transmission cycles. Further assessment of the risk of DENV and/or YFV spillback associated with *Ae. malayensis* should account for additional risk factors such as the presence of susceptible non-human primates and the probability of human-mediated introduction of arboviruses into the area.

Publication

Miot EF, Calvez E, Aubry F, Dabo F, Grandadam M, Marcombe S, Oke C, Logan JG, Brey PT, and Lambrechts L. Potential of the sylvatic mosquito *Aedes malayensis* to act as an arbovirus bridge vector in forested area of the Nakai district, Laos. Manuscript under review for publication in Parasites & Vectors.

Potential of peridomestic Aedes malayensis mosquitoes to transmit yellow fever virus in Singapore

Background

Following our evaluation of the vectorial capacity of a sylvatic population of *Ae. malayensis* in Laos (see Chapter 3), we extended our investigation to a peridomestic *Ae. malayensis* population found in urban parks of Singapore.

This *Ae. malayensis* population was previously colonized by our collaborators at Duke-NUS Medical School who suspected that it could contribute to "cryptic" arbovirus transmission. Although vector control measures in Singapore achieve very low densities of *Aedes* mosquitoes [10], arboviruses such as DENV, CHIKV, and ZIKV have re-emerged in Singapore in the last two decades [11-14]. Indeed, this peridomestic *Ae. malayensis* population was recently shown to be experimentally competent for DENV and CHIKV [15]. However, its vector competence for YFV had not been evaluated, and its propensity to bite humans was unknown.

Until now, the Asia-Pacific region has remained YFV-free. However, the increasing influx of travelers coming from endemic regions of Africa and South America where recent YFV outbreaks have occurred has significantly increased the risk of YFV introduction in places like Singapore, a major hub for trade and tourism [16,17]. YFV introduction into the Asia-Pacific region was confirmed in 2016 when 11 workers infected with YFV in Angola returned to China, although fortunately it did not subsequently result in local transmission of the virus [18].

Methods

To evaluate the ability of *Ae. malayensis* to contribute to YFV transmission in Singapore, we used the colony established in 2014 by our collaborators in Singapore. First, we measured the vector competence of this *Ae. malayensis* colony for YFV, using *Ae. aegypti* as a positive control. Mosquito were orally challenged with a low-passage YFV isolate belonging to the West African lineage. Second, I performed a small-scale field survey in several parks and a forested area of Singapore in March 2019 to evaluate the probability of host-vector contact between humans and *Ae. malayensis.* I sampled mosquitoes at 6 locations from 7 a.m. to 7 p.m. using a human-baited double-net trap: East Coast Park zone A (2 days), East Coast Park zone C (1 day), Mount Faber park (1 day), Clementi woods park (1 day), West Coast park (1 day), and a forested area in the northern part of the city in Sembawang (3 days). We also obtained data from the island-wide Gravitrap surveillance network implemented by Singapore National Environment Agency (NEA) to monitor the spatial and temporal variability of adult *Aedes* mosquito populations.

Results

Overall, the peridomestic *Ae. malayensis* population from Singapore was experimentally competent for YFV to a similar level as our *Ae. aegypti* control (Figure 13 &14).

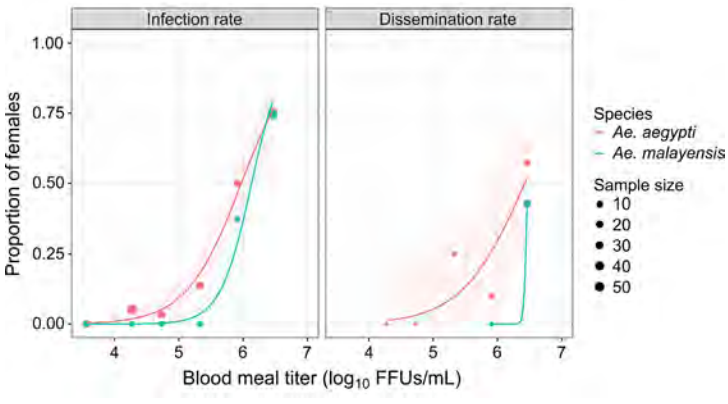


Figure 13: Peridomestic *Ae. malayensis* in Singapore are orally susceptible to YFV. Dose-response curves are shown for the *Ae. malayensis* population from Singapore under study and a control *Ae. aegypti* population. Infection rate is the proportion of blood-fed females testing YFV-positive 10–14 days post blood meal. Dissemination rate is the proportion of YFV-infected females with YFV-positive wings/legs or head 14 days post blood meal. The line represents the logistic regression of the data and the shaded area is the 95% confidence interval of the fit. The data shown are combined from two separate experiments.

During the small-scale field survey, *Ae. malayensis* was caught in the human-baited trap in one of the urban parks (Sembawang). The most abundant species collected was *Ae. albopictus* (89%) followed by *Ae. malayensis* (9%). Moreover, out of the 504,771 mosquitoes collected in Gravitraps, the predominant species was *Ae. aegypti* (80%), but a total of 1,931 Ae. malayensis were caught in 1,741 traps (Figure 15).

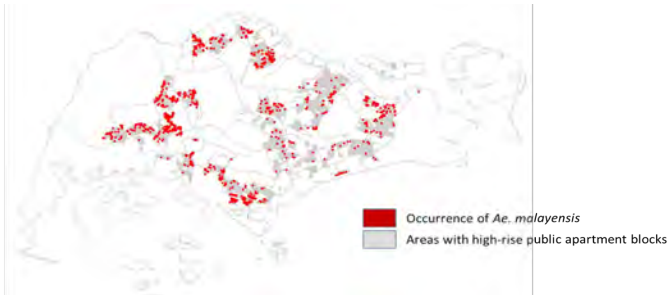


Figure 15: Distribution of *Aedes* malayensis in high-rise apartment blocks in Singapore. Map based on data collected from the island-wide Gravitrap surveillance network in 2018.

Together, this study provided evidence that peridomestic *Ae. malayensis* mosquitoes in Singapore are competent vectors for YFV, which may not only engage in contact with humans in urban parks but also breed in domestic settings such as housing blocks. Therefore, we conclude that Ae. malayensis could contribute to YFV transmission in Singapore if the virus were to be introduced.

Publication

Miot EF, Aubry F, Dabo S, Mendenhall IH, Marcombe S, Tan CH, et al. A peridomestic *Aedes malayensis* population in Singapore can transmit yellow fever virus. PLoS Negl Trop Dis. 2019;13: e0007783. doi:10.1371/journal.pntd.0007783

References

1. Rueda LM, Vongphayloth K, Pecor JE, Sutherland IW, Hii J, Debboun M, et al. Mosquito Fauna of Lao People's Democratic Republic, with Special Emphasis on the Adult and Larval Surveillance at Nakai District, Khammuane Province. US Army Med Dep J. 2015;: 25–32.

2. Huang Y-M. Contributions to the Mosquito Fauna of Southeast Asia. XIV. The Subgenus Stegomyia of Aedes in Southeast Asia I - The Scutellaris Group of Species. Contr Am Entomol Inst. 1972;: 1–109.

3. Tewari SC, Hiriyan J, Reuben R. Epidemiology of subperiodic Wuchereria bancrofti infection in the Nicobar Islands, India. Trans R Soc Trop Med Hyg. 1995;89: 163–166.

4. Rattanarithikul R, Harbach RE, Harrison BA, Panthusiri P, Coleman RE, Richardson JH. Illustrated keys to the mosquitoes of Thailand. VI. Tribe Aedini. Southeast Asian J Trop Med Public Health. 2010;41 Suppl 1: 1–225.

5. Kramer LD, Ebel GD. Dynamics of flavivirus infection in mosquitoes. Adv Virus Res. 2003;60: 187–232.

6. Vasilakis N, Cardosa J, Hanley KA, Holmes EC, Weaver SC. Fever from the forest: prospects for the continued emergence of sylvatic dengue virus and its impact on public health. Nat Rev Microbiol. 2011;9: 532–541. doi:10.1038/nrmicro2595

8. Bryant JE, Holmes EC, Barrett ADT. Out of Africa: a molecular perspective on the introduction of yellow fever virus into the Americas. PLoS Pathog. 2007;3: e75. doi:10.1371/journal.ppat.0030075

9. Tangena J-AA, Thammavong P, Hiscox A, Lindsay SW, Brey PT. The Human-Baited Double Net Trap: An Alternative to Human Landing Catches for Collecting Outdoor Biting Mosquitoes in Lao PDR. Hansen IA, editor. PLOS ONE. Public Library of Science; 2015;10: e0138735. doi:10.1371/journal.pone.0138735

10. Egger JR, Ooi EE, Kelly DW, Woolhouse ME, Davies CR, Coleman PG. Reconstructing historical changes in the force of infection of dengue fever in Singapore: implications for surveillance and control. Bull World Health Organ. World Health Organization; 2008;86: 187–196. doi:10.2471/BLT.07.040170

11. Hapuarachchi HC, Koo C, Rajarethinam J, Chong C-S, Lin C, Yap G, et al. Epidemic resurgence of dengue fever in Singapore in 2013-2014: A virological and entomological perspective. BMC Infect Dis. BioMed Central; 2016;16: 300. doi:10.1186/s12879-016-1606-z

12. Koh BKW, Ng L-C, Kita Y, Tang CS, Ang LW, Wong KY, et al. The 2005 dengue epidemic in Singapore: epidemiology, prevention and control. Ann Acad Med Singap. 2008;37: 538–545.

13. Leo YS, Chow ALP, Tan LK, Lye DC, Lin L, Ng LC. Chikungunya outbreak, Singapore, 2008. Emerging Infect Dis. 2009;15: 836–837. doi:10.3201/eid1505.081390

14. Maurer-Stroh S, Mak T-M, Ng Y-K, Phuah S-P, Huber RG, Marzinek JK, et al. South-east Asian Zika virus strain linked to cluster of cases in Singapore, August 2016. Euro Surveill. European Centre for Disease Prevention and Control; 2016;21: 1232. doi:10.2807/1560-7917.ES.2016.21.38.30347

15. Mendenhall IH, Manuel M, Moorthy M, Lee TTM, Low DHW, Missé D, et al. Peridomestic *Aedes malayensis* and *Aedes albopictus* are capable vectors of arboviruses in cities. Apperson C, editor. PLoS Negl Trop Dis. 2017;11: e0005667. doi:10.1371/journal.pntd.0005667

16. Gubler DJ. Pandemic yellow fever: a potential threat to global health via travelers. J Travel Med. 2018;25: 160. doi:10.1093/jtm/tay097

17. Butler D. Fears rise over yellow fever's next move. Nature. 2016.: 155–156. doi:10.1038/532155a

18. World Health Organization (WHO). Emergencies preparedness, response: Yellow Fever – China. In: WHO [Internet]. World Health Organization; 22 Apr 2016 [cited 3 Apr 2019]. Available: http://www.who.int/csr/don/22-april-2016-yellow-fever-china/en/

Vaccine Preventable Diseases Laboratory *Lao-Lux joint Lab*

Head of Laboratory:
Professor Claude P. Muller, Scientific Director

Scientists:
Dr. Antony Black, Responsible of the Lab
Dr. Phonethipsavanh Nouanthong
Dr. Siriphone Virachith

Junior Scientists:
Dr. Kinnaly Xaydalasouk
Dr. Vilaysone Khounvisith

Technicians:
Latdavone Khenkha
Bounta Vongphachanh
Nouna Innoula
Nampherng Xayyavong

Visiting scientists from Department of Infection and Immunity, Luxembourg
1. Lisa Hefele
2. Franziska Fuchs
3. Janko van Beek

Projects

- Immunity levels to poliovirus in Lao children and adults before the vaccine-derived poliovirus outbreak: a retrospective study
- Knowledge, attitudes and practices regarding vaccination among healthcare workers in Lao PDR
- Hepatitis A virus in Lao People's Democratic Republic
- Cross-species transmission of poultry pathogens in backyard farms: ducks as carriers of chicken viruses

Executive summary

The LaoLux Laboratory (LLL) aims to build capacity for investigations of human and animal infectious diseases that are of relevance for Lao PDR. All studies are conducted in close collaboration with local partners and focus mainly on the molecular epidemiology and seroprevalence of vaccine-preventable infectious diseases, as well as animal and zoonotic diseases. These studies have importance for stakeholders in public and animal health by providing estimates about the burden of specific infections, by promoting outbreak control and vaccination programmes, by improving animal health and productivity, and by proposing measures to optimize national health strategies. Our evidence-based results and recommendations are communicated to stake-holders and partners in the form of written and oral reports and policy briefs.

In this year's report, we present our serostudy of Polio virus. Emergence of vaccine-derived poliovirus has occurred in Lao PDR in recent years. It can only be prevented by high vaccination coverage and adequate disease surveillance. We find that adults born before the systematic introduction of the polio vaccine in Lao PDR have a lower seroprevalence of polio antibodies. Thus, in addition to the routine childhood vaccination and in the interest of an efficient use of resources, we recommend that at least health care workers should be systematically vaccinated in a nationwide programme.

We also detail a study regarding knowledge, attitude and practice towards vaccines in Lao healthcare workers. This important sub-group are at risk of infection from patients and also can transmit infections to patients. Furthermore, they can be an important source of information on vaccines for the general public. Our findings show a mixed knowledge, fair attitudes and fair practices regarding vaccination. Receiving advice from colleagues and having a free vaccine improved vaccine uptake. Vaccine promotion programs for HCW should take these factors into consideration.

Our study on hepatitis A virus (HAV) seroprevalence in Xiengkhouang province and Vientiane Capital showed evidence of a high level of previous infection in adults. This disease is related to poor sanitation, and evidence of high history of infection in those aged above 20 years old suggests poor sanitation in the past. Lower seroprevalence levels in young Lao adolescents and children may reflect improved sanitation in recent years. However, children in Xiengkhouang province had a higher seroprevalence than those in Vientiane Capital, indicating poorer sanitation in the rural areas. We suggest improved surveillance, awareness and sanitation.

Our collaboration with the Faculty of Agriculture at the National University of Laos continued with a study of poultry pathogens in backyard farms. In this study, we found high rates of avian viruses and demonstrated the co-circulation of diverse strains of coronaviruses and chicken anemia viruses. Interestingly, phylogenetic analysis suggested that there is cross-species transmission of viruses between chickens and ducks. We suggest ways to improve the control of avian viruses such as separation of bird species and tailored vaccination programs which would benefit the health of the birds and the economic sustainability of the smallholders.

Other ongoing and completed studies that will be reported in full in the report next year include investigation hepatitis B virus (HBV) infection in Lao dentists which showed that improvements in hygiene and awareness are warranted among Lao dentists. The results were reported to the Lao Dental Association and the data are being written as a manuscript. We also investigated the impact of the HBV vaccine introduction on infection of Lao adolescents and demonstrate a significant reduction in exposure and chronic infection rates following vaccine introduction. However, there remains a need to improve vaccine coverage, particularly of the HepB birth dose.

Human papillomavirus (HPV) can cause cervical cancer in infected women and Lao PDR has one of the highest rates of this cancer worldwide.

In an ongoing study, we are investigating the genotype distribution of HPV in healthy women and aim to determine the prevalence of high-risk genotypes. Related to this, we are investigating the seroprevalence of antibodies against high-risk HPV genotypes in vaccinated and unvaccinated adolescent Lao girls, in order to gain insight into natural exposure levels and vaccine immunogenicity.

From March to May, 2019, we were joined by Diana Cheung, a medical student from New York Institute of Technology, who did a project on "Knowledge and attitudes towards liver cancer and liver cancer screening among chronic HBV and chronic HCV patients in Vientiane, Lao PDR: a cross sectional study." We are also joined by Khamsai Khamphavong from the Lao Military Institute of Disease Prevention who will stay in the laboratory for 10 months to receive training in laboratory techniques and research. Thongchanh Khammounneurn, a Biomedical Masters student from the University of Health Sciences, completed her thesis on "Monitoring of immune response to HBV vaccination in Lao Red Cross and Blood Centre staff in Lao PDR". Franziska Fuchs, a Medical student from Saarland University, investigated "Knowledge, attitude and practice survey among healthcare workers and pregnant women and immunity status on ToRCH-infections during antenatal care in Lao PDR"

Vilaysone Khounvisith, from our laboratory, visited the Luxembourg Institute of Health and the Laboratoire National de Santé in Luxembourg for 2 months and received training in laboratory techniques and data analysis.

Financial support

In 2019, the laboratory was largely funded by the "PARECIDS II" grant from the Government of the Grand Duchy of Luxembourg and operates in close partnership with the Infectious Disease Research Unit at the Luxembourg Institute of Health, Luxembourg. A "Lao Equity through policy Analysis and Research Networks" (LEARN) research grant was awarded to Dr. Phonethipsavanh Nouanthong in 2018 to investigate the immunogenicity of human papillomavirus in Lao girls. We also receive support from ARBOSHIELD grant to facilitate training of Lao military staff.

Our partners include Luxembourg Development Cooperation, Lao National Immunisation Programme, Lao University of Health Sciences, Faculty of Agriculture at the National University of Laos, Lao Tropical and Public Health Institute, Lao Red Cross and various hospitals nationwide.

Meeting presentations

7th Asian Vaccine Conference, Yangon, 13-15th September

"Immunogenicity of human papillomavirus vaccine among vaccinated adolescent girls in Lao PDR; a pilot study" Poster

"Timeliness of the pentavalent childhood vaccine in selected health care facilities in Bolikhamxay province" Poster

"Lasting benefit of infant hepatitis B vaccination in adolescents in the Lao People's Democratic Republic." Poster

"Seroprotection at different levels of the health care system after routine vaccination with DTPw-HepB-Hib in Lao PDR." Poster

13th Vaccine Congress, Bangkok, 15-18th September

"Seroprevalence of anti-tetanus antibodies in mothers and cord blood and associated factors in health-care settings in Lao People's Democratic Republic" Poster

"High seroprevalence of Foot and Mouth Disease in Laos" Poster

"Timeliness of the pentavalent childhood vaccine in selected health care facilities in Bolikhamxay province" Poster

"Lasting benefit of infant hepatitis B vaccination in adolescents in the Lao People's Democratic Republic." Oral presentation

Adolescent Research Day and National Health Research Forum 2019, Vientiane Capital, 17-18th October

"Positive impact of hepatitis B vaccination in Lao PDR: Hepatitis B seroprevalence in adolescents" Poster

11th International Conference on Public Health among Greater Mekong Sub-Regional countries, Vientiane, 18-19th October

"Immunity against Human Paillomavirus in vaccinated Lao adolescent girls" Oral presentation

"Moving forward to prevent hepatits B infection among healthy blood donors in Lao PDR" Poster

The Liver Meeting, 8-12th November, Boston, USA

"Knowledge and attitude towards liver cancer and liver cancer screening among HBV and HCV patients in Vientiane, Lao PDR" Poster

Mekong Hepatitis Symposium, 27-29th November, Vientiane

"Knowledge and attitude towards liver cancer and liver cancer screening among HBV and HCV patients in Vientiane, Lao PDR: a cross sectional study" Oral presentation

Pasteur Institute Scientific Conference, Ho Chi Minh, 6th December

"Vaccine-preventable disease laboratory at Institut Pasteur du Laos; impact and immunogenicity of infant and maternal vaccination." Oral presentation

Teaching and training activities

- Taught at Institute Pasteur International Workshop ""Measles and Rubella Elimination: Options for Public Health Interventions", Ho Chi Minh, 2-5th December
- Seminar given to Lao Military staff on vaccines
- Short course on "Viral Hepatitis" given to Lao Tropical Public Health Institute students, November
- Organised training on microscope use and maintenance for veterinary staff from Xiengkhoung iVet school, following donation of 2 microscopes

Manuscripts published 2019

Seroprotection at different levels of the health care system after routine vaccination with DTPw-HepB-Hib in Lao PDR. Hefele L, Syphan S, Xayavong D, Homsana A, Kleine D, Chanthavilay P, Nouanthong P, Xaydalasouk K, Phathammavong O, Billamay S, Xeuatvongsa A, Reinharz D, Muller CP, Black AP. Clin Infect Dis. 2019 Feb 19.

Knowledge, attitudes, and practices regarding vaccination among healthcare workers in Lao PDR. Sengchaleun V, Khampanisong P, Aye-Soukhathammavong P, Reinharz D, Black AP. Southeast Asian J Trop Med Public Health, March 2019.

Varicella zoster and fever rash surveillance in Lao People's Democratic Republic
Nouanthong P, Hübschen JM, Billamay S, Mongkhoune S, Vilivong K, Khounvisith V, Sinner R, Grandadam M, Phonekeo D, Black AP, Muller CP. BMC Infect Dis. 2019 May 8;19(1):392.

Cross-species transmission of poultry pathogens in backyard farms: ducks as carriers of chicken viruses. Pauly M, Snoeck CJ, Phoutana V, Keosengthong A, Sausy A, Khenkha L, Nouanthong P, Samountry B, Jutavijittum P, Vilivong K, Hübschen JM, Black AP, Pommasichan S, Muller CP. Avian Pathol. 2019 Jun 14.

High prevalence of helminth infections in mother-child pairs from three central provinces of Lao People's Democratic Republic. Pauly M, Sayasinh K, Muller CP, Sayasone S, Black AP. Parasite Epidemiology and Control. 2019

Seroprevalence of anti-tetanus antibodies in mothers and cord blood and associated factors in health-care settings in Lao People's Democratic Republic. Ounnavong P, Chanthavilay P, Khampanisong P, Reinharz D, Muller CP, Black AP. Vaccine. 2019

ສະຫຼຸບການປະຕິບັດວຽກງານ

ຫ້ອງທົດລອງລາວລຸກແລັບ ມີຈຸດປະສົງເພື່ອ ສ້າງຄວາມອາດສາ ມາດໃນການກວດຫາພະຍາດທີ່ຕິດເຊື້ອໃນຄົນ ແລະ ສັດທີ່ມີໃນ ສປປ ລາວ. ທຸກໆການສຶກສາແມ່ນໄດ້ເຮັດວຽກຮ່ວມກັບ ຄູ່ຮ່ວມ ພັດທະນາ ແລະ ເນັ້ນຫນັກສະເພາະດ້ານການລະບາດວິທະຍາ ທາງດ້ານໂມເລກຸນ ແລະ ອັດຕາການຊຸກຊຸມ ຂອງພະຍາດທີ່ສາມາດ ປ້ອງກັນໄດ້ດ້ວຍວັກຊີນ, ລວມໄປເຖິງພະຍາດທີ່ມີໃນສັດ ແລະ ພະ ຍາດທີ່ຕິດຕໍ່ຈາກສັດສູ່ຄົນນຳອີກດ້ວຍ. ການສຶກສາເຫຼົ່ານີ້ ມີຄວາມ ສຳຄັນສຳລັບ ຜູ້ທີ່ມີສ່ວນຮ່ວມໃນຊຸມຊົນ ແລະ ສຸຂະພາບຂອງສັດ ໂດຍໃຫ້ການປະເມີນກ່ຽວກັບບັນຫາທີ່ສະເພາະເຈາະຈົງຂອງ ການຕິດເຊື້ອຕ່າງໆ, ໂດຍສົ່ງເສີມການຄວບຄຸມການລະບາດ ແລະ ໂຄງການສັກວັກຊີນຕ່າງໆ, ພ້ອມທັງປັບປຸງດ້ານສຸຂະພາບຂອງ ສັດ ແລະ ການຜະລິດ, ແລະ ການສະເໜີມາດຕາການເພື່ອປັບປຸງຍຸດ ທະສາດດ້ານສຸຂະພາບແຫ່ງຊາດໃຫ້ແທດເໝາະ. ຜົນການສຶກສາ ແລະ ການໃຫ້ຄຳແນະນຳຕ່າງໆຂອງພວກເຮົາຍັງໄດ້ມີການສົ່ງຕໍ່ ປຶກສາ ຫາລືກັນລະຫວ່າງຜູ້ທີ່ຮ່ວມການສຶກສາ ແລະ ຄູ່ຮ່ວມງານ ຈາກພາກສ່ວນອື່ນໆ ໃນຮູບແບບຂອງບົດລາຍງານ ແລະ ການ ລາຍງານໂດຍການນຳສະເໜີ ແລະ ການເຮັດນະໂຍບາຍແບບ ສັງເຂບນຳອີກດ້ວຍ.

ບົດລາຍງານໃນປີນີ້, ພວກເຮົານຳສະເໜີຂໍ້ມູນ ຈາກບົດການສຶກ ສາທາງເຊຣອມວິທະຍາຂອງ ໄວຣັດໂປຣິໂອ. ການລະບາດຂອງ ເຊື້ອໄວຣັສໂປລິໂອທີ່ມາຈາກວັກຊີນ ແມ່ນໄດ້ເກີດຂຶ້ນໃນ ສປປ ລາວ ຫວ່າງ ມໍ່ໆມານີ້. ມັນສາມາດປ້ອງກັນໄດ້ດ້ວຍການໃຫ້ວັກຊີນ ທີ່ທົ່ວເຖິງກັນ ແລະ ມີການເຝົ້າລະວັງພະຍາດໃນທົ່ວທຸກບ່ອນ. ພວກເຮົາພົບວ່າຜູ້ໃຫ່ຍທີ່ເກີດກ່ອນການລິເລີ່ມການໃຫ້ວັກຊີນ ໂປລິໂອແບບເປັນລະບົບ, ສປປ ລາວແມ່ນພົບວ່າມີອັດຕາຊຸກຊຸມ ທາງເຊຣອມວິທະຍາ ຂອງທາດການຕ້ານໂປລິໂອ ທີ່ຕ່ຳຫຼາຍ. ສະນັ້ນ, ນອກຈາກການໃຫ້ວັກຊີນໃນເດັກຢ່າງສະຫມ່ຳສະເໜີແລ້ວຍັງ ຕ້ອງເອົາໃຈໃສ່ທັງແຫລ່ງຂອງການນຳໃຊ້ຢ່າງມີປະສິດທິພາບພ້ອມ, ພວກເຮົາຍັງແນະນຳວ່າ ຢ່າງຫນ້ອຍແພດຫມໍແມ່ນຕ້ອງໄດ້ຮັບວັກ ຊີນ ຢ່າງເປັນລະບົບໃນທົ່ວປະເທດນຳອີກດ້ວຍ.

ພວກເຮົາຍັງໄດ້ລົງເລິກການສຶກສາທີ່ເນັ້ນເຖິງຄວາມຮູ້,ທັດ ສະນະຄະຕິ ແລະ ການປະຕິບັດຕົວຈິງຕໍ່ວັກຊີນໃນ ພະນັກງານ ແພດຫມໍໃນລາວ. ມັນມີຄວາມຈຳເປັນໃນບາງກຸ່ມທີ່ມີຄວາມ ສ່ຽງ ໃນການຕິດເຊື້ອຈາກຄົນເຈັບ ແລະ ຍັງເປັນແຫລ່ງຂອງ ການສົ່ງຕໍ່ເຊື້ອໄປສູ່ຄົນເຈັບນຳອີກດ້ວຍ. ນອກເຫນືອໄປກວ່ານັ້ນ, ພວກເຂົາຍັງເປັນແຫລ່ງສຳຄັນໃນການໃຫ້ຂໍ້ມູນວັກຊີນໃຫ້ແກ່ ປະຊາຊົນທົ່ວໄປນຳອີກ. ການຄົ້ນພົບຂອງພວກເຮົາຍັງໄດເວົ້າ ເຖິງຄວາມຮູ້, ທັດສະນະຄະຕິ ແລະ ການປະຕິບັດທີ່ຢູ່ໃນລະດັບ ທີ່ປານກາງຕໍ່ການໃຫ້ວັກຊີນ. ການໄດ້ຮັບຄຳແນະນຳຈາກເພື່ອນຮ່ວມ ງານ ແລະ ການໄດ້ຮັບວັກຊີນໂດຍບໍ່ໄດ້ເສຍຄ່າໃຊ້ຈ່າຍ ກໍຍັງຍົກ ລະດັບຂອງວັກຊີນນຳອີກດ້ວຍ. ໂຄງການສົ່ງເສີມການໃຫ້ວັກຊີນສຳ ລັບພະນັກງານແພດຫມໍແມ່ນຕ້ອງໄດ້ເບິ່ງປັດໃຈເຫຼົ່ານີ້ ເພື່ອນຳໄປພິ ຈາລະນາເພີ່ມຕື່ມ.

ການສຶກສາຂອງພວກເຮົາກ່ຽວກັບໄວຣັດຕັບອັກເສບເອ (HAV) ຢູ່ແຂວງຊຽງຂວາງແລະນະຄອນຫຼວງວຽງຈັນໄດ້ສະແດງໃຫ້ ເຫັນເຖິງອັດຕາຊຸກຊຸມຂອງການຕິດເຊື້ອທີ່ສູງຫຼາຍໃນຜູ້ໃຫຍ່. ພະຍດນີ້ກ່ຽວຂ້ອງກັບການສຸຂະອານາໄມ ທີ່ບໍ່ສະອາດ, ແລະ ສາ ເຫດຂອງອັດຕາຊຸກຊຸມສູງໃນຜູ້ທີ່ມີອາຍຸຫລາຍກວ່າ 20 ປີຍັງຊີ້ ໃຫ້ ເຫັນເຖິງສຸຂະອະນາໄມບໍ່ສະອາດໃນເມື່ອກ່ອນ. ນອກຈາກນີ້ອັດ ຕາການຊຸກຊຸມໃນໄວໜຸ່ມ ແລະ ເດັກນ້ອຍລາວທີ່ຕ່ຳນັ້ນອາດຈະ ສະທ້ອນໃຫ້ເຫັນເຖິງສຸຂະອະນາໄມທີ່ດີຂຶ້ນໃນປະຈຸບັນ. ເຖິງຢ່າງໃດກໍ່ຕາມ, ເດັກນ້ອຍທີ່ຢູ່ແຂວງຊຽງຂວາງກໍມີອັດຕາ ຊຸກຊຸມຂອງການເກີດພະຍາດສູງກວ່າເດັກນ້ອຍທີ່ອາໄສຢູ່ໃນນະ ຄອນຫຼວງວຽງຈັນ, ເຊິ່ງສະແດງເຖິງສຸຂະອະນາໄມທີ່ບໍ່ສະອາດ ໃນຂົງເຂດຊົນນະບົດ. ພວກເຮົາແນະນຳໃຫ້ມີການປັບປຸງການເຝົ້າ ລະວັງ, ຍົກລະດັບຄວາມຮັບຮູ້ຄວາມເຂົ້າໃຈ ແລະ ເອົາໃຈໃສ່ດ້ານສຸ ຂະອະນາໄມໃຫ້ຫລາຍຂຶ້ນ.

ການຮ່ວມມືຂອງພວກເຮົາ ກັບຄະນະກະເສດສາດ, ມະຫາວິທະ ຍາໄລແຫ່ງຊາດໄດ້ສືບຕໍ່ກັບການສຶກສາເຊື້ອພະຍາດ ທີ່ຢູ່ໃນສັດປີກ ທີ່ຢູ່ໃນຟາມ. ການສຶກສາໃນຄັ້ງນີ້, ພວກເຮົາພົບອັດຕາຂອງ ເຊື້ອ ໄວຣັດໃນສັດປີກຫຼາຍ ແລະ ສະແດງໃຫ້ເຫັນເຖິງການກະຈາຍ ທີ່ ຫຼາກຫຼາຍສາຍພັນຂອງເຊື້ອໄວຣັດໂຄໂລນ້າ ແລະ ໂລກເລືອດ ຈາງໃນໄກ່. ສິ່ງທີ່ຫນ້າສົນໃຈໄປກວ່ານັ້ນ, ການວິເຄາະທາງດ້ານ ສາຍພັນທຸກຳຍັງເຫັນວ່າມີການສົ່ງຕໍ່ເຊື້ອໄວຣັດຂ້າມສາຍພັນ ລະຫວ່າງໄກ່ແລະເປັດ. ພວກເຮົາຍັງໄດ້ແນະນຳວິທີການໃນການ ປັບປຸງຄວບຄຸມໄວຣັດສັດປີກເຊັ່ນວ່າການແຍກຊະນິດຂອງສັດປີກ ແລະ ຄວນມີຕາຕະລາງສັກຢາໃຫ້ສັດປີກຕາມຄວາມຫມາະສົມ ເພື່ອ ໃຫ້ໄດ້ຮັບຜົນປະໂຫຍດ ຕ່ຳສຸຂະພາບສັດປີກແລະພັດທະນາເສດຖະ ກິດຂະໜາດນ້ອຍໃຫ້ຍືນຍົງອີກດ້ວຍ.

ການສຶກສາອື່ນໆທີ່ຍັງດຳເນີນການຢູ່ ແລະ ການສຶກສາທີ່ໄດ້ສຳ ເລັດໄປແລ້ວນັ້ນຈະໄດ້ຖືກລາຍງານໃນບົດລາຍງານແບບສະບັບ ສົມບູນໃນປີຫນ້າ ໃນນັ້ນລວມມີການສຶກສາຂອງພວກເຮົາຕໍ່ ກັບການຕິດເຊື້ອໄວຣັດຕັບອັກເສບບີໃນທັນຕະແພດລາວໄດ້ສະ ແດງໃຫ້ເຫັນເຖິງ ການປັບປຸງດ້ານສຸຂະອະນາໄມ ແລະ ຄວາມຕື່ນ ຕົວໃນທັນຕະແພດລາວນຳຕື່ມ. ຜົນຂອງການສຶກສາໄດ້ຖືກລາຍ ງານໃຫ້ສະມາຄົມທັນຕະແພດລາວ ແລະ ຂໍ້ມູນແມ່ນໄດ້ຖືກຂຽນ ໄວ້ເປັນທີ່ຮຽບຮ້ອຍ. ພວກເຮົາຍັງໄດ້ຊອກຫາຜົນຂອງການໃຫ້ ວັກຊີນໄວຣັດຕັບອັກເສບບີ ໃນໄວໜຸ່ມລາວທີ່ຕິດເຊື້ອ ແລະ ສະແດງໃຫ້ເຫັນເຖິງການຫຼຸດລົງຢ່າງເຫັນໄດ້ຊັດເຈນຂອງອັດຕາ ການເປັນພະຍາດແບບຊຳເຮື້ອ ແລະ ການສຳພັດກັບເຊື້ອລວມໄປ ເຖິງການນຳໃຊ້ວັກຊີນນຳອີກດ້ວຍ.ເຖິງຢ່າງໃດກໍຕາມ, ກໍຍັງມີ ຄວາມຈຳເປັນໃນການປັບປຸງອັດຕາການປົກຄຸມຂອງການໃຫ້

ວັກຊີນ, ໂດຍສະເພາະແມ່ນການໃຫ້ວັກຊີນໄວຣັດຕັບອັກເສບບີ ເຂັ້ມທຳອິດຫລັງຈາກເກີດ. ຂໍ້ມູນໄດ້ຖືກນຳມາຂຽນ ແລະ ຕີພິມ. ນະໂຍບາຍໂດຍສັງເຂບແມ່ນໄດ້ຖືກຂຽນອອກມາສຳລັບການສື່ສານ ກັບພາກສ່ວນທີ່ກ່ຽວຂ້ອງ.

ເຊື້ອຈຸລະໂລກ human papillomavirus (HPV) ແມ່ນ ເຊື້ອສາເຫດທີ່ເຮັດໃຫ້ເກີດມະເຮັງປາກມົດລູກໃນແມ່ຍິງທີ່ ຕິດເຊື້ອ ແລະ ໃນທົ່ວໂລກ ສປປ ລາວ ແມ່ນໜຶ່ງໃນປະ ເທດທີ່ມີ ອັດຕາການຕິດເຊື້ອສູງ.ໃນການສຶກສາທີ່ກຳລັງດຳເນີນຢູ່, ພວກເຮົາ ໄດ້ຊອກຫາການແຜ່ກະຈາຍພັນທຸກຳຂອງສາຍພັນ HPV ໃນ ແມ່ຍິງທີ່ມີສຸຂະພາບດີແລະມີຈຸດປະສົງເພື່ອກຳນົດອັດຕາການ ແຜ່ກະຈາຍຂອງເຊື້ອໄວຣັດໃນກຸ່ມສາຍພັນທີ່ມີຄວາມສ່ຽງສູງ ທີ່ກໍ່ໃຫ້ເກີດມະເຮັງ. ນອກຈາກນີ້ ຍັງມີການສຶກສາທີ່ກ່ຽວຂ້ອງກັນ ເຊິ່ງ ພວກເຮົາກຳລັງດຳເນີນການຄົ້ນຄວ້າ ກ່ຽວກັບ ອັດຕາການ ຊຸກຊຸມຂອງທາດພາຍຕ້ານຕໍ່ກັບເຊື້ອໄວຣັດ HPV ໃນເດັກຍິງລາວ ທີ່ໄດ້ຮັບການສັກຢາກັນພະຍາດ ແລະ ບໍ່ໄດ້ຮັບການສັກຢາປ້ອງກັນ ມະເຮັງປາກມົດລູກ, ເພື່ອໃຫ້ຮູ້ເຖິງລະດັບພູມຄຸ້ມກັນທີ່ສ້າງຂຶ້ນ ຈາກການຕິດເຊື້ອໂດຍທຳມະຊາດ ແລະ ພູມຕ້ານທານທີ່ເກີດຈາກ ການໄດ້ຮັບວັກຊີນ.

ເດືອນມີນາຫາ ເດືອນພຶດສະພາ ປີ 2019, ພວກເຮົາໄດ້ເຮັດ ວຽກຮ່ວມກັບ ດຣ. ໄດອານ້າ ຈຶ່ງ, ທີ່ເປັນນັກສຶກສາແພດຢູ່ສະຖາບັນ ເທັກໂນໂລຢີນິວຢອກ, ຜູ້ທີ່ເຮັດໂຄງການ ຄວາມຮູ້ ແລະ ທັດ ສະນະຄະຕິ ຕໍ່ກັບມະເຮັງຕັບ ແລະ ການກວດຫາມະເຮັງຕັບ ໃນ ກຸ່ມຄົນເຈັບທີ່ເປັນໄວຣັດຕັບອັກເສບບີ ແລະ ຊີແບບຊຳເຮື້ອໃນ ນະຄອນຫຼວງວຽງຈັນ, ສປປ ລາວ: ການສຶກສາແບບຕັດຂວາງ. ພວກເຮົາຍັງໄດ້ຮັບເອົາ ທ. ຄຳໃສ ຄຳພາວົງ ເຊິ່ງມາຈາກ ສະຖາບັນ ປ້ອງກັນພະຍາດທະຫານລາວທີ່ຈະມາຢູ່ຫ້ອງທົດລອງເປັນເວລາ 10 ເດືອນ ເຊິ່ງຈະໄດ້ຮັບການຝຶກອົບຮົມທາງດ້ານເທັກນິກຫ້ອງທົດລອງ ແລະ ເຮັດການຄົ້ນຄ້ວາ. ນ ທອງຈັນ ຄຳມູນນອນ, ເປັນນັກສຶກສາ ປະລິນຍາໂທດ້ານຊີວະການແພດຈາກມະຫາວິທະຍາ ໄລສຸຂະພາບ, ເຊິ່ງໄດ້ສຳເລັດການຄົ້ນຄ້ວາວິໄຈການຕິດຕາມການ ຕອບສະໜອງພູມຄຸ້ມກັນການໃຫ້ວັກຊີນໄວຣັດຕັບອັກເສບບີໃນພະນັກງານກ າແດງລາວ ແລະ ສູນເລືອດໃນ ສປປ ລາວ. ນາງ ແຟນຊິດກ້າຟູກ ນັກສຶກສາການແພດຈາກ ມະຫາວິທະຍາໄລຊາ ແລນ, ເຊິ່ງໄດ້ເຂົ້າ ມາຮ່ວມໂຄງການກັບພວກເຮົາ ເປັນເວລາ 8 ເດືອນ ພາຍໃຕ້ໂຄງການ ຄວາມຮູ້ ທັດສະນະຄະຕິ ແລະ ການປະຕິບັດທີ່ສຳຫລວດໃນບັນດາ ແພດໝໍ ແລະ ແມ່ຍິງຖືພາ ແລະ ພູມຄຸ້ມກັນດ້ານຊີມເຊື້ອພະຍາດ TORCH ໃນໄລຍະຝາກທ້ອງຢູ່ ສປປ ລາວ.

ດຣ. ວິໄລສອນ ຂຸນວິສິດ, ຈາກຫ້ອງທົດລອງຂອງພວກເຮົາ ,ໄດ້ເຂົ້າຢ້ຽມຢາມສະຖາບັນ ດ້ານສຸຂະພາບລັກຊຳເບີກ ແລະ ຫ້ອງທົດລອງສຸຂະພາບແຫ່ງຊາດ ປະເທດລັກຊຳເບີກ, ເປັນເວລາ 2 ເດືອນ ແລະ ໄດ້ຮັບການຝຶກອົບຮົມທາງດ້ານເທັກນິກໃນຫ້ອງທົດ ລອງ ແລະ ການວິເຄາະຂໍ້ມູນນຳອີກດ້ວຍ.

ຜູ້ສະໜັບສະໜູນດ້ານການເງິນ

ໃນປີ 2019, ຫ້ອງທົດລອງສ່ວນໃຫຍ່ແມ່ນໄດ້ຮັບທຶນຊ່ວຍ ເຫຼືອ ຈາກ PARECIDS ຈາກລັດຖະບານຂອງລັກຊຳເບີກ ແລະ ເຮັດວຽກຮ່ວມມືຢ່າງໃກ້ຊິດກັບໜ່ວຍງານທີ່ຄົ້ນຄວ້າພະຍາດຊຶມ ເຊື້ອຢູ່ສະຖາບັນດ້ານສຸຂະພາບລັກຊຳເບີກ. ການເຂົ້າເຖິງດ້ານສຸຂະ ພາບໃຫ້ສະເໜີພາບ ແລະ ທົ່ວເຖິງ ຜ່ານການວິເຄາະທາງນະ ໂຍບາຍ ແລະ ເຄືອຂ່າຍການຄົ້ນຄວ້າ LEARN ທຶນການຄົ້ນຄວ້າ ແມ່ນໄດ້ຖືກມອບໃຫ້ ດຣ ພອນທິບ ສະຫວັນ ນອນທອງໃນປີ 2018 ເພື່ອເຮັດການວິໄຈລະດັບທາດພາຍຕ້ານຂອງ ໄວຣັດ ທີ່ກໍ່ ເກີດມະເຮັງປາກມົດລູກ ໃນເດັກຜູ້ຍິງ ໃນລາວ. ພວກເຮົາຍັງໄດ້ຮັບ ທຶນສະໜັບສະໜູນຈາກ ARBOSHIELD ເພື່ອອຳນວຍການ ຝຶກອົບຮົມໃຫ່ແກ່ພະນັກງານທະຫານ.

ຄຸ່ຮ່ວມງານຂອງເຮົາຍັງລວມເຖິງຄຸ່ຮ່ວມພັດທະນາລັກຊຳເບີກ, ໂຄງການສັກຢາກັນພະຍາດແຫ່ງຊາດ, ມະຫາວິທະຍາໄລ ວິທະຍາ ສາດສຸຂະພາບ, ຄະນະກະເສດສາດມະຫາວິທະຍາໄລແຫ່ງຊາດລາວ, ສະຖາບັນການແພດເຂດຮ້ອນ ແລະ ສາທາລະນະສຸກສາດ, ອົງການ ກາແດງລາວ ແລະ ໂຮງໝໍຫລາຍໆແຫ່ງໃນທົ່ວປະເທດ.

ການສະເໜີບົດໃນກອງປະຊຸມຕ່າງໆ

ກອງປະຊຸມວັກຊີນອາຊຽນຄັ້ງທີ 7, ຢ່າງກຸ້ງ, ວັນທີ 13-15 ເດືອນກັນຍາ.
"ການສ້າງທາດພາຍຕ້ານຕໍ່ວັກຊີນກັນພະຍາດມະເຮັງປາກມົດລູກ ໃນກຸ່ມເດັກຜູ້ຍິງໄວໜຸ່ມທີ່ໄດ້ຮັບການສັກວັກຊີນໃນ ສປປ ລາວ; ການສຶກສາເບື້ອງຕົ້ນ" - ສະເໜີເປັນໂພສເຕີ

"ຊ່ວງໄລຍະເວລາຂອງວັກຊີນ Pentavalent ໃນເດັກນ້ອຍທີ່ໄດ້ ຖືກເລືອກມາຈາກໂຮງໝໍໃນແຂວງບໍລິຄຳໄຊ" - ສະເໜີເປັນ ໂພສ ເຕີ

"ຜົນດີຂອງການໄດ້ຮັບວັກຊີນກັນພະຍາດຕັບອັກເສບບີໃນເດັກໄວ ໜຸ່ມໃນ ສປປ ລາວ" - ສະເໜີເປັນໂພສເຕີ
"ລະດັບທາດພາຍຕ້ານທີ່ແຕກຕ່າງກັນໃນລະບົບສາທາລະນະສຸກ ຫຼັງຈາກໄດ້ຮັບວັກຊີນ DTPw-HepB-Hib ໃນ ສປປ ລາວ"- ສະເໜີເປັນໂພສເຕີ

ກອງປະຊຸມອັກຊີນຄັ້ງທີ 13, ບາງກອກ, ວັນທີ 15-18 ເດືອນກັນຍາ.

"ອັດຕາຊຸກຊຸມທາງດ້ານເຊຣອມຂອງທາດກາຍຕ້ານພະຍາດບາດທະຍັກໃນແມ່ ແລະ ເລືອດຢູ່ສາຍດື ແລະ ປັດໄຈຮ່ວມອື່ນໆໃນບ່ອນໃຫ້ບໍລິການສາທາລະນະສຸກໃນ ສປປ ລາວ" - ສະເໜີເປັນໂພສເຕີ

"ອັດຕາຊຸກຊຸມທາງດ້ານເຊຣອມວິທະຍາ ຂອງພະຍາດ ປາກເປື່ອຍ ລີ້ງເລັບ ທີ່ສູງ ໃນ ສປປ ລາວ" - ສະເໜີເປັນໂພສເຕີ

"ຊ່ວງໄລຍະເວລາຂອງວັກຊີນ Pentavalent ໃນເດັກນ້ອຍທີ່ໄດ້ ຖືກເລືອກມາຈາກໂຮງໝໍໃນແຂວງບໍລິຄຳໄຊ"-ສະເໜີເປັນໂພສເຕີ

"ຜົນດີຂອງການໄດ້ຮັບວັກຊີນກັນພະຍາດຕັບອັກເສບບີໃນເດັກໄວໜຸ່ມໃນ ສປປ ລາວ"- ສະເໜີເປັນໂພສເຕີ

ການຄົ້ນຄວ້າທາງວິໄຈ ເພື່ອສົ່ງເສີມສຸຂະພາບໄວຈະເລີນພັນ ແລະ ກອງປະຊຸມການຄົ້ນຄວ້າທາງດ້ານສຸຂະພາບແຫ່ງຊາດ, ວຽງຈັນ, ວັນທີ 17-18 ເດືອນຕຸລາ.

"ຜົນດີຂອງການໄດ້ຮັບວັກຊີນໄວຣັດຕັບອັກເສບບີ ໃນ ສປປ ລາວ: ອັດຕາຊຸກຊຸມທາງເຊຣອມວິທະຍາພະຍາດຕັບອັກເສບບີໃນໄວໜຸ່ມ" - ສະເໜີເປັນໂພສເຕີ

ກອງປະຊຸມລະດົບສາກົນດ້ານສາທາລະນະສຸກສາດໃນບັນດາປະເທດທີ່ຢູ່ລະດັບພາກພື້ນແມ່ນ້ຳຂອງ ຄັ້ງທີ11, ວຽງຈັນ, ວັນທີ 18-19 ເດືອນຕຸລາ.

"ພູມຄຸ້ມກັນໃນເດັກໄວໜຸ່ມຍິງລາວທີ່ໄດ້ຮັບການສັກວັກຊີນກັນພະຍາດມະເຮັ້ງປາກມົດລູກ"- ນຳສະເໜີເປັນປົດເວົ້າ

"ການກ້າວໄປຂ້າງໜ້າເພື່ອປ້ອງກັນການຕິດເຊື້ອໄວຣັດຕັບອັກເສບບີ ໃນຜູ້ບໍລິຈາກເລືອດ ໃນ ສປປ ລາວ" ສະເໜີເປັນໂພສເຕີ

ງານປະຊຸມພະຍາດໄວຣັດຕັບອັກເສບບັນດາປະເທດແມ່ນ້ຳຂອງ, ວຽງຈັນ, ວັນທີ 27-29 ເດືອນພະຈິກ.

"ຄວາມຮູ້ ແລະ ທັດສະນະຄະຕິ - ນຳສະເໜີໂດຍຂົ້ນເວົ້າຕໍ່ກັບມະເຮັ້ງຕັບ ແລະ ການກວດກັ່ນຕອງມະເຮັ້ງຕັບໃນກຸ່ມຄົນເຈັບທີ່ຕິດເຊື້ອໄວຣັດຕັບອັກເສບບີ ແລະ ຊີ, ໃນ ສປປ ລາວ: ການສຶກສາແບບຕັດຂວາງ"- ນຳສະເໜີເປັນປົດເວົ້າ

ກອງປະຊຸມທາງດ້ານວິທະຍາສາດຂອງສະຖາບັນປັສເຕີ, ໂຮ່ຈີມິນ, ວັນທີ 6 ເດືອນທັນວາ.

"ຫ້ອງທົດລອງພະຍາດທີ່ປ້ອງກັນໄດ້ດ້ວຍວັກຊີນທີ່ສະຖາບັນປັສເຕີ ລາວ; ຜົນ ແລະ ພູມຄຸ້ມກັນຕ່ຳການໃຫ້ວັກຊີນຂອງເດັກ ແລະ ແມ່"- ນຳສະເໜີເປັນປົດເວົ້າ

ການສິດສອນ ແລະ ການຝຶກອົບຮົມ

- ສອນທີ່ງານສຳມະນາສະຖາບັນປັສເຕີສາກົນ ການລົບລ້າງຫມາກາແດງນ້ອຍແລະຫມາກແດງໃຫຍ່: ທາງເລືອກສຳລັບການປ້ອງກັນທາງສາທາລະນະສຸກສາດ, ໂຮ່ຈີມິນ, ວັນທີ 2-5 ເດືອນທັນວາ

- ໄດ້ເຮັດສຳມະນາກ່ຽວກັບວັກຊີນໃຫ້ແກ່ພະນັກງານແພດທະຫານ ຫລັກສູດໄລຍະສັ້ນ ໄວຣັດຕັບອັກເສບ, ພູມຄຸ້ມກັນ ແລະ ວັກຊີນ ໃຫ້ແກ່ນັກສຶກສາສະຖາບັນສາທາລະນະສຸກສາດການແພດ ເຂດຮ້ອນລາວ, ໃນເດືອນພະຈິກ

- ການຈັດຕັ້ງການຝຶກອົບຮົມໃນການນຳໃຊ້ກ້ອງຈຸລະທັດການບຳລຸງຮັກສາ ສຳລັບພະນັກງານສັດຕະວະແພດຈາກແຂວງຊຽງຂວາງ ໂຮງ ຮຽນ ໄອເວັດ, ພ້ອມທັງມອບກ້ອງຈຸລະທັດຈຳນວນ 2 ອັນ.

ການຝຶກອົບຮົມທີ່ໄດ້ເຂົ້າຮ່ວມ

ປີ ຕິ ພິ ມ ປີ 2019

ລະດັບທາດກາຍຕ້ານທີ່ແຕກຕ່າງກັນຂອງລະບົບສາທາລະນະສຸກພາຍຫລັງທີ່ໄດ້ມີການໃຫ້ວັກຊີນ DTPw-HepB-Hib ໃນ ສປປ ລາວ. Hefele L, Syphan S, Xayavong D, Homsana A, Kleine D, Chanthavilay P, Nouanthong P, Xaydalasouk K, Phathammavong O, Billamay S, Xeuatvongsa A, Reinharz D, Muller CP, Black AP. Clin Infect Dis. 2019 ກຸມພາ 19

ຄວາມຮູ້, ທັດສະນະຄະຕິ ແລະ ການປະຕິບັດ ຕໍ່ການໃຫ້ວັກຊີນໃນພະນັກງານແພດໃນ ສປປ ລາວ. Sengchaleun V, Khampanisong P, Aye-Soukhathammavong P, Reinharz D, Black AP. Southeast Asian J Trop Med Public Health, ມີນາ 2019.

ການເຝົ້າລະວັງພະຍາດອີສຸກອີໃສ ແລະ ໄຂ້ອອກຕຸ່ມໃນ ສປປ ລາວ. Nouanthong P, H bschen JM, Billamay S, Mongkhoune S, Vilivong K, Khounvisith V, Sinner R, Grandadam M, Phonekeo D, Black AP, Muller CP. BMC Infect Dis. 2019 ພຶດສະພາ 8;19(1):392.

ການຕິດຕໍ່ເຊື້ອພະຍາດ ແບບຂ້າມສາຍພັນໃນສັດປີກ ໃນຟາມລ້ຽງສັດແບບຈຸລະພາກ: ເປັດທີ່ຖືເຊື້ອໄວຣັດຈາກໄກ່. Pauly M, Snoeck CJ, Phoutana V, Keosengthong A, Sausy A, Khenkha L, Nouanthong P, Samountry B, Jutavijittum P, Vilivong K, H bschen JM, Black AP, Pommasichan S, Muller CP. Avian Pathol. 2019 ມິຖຸນາ 14.

ການຕິດຕໍ່ເຊື້ອແມ່ກາຝາກສູງໃນຄູ່ແມ່-ລູກ ຈາກສາມໂຮງໝໍສູນກາງ ໃນ ສປປ ລາວ. Pauly M, Sayasinh K, Muller CP, Sayasone S, Black AP. Parasite Epidemiology and Control. 2019

ອັດຕາຊຸກຊຸມທາງເຊຣອມວິທະຍາຂອງທາດກາຍຕ້ານພະຍາດບາດທະຍັກໃນ ແມ່ ແລະ ເລືອດຈາກສາຍແຮ່ຂອງລູກ ແລະ ປັດໄຈອື່ນໆ ທີ່ກ່ຽວຂ້ອງຂອງການເບິ່ງແຍງສຸຂະພາບໃນ ສປປ ລາວ
Ounnavong P, Chanthavilay P, Khampanisong P, Reinharz D, Muller CP, Black AP.

ພະນັກງານ

- ໂຄລດ ພີ. ມູນເລີ, ຫົວໜ້າ
- ແອນໂທນີ ແບລັກ, ນັກວິທະຍາສາດ ແລະ ຜູ້ຕາງໜ້າຮັບຜິດຊອບຫ້ອງທົດລອງ
- ພອນທິບສະຫວັນ ນວນທອງ, ນັກວິທະຍາສາດ
- ສີລິພອນ ວິຣະຈິດ, ນັກວິທະຍາສາດ
- ກິນນາລີ ໄຊດາລາສຸກ, ນັກວິທະຍາສາດ
- ວິໄລສອນ ຂຸນວິສິດ, ນັກວິທະຍາສາດ
- ລັດດາວອນ ແຄນຂາ, ເທັກນິກການແພດ
- ບຸນຕາ ວົງພະຈັນ, ເທັກນິກການແພດ

ນັກວິທະຍາສາດເຂົ້າຢ້ຽມຢາມຈາກພະແນກຂອງການຕິດເຊື້ອ ແລະ ພູມຕ້ານ,ລັກຊໍາເບີກ.

- ລີຊ້າ ເຫຟາລີ
- ແຟນຊິສກ້າ ຟຸກ
- ຍັງໂກ ຟານ ເບກ

Immunity levels to poliovirus in Lao children and adults before the vaccine-derived poliovirus outbreak: a retrospective study

Project coordinators: Maude Pauly, Claude Muller
Staff Members: Phonethipsavanh Nouanthong

Background

Large-scale vaccination campaigns eliminated wild poliovirus (WPV) in most countries of the world and WPV remains endemic only in Pakistan, Nigeria and Afghanistan. In the Lao People's Democratic Republic (Lao PDR), oral polio vaccine (OPV) was introduced in the early eighties, and vaccination was expanded to the whole country in the nineties. In 1996, the last case of WPV was reported in Lao PDR and in 2000, the Western Pacific Region, including Lao PDR, was certified polio-free.

Despite its ground-breaking role in the eradication of poliovirus (PV), OPV (containing live attenuated PV strains) has some important drawbacks. A small proportion of vaccinees develop vaccine-associated paralytic poliomyelitis (VAPP).

In countries with suboptimal vaccination coverage levels and weak acute flaccid paralysis (AFP) surveillance, excreted vaccine-virus can replicate and circulate for a prolonged time. Within less than a year of circulation, vaccine viruses may accumulate genetic mutations and neurovirulent vaccine-derived PV (VDPV) may emerge.

At the end of 2015, VDPV type 1 strains emerged in Lao PDR and caused paralysis in 11 individuals (last case in January 2016) in the three neighbouring provinces Bolikhamxay, Xaisomboun and Vientiane Province (see also Fig 1). Most of the affected were male; 4 were below the age of 18 months, 4 between 4 and 15 years, and 2 were in their forties. Only a one-year old child had completed the three OPV vaccinations. The others had only one or no dose of OPV. Virtually all belonged to the ethnic group of the Hmong. Circulation of the VDPV (cVDPV) was confirmed by epidemiological investigations and molecular analyses. By the beginning of 2016, the outbreak was declared a public health emergency by the Prime Minister and large vaccination campaigns with trivalent OPV were launched and surveillance was intensified throughout the country.

To reduce the risk of cVDPV emergence and in compliance with the Polio End Game Strategy, a switch from trivalent (containing type 1, 2 and 3 vaccine strains) to bivalent (containing type 1 and 3 vaccine strains) OPV was globally implemented in 2016. In Lao PDR, the new recommendations, introduction of bivalent OPV and of inactivated vaccine (IPV) as an adjunct to routine immunization with OPV will soon be implemented.

Currently, PV surveillance relies mainly on the reporting of AFP and in Lao PDR, reported rates of AFP reached almost the recommended minimum required for PV-free countries (i.e. one per 100 000 children below 15 years of age). However, PV surveillance remains challenging as silent circulation of WPV and VDPV is frequent.

Not enough is known in Lao PDR about immunity levels against PV to understand the reasons for the recent VDPV outbreak. We investigated five large cohorts with different epidemiological, demographic, exposure and vaccination histories in order to provide important public health information about polio epidemiology.

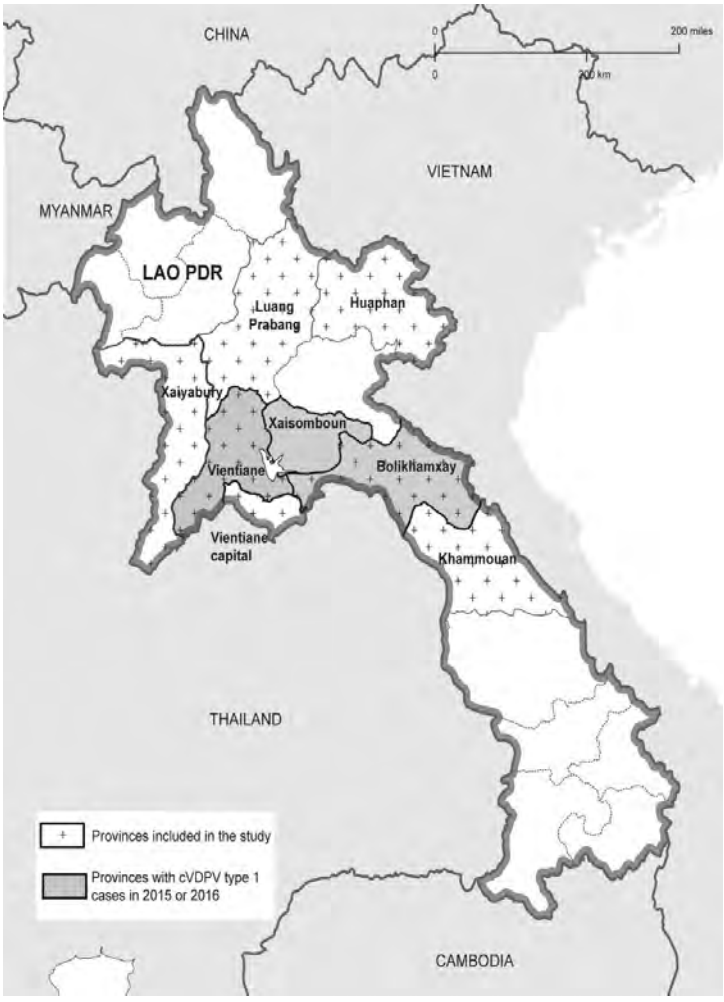


Fig 1. Lao provinces. Provinces affected by the cVDPV type 1 outbreak are represented in grey and provinces included in the study are dotted.

Results

Seroprevalence in children (Cohorts 1-3)

97.6% of the children with full vaccination records and aged less than 3.5 years had IgG antibodies against PV by ELISA (Cohort 1). Neither sex or age, nor birthplace or nutritional status influenced PV immune status. However, seropositivity rates differed significantly between the 3 provinces ($\chi 2=14.7$, df=2, p<0.01; Table 1).

An anti-PV antibody seroprevalence of 98.8% was determined in children aged less than 5 years from remote districts in Huaphan (Cohort 2).

Neither age or sex, nor distance to next health care facility had a significant effect on PV immune status (Table 1). In another cohort of children with unknown vaccination status from less marginalized communities (Cohort 3), antibody seroprevalence was somewhat lower with 92.3%. Similar to what was found for cohort 1, significantly lower immunity rates were also found for this cohort in Bolikhamxay (84.1%) than in the two other provinces (Luang Prabang: 95.4%, OR=3.9, 95%CI=1.4-10.6, p=0.01; Vientiane: 96.3%, OR=4.9, 95%CI=1.4-18, p=0.007). Children aged less than 1 year were significantly more likely to have anti-PV antibodies than children aged 5 to 9 years (97.6% versus 88.6%, p=0.007) and anti-PV antibody levels were negatively correlated with age (Pearson's r=-0.4; 95%CI=-0.5 and -0.3; t=-7.1, df=318, p<0.001).

Seroprevalence in adults (Cohorts 4 and 5)

81.7% of the blood donors (Cohort 4) had antibodies against PV by ELISA. Seroprevalence rates ranged from 81.1 to 83.3% in the 4 provinces with no significant differences. Participants born after the introduction of OPV into the national immunization program in 1979 were significantly more likely to be seropositive: only 69.2% of the birth cohort 1958-1977 were seropositive, compared to 82.7 and 85.1% of the younger birth cohorts (1978-1988: OR=2.1, 95%CI=1.1-4, p=0.028; 1989-1998: OR=2.5, 95%CI=1.4-4.6, p=0.003) (Table 1, Fig 2A). Anti-PV antibody levels correlated negatively with age (Pearson's r=-0.1; 95%CI=-0.2 and 0; t=-3.6, df=526, p= 0.001) (Table 1, Fig 2B). Mean antibody levels were significantly lower in blood donors (47.6, 95%CI=18.2-69.6) than in the fully vaccinated children (79.1; 95%CI=47.7-79.1) (t=-14.3, df=1031.5, p-value<0.001; Fig 2C).

Overall, a lower seroprevalence was determined for HCW (Cohort 5) than for blood donors: only 71.9% of the HCW had antibodies against PV by ELISA. Also in this adult cohort, the antibody seroprevalence decreased with age from 79.7% (birth year 1989-1998), to 74.3% (birth year 1978-1988), to 69% (birth year 1958-1977), but between the youngest and the oldest cohort there was only a trend to significance (p=0.067).

Anti-PV antibody levels were negatively correlated with age (Pearson's r=-0.2; 95%CI=-0.2 and -0.1; t=-2.9, df=698, p-value=0.004). The other recorded risk factors were not significant (Table 1).

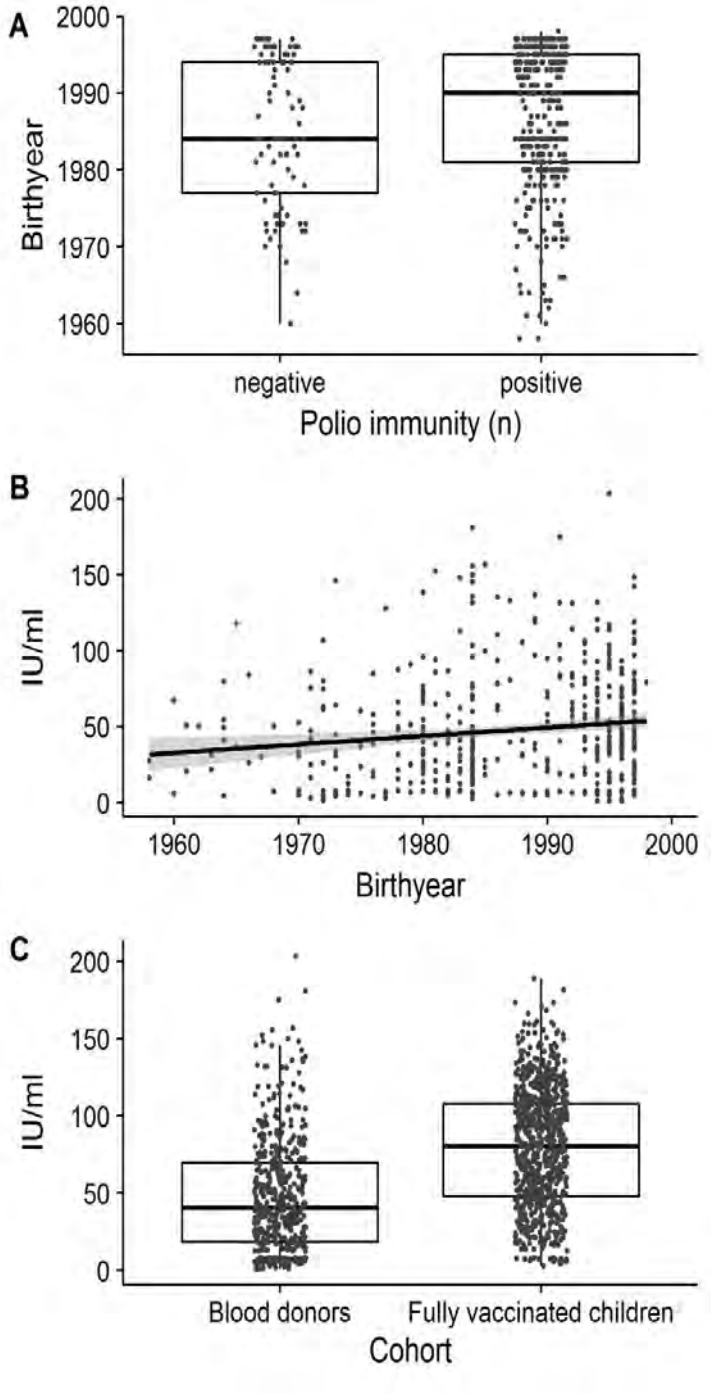


Fig 2. Age-related differences in poliovirus immunity as determined by ELISA

(A) Differences in age between seronegative and seropositive blood donors and (B) age-stratified anti- poliovirus antibody levels in blood donors. The regression line and the confidence interval (shaded) are shown. (C) Differences in median antibody levels between fully vaccinated children (Cohort 1) and blood donors (Cohort 4).

Discussion

To reduce the risk of WPV re-importation in Lao PDR, the immunization services were strengthened nationwide. In 2015, the OPV vaccination coverage was estimated at 89% for 12-23 months old children. Nevertheless, in the same year, the country experienced a cVDPV type 1 outbreak.

We find here that overall well above 90% of children had antibodies against PV. This was true even for a rural cohort of children with unknown vaccination status. This high seroprevalence is also largely due to a very high vaccine efficacy: 97.6% of fully vaccinated children were positive for anti-PV antibodies by ELISA. Although weak vaccine responses to OPV have been reported in chronically malnourished children, we observed no effect of malnutrition on antibody levels. Also no significant difference in PV immunity was found between children born at home and children born in hospital settings. Remarkably, the cohort of children from remote districts in Huaphan were equally well protected as the fully vaccinated children, and distance to the next health care facility had no negative impact on their immune status. This suggests a high efficiency of outreach vaccination activities.

However, significantly lower seropositivity rates were determined in Bolikhamxay, both for fully vaccinated children (94.5%) and children with unknown OPV status (84.1%). This is also the province which notified the first paralytic cases during the outbreak. Deficiencies related to vaccine management may also have influenced OPV efficacy.

In our adult cohorts, we found that only 81.7% of the Lao blood donors and 71.9% of the HCW had anti-PV antibodies by ELISA. There was no significant difference between blood donors from different provinces and between HCW from central, provincial or district hospitals or with different clinical and non-clinical positions. In both adult cohorts, older participants and particularly those born before the introduction of OPV in the country had significantly lower anti-PV antibody levels and, were significantly less likely to be seropositive (Figs 2A and B) than younger adults or the children of Cohort 1 and 2. Adults born before the eighties did not undergo routine vaccination and were also less likely to participate in SIAs. The significant age-dependent decrease in total anti-PV antibodies determined by ELISA among all adult cohorts could thus be a reflection of the lower vaccination coverage in this age group, or of the waning immunity after vaccination or exposure to VDPV or WPV. This may explain why four of the eleven reported paralytic cases occurred among individuals above the age of 14 years.

Thus, our seroprevalence results are in line with what was observed during the outbreak. However, a limitation of our cohorts was that for ethical reasons we were unable to collect explicit information on ethnicity, while ethnic minority communities were most affected by the outbreak. These and adult risk groups such as HCWs, and regions with weak vaccination programs should be primarily targeted by future supplementary vaccination activities.

These data have been published as a manuscript in Plos One, and reported to the National Immunization Technical Advisory Group and the National Immunization Programme in the form of a policy brief. The data were also presented as a poster at the Global Vaccine Research Forum in Bangkok, 2018.

Table 1. Association between socioeconomic characteristics and anti-polio IgG seropositivity as determined by ELISA for each study cohort.

# Cohort	Factors (significance level)	Levels	% of Total	Anti-Polio seroprevalence	
				n (%)[a]	n (%)[b]
#1 Fully vaccinated children (N=806)	Total Cohort		100	787/806 (97.6)	757/776 (97.6)
	Province (**)	Bolikhamxay	26.1	199/210 (94.8)	188/199 (94.5)
		Khammouane	42.9	345/346 (99.7)	338/339 (99.7)
		Vientiane	31	243/250 (97.2)	231/238 (97.1)
	Age group	≤ 1 year	8.9	70/72 (97.2)	69/71 (97.2)
		1 year > x ≥ 2 years	52.1	408/420 (97.1)	391/403 (97)
		2 years > x ≥ 3.5 years	39	309/314 (98.4)	297/302 (98.3)
	Sex	Female	51.4	405/414 (97.8)	389/398 (97.7)
		Male	48.6	382/392 (97.5)	368/378 (97.4)
	Birthplace	Home	30	239/242 (98.8)	230/233 (98.7)
		Hospital	70	548/564 (97.2)	527/543 (97.1)
	WHZ	≥ -2	89.1	699/718 (97.4)	672/691 (97.3)
		< -2	8.1	65/65 (100)	62/62 (100)
		Unknown	2.8	N/A	N/A
	HAZ	≥ -2	57	448/459 (97.6)	430/441 (97.5)
		< -2	39	307/315 (97.5)	297/305 (97.4)
		Unknown	4	N/A	N/A

	WAZ	≥ -2	78.5	616/633 (97.3)	592/609 (97.2)
		< -2	20.8	166/168 (98.8)	160/162 (98.8)
		Unknown	0.7	N/A	N/A
#2 Children from remote areas (N=90)	Total Cohort		100	88/90 (97.8)	84/85 (98.8)
	District	Xam Tai	58.9	51/53 (96.2)	49/50 (98)
		Kuan	41.1	37/37 (100)	35/35 (100)
	Age group	1-2 years	16.7	15/15 (100)	15/15 (100)
		2 years ≥ x > 3years	22.2	19/20 (95)	18/19 (94.7)
		3 years ≥ x ≥ 5 years	61.1	54/55 (98.2)	51/51 (100)
	Sex	Female	43.3	39/39 (100)	36/36 (100)
		Male	56.7	49/51 (96.1)	48/49 (98)
#3 Children with unknown vaccination status (N=320)	Total Cohort		100	297/320 (92.8)	277/300 (92.3)
	Province (*)	Bolikhamxay	30.6	84/98 (85.7)	74/88 (84.1)
		Luang Prabang	43.8	134/140 (95.7)	124/130 (95.4)
		Vientiane	25.6	79/82 (96.3)	79/82 (96.3)
	Age group (**)	≤ 1 year	39.1	122/125 (97.6)	122/125 (97.6)
		5 years ≥ x ≥ 9 years	60.9	175/195 (89.7)	155/175 (88.6)
	Sex	Female	51.3	152/164 (92.7)	146/158 (92.4)
		Male	48.7	145/156 (93)	131/142 (92.3)
#4 Blood donors (N=528)	Total Cohort		100	441/528 (83.5)	389/476 (81.7)
	Province	Huaphan	15.3	67/81 (82.7)	60/74 (81.1)
		Khammouane	48.7	215/257 (83.7)	185/227 (81.5)
		Vientiane	25.8	113/136 (83.1)	104/127 (81.9)
		Xaiyabury	10.2	46/54 (85.2)	40/48 (83.3)
	Age group (**)	1989-1998	50.4	230/266 (86.5)	206/242 (85.1)
		1978-1988	32.4	144/171 (84.2)	129/156 (82.7)
		1958-1977	17.2	67/91 (73.6)	54/78 (69.2)
	Sex	Female	38.8	175/205 (85.4)	162/192 (84.4)
		Male	61.2	266/323 (82.4)	227/284 (80)
#5 Healthcare workers (N=700)	Total Cohort		100	536/700 (76.6)	420/584 (71.9)
	Location hospital	Central or provincial	72.7	388/509 (76.2)	304/425 (71.5)
		District	27.3	148/191 (77.5)	116/159 (73)
	Age group	1989-1998	12.4	72/87 (82.8)	59/74 (79.7)
		1978-1988	29.3	160/205 (78.1)	130/175 (74.3)
		1958-1977	58.3	304/408 (74.5)	231/335 (69)
	Sex	Female	78.7	429/551 (77.9)	335/457 (73.3)
		Male	21.3	107/149 (71.8)	85/127 (66.9)
	Position	Lab technician	7.3	41/51 (80.4)	35/45 (77.8)
		Medical doctor	21	112/147 (76.2)	90/125 (72)
		Nurse	53.9	287/377 (76.1)	219/309 (70.9)
		Others	12.1	64/85 (75.3)	51/72 (70.8)
		Specialist	5.7	32/40 (80)	25/33 (75.8)

[a]Complete dataset, borderlines being considered positive
[b]Dataset without borderline samples
*significant effect on anti-poliovirus antibody seroprevalence (p-value between 0.05 and 0.01)
**highly significant effect on anti-poliovirus antibody seroprevalence (p<0.01)

Knowledge, attitudes and practices regarding vaccination among healthcare workers in Lao PDR

Project coordinators: Antony Black
Staff members: Viengsamay Sengchaleun, Phonepaseuth Khampanisong

Background

Health care workers (HCW) are at increased risk for infection due to vaccine-preventable diseases (VPD) because of their contact with infected patients and contaminated equipment and environments. HCW may also transmit infection to other staff and patients.

Outbreaks of VPD are common in health-care settings, even in countries with well-established immunization programs. Vaccination of HCW is important to prevent some nosocomial infections.

The World Health Organization recommends HCW should be vaccinated against hepatitis B, polio, diphtheria, measles, rubella, meningococcal, influenza and varicella.

In Lao PDR hepatitis B virus (HBV) infection is endemic; approximately 45% of the general population has been exposed to HBV and 8–10% of the adult population is chronically infected. Outbreaks of other VPD continue to occur due to a combination of low vaccine coverage and poor vaccine immunogenicity. Our previous study found Lao HCW had low levels of protective antibodies against VPD.

Given the low vaccination coverage and rate of people with vaccination induced protective antibodies among general population in Lao PDR, HCW vaccination is important. Several knowledge, attitudes and practices (KAP) studies among HCW have been done in the USA, UK and China to evaluate the relationship between knowledge and influenza vaccination.

We aimed to determine the KAP regarding vaccination among HCW in Lao PDR in order to inform VPD control programs to improve vaccination rate among HCW.

The survey was done on 400 participants from 4 central hospitals in Vientiane Capital (Settathirat, Mitthaphab, Mahosot and Children's Hospital) and four provincial hospitals (Vientiane, Khammouan, Bolikhamxay and Houaphan). Subjects recruited for the study were physicians (internists, paediatricians, surgeons, obstetricians), midwives, nurses, dentists, lab technicians, administrators and pharmacists.

Results

Socio-demographics characteristics

The mean age (range) of study subjects was 38 (21 to 64) years; 74.7% were female. 40.5% of study subjects were nurses. The mean number of years worked at a hospital was 14. 49.5% of subjects had completed vocational training without further studies (Table 1).

Table 1: Socio-demographic characteristics of study subjects

Variables	n (%)
Gender	
Female	299 (74.7)
Age (in years)	
21-40	246 (61.5)
41-61	154 (38.5)
Profession	
Nurse	162 (40.5)
Administrator	51 (12.7)
Internist	46 (11.5)
Anesthesiologist	22 (5.5)
Pharmacist	21 (5.2)
Dentist	19 (4.7)
Lab technician	19 (4.7)
Pediatrician	16 (4.0)
Surgeon	16 (4.0)
Physical therapist	8 (2.0)
Obstetrician/ Gynecologist	8 (2.0)
Radiologist	6 (1.5)
Otolaryngologist/ophthalmologist	6 (1.5)
Duration of work (years)	
1-10	191 (47.7)
10-41	209 (52.3)
Education	
Vocational training	198 (49.5)
Graduate or higher	202 (50.5)

Knowledge on vaccination

88.7% of study subjects were aware of the existence of an HBV vaccine and 88.2% of the polio vaccines. Eighty-four percent of subjects knew vaccines can have side-effects. 13.5% of subjects believed vaccines can be replaced by traditional medicine. 21.5% of subjects believed a single vaccine dose always provides life-long immunity (Table 2).

Table 2: Knowledge about vaccination among study subjects

Knowledge about vaccination	n (%)
Vaccination can prevent some diseases	382 (95.5)
Which diseases can be prevented by vaccination	
Hepatitis B[a]	355 (88.7)
Hepatitis C[b]	146 (36.5)
Polio[a]	353 (88.2)
Malaria[b]	39 (9.7)
HIV[b]	19 (4.7)
It is recommended not to administer a vaccine to a child if they have:	
A severe allergic reaction to a previous dose[a]	355 (88.7)
Malnutrition[b]	26 (6.5)
A fracture[b]	18 (4.5)
None of above[b]	1 (0.2)
Vaccination can have side-effects	
	336 (84.0)
If yes, which side-effects	
Fever[a]	303 (90.2)
Headache[a]	98 (29.2)
Paralyze[b]	246 (73.2)
Rash[a]	24 (7.1)
Epistaxis[b]	322 (95.8)
Some vaccines cause disease	151 (37.7)
One dose of vaccine provides life-long immunity	258 (64.5)

a: correct answer; b: incorrect answer

Awareness of diseases and attitude toward vaccination

Seventy-nine percent of subjects were concerned about contracting a disease while at work; 63.6% of subjects were concerned about contracting HBV infection, 61.0% were concerned about contracting tuberculosis and 56.6% were concerned about contracting HIV infection. 15.5% of subjects were unconcerned about contracting infection from their patients; of these, 93.5% said they knew how to protect themselves but only 6.5% of them mentioned vaccination as a method of protecting themselves.

Ninety-seven percent of subjects stated HCW should be vaccinated; but only 70.3% stated HCW should be vaccinated against HBV. 86.2% of subjects believed vaccinations were effective in preventing disease, 7.8% were not sure and 6.0% believed vaccinations were not effective in preventing disease; of those, 50.0% stated the reason for this belief was they felt vaccines were not stored correctly. Ninety-five percent of the subjects would recommend vaccinations for their colleagues and 88.2% would recommend vaccinations for their patients. However, only 48.5%of subjects stated they intended to get vaccinations in the future.

Practice

Ninety percent of subjects reported having been vaccinated against at least one disease since starting work in the hospital with the main motivation being self-protection. Among those who had not been vaccinated, the most frequent reason given for not being vaccinated was fear of adverse reactions (Table 3). Of those who had been vaccinated, 73.5% had been vaccinated against HBV. 66.9% of subjects knew their HBV immunity status. Some subject had been previously vaccinated against polio virus, tetanus, diphtheria or seasonal influenza; these depended on the availability of vaccines and presence of epidemics where they lived.

Table 3. Reasons given by study subjects for receiving or avoiding vaccinations.

Reasons given for receiving a vaccination (n = 367)	n (%)
Self-protection	361 (98.4)
Protection of patients	121 (33.0)
Protection of family	149 (40.5)
Reasons given for avoiding a vaccination (n = 31)	**n (%)**
Fear of an adverse reaction	10 (32.2)
Never offered a vaccine	9 (29.0)
Disease not perceived to be serious	7 (22.6)
Fear of the injection	3 (9.7)
Belief vaccine does not provide sufficient protection	1 (3.2)
Received a vaccine before coming to work at the hospital	1 (3.2)

Information sources and hospital policies

80.5% of study subjects received information about vaccinations from their colleagues and 43.7% received information via their professional studies. 71.7% of study subjects stated their hospitals held information seminars about vaccines and 89.7% stated they always received information from their hospitals during an outbreak.

Eighty-one percent of study subjects stated some vaccinations were provided free at their hospitals, including the influenza vaccine, HBV vaccine, diphtheria vaccine, polio vaccine, tetanus vaccine, measles vaccine, pertussis vaccine, Japanese encephalitis vaccine and rubella vaccine; this varied by hospital.

Factors associated with a good knowledge about vaccinations after multivariate analysis were: working in a clinical field rather than working in administration (OR: 2.0; 95% CI: 1.2-3.4; p>0.05) and duration of hospital work >10 years (OR: 1.7; 95% CI: 1.1-2.5; p>0.05). Receiving information about vaccinations from colleagues was significantly associated with a favorable attitude toward vaccination (OR: 3.2; 95% CI: 1.4-7.7; p>0.01). Working in a hospital providing free vaccinations was significantly associated with vaccination practice (OR: 3.5; 95% CI: 1.4-8.5; p<0.01). Having a positive attitude about vaccination was significantly associated with practices regarding vaccination (OR: 2.4; 95% CI: 1.1-5.5; p<0.05).

Discussion

The results of this study must be understood in the context of vaccinations among HCW in the Lao PDR. There is currently no national policy in Lao PDR regarding HCW vaccinations, unlike many other countries. A previous study found 53% of HCW in Lao PDR had protective antibodies against HBV infection, the immunity was derived mostly from previous infection, rather than vaccination. Indeed, only 21% had anti-HBs antibodies without anti-HBc antibodies, indicative of HBV vaccination. Childhood HBV vaccination was only introduced into Lao PDR in 2001.

Vaccination rates among HCW in Lao PDR can be improved by focusing on factors in our study significantly associated with HCW vaccination uptake, such as receiving recommendations by colleagues to be vaccinated and receiving the vaccine for free.

Having a good attitude about vaccination was significantly associated with being vaccinated in our study, similar to a study from Shropshire, United Kingdom that found that the most common reason for being vaccinated against influenza among HCW was believing vaccination to be effective.

A statistically significant association was seen between receiving information from colleagues and being vaccinated. Colleagues played an important role in vaccination decisions. A study from the United States, reported the 95% of surveyed parents of children aged ≤18 months accepted advice about vaccination decisions from a "people network" (friend, physician and family). This suggests giving HCW the opportunity to discuss vaccine with colleagues along with receiving information though seminars may improve vaccine uptake among HCW. Discussing the potential side effects and benefits of vaccination may also improve uptake among HCW. Misconceptions can also be corrected as seen in study subjects, such as the belief that traditional medicine is as effective in disease prevention as vaccination or a single dose of a vaccine provides life-long protection.

Free vaccination and convenient vaccination should also help improve vaccination uptake by HCW. The hepatitis

B vaccine usually costs around 10-20USD per dose in Lao PDR, where many workers received an income of around 200USD per month.

Conclusion

Vaccination is the most effective way to prevent VPD among HCW. Attitudes about vaccination affects vaccination practices. Receiving advice from colleagues and having a free vaccine also improve vaccine uptake. Vaccine promotion programs for HCW should take these factors into consideration. Further studies are needed to determine if implementation of programs considering these factors can improve VPD morbidity among HCW in Lao PDR. These data have been published as a manuscript in Southeast Asian Journal of Tropical Medicine and Public Health and were reported as a Masters Thesis for a student from the Lao Tropical and Public Health Institute.

Hepatitis A virus in Lao People's Democratic Republic

Project coordinators: Antony Black
Staff member: Vilaysone Khounvisith

Background

Hepatitis A virus is a global public health problem, with approximately 1.5 million clinical cases of hepatitis A estimated worldwide annually. The incidence of infection has a strong relation with sanitary and environmental conditions and the level of socioeconomic development. Hepatitis A has a particularly high rate of endemicity in underdeveloped countries with poor sanitation, as it is often passed through the faecal-oral route from contaminated food or water. In Lao PDR, little is known about the disease. Several outbreaks of the disease have been reported, such as a major one that occurred in 2016 in Xiengkhuang Province (North of Lao PDR). Therefore, we investigated the age-stratified HAV seroprevalence in Xiengkhouang province and Vientiane Capital in Lao PDR. We also determined risk-factors for HAV infection.

Results

1195 participant were included, 400 from Xiengkhoung province and 795 from Vientiane Capital. Overall 62% of participants were anti-HAV positive in Xiengkhouang province compared to 45.5% in Vientiane Capital. In Xiengkhouang, 23.7% of 5 to 10 years olds were already seropositive compared to 5% in Vientiane. A dramatic increase in seroprevalence occurred between the 15 to 20 and the 21 to 30-year-old age groups (35.7% to 62.4%, Xiengkhouang and 11.5 to 69.7%, Vientiane) until essentially all older adults were positive in both locations. The main risk factors for HAV-antibodies were age, non Lao-Tai ethnicity and food-related.

This study showed significant exposure to HAV in Lao PDR. The majority of the adult population have been exposed, probably during their early lives before general sanitation had improved. Currently, some exposure still begins early, particularly in rural areas with lesser sanitation, whilst progress has been made in Vientiane Capital.

As expected, the rate of past infections increased steadily with age. In the participants above 30 years of age, more than 80% had previous HAV infection in both the rural setting of Xiengkhouang and the urban setting of Vientiane Capital. In the capital, there was a conspicuous difference between cohorts born before and after 1996. This may reflect infections of these individuals during childhood and an improvement in sanitation in the late 1990s. Similarly, in neighbouring Thailand, improved sanitation resulted in a reduction in childhood infections and therefore a shift in the age distribution of anti-HAV positive individuals. The age at which 50% of the population are anti-HAV positive in Xiengkhouang is about 20 years.

Policy and impact

Awareness campaigns regarding the risk of HAV infection should be conducted e.g. in schools and villages. Furthermore, improvements in water sanitation, in particular in the rural districts, and better food hygiene are warranted. Our study also suggests that HAV infections are under-reported. Improved reporting would provide guidance for targeted interventions to further reduce HAV infections. These data have been formulated as a policy brief and a manuscript for submission to an international journal.

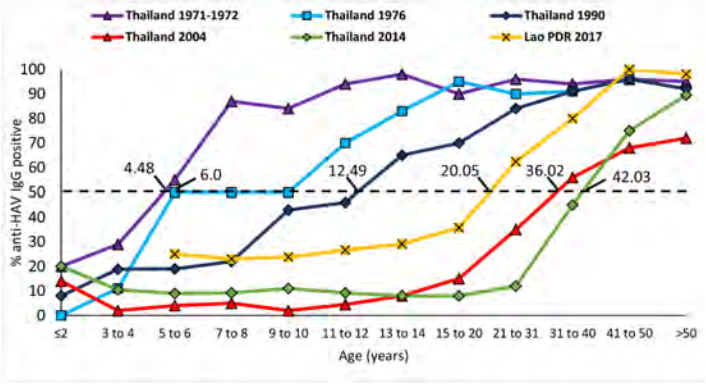


Figure 1. Age-stratified anti-HAV IgG seroprevalence data from Xiengkhouang, Lao PDR in 2017 compared to Thailand from 1971/1972 to 2014. Dotted line corresponds to the age of 50% seropositivity. Adapted from Sa-Nguanmoo *et al.* 2016.

Cross-species transmission of poultry pathogens in backyard farms: ducks as carriers of chicken viruses

Project coordinators: Maude Pauly, Claude Muller
Staff members: Latdavone Khenkha, Phonethipsavanh Nouanthong

Background

In Southeast Asia, many risk factors facilitate the cross-species transmission of poultry diseases: large populations of wild and domestic birds, the omnipresence of extensive agriculture, and popular live bird markets. In particular, the village or backyard production system is challenged by enzootic avian diseases. Low hygienic standards, mixing of poultry species and production stages, low-quality feed, uncontrolled bird trade, lack of bird containment and limited access to veterinary and diagnostic laboratory services are drivers for pathogen circulation and cross-species transmission.

Worldwide, the broad range of susceptible hosts and transmission routes represent major challenges to the control of avian viruses. Virtually all bird species are susceptible to Newcastle disease virus (NDV) infection, and wild birds can be a source of infection for domestic poultry and *vice versa.*

Chickens are the only natural host of chicken anaemia virus (CAV), but other avian and mammalian species may contribute to its spread. Although CAV is mainly transmitted by the faecal-oral route, vertical transmission dramatically impacts the viability of the progeny. Unlike CAV, several bird species are susceptible to infectious bronchitis virus (IBV), an avian coronavirus (CoV). However, other avian CoV strains (e.g. duck CoV) are more species-specific. The emergence of novel recombinants and strains, sometimes in unusual host species, as well as the prevailing lack of pathognomonic signs, further complicates the diagnosis of avian diseases. The clinical course of many avian diseases depends on strain- and host-related factors. Depending on the strain, IBV replicates in the respiratory, urogenital or gastrointestinal tracts of symptomatic or even asymptomatic birds. Inapparent infections are also typical for some pathotypes of NDV, while others lead to high morbidity and mortality.

Vaccination against avian viruses is challenging. Adequate storage and administration of vaccines are difficult in remote settings. Moreover, the protection is short-lived, weak and type-specific, and the vaccines are mostly licenced only for few host species. Due to the high antigenic diversity, the propensity for recombination and the lack of universal cross-protection, continuous adaptation of vaccine strains to circulating IBV strains is required. These features of avian viruses represent important challenges to backyard farming in rural Southeast Asia.

In Lao People's Democratic Republic (Lao PDR), poultry rearing is an important source of subsistence, income and high-quality nutritional protein. To assess viral evolution, host range and transmission routes in backyard farms, chickens and ducks from rural areas in Lao PDR were screened for viruses with a broad host range (i.e. NDV and CoV), as well as for a virus with a restricted host range (i.e. CAV).

Results

Table 1. Description of sample datasets.

		Complete dataset	Oral/tracheal swabs (OS)	Cloacal swabs (CS)	Year 2011 (CS)	Year 2014 (OS)	Year 2015 (OS and CS)
		n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Total		619 (100)	418 (100)	407 (100)	201 (100)	212 (100)	206 (100)
District	Xaythani	469 (75.8)	418 (100)	257 (63.2)	51 (25.4)	212 (100)	206 (100)
	Sikhottabong	150 (24.2)	0 (0)	150 (36.9)	150 (74.6)	0	0
Poultry species	Chicken	297 (48.0)	237 (56.7)	183 (45.0)	60 (29.9)	114 (53.8)	123 (59.7)
	Duck	309 (49.9)	168 (40.2)	220 (54.1)	141 (70.1)	89 (42.0)	79 (38.3)
	Other	13 (2.1)	13 (3.1)	4 (1.0)	0 (0)	9 (4.3)	4 (1.9)
Sex	Female	262 (62.7)	262 (62.7)	141 (68.4)		121 (57.1)	141 (68.5)
	Male	156 (37.3)	156 (37.3)	65 (31.6)		91 (42.9)	65 (31.6)
	not available	201	0	201	201	0	0
Age class	Adult	263 (62.9)	263 (62.9)	159 (77.2)		104 (49.1)	159 (77.2)
	Young	155 (37.1)	155 (37.1)	47 (22.8)		108 (50.9)	47 (22.8)
	not available	201	0	201	201	0	0

Table 2. Virus detection rates according to sample type and collection year.

	Oral/tracheal swabs (OS)	Cloacal swabs (CS)	Year 2011 (CS)	Year 2014 (OS)	Year 2015 (OS)	Year 2015 (CS)
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
CoV	26 (6.2)	156 (38.3)	115 (57.2)	6 (2.8)	20 (9.7)	41 (19.9)
CAV	67* (16.1)	7 (1.7)	2 (1.0)	24* (11.4)	43 (20.9)	5 (2.4)

*1 sample not tested

Results

CoV detection

Overall, 6.4% (26/404) of the oral/tracheal swabs and 38.7% (156/403) of the cloacal swabs were positive for CoV. High CoV positivity rates were found in cloacal swabs of chickens (27.3%) and ducks (48.2%). Detection rates in the oral/tracheal swabs were significantly lower (9.3% and 2.4%) for both host species. While CoV RNA was consistently found in faecal swabs from every village, viral RNA was only sporadically detected in oral/tracheal swabs. Significantly higher circulation was found in juveniles than in adults (oral/tracheal swabs: 9.7% versus 4.4%, $P = 0.04$; cloacal swabs: 46.8% versus 12.3%, $P < 0.01$). IBV-like and duck CoV-like strains were detected in both chickens and ducks. However, IBV-like strains

were mostly detected in chickens, and duck CoV-like strains mostly in duck samples ($P < 0.001$).

The most suitable specimen for virus detection depended on the viral strain. IBV-like strains were detected in both swabs of chickens and ducks. In contrast, duck CoV-like strains were detected only in the oral/tracheal swab of a single duck. Only in 2015, both swab types were collected: although overall 9.9% of the oral/tracheal and swabs 20.3% of the cloacal swabs were positive, CoV RNA was detected in both sample types only in 11 cases. Whenever good quality sequences of the partial viral genomes were obtained from both sample types, the nucleotide sequences were 100% identical. The results of the CoV screening are summarized in Table 2.

CAV detection

Overall, 11.6% (47/404) of the oral/tracheal swabs and 1.7% (7/403) of the cloacal swabs were positive for CAV with an apparent local outbreak pattern: CAV positivity was limited to approximately half of the villages (3/6 in 2011 and 4/6 in 2015; no village-related data available for 2014). Positivity was higher in juveniles than in adults (oral/tracheal swabs: 17.5% versus 8%, $P < 0.01$; for cloacal swabs: 6.4% versus 1.3%, $P > 0.05$). Although CAV DNA detection predominated in respiratory secretions of chickens (18.1%, 43/237), viral DNA was also detected in oral/tracheal swabs of a few ducks (4/167, 2.4%). Two of the positive ducks were from mixed-species farms located in the same village. While CAV DNA was detected in none of the chicken samples collected in these farms, viral DNA was detected in chickens from other farms in the village. In 2015, CAV DNA was only detected in the two sample types of three birds and the nucleotide sequences obtained from both sample types were 100% identical. The results of CAV screening are summarized in Table 2.

NDV detection

While anti-NDV antibodies were detected in 86.9% (107/123) of chicken sera with no difference in seroprevalence between juvenile and adult birds, virus RNA was detected in none of the swabs.

Viral strain characterization

BLAST and phylogenetic analyses of the partial RdRp gene of CoV, revealed that diverse avian CoV strains co-circulated in rural Lao PDR. A strain clustering with known duck CoV strains was detected in cloacal swabs of 81 ducks and 10 chickens that were collected in 2011 in different villages in both districts. Interestingly, the positive chickens were not from the same farms or villages as the positive ducks. Two different duck CoV strains were detected in cloacal and tracheal swabs of ducks in 2015. Although most Lao IBV strains were obtained from cloacal and tracheal swabs of chickens, some were also detected in duck samples (e.g. AvCoV/duck/Lao PDR/LAO15_A_11205/2015_MH496841).

All positive ducks were from mixed-species farms or at least from villages with IBV-positive chickens. The vast majority formed a cluster together with the reference strains XDC-2, A2, CK/CH/LLN/111169 and GX-NN09032 (accession numbers: KM213963, EU526388, KF411040 and JX897900) from chickens in China. Another Lao strain detected in only one chicken in 2015 belonged to another cluster (AvCoV/duck/Lao PDR/LAO15_A_112551/2015_MH496833) and was most closely related to the reference strain SAIBK from China (accession number: DQ288927). All attempts to sequence the S1 gene of the duck CoV-like strains failed. However, partial S1 gene sequences were successfully obtained from a subset of the IBV-like positive tracheal/oral and cloacal swabs.

Phylogenetic analyses showed that the Lao IBV-like strains obtained from chickens and ducks may be classified as lineages GI-1, GI-13, GI-19 and GI-25. Most, but not all, of those lineages include strains from China or India. Lao CAV strains clustered in three of the eight recognized CAV lineages, namely lineages 6, 7 and 8. Almost all CAV sequences were obtained from oral/tracheal swabs and, in 2015, most were obtained from samples collected in a single village where strains from lineages 7 and 8 co-circulated. A CAV sequence (MH497005) could only be obtained from one duck sample from the 2014 cohort and it was closely related to the chicken strains. As neither village- nor

farm-related information was collected in 2014, , it remains unknown whether this duck was raised with CAV-positive chickens. Interestingly, one Lao CAV strain was distantly related to the recognized lineage 1 (i.e. MH496999), but only a relatively small sequence was obtained from this strain (i.e. 578 bp). The vast majority of the Lao strains clustered with sequences from Asia (e.g. China, India, Cambodia and Taiwan).

Discussion

We previously found that 90% of the smallholders in Vientiane Province own poultry with the majority (64%) rearing both chickens and ducks (data not shown). Here, we show high circulation rates of NDV, CoV and CAV, challenging the livelihoods of (semi-)subsistence farmers.

It was difficult to compare our results from Lao PDR to other countries in the region due to the dearth of recent high-quality baseline data on avian diseases in Southeast Asia. It is known that certain avian CoV strains of galliform and non-galliform birds are closely related. Host tropism of CoV depends on the virus strain. In line with previous reports, IBV-like strains were detected in Lao chickens and ducks. Most interestingly, duck CoV-like strains were also detected in both poultry species. Domestic ducks are susceptible to CoV related to viruses of wild waterfowls, which can shed diverse Gamma- and Deltacoronaviruses at high rates. Hence, wild and domestic Anseriformes birds may play a role as mixing vessels for diverse CoV strains. In return, chickens probably also represent a minor infection source of duck CoV. Co-circulation of IBV-like and duck CoV-like strains in both species raises concerns for the generation of new CoV variants through recombination.

In contrast to avian CoV, CAV circulation was so far mainly observed in chickens. Interestingly, however, CAV DNA was also detected in a few Lao ducks. It is likely that ducks serve as biological, or at least mechanical, carriers of diverse avian CoV, but also CAV. As most backyard poultry was free-roaming, there are also cross-species interactions with poultry from neighbouring farms and with wild birds. This, coupled with the limited sample size and unknown geographical or commercial origin of the poultry, hindered identification of the infection source.

While the infection status of biological carriers needs confirmation by virus isolation and whole genome sequencing, mechanical carriers certainly contribute to the viral spread by contaminating the environment. Genetic characterization of numerous Lao CoV and CAV strains from chickens and ducks was attempted. Almost all Lao strains were closely related to strains from Asia, but, as observed previously, the phylogenetic analyses revealed no clear geographic clustering. Most interestingly, the partial genomes from virus strains detected in ducks and chickens were closely related, which further highlights the high risk of cross-species transmission in this setting.

The current nomenclature of IBV genotypes is based on the complete S1 unit of the spike gene characterized by a high frequency of random mutations. This, as well as low viral loads in the samples, explains why the molecular characterization of the S1 unit of all Lao duck CoV and many IBV failed. In addition, recombination between IBV and duck CoV, favoured by their co-circulation, may also be responsible for sequencing failure. By referring to IBV-like and duck CoV-like strains, we highlight the uncertainty that remains with regard to the strain classifications as viral genomes were only partially sequenced. In the future, unbiased full genome sequencing is needed to confirm not only strain classification, but also to identify possible recombination events.

CoV and CAV co-circulated in approximately 30% of the villages, and nucleic acids of both viruses were detected in four young chickens. Even if none of the Lao poultry was obviously symptomatic, co-circulation of both viruses often increases disease severity. While persistent infections with asymptomatic shedding are typical for young poultry, older birds tend to be resistant to clinical disease, but remain susceptible to infection. Silent shedding complicates diagnosis and facilitates prolonged co-circulation of different viral strains. The latter represents a recognized risk factor for the emergence of

novel strains and of recombinants adapted to new host species.

The impact of the circulating CAV or IBV strains on duck production remains unknown. While most reports suggest low pathogenicity of IBV for ducks, a novel IBV strain with clear pathogenicity for ducks was detected in China, also highlighting the need for new IBV vaccines and continuous surveillance. Although detection rates of CAV were highest in tracheal swabs, CAV detection was successful usually only in one of the two swab types, likely due to differences in infection stages and/or viral strain. Thus, both swab types should be tested for CAV to diagnose an infection or assess virus prevalence with high confidence in all host species, including ducks. A similar approach is already recommended for IBV surveillance, but may not be necessary for duck CoV. In fact, this avian CoV was mainly detected in faecal samples.

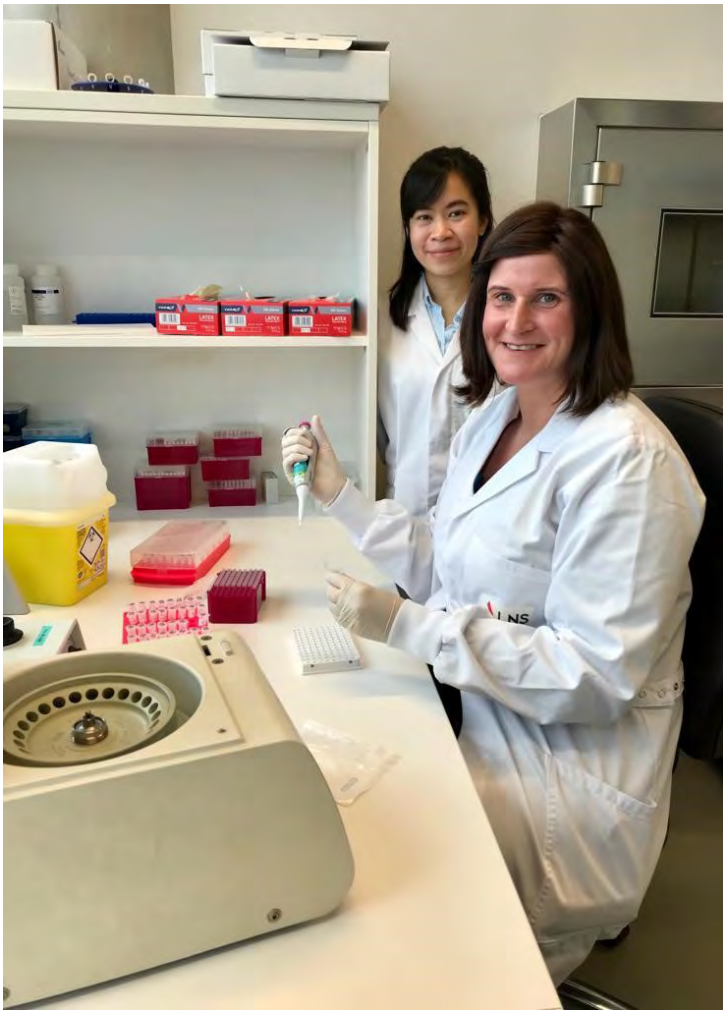
Cross-species transmission of NDV could not be evaluated in this study. None of the swab samples were positive by multiplex real-time RT–PCR targeting both NDV class I and class II strains. Since CoV, another RNA virus, was successfully detected in the same samples, it is unlikely that sample transport and storage conditions affected NDV detection adversely. However, serological evidence of NDV circulation among Lao chickens was obtained. As the ELISA is only suitable for screening chickens, applying an ELISA with a broader host range would be important to assess the role of ducks in NDV epidemiology in Lao PDR.

Conclusion

Our study shows that backyard farming of mixed poultry species facilitates cross-species transmission and represents an ideal breeding ground for new virus strains. To reduce the risk of infection in backyard settings, improved biosecurity, bird containment, separation of poultry species and sick birds, tailored vaccination programmes and continuous surveillance are warranted. Improving the control of avian viruses will not only benefit the birds, but will also contribute to the economic sustainability of smallholders. This study was published in the journal Avian Pathology and communicated with our partners at the Faculty of Agriculture, National University of Laos.

Phylogenetic analysis of the partial RNA-dependent RNA polymerase gene of avian Gammacoronaviruses Bayesian analyses of a 329 nt long alignment comprising unique, partial RNA-dependent RNA polymerase (RdRp) gene sequences of 208 coronavirus (CoV) strains. All available RdRp sequences from reference strains of Infectious bronchitis virus (IBV) were included (in bold and italic) as well as a selection of unique GenBank sequences. The phylogenetic relationship of every unique Lao sequence (in red) to GenBank sequences is shown. When identical sequences were retrieved from several samples, this is shown by indicating the frequency of detection behind the strain name (e.g. "*8"). The sample material from which the sequences were obtained is also displayed (CS: cloacal swab and OC: oral/tracheal swab). Only the posterior probability (pp) values of well supported nodes (pp>0.7) are shown. The sequences were named, if the information was available, according to the following nomenclature: AvCoV/host/country/specimen id/year_ Genbank accession number. For the IBV reference stains also the lineage as determined before based on complete spike sequences is displayed (e.g. "GVI-9").

Bayesian analyses of a 977 nt long alignment comprising unique, partial VP1 gene sequences of 129 chicken anemia virus (CAV) strains. The selection of GenBank sequences included in the analysis and also the CAV classification are based on a recent publication. The phylogenetic relationship of every unique Lao sequence (in red) to GenBank sequences is shown. Only the posterior probability (pp) values of well supported nodes (pp>0.7) are shown. The sequences were named, if the information was available, according to the following nomenclature: Genbank accession number|country|year.



Vilaysone (in background) receiving training on molecular techniques at Laboratoire National de Sante in Luxembourg.

Vilaysone and the team at Luxembourg Institute of Health, Luxembourg.

Vilaysone has PCR and ELISA training at Luxembourg Institute of Health, Luxembourg Human papillomavirus genotype distribution in Lao PDR. Cervical sample collection in 4 provincial hospitals

Parasitology Laboratory

Lao-Japan joint Lab

The aims of the Parasitology Laboratory are to carry out research and training in the area of parasitology to better understand parasitic diseases affecting the Lao population and to propose ways to mitigate possible infections, and to provide technical support to the national-level institutions in the areas of malaria and other parasitic diseases.

Visiting Scientists:
Phonepadith XANGSAYARATH, MD, PhD
Masami NAKATSU, PhD
Emilie MATSUMOTO-TAKAHASHI, PhD
Satoru KAWAI, PhD
Megumi SATO, PhD
Masamine JIMBA, MD, PhD
Jun KOBAYASHI, MD, PhD
Toshihiro MITA, MD, PhD
Takashi KUMAGAI, PhD
Satoshi NAKAMURA, PhD
Daisuke NONAKA, PhD
Ken Ing Cherng ONG

Head of Laboratory:
Shigeyuki KANO, MD, PhD

Scientist:
Moritoshi IWAGAMI, PhD, Laboratory Manager

Junior Scientists:
Phonepadith KHATTIGNAVONG, MD
Sengdeuane KEOMALAPHET, MD
Phoyphaylinh PRASAYASITH, MD

Technicians:
Pheovaly SOUNDALA
Sonesimmaly SANNIKONE

Trainees:
Intong PHENGSOUVANH (July 2018-June 2019)
Anousone KIENGPASA (July 2019-June 2020)

PhD Students:
Emiri TAKAHASHI
Phoutnalong VILY
Hitomi ARAKI

Volunteer:
Takashi SEKINE, JICA Short Term Volunteer (March 2019-March 2020)

Projects

- SATREPS
- Arboshield
- BioLao Plus
- 5-Aminolevlinic acid (5-ALA) Asymptomatic Malaria

Executive summary

Parasitology Laboratory had conducted the JICA/AMED SATREPS project for parasitic diseases (malaria, schistosomiasis, opisthorchiasis) since 2014, with close collaboration with Center of Malariology, Parasitology and Entomology (CMPE), Ministry of Health, Lao PDR, local partners in provinces, WHO Laos, National Center for Global Health and Medicine (NCGM), Tokyo, Japan and other partners. This project aimed to develop high-sensitive diagnostic methods, such as PCR, LAMP, ELISA for the parasitic diseases, to identify precise endemicity of the diseases, to monitor drug resistant malaria, to build capacity for investigations of the diseases, and to provide evidence and advises for control and elimination of the diseases.

This five-years collaborative project was terminated on April 2019. A total of 14 scientific papers were published in international journals. Some of the outcomes of the project were utilized for national guidelines for control and elimination of malaria and schistosomiasis in Lao PDR. One of the important achievements of the project is human resource development. On 2019, one staff member from CMPE who was a member of the project, obtained his PhD under the scheme of the SATREPS project. Our lab staff supported his study on malaria among Lao military personnel.

A major focus of our research is drug resistant malaria, especially artemisinin resistance. Artemisinin resistant malaria has been a major obstacle for malaria control and elimination in the country. In this report, we will update the situation of artemisinin resistance. In 2018, we showed that artemisinin resistant malaria has rapidly spread from south to north and already reached to the northern most province (Phongsaly). In our recent study, we found that prevalence of artemisinin resistance in 2017 decreased, comparing with that in 2015 in Savannakhet, Salavan, Sekong and Attapeu Provinces.

Arboshield Project continues to improve capacity of surveillance and diagnosis of vector-borne diseases. Role of Parasitology lab in this project is to improve malaria diagnosis for civil sector and military sector through training and quality assessment.

After the SATREPS project, Parasitology Lab starts new malaria research project named “5-Aminolevlinic acid (5-ALA) Asymptomatic Malaria Project” on October 2019. 5-ALA is a health food supplement produced by neopharma Japan Co. Ltd. Some studies showed that 5-ALA has an efficacy to kill or inhibit *Plasmodium* growth both in vitro and in vivo. In this new project, we will evaluate the efficacy to kill or inhibit *Plasmodium* growth among asymptomatic *Plasmodium* carriers in malaria high endemic villages in Savannakhet Province. If this supplement is effective for asymptomatic *Plasmodium* carriers, this will be an effective tool to eliminate asymptomatic *Plasmodium* carriers in the endemic areas.

ສະຫຼຸບການປະຕິບັດວຽກງານ

ຫ້ອງວິເຄາະກາຝາກວິທະຍາ ໄດ້ດຳເນີນໂຄງການ JICA/AMED SATREPS ກ່ຽວກັບພະຍາດກາຝາກ (ໄຂ້ມາລາເຣຍ, ພະຍາດໃບໄມ້ໃນເລືອດ ແລະ ພະຍາດໃບໄມ້ໃນຕັບ) ນັບຕັ້ງແຕ່ປີ 2014 ເປັນຕົ້ນມາ ໂດຍມີການຮ່ວມມືຢ່າງໃກ້ຊິດກັບສູນໄຂ້ຍຸງ, ແມ່ກາຝາກ ແລະ ແມງໄມ້ (CMPE), ກະຊວງສາທາລະນະສຸກ, ສປປ ລາວ, ຄູ່ຮ່ວມມືຂັ້ນທ້ອງຖິ່ນ ໃນແຕ່ລະແຂວງ, ອົງການອະນາໄມໂລກ ປະຈຳລາວ, ສູນການແພດ ແລະ ສຸຂະພາບ ແຫ່ງຊາດ, ໂຕກຽວ, ປະເທດຍີ່ປຸ່ນ (NCGM) ພ້ອມດ້ວຍຄູ່ຮ່ວມມືອື່ນໆອີກ.

ຈຸດປະສົງຂອງໂຄງການນີ້ແມ່ນແນໃສ່ການພັດທະນາເຕັກນິກການບົ່ງມະຕິທີ່ມີຄວາມແມ່ນຍຳສູງເຊັ່ນ: PCR, LAMP, ELISA ເພື່ອບົ່ງມະຕິພະຍາດກາຝາກຕ່າງໆ, ຊອກຫາການລະບາດຂອງພະຍາດຢ່າງລະອຽດ, ຕິດຕາມການຕ້ານຕ່ຳຢາຂອງໄຂ້ມາລາເຣຍ, ສ້າງຄວາມອາດສາມາດໃນການກວດຊອກຫາພະຍາດ, ໃຫ້ຂໍ້ມູນຫຼັກຖານ ແລະ ຄຳແນະນຳເພື່ອຄວບຄຸມພ້ອມທັງລົບລ້າງບັນດາພະຍາດດັ່ງ ກ່າວນຳອີກ.

ໂຄງການຮ່ວມມື 5 ປີ ນີ້ແມ່ນໄດ້ສິ້ນສຸດລົງໃນເດືອນເມສາ 2019 ທີ່ຜ່ານມາ. ໃນນັ້ນລວມມີບົດຄົ້ນຄວ້າວິທະຍາສາດທັງໝົດ 14 ບົດທີ່ໄດ້ຮັບການຕີພິມໃນວາລະສານລະດັບສາກົນ ຫຼາຍສະບັບ. ຜົນໄດ້ຮັບຂອງໂຄງການໄດ້ຖືກນຳໃຊ້ເຂົ້າໃນຄູ່ມືແຫ່ງຊາດເພື່ອຄວບຄຸມ ແລະ ລົບລ້າງໄຂ້ມາລາເຣຍ ແລະ ພະຍາດໃບໄມ້ໃນເລືອດ ຈາກ ສປປ ລາວ.

ໃນນີ້ໜຶ່ງໃນໝາກຜົນສຳເລັດທີ່ສຳຄັນຂອງໂຄງການແມ່ນການພັດທະນາບຸກຄະລາກອນ. ໃນປີ 2019 ນີ້, ພະນັກງານຈາກສູນໄຂ້ຍຸງ, ແມ່ກາຝາກ ແລະ ແມງໄມ້ (CMPE) ຈຳນວນ 1 ທ່ານ ເຊິ່ງກໍ່ເປັນໜຶ່ງໃນສະມາຊິກຂອງໂຄງການ SATREPS ໄດ້ຮຽນຈົບປະລິນຍາເອກພາຍໃຕ້ໂຄງການນີ້ ໂດຍພະນັກງານຈາກຫ້ອງວິເຄາະຂອງພວກເຮົາກໍ່ໄດ້ປະກອບສ່ວນເຂົ້າໃນການຄົ້ນຄວ້າຂອງລາວກ່ຽວກັບ ໄຂ້ມາລາເຣຍຢູ່ໃນບັນດາບຸກຄະລາກອນຂອງກອງທັບລາວ.

ເນັ້ນໜັກຂອງການຄົ້ນຄວ້າຂອງພວກເຮົາແມ່ນແນໃສ່ການຕ້ານຕ່ຳຢາຂອງເຊື້ອໄຂ້ມາລາເຣຍ ໂດຍສະເພາະແລ້ວແມ່ນການຕ້ານຕ່ຳຢາອາກເຕມິຊິນິນ ເຊິ່ງເປັນອຸປະສັກອັນໃຫຍ່ຫຼວງສຳລັບການຄວບຄຸມ ແລະ ລົບລ້າງໄຂ້ມາລາເຣຍຈາກ ສປປ ລາວ. ໃນບົດລາຍງານສະບັບນີ້, ພວກເຮົາຈະໃຫ້ຮູ້ສະພາບຂອງການຕ້ານຕ່ຳຢາອາກເຕມິຊິນິນຂອງໄຂ້ມາລາເຣຍ.ໃນປີຜ່ານມາພວກເຮົາໄດ້ນຳສະເໜີວ່າ ການຕ້ານຕ່ຳຢາອາກເຕມິຊິນິນຂອງໄຂ້ມາລາເຣຍໄດ້ແຜ່ຂະຫຍາຍຈາກພາກໃຕ້ໄປສູ່ພາກເໜືອຂອງປະເທດໂດຍພົບຢູ່ແຂວງຜົ້ງສາລີ ເຊິ່ງເປັນແຂວງທີ່ຢູ່ເໜືອສຸດຂອງ ສປປ ລາວ. ການຄົ້ນຄວ້າຂອງພວກເຮົາຍັງຍົກໃຫ້ເຫັນວ່າ ອັດຕາຊຸກຊຸມຂອງການຕ້ານຕ່ຳຢາອາກເຕມິຊິນິນຂອງໄຂ້ມາລາເຣຍຢູ່ແຂວງສະຫວັນນະເຂດ, ສາລະວັນ, ເຊກອງ ແລະ ອັດຕະປື ໃນປີ 2017 ຫຼຸດລົງເມື່ອປຽບທຽບກັບປີ 2015 ທີ່ຜ່ານມາ.

ໂຄງການ Arboshield ຈະສືບຕໍ່ປັບປຸງຄວາມອາດສາມາດຂອງການເຝົ້າລະວັງ ແລະ ການຕ່ຳມະຕິພະຍາດທີ່ມີແມງໄມ້ເປັນພາຫະນຳເຊື້ອ. ນອກຈາກນັ້ນ, ພາລະບົດບາດຂອງຫ້ອງວິເຄາະກາຝາກວິທະຍາສຳລັບໂຄງການນີ້ແມ່ນເພື່ອປັບປຸງການຕ່ຳມະຕິໄຂ້ມາລາເຣຍໃຫ້ແກ່ພາກສ່ວນພົນລະເຮືອນ ແລະ ກອງທັບໂດຍຜ່ານການຝຶກອົບຮົມ ແລະ ການປະເມີນຄຸນນະພາບ.

ພາຍຫຼັງສຳເລັດໂຄງການ SATREPS, ຫ້ອງວິເຄາະກາຝາກວິທະຍາໄດ້ເລີ່ມໂຄງການຄົ້ນຄວ້າໄຂ້ມາລາເຣຍອັນໃໝ່ຊື່ວ່າ "5-Aminolevlinic acid (5-ALA) Asymptomatic Malaria Project" ໃນເດືອນ ຕຸລາ 2019. 5-ALA ນີ້ແມ່ນອາຫານເສີມທີ່ຜະລິດຈາກບໍລິສັດ ນິໂອຟາມາ ປະເທດຍີ່ປຸ່ນ ເຊິ່ງມີບົດຄົ້ນຄວ້າຫລາຍບົດໄດ້ຍົກໃຫ້ເຫັນວ່າ 5-ALA ມີປະສິດທິພາບ ໃນການຂ້າເຊື້ອຫຼືຢັບຢັ້ງການຈະເລີນເຕີບໂຕຂອງເຊື້ອ *Plasmodium* ທັງໃນການທົດລອງໃນຫຼອດແກ້ວ (in vitro) ແລະ ການສຶກສາໃນສັດທົດລອງ (in vivo). ໃນໂຄງການໃໝ່ນີ້, ພວກເຮົາຈະປະເມີນປະສິດທິພາບໃນການຂ້າເຊື້ອ ຫຼື ການຢັບຢັ້ງການຈະເລີນເຕີບໂຕຂອງເຊື້ອ *Plasmodium* ໃນຄົນທີ່ຖືເຊື້ອໄຂ້ມາລາ ເຣຍແຕ່ບໍ່ສະແດງອາການໃນບັນດາບ້ານທີ່ມີອັດຕາຊຸກຊຸມຂອງເຊື້ອສູງຢູ່ແຂວງສະຫວັນນະເຂດ. ຖ້າຫາກ 5-ALA ມີປະສິດທິພາບດີຕໍ່ກັບຜູ້ທີ່ຖືເຊື້ອໄຂ້ມາລາເຣຍແຕ່ບໍ່ສະແດງອາການນີ້ຈະເປັນວິທີທີ່ມີປະສິດທິພາບຕໍ່ການລົບລ້າງໄຂ້ມາລາເຣຍສຳລັບຄົນທີ່ຖືເຊື້ອ *Plasmodium* ແຕ່ບໍ່ມີອາການສະແດງໃນເຂດທີ່ມີການບົ່ມເຊື້ອຂອງພະຍາດໄດ້.

SATREPS Project: Project for Development of Innovative Research Techniques in the Genetic Epidemiology of Malaria and Other Parasitic Diseases in the Lao PDR for Containment of Their Expanding Endemicity

Project Coordinator: Dr. Shigeyuki KANO

Staff members:
Moritoshi IWAGAMI, Phonepadith KHATTIGNAVONG, Sengdeuane KEOMALAPHET, Phoyphaylinh PRASAYASITH, Pheovaly SOUNDALA, Sonesimmaly SANNIKONE, and other visiting scientists and students.

Background

Malaria, Schistosomiasis (*Schistosoma mekongi*), and Opisthorchiasis (*Opisthorchis viverrini*) are a tremendous health burden on the people of the Lao PDR. Although significant reductions in malaria transmission have been reported due to the large-scale distribution of insecticide-treated bed nets (ITNs) through the

Global Fund to Fight AIDS, Tuberculosis and Malaria, strategies based on the scientific evidence have not been developed to deal with the genetic variations in parasite and vector populations, and drug-resistant malaria. Recently, artemisinin-resistant malaria was reported in southern provinces [1, 2]. Therefore, it is necessary to survey other provinces, especially the five southern provinces of the country, to monitor and contain the spread of drug-resistant malaria. The Lao Ministry of Health and the WHO have set a goal of eliminating malaria by 2030. To achieve this goal, we have to understand the real malaria situation, including drug-resistant malaria, prevalence of glucose-6-phosphate dehydrogenase (G6PD) deficiency, the prevalence of asymptomatic *Plasmodium* carriers, malaria situation among mobile and migrant populations, and develop effective elimination strategies.

Significant progress has been made in the past decades in the reduction of the prevalence of Schistosomiasis (*S. mekongi*) in the endemic areas (approximately 200 villages) in Khong district and Mounlapamok district, Champasak province, Lao PDR, through preventive chemotherapy using praziquantel as the drug of choice once a year as well as health education in the community. However, current monitoring methods rely on the Kato-Katz stool examination whose sensitivity may not be sufficient to detect light intensity *S. mekongi* infections. In 2017, WHO adopted a new strategy that accelerates the elimination of Asian schistosomiasis in the Western Pacific Region, i.e., transmission interruption by 2025 and eradication by 2030.

One criteria of transmission interruption is "*no new case of animal infection*." To achieve this goal, we need sensitive detection methods, such as DNA diagnostic methods (PCR and LAMP) and serological methods (ELISA), to monitor the prevalence of the disease precisely in the endemic areas.

Since Opisthorchiasis (*O. viverrini*) is localized to the Lao PDR and surrounding countries, it is recognized as a neglected tropical diseases. Nevertheless, the prevalence of opisthorchiasis is estimated to be as high as 15–54% in the Lao PDR. Little information on the molecular/genetic epidemiology of opisthorchiasis is available to develop effective measures for prevention and diagnosis of the disease.

The government of the Lao PDR requested the Japan International Cooperation Agency (JICA) to establish the Lao–Japan Joint Laboratory within Institut Pasteur du Laos (IPL) to conduct highly technological research on malaria parasites (*Plasmodium falciparum, P. vivax,* and the monkey malaria parasite *P. knowlesi*) and human trematodiases (*S. mekongi* and *O. viverrini*). The joint research will concentrate on genetic epidemiological studies to detect and control the emergence and dissemination of these parasitic diseases. The project also contributes to the capacity development of researchers and technicians in the Lao PDR through training in field and lab work, seminars, and career development.

In order to carry out this project, the IPL in collaborating with the National Center for Global Health and Medicine (NCGM), Tokyo, Japan, Tokyo Medical and Dental University (TMDU), University of the Rukyus, Okinawa, Japan, the Center of Malariology, Parasitology and Entomology (CMPE), the Lao Tropical and Public Health Institute (Lao TPHI), and other Departments of the Ministry of Health, Lao PDR.

Objectives

The objectives of this project are (1) to develop more convenient and accurate methods (PCR methods, LAMP methods, etc.) for diagnosis of the diseases, (2) to monitor the temporal and spatial epidemiological situations of pathogens and vectors of the diseases, (3) to analyze mechanisms of emergence and expansion of drug-resistant malaria, especially artemisinin resistance, and (4) to analyze the G6PD activity of the Lao population for evaluation of the possible usage of primaquine [3, 4], utilizing molecular biological techniques. Based on the scientific evidence obtained by this project, health education for the people will be strengthened and the endemicity of the diseases will be monitored together with the local Lao Ministry of Health officials. Research results will also be utilized in government services for the sustainable development of the Lao PDR.

Study period of the project

Five years (May 2014 to April 2019)

Project study sites

Malaria:
Savannakhet province, Salavan province, Sekong province, Attapeu province, Champasak province, Khammouane province, Phongsaly province, Luang Prabang province.

Schistosomiasis *(S. mekongi)*:
Khong district and Mounlapamok district, Champasak province.

Opisthorchiasis *(O. viverrini)*:
Khammouane province, Champasak province.

Ethical clearance

The SATREPS project was approved by the National Ethic Committee for Health Research, the Ministry of Health, Lao PDR from 2014 to present (extended each year).

Activities and Results in November 2018 to April 2019

Prevalence and distribution of artemisinin resistant *P. falciparum* in Lao PDR

Artemisinin based combination therapies (ACTs) have been used as a first line treatment for uncomplicated malaria in Lao PDR since 2005. However, artemisinin resistant *P. falciparum* was first reported in 2013 and is indeed threatening malaria elimination by 2030. It is reported that mutations of *K13* gene in *P. falciparum* are associated with artemisinin resistance and can be used as a molecular marker for monitoring artemisinin resistant *P. falciparum*. The aim of the study is to assess the prevalence and distribution of the *K13* mutations in Lao PDR.

Malaria patient blood samples were collected from malaria patients or malaria suspected patients in 156 healthcare facilities in the five southern provinces from October 2015 to June 2017, and the northern most province from November to December 2017 (Table 1). In 2015, only malaria positive samples were collected, whereas in 2016 and 2017, both malaria positive and negative samples were collected. "Malaria positive" means that patients were diagnosed as malaria by microscopy or malaria rapid diagnostic test (RDT). On the other hand, "malaria negative" means that patients were not diagnosed as malaria by microscopy or malaria RDT, although they showed malaria like signs or symptoms.

Real-time nested PCR was performed to identify *Plasmodium* species using malaria positive samples (Table 2). Proportion of *Plasmodium* species was heterogeneous by province or by year. For example, in Savannakhet, *P. falciparum* was predominant species while in Sekong, *P. vivax* was predominant species. Direct DNA sequencing was performed to detect the *K13* mutations in isolates of *P. falciparum* (Table 3). Data of the *K13* mutations of *P. falciparum* in the five southern provinces from October 2015 to April 2016 and the northern most province in 2017 was published in Malaria Journal (Iwagami et al., 2018). Recently, the *K13* mutation analysis of *P. falciparum* collected in the five southern provinces from November 2016 to May (or June) 2017 has just finished. Most of mutations were C580Y mutation. Surprisingly, prevalence of artemisinin resistant mutation decreased from 2015 to 2017, except Champasak.

However, a caution is needed for these results because the sample sizes drastically decreased by year and a sampling bias might exist. For example, in Champasak, the sample sizes drastically decreased although reported number of malaria cases in Champasak did not drastically changed.

Summary of malaria patient blood sampling from public health care facilities in 2019

The objective of malaria patient blood sampling is to monitor the distribution and frequency of drug resistant malaria in Lao PDR. We collected 2,870 dried blood

samples on filter papers (FTA™ Classic Card, GE Healthcare Life Sciences, Whatman™, UK) in 2019. The number of blood samples and sampling period in each province are shown in Table 1. The dried blood samples (n=2,472) were collected from malaria patients who visited 156 public health care facilities in the five southern provinces (Savannakhet, Salavan, Sekong, Attapeu, and Champasak) on February 2019. Malaria patients mean that the patients were diagnosed as malaria by either the RDTs or microscopy at health care facilities. On the contrary, the dried blood samples (n=398) were collected from malaria suspected patients who visited eight public health care facilities in Phongsaly, the northern most province on January 2019. Malaria suspected patients consist of both malaria patients confirmed by either the RDTs or microscopy and malaria negative people confirmed by the tests even though they manifested malaria like signs and symptoms.

The blood samples are now being analyzed by nested real-time PCR for identification of *Plasmodium* species. Mutation(s) of artemisinin resistant gene (the *K13* gene) is also being examined using *P. falciparum* positive samples at IPL and NCGM.

Training course on basic and molecular parasitology at the National Center for Global Health Medicine and (NCGM), Tokyo, Japan

In 2019, two Junior Scientists in Parasitology lab attended a training course on basic and molecular parasitology at NCGM, Tokyo, and Tokyo Medical and Dental University (TMDU), Tokyo, Japan. Dr. Phonepadith KATTIGNAVING attended the training course on 13th January to 2nd February 2019 and Dr. Phoyphaylinh PRASAYASITH attended the training course on 13th January to 16th February 2019. At NCGM, they studied malaria diagnosis by microscopy (all four human malaria species and one simian malaria parasite: *P. knowlesi*) and DNA diagnosis by PCR, LAMP and DNA sequencing including drug resistant gene analysis. Dr. Phoyphaylinh also studied how to design PCR primers and technique of cloning of PCR product using plasmid DNA and competent cell (*E. coli*). At TMDU, they studied ELISA technique for *Schistosoma mekongi* and how to maintain *S. japonicum* in laboratory setting using mice and snail host: *Oncomelania hupensis nosophora*

Completed PhD course on public health at University of the Ryukyus

Mr. Phoutnalong VILAY, CMPE completed PhD course on public health and obtained his PhD from University of the Ryukyus, Okinawa, Japan under the supervision of Professor Jun KOBAYASHI and Associate Professor Daisuke NONAKA on September 2019. The SATREPS project supported his study in Japan through JICA. He conducted malaria study among Lao Military personnel in Attapeu and Champasak provinces (Vilay et al., Trop Med Health, 47:11, 2019). His malaria PCR was conducted at IPL under the supervision of Parasitology lab staff. Ms. Emiri TAKAHASHI also completed PhD course on public health and obtained her PhD from University of the Ryukyus, Okinawa, Japan under the supervision of Professor Jun KOBAYASHI and Associate Professor Daisuke NONAKA on 2019. She conducted malaria study about medication adherence in Savannakhet province (Takahashi et al., Trop Med Health, 46:44, 2018).

The SATREPS Project Final Meeting

On 2nd April 2019, JICA/AMED SATREPS Project Final Dissemination Meeting was held at Lao Plaza Hotel. Dr. Shigeyuki KANO, chief advisor of the SATREPS project and Dr. Rattanaxay PHETSOUVANH, Director General of Department of Communicable Disease Control (DCDC), Ministry of Health were cochaired the meeting. Dr. Bouasy HONGVANTHONG, Project Director of the SATREPS, Former Director of Center of Malariology, Parasitology, and Entomology (CMPE), Ministry of Health, Dr. Viengxay VANISAVETH, Acting Director of CMPE, Dr. Paul BREY, Project Manager of the SATREPS, Director of IPL, and other members of the SATREPS project both Japan and Laos participated in the meeting. Staff members from Provincial Health Department, WHO Laos Office, JICA Laos Office and Japan Embassy were also attended. Project members of Parasitology lab presented seven presentations about outcomes of the five-years project including malaria, schistosomiasis and opisthorchiasis. Finally, Dr. Rattanaxay summarized the

outcomes of the SATREPS project.

A handover ceremony of Eiken LAMP machine was also held at the end of the meeting. Eiken Chemical, Co. Ltd, Japan donated five LAMP machines for Lao PDR to accelerate malaria elimination by 2030. The five LAMP machines will be installed in health care facilities (District Hospitals) in malaria high endemic districts in the five southern provinces.

Partners

- Center of Malariology, Parasitology and Entomology (CMPE), Ministry of Health, Vientiane Capital, Lao PDR
- Lao Tropical and Public Health Institute (Lao TPHI), Ministry of Health, Vientiane Capital, Lao PDR
- Department of Communicable Diseases Control (DCDC), Ministry of Health, Vientiane Capital, Lao PDR
- National Center for Laboratory and Epidemiology (NCLE), Ministry of Health, Vientiane Capital, Lao PDR
- Department of Training and Research (DTR), Ministry of Health, Vientiane Capital, Lao PDR
- Department of Hygiene and Health Promotion (DHHP), Ministry of Health, Vientiane Capital, Lao PDR
- National Center for Global Health and Medicine (NCGM), Tokyo, Japan
- Department of Community and Global Health, School of International Health, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan
- Department of Global Health, Graduate School of Health Sciences, University of the Ryukyus, Okinawa, Japan
- Department of Molecular and Cellular Parasitology, Juntendo University School of Medicine, Tokyo, Japan
- Section of Environmental Parasitology, Department of International Health Development, Division of Public Health, Graduate School, Tokyo Medical and Dental University, Tokyo, Japan.

Acknowledgements / Funding

We wish to thank Dr. Bouasy HONGVANTHONG, Former Director of the Center of Malariology, Parasitology and Entomology (CMPE), the Ministry of Health, Lao PDR, and Project director of SATREPS for his kind support of this project. We thank Dr. Tiengkham PONGVONGSA, Deputy Director, Savannakhet Provincial Health Department, Lao PDR. We also thank the staff of the CMPE, the Lao TPHI, Provincial Health Departments, Provincial Hospitals, District Hospitals, and Health Centers, the Ministry of Health, for supporting our field surveys and training courses.

This work is financially supported by the SATREPS (Science and Technology Research Partnership for Sustainable Development) project from the Japan International Cooperation Agency (JICA) and the Japan Agency for Medical Research and Development (AMED).

References

1. Ashley EA, Dhorda M, Fairhurst RM, Amaratunga C, Lim P, Suon S, et al. Spread of artemisinin resistance in *Plasmodium falciparum* malaria. *New England Journal of Medicine*, 371: 411–423, 2014.
2. Ménard D, Khim N, Beghain J, Adegnika A, Alam M, Amodu O, et al. A Worldwide Map of *Plasmodium falciparum* Arteminisin Resistance. *New England Journal of Medicine*, 374:2453–2464, 2016.
3. Howes RE, Dewi M, Piel FB, Monteiro WM, Battle KE, Messina JP, et al. Spatial distribution of G6PD deficiency variants across malaria-endemic regions, *Malaria Journal*, 12: 418, 2013.
4. Ong KIC, Kosugi H, Thoeun S, Araki H, Thandar MM, Iwagami M, et al. Systematic review of the clinical manifestations of glucose-6-phosphate dehydrogenase deficiency in the Greater Mekong Subregion: implications for malaria elimination and beyond. *BMJ Global Health*, 2: e000415, 2017.

Arboshield Project

Project Coordinator: Dr. Darouny PHONEKEO

Assistant Coordinator:
Dr. Phonepadith KHATTIGNAVONG

Consultant: Dr. Philippe CAVAILLER

Staff members in Parasitology Lab:
Dr. Moritoshi IWAGAMI, Dr. Sengdeuane KEOMALAPHET, Phoyphaylinh PRASAYASITH, Pheovaly SOUNDALA, Sonesimmaly SANNIKONE

Finance and administration:
Dr. Antoine des GRAVIERS and Ms. Phouvanhnamalee VILAYSOUK, Sonesimmaly SANNIKONE

Arboshield is a three-years training program on surveillance and diagnosis of vector-borne diseases, organized by IPL. Roles of the Lao-Japan Parasitology lab in this program are giving a training course on malaria for trainees (n=24 trainees/year) from civil sector (one third) and military sector (two third), and quality assessment of malaria diagnosis in some military provincial hospitals, which participate in Arboshield project (Table 4). Blood samples collected from malaria suspected patients in the military provincial hospitals were transported to IPL, and were diagnosed by malaria RDT and PCR. The summary of malaria diagnosis conducted by IPL were shown in Table 4.

BioLao Plus Project

Project Coordinators: Dr. Virginie POMMELET

Partners:
Vientiane, Laos: Institut Pasteur du Laos (IPL)
Singapore: Naval Medical Research Center - Asia (NMRC - A)
Vientiane, Laos: Microbiology Laboratory of Mahosot Hospital

Staff members in Parasitology Lab, IPL:
Dr. Moritoshi IWAGAMI, Dr. Sengdeuane KEOMALAPHET, Ms. Pheovaly SOUNDALA, and Mr. Takashi SEKINE

Background

The BioLao Plus Project, "an integrated approach to detect emerging pathogens and assess the infectious disease burden of a remote area in Khammouane province in the Lao PDR" was initiated by IPL in September 2017 with financial support from Naval Medical Research Center - Asia (NMRC-A). This project was the first opportunity to bring together IPL's 5 laboratories' expertise in one field site. A role of the Lao-Japan Parasitology lab in this program is to investigate situation of parasitic diseases (malaria and intestinal helminth) among villagers at study site villages in Nakai district, Khammouane province.

Objective

To detect emerging and reemerging pathogens and assess the infectious disease burden of a remote area in Khammouane province, Lao PDR and to provide evidence (human and vector data) and ways to mitigate possible infections to health authorities and the villagers.

Methodology

Blood samples and stool samples were collected from the study participants for malaria and helminthiasis detection, respectively. Malaria RDT and stool examination by Kato-Katz method were performed on site, whereas malaria PCR was performed at IPL using dried spot blood samples on filter papers.

Results and Discussion

Malaria study: A total of 191 blood samples were examined by malaria RDT and PCR. All of them were malaria negative in this study. No malaria case was detected in this study. However, IPL entomology team found *Anopheles* mosquitoes in the study villages. This finding suggests that if certain number of malaria patients or asymptomatic *Plasmodium* carriers are introduced into the villages, malaria transmission might occur among the villagers.

Helminthiasis study: One stool sample was collected from 126 participants and two stool sample were collected from 50 participants. Microscopic examination by Kato-Katz thick smear method revealed that multiple species infection was found in this study. Two species infection was 30.2% (38/126), and three species infection was 11.1% (14/126), whereas single species infection was 38.9% (49/126). Only 19.8% (25/126) was helminthiasis free in this study participants. Predominant species was hookworm (60.3%: 76/126), and the second predominant species was *Ascaris lumbricoides* (50.8%: 64/126). In this study, *Trichuris trichuria* (11.1%: 14/126), Taenia (6.3%: 8/126) and *Opisthorchis viverrini* (3.2%: 4/126) were also detected. Most of the infections were categorized either light or moderate intensity infections. However, there is a risk of malnutrition among children if it becomes a heavy intensity infection. Therefore, installation and proper use of latrine(s) for each village would be needed to improve their hygiene status and reduce the rate of infections.

Financial Support

This project was financially supported by NMRCA.

5-Aminolevlinic acid asymptomatic malaria project

Double blind, parallel, randomized, placebo controlled research to evaluate safety and efficacy of the 5-aminolevulinic phosphate (5-ALA PO4), sodium ferrous citrate (SFC) and zinc (Zn) with asymptomatic malaria parasite carriers

Principal Investigator:
Dr. Mayfong Mayxay, University of Health Sciences, Lao PDR

Members of the project:
Moritoshi IWAGAMI, Phonepadith KHATTIGNAVONG, Sengdeuane KEOMALAPHET, Phoyphaylinh PRASAYASITH, Pheovaly SOUNDALA, Sonesimmaly SANNIKONE and Shigeyuki KANO

Background

Malaria morbidity and mortality have decreased in Lao PDR due to extensive efforts by the Lao Government and international organizations, such as World Health Organization (WHO), The Global Fund to Fight AIDS, Tuberculosis and Malaria. Recently, the Lao Ministry of Health and WHO have adopted a goal to achieve elimination of malaria by 2030. However, several studies demonstrated that there were asymptomatic *Plasmodium* carriers in the malaria endemic areas in Lao PDR. Most of them were adult population who had histories of malaria episodes, and were engaged on forest related occupations. Some studies also suggested that asymptomatic *Plasmodium* carriers can be a reservoir for transmission of malaria by *Anopheles* mosquitos. However, such people will never take any antimalarial medicines until they become symptomatic. In addition, most of asymptomatic *Plasmodium* infections cannot be detected by standard diagnostic methods (microscopy and rapid diagnostic test: RDT) that are available in the endemic areas. Current malaria control and elimination strategy in Lao PDR is targeting for symptomatic malaria patients. Therefore, to accelerate elimination of malaria in Lao PDR, new effective strategy for targeting asymptomatic *Plasmodium* carriers is urgently needed in the endemic areas. 5-aminolevulinic acid (5-ALA), which is produced by neopharma Japan Co., Ltd., as a health food supplement commercially available in Japan, Philippines, Vietnam and UAE, is a natural precursor of heme in all animals. It is a non-protein amino acid synthesized in mitochondria and through activity of cytochrome C oxidase is involved in the electron transport chain. It was found from pre-clinical studies that sodium ferrous citrate (SFC) enhanced *Plasmodium falciparum*-killing potency of 5-ALA and significantly inhibited the parasite growth both in vitro and in vivo [1, 2]. These novel findings may lead us to develop a new functional health supplement containing antimalarial activity using 5-ALA. Moreover, 5-ALA is being sold as health food supplement, which has functional claim "5-ALA supports to bring higher fasting blood glucose levels closer to normal" in Japan [3, 4].

In this study, we will evaluate acceptability, safety and efficacy of 5-ALA phosphate (PO4) with SFC and Zn for asymptomatic *Plasmodium* carriers in malaria endemic villages, Nong district, Savannakhet province, Lao PDR for one year. Efficacy of 5-ALA PO4 to *Plasmodium* infection will be examined by reduction of *Plasmodium* DNA positivity rate by PCR, compering to that of Placebo group (only SFC and Zn). Since Zn deficiency is also a serious health problem in Lao PDR, participants of this study will take Zn as well to enhance a benefit for the study participants. In addition to evaluate efficacy of 5-ALA PO4 to *Plasmodium* infection, we will evaluate level of HbA1c, which is one of markers of type 2 diabetes. It is reported that type 2 diabetes increases risk for malaria infection [5]. Expected outcomes will contribute for malaria elimination and type 2 diabetes control in Lao PDR.

Objectives

- To assess influence of 5-aminolevulinic acid phosphate (5-ALA PO4), sodium ferrous citrate (SFC) and zinc (Zn) to *Plasmodium* (*Plasmodium* DNA detected by PCR) in asymptomatic *Plasmodium* carriers.
- To assess the acceptability and safety of 5-ALA PO4 among Lao villagers who carry malaria parasites without symptoms as detected by PCR.
- To investigate the HbA1c level in asymptomatic malaria parasite carriers after administrations of 5-ALA PO4, SFC and Zn for daily usage.

Study period of the project

Two years (October 2019- September 2021)

Ethical approval

This study proposal was reviewed and approved by the Ethic Committee (No. 187), University of Health Sciences, Ministry of Health, Lao PDR on 26th June 2019 and the Institutional Review Board for Clinical Research (No. NCGM-G-003300-00), National Center for Global Health and Medicine (NCGM), Japan on 27th September 2019. A permission of importation of 5-ALA PO4 (No. 9330) was also obtained from Department of Food and Drug, Ministry of Health, Lao PDR on 20th September 2019.

Methodology

After having provided informed consent, potential participants will be enrolled into a screening during which all inclusion/exclusion criteria, including laboratory assessments will be checked for eligibility. If full eligibility is confirmed, the participants will be randomized to three arms: Arm 1: 5-ALA PO4 25 mg/day + SFC 28.7 mg + zinc 10 mg (12 months), Arm 2: Placebo + SFC 28.7 mg + zinc 10 mg (3, 6 or 9 months*) and then 5-ALA PO4 25 mg/day + SFC 28.7 mg + zinc 10 mg (3, 6 or 9 months) or Arm 3: SFC 28.7 mg + zinc 10 mg (12 months). Interim Analysis will be carried out at 3, 6 or 9 months (Figure 1).

*When statistical difference of *Plasmodium* DNA positivity rate between Arm 1 and Arm 2 is observed at 3 months, Arm 2 participants will take 5-ALA PO4 25 mg/day + SFC 28.7 mg + zinc 10 mg from 3 months through the end of the study period. If no statistical difference between the 2 groups is observed at 3 months and statistical difference is observed at 6 months, Arm 2 participants will take 5-ALA PO4 25 mg/day + SFC 28.7 mg + zinc 10 mg from 6 months through the end of the study period. If no statistical difference between the 2 groups is observed at 6 months and statistical diference is observed at 9 months, Arm 2 participants will take 5-ALA PO4 25 mg/day + SFC 28.7 mg + zinc 10 mg from 9 months through the end of the study period. If no statistical difference between the 2 groups is observed at 9 months, Arm 2 participants will take only SFC 28.7 mg + zinc 10 mg for 12 months (entire the study period).

Clearance of *Plasmodium* DNA [Time Frame: 1, 2, 3, 6, 9 and 12 months] defined as the positive rate of blood stage of *Plasmodium* among participants confirmed by PCR and HbA1c defined as the actual value from blood

confirmed by handy HbA1c monitoring device within all-time periods are provided in Table 1. Blood sample (maximum 800μL per sampling) will be collected using lancet, syringe, needle and preserved on filter paper.

Follow up surveys will be conducted at 2 months later at the end of the administration of 5-ALA or Placebo. In the follow up survey, blood sample will be collected and examined by PCR for checking *Plasmodium* DNA.

Current situation (as of 31st October 2019)

The first field survey for screening of asymptomatic *Plasmodium* carriers started on 20th October 2019 (until 14th November 2019). More than 1,100 blood samples were collected from adult participants (age: 18-65 years-old) in malaria endemic villages, Nong district, Savannakhet province. Those who had any signs and symptoms of malaria, and pregnant ladies were excluded from the screening. PCR screening for detecting *Plasmodium* infection will be performed at IPL and NCGM. Administration of 5-ALA PO4 to asymptomatic *Plasmodium* carriers will be performed by the team of University of Health Sciences leaded by Dr. Mayfong Mayxay. Roles of IPL in this project is laboratory analyses (DNA detection by PCR or LAMP methods) and support the field works.

Financial support

This work is financially supported by neopharma Japan Co. Ltd.

References

1. Komatsuya K, Hata M, Balogun EO, Hikosaka K, Suzuki S, Takahashi K, Tanaka T, Nakajima M, Ogura S, Sato S, Kita K. Synergy of ferrous ion on 5-aminolevulinic acid-mediated growth inhibition of *Plasmodium falciparum*. *Journal of Biochemistry*, 154(6): 501-504, 2013.

2. Suzuki S, Hikosaka K, Balogun EO, Komatsuya K, Niikura M, Kobayashi F, Takahashi K, Tanaka T, Nakajima M, Kita K. In vivo curative and protective potential of orally administered 5-aminolevulinic acid plus ferrous ion against malaria. *Antimicrobial Agents and Chemotherapy*, 59(11): 6960-6967, 2015.

3. Higashikawa F, Noda M, Awaya T, Tanaka T, Sugiyama M. 5-aminolevulinic acid, a precursor of heme, reduces both fasting and postprandial glucose levels in mildly hyperglycemic subjects. *Nutrition*, 29(7-8): 1030-10306, 2013.

4. Al-Saber F, Aldosari W, Alselaiti M, Khalfan H, Kaladari A, Khan G, Harb G, Rehani R, Kudo S, Koda A, Tanaka T, Nakajima M, Darwish A. The Safety and Tolerability of 5-Aminolevulinic Acid Phosphate with Sodium Ferrous Citrate in Patients with Type 2 Diabetes Mellitus in Bahrain. *Journal of Diabetes Research*, 2016: 8294805, 2016.

5. Danquah I, Bedu-Addo G, Mockenhaupt FP. Type 2 diabetes mellitus and increased risk for malaria infection. *Emerging Infectious Diseases*, 16(10): 1601-1604, 2010.

Scientific communications

Publications:

Ong KIC, Iwagami M, Araki H, Khattignavong P, Soundala P, Keomalaphet S, Prasayasith P, Lorpachan L, Xangsayalath P, Pongvongsa T, Hongvanthong B, Brey PT, Kano S, Jimba M. Prevalence of G6PD Viangchan variant in malaria endemic areas in Lao PDR: an implication for malaria elimination by 2030, *Malaria Journal*, 18: 75, 2019.

Vilay P, Nonaka D, Senamonty P, Lao M, Iwagami M, Kobayashi J, Hernandez PM, Phrasisombath K, Kounnavong S, Hongvanthong B, Brey PT, Kano S. Malaria prevalence, knowledge, perception, preventive and treatment behaviour among military in Champasack and Attapeu provinces, Lao PDR: a mixed methods study, *Tropical Medicine and Health*, 47: 11, 2019.

Pongvongsa T, Nonaka D, Iwagami M, Soundala P, Khattignavong P, Xangsayarath P, Nishimoto F, Kobayashi J, Hongvanthong B, Brey P, Kano S. Malaria among foreign migrant workers in Savannakhet Province, Lao PDR: a cross-sectional study, *Tropical Medicine and Health,* 47: 10, 2019.

Iwagami M, Nakatsu M, Khattignavong P, Soundala P, Keomalaphet S, Lorpachan L, Xangsayalath P, Matsumoto-Takahashi E, Pommelet V, Hongvanthong B, Brey PT, Kano S. Heterogeneous distribution of *K13* mutations in *Plasmodium falciparum* in Lao PDR. *Malaria Journal,* 17: 483, 2018.

Takahashi E, Nonaka D, Iwagami M, Vilay P, Chanthakoumane K, Kobayashi J, Pongvongsa T, Kounnavong S, Hongvanthong B, Brey PT, Kano S. Patients' adherence to artemisinin-based combination therapy and healthcare workers' perception and practice in Savannakhet province, Lao PDR, *Tropical Medicine and Health*, 46: 44, 2018.

Iwagami M, Tangpukdee N, Wilairatana P, Krudsood S, Dao LD, Nakazawa S, Sinuon M, Socheat D, Yasuoka J, Jimba M, Watanabe H, Kobayashi J, Toma H, Vanisaveth V, Hongvanthong V, Brey PT, Kano S. *Pfcrt* genotypes and microsatellite DNA polymorphisms flanking the gene on *Plasmodium falciparum* suggest different chloroquine selective pressure among populations in the Greater Mekong Subregion, *Parasitology International*, 67: 816-823, 2018.

Vincent JP, Komaki-Yasuda K, Iwagami M, Kawai S, Kano S. Combination of PURE-DNA extraction and LAMP-DNA amplification methods for accurate malaria diagnosis on dried blood spots, *Malaria Journal*, 17: 373, 2018.

Iwagami M, Nakatsu M, Khattignavong P, Soundala P, Lorphachan L, Keomalaphet S, Xangsayalath P, Kawai S, Hongvanthong B, Brey PT, Kano S. First confirmed case of human infection with *Plasmodium knowlesi* in Lao PDR, *PLOS Neglected Tropical Diseases,* 12(3): e0006244, 2018.

Araki H, Ken Ong KIC, Lorphachan L, Soundala P, Iwagami M, Shibanuma A, Hongvanthong B, Brey PT, Kano S, Jimba M. Mothers' *Opisthorchis viverrini* infection status and raw fish dish consumption in Lao People's Democratic Republic: determinants of child infection status, *Tropical Medicine and Health*, 46: 29, 2018.

Iwagami M, Keomalaphet S, Khattignavong P, Soundala P, Lorphachan L, Matsumoto-Takahashi E, Strobel M, Reinharz D, Phommasansack M, Hongvanthong B, Brey PT, Kano S. The detection of cryptic *Plasmodium* infection among villagers in Attapeu province, Lao PDR. *PLOS Neglected Tropical Diseases*, 11(12): e0006148, 2017.

Inthavong N, Nonaka D, Kounnavong S, Iwagami M, Phommala S, Kobayashi J, Hongvanthong B, Pongvongsa T, Brey PT, Kano S. Individual and household factors associated with incidences of village malaria in Xepon district, Savannakhet province, Lao PDR. *Tropical Medicine and Health*, 45: 36, 2017.

Ong KIC, Kosugi H, Thoeun S, Araki H, Thandar MM, Iwagami M, Hongvanthong B, Brey PT, Kano S, Jimba M. Systematic review of the clinical manifestations of glucose-6-phosphate dehydrogenase deficiency in the Greater Mekong Subregion: implications for malaria elimination and beyond. *BMJ Global Health*, 2: e000415, 2017.

Ménard D, Khim N, Beghain J, Adegnika AA, Shafiul-Alam M, Amodu O, Rahim-Awab G, Barnadas C, Berry A, Boum Y, Bustos MD, Cao J, Chen JH, Collet L, Cui L, Thakur GD, Dieye A, Djallé D, Dorkenoo MA, Eboumbou-Moukoko CE, Espino FE, Fandeur T, Ferreira-da-Cruz MF, Fola AA, Fuehrer HP, Hassan AM, Herrera S, Hongvanthong B, Houzé S, Ibrahim ML, Jahirul-Karim M, Jiang L, Kano S, Ali-Khan W, Khanthavong M, Kremsner PG, Lacerda M, Leang R, Leelawong M, Li M, Lin K, Mazarati JB, Ménard S, Morlais I, Muhindo-Mavoko H, Musset L, Na-Bangchang K, Nambozi M, Niaré K, Noedl H, Ouédraogo JB, Pillai DR, Pradines B, Quang-Phuc B, Ramharter M, Randrianarivelojosia M, Sattabongkot J, Sheikh-Omar A, Silué KD, Sirima SB,

Sutherland C, Syafruddin D, Tahar R, Tang LH, Touré OA, Tshibangu-wa-Tshibangu P, Vigan-Womas I, Warsame M, Wini L, Zakeri S, Kim S, Eam R, Berne L, Khean C, Chy S, Ken M, Loch K, Canier L, Duru V, Legrand E, Barale JC, Stokes B, Straimer J, Witkowski B, Fidock DA, Rogier C, Ringwald P, Ariey F, Mercereau-Puijalon O; KARMA Consortium. A Worldwide Map of *Plasmodium falciparum* Artemisinin Resistance. *New England Journal of Medicine.* 374: 2453–2464, 2016.

Pongvongsa T, Nonaka D, Iwagami M, Nakatsu M, Phongmany P, Nishimoto F, Kobayashi J, Hongvanthong B, Brey PT, Moji K, Mita T, Kano S. Household clustering of asymptomatic malaria infections in Xepon district, Savannakhet province, Lao PDR. *Malaria Journal*. 15: 508, 2016.

Oral presentations:

1. Moritoshi Iwagami, Phonepadith Khattignavong, Mayfong Mayxay, Paul T. Brey, Shigeyuki Kano, Development of new clinical research beyond the SATREPS project, 13th National Health Research Forum 2019, Vientiane, Done Chanh Palace Hotel, Lao PDR, 15th-17th October 2019.

2. Masami Nakatsu, Moritoshi Iwagami, Sengdeuane Keomalaphet, Phonepadith Khattignavong, Pheovaly Soundala, Lavy Lorphachan, Bouasy Hongvanthong, Brey Paul, Shigeyuki Kano, Analysis of artemisinin-resistant gene of *Plasmodium falciparum* in Phongsaly, the northern-most province in Laos, The 88th Annual Meeting of Japanese Society of Parasitology, Nagasaki University, Nagasaki city, Japan, 15th -16th, March 2019.

3. Takashi Kumagai, Moritoshi Iwagami, Emilie Matsumoto-Takahashi, Keomalaphet Sengdeuane, Phonepadith Khattignavong, Lavy Lorphachan, Pheovaly Soundala, Bouasy Hongvanthong, Kei Oyoshi, Yousei Mizukami, Yoshinobu Sasaki, Nobuo Ohta, Paul T. Brey, Shigeyuki Kano, Risk mapping of Schistosomiasis mekongi using LAMP method and earth observation satellite data, The 88th Annual Meeting of Japanese Society of Parasitology, Nagasaki University, Nagasaki city, Japan, 15th -16th, March 2019

4. Emilie Matsumoto-Takahashi, Takashi Kumagai, Moritoshi Iwagami, Yoshinobu Sasaki, Yousei Mizukami, Kei Oyoshi, Shigeyuki Kano, Impact of precipitation on schistosomiasis mekongi in Lao PDR: Spatial epidemiology using earth observation satellite data, The 88th Annual Meeting of Japanese Society of Parasitology, Nagasaki University, Nagasaki city, Japan, 15th -16th, March 2019

Poster presentation:

1. Ken Ing Cherng Ong, Phonepadith Khattignavong, Sengdeuane Keomalaphet, Moritoshi Iwagami, Bouasy Hongvanthong, Paul T. Brey, Shigeyuki Kano, Masamine Jimba. Listening to the voices of the vulnerable: a mixed methods study on health-seeking behaviors in a malaria endemic district in Lao People's Democratic Republic. The 68th Annual Meeting of American Society of Tropical Medicine and Hygiene, Gaylord National Resort and Convention Center National Harbor, Maryland, USA, November 21st -24th, 2019.

Table 1. List of dried blood samples on filter paper collected from malaria patients and suspected patients in five southern province and one northern most province

Sampling period	Sampling time	Location	Type of blood samples	No. of samples
2015 Oct - 2016 April	2016 April	5 Southern Provinces	Malaria Positive only	2,409
2016 May-Oct or Nov	2016 Oct-Nov	5 Southern Provinces	Both Malaria Positive and Negative	10,813
2016 Nov - 2017 May or June	2017 May-June	5 Southern Provinces	Both Malaria Positive and Negative	14,453
2017 Nov-Dec	2017 Dec	Phongsaly	Both Malaria Positive and Negative	98
2017 June-2018 August	2018 August	4 Southern Provinces*	Malaria Positive only	2459
2018 Jan-2019 Jan	2019 January	Phongsaly	Both Malaria Positive and Negative	398
2018 August-2019 February	2019 February	5 Southern Provinces	Malaria Positive only	2472
Total				**33,102**

Malaria diagnoses was performed by microscopy or RDT.
*Blood samples were not collected from Attapeu because of flooding in July 2018.

Table 2. Summary of *Plasmodium* species in five Southern Provinces 2015-2017

Province	Year					
	2015		2016		2017	
	No.	%	No.	%	No.	%
Savannakhet						
Pf	258	63.1	155	66.5	263	83.0
Pf+Pv	13	3.2	25	10.7	31	9.8
Pv	136	33.3	50	21.5	21	6.6
Pm	1	0.2	3	1.3	2	0.6
Po	1	0.2	0	0.0	0	0.0
Sub-Total	**409**	**100.0**	**233**	**100.0**	**317**	**100.0**
Salavan						
Pf	211	56.7	50	58.1	74	70.5
Pf+Pv	17	4.6	2	2.3	1	1.0
Pv	132	35.5	34	39.5	30	28.6
Pm	3	0.8	0	0.0	0	0.0
Po	9	2.4	0	0.0	0	0.0
Sub-Total	**372**	**100.0**	**86**	**100.0**	**105**	**100.0**
Sekong						
Pf	127	28.7	17	6.5	10	9.3
Pf+Pv	35	7.9	20	7.7	0	0.0
Pv	280	63.3	223	85.8	98	90.7
Pm	0	0.0	0	0.0	0	0.0
Po	0	0.0	0	0.0	0	0.0
Sub-Total	**442**	**100.0**	**260**	**100.0**	**108**	**100.0**
Champasak						
Pf	391	53.2	85	26.7	58	45.7
Pf+Pv	34	4.6	51	16.0	5	3.9
Pv	309	42.0	182	57.2	64	50.4
Pm	0	0.0	0	0.0	0	0.0
Po	1	0.1	0	0.0	0	0.0
Sub-Total	**735**	**100.0**	**318**	**100.0**	**127**	**100.0**
Attapeu						
Pf	120	44.1	27	26.7	57	54.3
Pf+Pv	11	4.0	2	2.0	4	3.8
Pv	140	51.5	70	69.3	43	41.0
Pm	1	0.4	1	1.0	1	1.0
Po	0	0.0	0	0.0	0	0.0
Pk	0	0.0	1	1.0	0	0.0
Sub-Total	**272**	**100.0**	**101**	**100.0**	**105**	**100.0**
Grand-Total	**2,230**		**998**		**762**	

Pf: *P. falciparum*; Pv: *P. vivax*; Pm: *P. malariae*; Po: *P. ovale*; Pk: *P. knowlesi*

Sampling period:
2015: October 2015 – April 2016
2016: May – October or November 2016
2017: November 2016 – May or June 2017

Table 3. Prevalence of artemisinin resistant mutation in *K13* gene in *P. falciparum* in five Southern Provinces 2015-2017

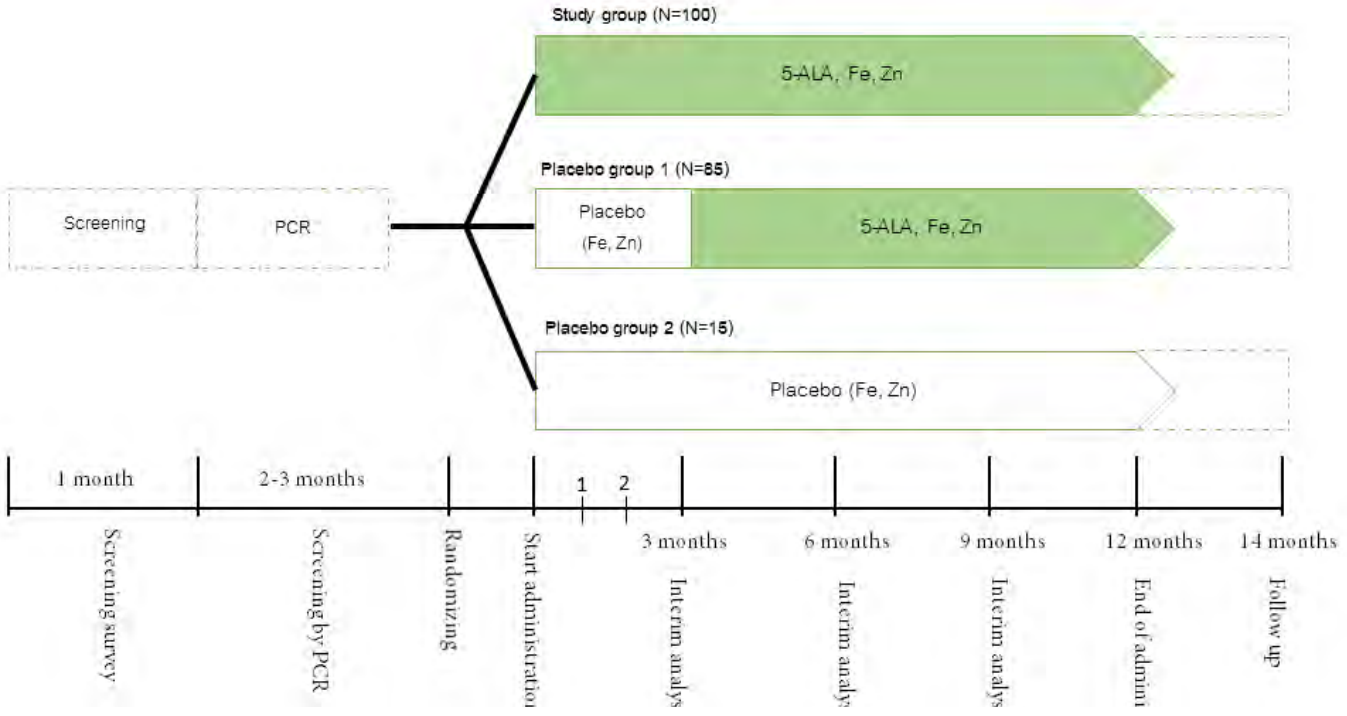
Province	Year					
	2015		2016		2017	
	No.	%	No.	%	No.	%
Savannakhet						
Wild Type	184	72.2	131	78.4	248	94.3
Resistant Type	71	27.8	36	21.6	15	5.7
Sub-Total	**255**	**100.0**	**167**	**100.0**	**263**	**100.0**
Salavan						
Wild Type	90	41.1	26	52.0	50	70.4
Resistant Type	129	58.9	24	48.0	21	29.6
Sub-Total	**219**	**100.0**	**50**	**100.0**	**71**	**100.0**
Sekong						
Wild Type	97	60.6	13	43.3	4	44.4
Resistant Type	63	39.4	17	56.7	5	55.6
Sub-Total	**160**	**100.0**	**30**	**100.0**	**9**	**100.0**
Champasak						
Wild Type	115	27.3	48	37.2	14	23.0
Resistant Type	306	72.7	81	62.8	47	77.0
Sub-Total	**421**	**100.0**	**129**	**100.0**	**61**	**100.0**
Attapeu						
Wild Type	39	30.0	4	15.4	28	57.1
Resistant Type	91	70.0	22	84.6	21	42.9
Sub-Total	**130**	**100.0**	**26**	**100.0**	**49**	**100.0**
Grand-Total	**1,185**		**402**		**453**	

Sampling period
2015: October 2015 – April 2016
2016: May-October or November 2016
2017: November 2016 - May or June 2017

Table 4. Summary of blood sample testing by malaria RDT and PCR

Institute	Location	No. of malaria suspected	RDT Positive	PCR Positive
Institute of Preventative Medicine	Vientiane Capital	0	0	0
103 Hospital	Vientiane Capital	28	3	5
101 Hospital	Xiangkhouang Province	17	3	0
107 Hospital	Luang Prabang Province	27	3	0
108 Hospital	Vang Vieng, Vientiane Province	6	0	0
106 Hospital	Champasak Province	63	1	3
109 Hospital	Savannakhet Province	74	9	5
Total		215	19	13

Figure 1. Scheme of the 5-ALA Malaria study



ARBOSHIELD

Surveillance of Arthropod-borne Diseases in Military Facilities in Lao People's Democratic Republic.

Project Coordinator:
Dr. Darouny Phonekeo

Background

The main goal of Arboshield is to strengthen the capacity of the Lao Military and Civilian Medical Departments in the areas of vector-borne diseases surveillance, outbreak detection, and response. This three-year project initiated in November 2016, includes several components: a training curriculum, the support of arthropod-borne diseases surveillance in 7 military facilities, and the implementation of a quality assurance process.

The training curriculum includes four modules: "Introduction of infectious diseases surveillance system" (delivered initially in November 2016), "Biosafety and Biosecurity" (February 2017), "Introduction to arthropod-borne diseases" (May 2017), and "Taxonomy and systems for the identification of mosquitoes, ticks, and sandflies" (August 2017). A first group of 25 participants joined the course during the first year of Arboshield. The course has been replicated two times: in November 2017 and 2018, with two additional groups of trainees. The audience includes both civilians and militaries. Civilian participants are coming from Borkeo and Xayabouly provinces.

Military participants are originating from the Military Academy, the Lao Army Institute for Disease Prevention, and from 6 military hospitals (Xieng Khouang, Luang Prabang, Vang Vieng, Savannakhet, Champasak and Vientiane municipality).

During the third quarter 2017, laboratory equipment (centrifuge, autoclave and combined freezer-refrigerator) have been donated and installed in all hospitals sites. Rapid diagnostic tests (RDT) for dengue and malaria, personal protective equipment, consumable for sample collection and transportation, and computer were additionally donated to the sites. Starting November 2017, the sites have started sending occasionally serum specimens to IPL for dengue PCR diagnostic. In August 2018, the hospital sites were requested to expand the scope of Arboshield surveillance to malaria, and to collect additional blood specimens on filter papers (to be shipped to IPL for PCR analysis using the dried blood technique).

Starting in February 2018, the IPL team has initiated on-site post-training assessments in the military sites. The objectives of these field visits were to: organize a refresher course with a focus on biosafety and biosecurity procedures, assess the local laboratory capacity, engage more actively the hospital teams in active surveillance, and proceed with the implementation of the quality assurance (QA) process. These visits allowed to develop a close relationship with the hospital teams, especially with the clinicians who were not the prime targets of the training workshops.

Regarding the status of the equipment, several minor problems have been reported by the hospital sites,

such as the breakage of the centrifuge covers in several hospitals, the failure of the autoclave in Vang Vieng with which need to be replaced, and the sudden breakdown of the freezer in Champasak. The lack of an electrical "grid" in Lao PDR and the basic electrical installations within the military hospitals seems to be the main underlying cause. Laboratory personnel are currently using the equipment routinely for the purpose of vector-borne diseases surveillance. To emphasize Biosafety measures, a washing machine was provided to each targeted laboratory for Laboratory coats cleaning. The staff is now well aware of the precautions and security procedures for specimen collection and transportation, and big efforts have been made, in all sites, to increase cleanliness, biosecurity, and biosafety.

Aside from the supervisory visits, the QA also includes bi-monthly monitoring calls to the hospital Arboshield focal points, conducted by IPL project assistant. These calls are aimed at enhancing surveillance, early identification (and resolution) of problem encountered on sentinel sites, and close monitoring of the epidemiological situation and early outbreak detection.

The implementation of the QA process had a significant, immediate, positive impact on Arboshield surveillance. The sites are now sending very regularly serum specimens for dengue and malaria PCR analyses. Additionally, the implementation of Arborshield activities resulted in the detection of an epidemic alert of Rickettsia disease in Vangvieng in June 2018 (with a follow-up field investigation conducted by IPL team), with the technical support of the Lao-Mahosot-Welcome Research Unit (LOMWU).

BioLao Plus

Epidemiology

2017-2019

Interviews of the villagers, sample collections

Project Coordinators:
Virginie Pommelet (Epidemiologist, IPL)
Jeff Hertz, (Research Entomologist,NMRCA)
Dr. Paul T.Brey, (Director,IPL)

Collaborators:
Scientists:
Dr Moritoshi Iwagami
Dr Marc Grandadam
Dr Khamsing Vongphailoth
Dr Sengdeuane Keomalaphet
Dr Thonglakhone Xaybounsou
Technicians:
Pheovaly Soundala
Thep Aksone Chindavong
Khaithong Lakeomany
Nothasine Phommavanh

Projects

- An integrated approach to detect emerging pathogens and assess the infectious disease burden of a remote area within Khammouane Province in The Lao PDR.

An integrated approach to detect emerging pathogens and assess the infectious disease burden of a remote area within Khammouane Province in The Lao PDR.

Project Coordinators:
Virginie Pommelet (Epidemiologist, IPL)
Jeff Hertz, (Research Entomologist, NMRCA)
Dr. Paul T.Brey, (Director, IPL)

Collaborators:
Dr Moritoshi Iwagami
Dr Marc Grandadam
Dr Khamsing Vongphailoth
Dr Sengdeuane Keomalaphet
Dr Thonglakhone Xaybounsou
Pheovaly Soundala
Thep Aksone Chindavong
Khaithong Lakeomany
Nothasine Phommavanh

Executive summary

The BiolaoPlus Project, « an integrated approach to detect emerging pathogens and assess the infectious disease burden of a remote area within Khammouane Province in the Lao PDR » was initiated at Institut Pasteur du Laos (IPL) in September 2017 with financial support from NMRC-A. This project was the first opportunity to bring together IPL's 5 laboratories' expertise in one field site. Furthermore, this project gave IPL the opportunity to collaborate with international partners such as the Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit (LOMWRU) in Laos, the Mahidol-Oxford Tropical Medecine Research Unit (MORU) in Bangkok and the Institut Pasteur in Paris (IPP).

The main objectives of this project were (i) to detect emerging and reemerging pathogens and assess the infectious disease burden of a remote area (ii) to provide evidence of (with human and vector data) and ways to mitigate possible infections.

The project had both a human and a vector biology component, which interacted and complemented each other.

Human component

Two hundred and two presumably healthy volunteers from 8 different villages aged 5 to 75 years were included in the study (sex ratio M/F of 1.7). The study population was mainly composed of young men, relying on agriculture and forestry activities, living in close contact with both wild and domesticated animals and highly exposed to arthropods.

Despite the presence of *Anopheles* mosquitoes in this area, no *Plasmodium* infections were detected in these healthy volunteers. The rate of infection with helminthiasis reached up to 80.2% (95%CI 73.1-87.2) with low intensity of infection. Unlike other studies in Khammouane Province, the rate of *Opistorchiasis viverrini* infection was very low, possibly related to cold temperatures of the water in these upland villages; hence, inadequate ecological conditions for intermediate snail hosts.

The prevalence of villagers with evidence of past exposure to rickettsiae (IgG positive) was very high, with an overall exposure rate of 80.4% (95%CI 73.7-87.1) to the three major rickettsial serogroups (scrub typhus orientia group (STG), typhus group rickettsiae (TG) and spotted fever group rickettsia (SFG)) in the 138 blood samples tested. Prevalence of STG-specific antibodies was predominant and reached up to 77.5% (95%CI 70.5-84.6) followed by 13.8% (95%CI 7.9-19.6) of SFG-specific antibodies and 6.5% (95%CI 2.3-10.7) of TG-specific antibodies. High exposure rates were found in all age groups suggesting an early and repeated exposure to these pathogens. IgG antibodies against *Ehrlichia chaffensis* and *Anaplasma phagocytophilum* were not detected in these samples.

Preliminary data using indirect ELISA testing suggested that the population of this study is exposed to a large diversity of vector-borne viruses. As such, exposure to flaviviruses was high in this population: 69.1% (95%CI 62.0-75.0) with 28.3% (95%CI 21.8-34.7) of exposure to viruses belonging to the Japanese encephalitis virus

Nevertheless, anti-flavivirus cross reactivity reached up to 30%. Indeed, serologic profile interpretation is known to be delicate because of inter-antigenic complex antibody cross reactivity within the *flavivirus genus* and requires labour-intensive extended plaque reduction neutralization tests (PRNT). An alternative ELISA, the DElTAa (Discriminant ELISA for Typing Arbovirus antibodies) has been set up during this project and is under validation to improve the capacity to discriminate the anti-arbovirus immune response. Preliminary results, confronted to PRNT, support a possible application of the DElTAa test to discriminate flavivirus (arbovirus) antigenic complex.

Vector biology component

This study described the abundance and diversity of putative arthropod vectors and assessed the risk of human exposure to known and unknown pathogens by collecting mosquitos and sandflies using human-baited double bed net (HDN) method and screened them for pathogens. A total of 51 species of mosquitoes were identified belonging to 9 genera: *Aedes* , *Anopheles*, *Armigeres*, *Culex*, *Heizmania*, *Malaya*, *Mansonia*, *Tripteroides*, and *Uranotaenia*. Four species belonging to the genus *Sergentomyia* of sandflies, 8 species of 5 tick genera, and 2 species of 2 flea genera were identified. The known mosquito vectors found in our study were: the dengue vector *Ae. albopictus* [1], the JEV vector *Cx. vishnui* s.l. [2] and the *lymphatic filariasis* vector *Ar. kesseli* [3].

Arboviral screening for alpha, flavi and phleboviruses in mosquitoes, ticks and sandflies using pan-flavi, pan-alpha and pan-phlebo RT-PCR, real-time PCR using dengue universal and JEV group primers and sequencing did not evidence any virus.

Ticks were screened for *Rickettsia spp, Neorickettsia sennetsu, Ehrilichia spp, Anaplasma spp. Coxiella burnetii* and *Leptospira spp.* by PCR. Unlike in previous studies done in the same area, none of these pathogens were identified. However, *Rickettsia spp* was identified in fleas collected during this study, which is consistent with the serological evidence of TG-specific antibodies in participants' blood samples.

With the objective to identify unknown spillover events, agnostic descriptions of the virome of mosquitoes and sandflies using deep sequencing were conducted in IPP. A total of 1,471 mosquito-borne and 55 sandfly-borne viral communities were sequenced and analyzed. The most abundant viral families identified among RNA viruses were those usually infecting plants and invertebrates while viral families able to infect vertebrates were in minority.

Viruses possibly infecting vertebrates belonged to the *Flaviviridae, Picornaviridae, Astroviridae, Hepeviridae, Phenuiviridae, Peribunyaviridae, Orthomyxoviridae, Rhabdoviridae* and *Reoviridae* families. Several viruses belonging to families putatively containing arboviruses were phylogenetically characterized. Among the *Phenuiviridae/phlebovirus* family, a complete L segment of a novel phlebovirus detected in *Culex vishnui* was assembled. Phylogenetic analyses placed it at the root of the clade formed by mosquito-and-sandfly-borne arbo-phlebiviruses. Further analyzes will be conducted.

The ORFs of a potentially zoonotic tick-borne virus, Jingmen tick virus-like virus (JMTV) previously identified in ticks collected in this area were obtained after deep sequencing and finishing by PCR-sanger sequencing. Past exposure to JMTV was then tested in blood samples from the BiolaoPlus study participants using Luciferase ImmunoPrecipitation System (LIPS), a high-throughput serological screening of antibodies against the external proteins of viruses. No past exposure was detected so far. Other target viruses will be expressed and used as antigens for LIPS.

Field missions

ການສຶກສາ ແບບປະສົມປະສານເພື່ອຊອກຫາເຊື້ອພະຍາດທີ່ເກີດຂຶ້ນໃໝ່ ແລະ ການປະເມີນອັດຕາການເກີດຂອງພະຍາດຊຶມເຊື້ອຢູ່ເຂດຫ່າງໄກສອກຫຼີກ ຂອງ ແຂວງຄຳມ່ວນ, ສປປ

ຜູ້ປະສານງານໂຄງການ:
Virginie Pommelet (ຊ່ຽວຊານລະບາດວິທະຍາ, ສະຖາບັນ ປັດສະເຕີ ລາວ)
Jeff Hertz, (ນັກຄົ້ນຄວ້າ ແມງໄມ້ວິທະຍາ, NMRCA)
Dr. Paul T.Brey, (ຫົວໜ້າ ສະຖາບັນ ປັດສະເຕີ ລາວ)

ຜູ້ຮ່ວມງານ:
ດຣ. ໂມລິໂຕຊິ ອິວະກະມິ
ດຣ. ແສງເດືອນ ແກ້ວມາລາເພັດ
ນາງ ແພວວາລີ ສຸນດາລາ
ດຣ. ມາກ ກຣັງອາດາມ
ດຣ. ທອງລະຄອນ ໄຊບຸນຊ
ນາງ ເທບອັກສອນ ຈິນດາວົງ
ດຣ. ຄຳສິງ ວົງໄພໂລດ
ທ້າວ ໄຄທອງ ຫຼ້າແກ້ວມະນີ
ທ້າວ ໂນ່ທະສິນ ພົມມະວັນ

ສະຫຼຸບການປະຕິບັດວຽກງານ

ໂຄງການ BioLao Plus ການສຶກສາແບບປະສົມປະສານ ເພື່ອຊອກຫາເຊື້ອພະຍາດທີ່ເກີດຂຶ້ນໃໝ່ ແລະ ການປະເມີນອັດຕາການເກີດຂອງພະຍາດຊຶມເຊື້ອຢູ່ເຂດຫ່າງໄກສອກຫຼີກ ຂອງ ແຂວງຄຳມ່ວນ, ສປປ ລາວ ໄດ້ຈັດຕັ້ງປະຕິບັດຢູ່ ສະຖາບັນ ປັດສະເຕີ ລາວ ໃນເດືອນ ກັນຍາ ປີ 2017 ໂດຍການສະໜັບສະໜຸນທາງດ້ານການເງິນຈາກ NMRC-A. ໂຄງການນີ້ ເປັນໂອກາດຄັ້ງທຳອິດທີ່ນຳເອົາຊ່ຽວຊານໃນ 5 ຫ້ອງວິເຄາະຂອງສະຖາບັນ ປັດສະເຕີ ລາວ ມາລົງເຮັດວຽກພາກສະໜາມດຽວກັນ. ນອກຈາກນີ້, ໂຄງການນີ້ ຍັງເປີດໂອກາດໃຫ້ສະຖາບັນ ປັດສະເຕີ ລາວ ໄດ້ຮ່ວມມືກັບຄູ່ຮ່ວມງານຕ່າງປະເທດເຊັ່ນ: ໜ່ວຍງານຄົ້ນຄວ້າ Lao-Oxford-Mahosot Hospital-Wellcome Trust (LOMWRU) ຢູ່ ລາວ ແລະ ໜ່ວຍງານຄົ້ນຄວ້າພະຍາດເຂດຮ້ອນ Mahidol-Oxford (MORU) ຢູ່ບາງກອກ ແລະ ສະຖາບັນ ປັດສະເຕີ ປາຣີ. ຈຸດປະສົງຫຼັກຂອງໂຄງການນີ້ (i) ແມ່ນເພື່ອຊອກຫາເຊື້ອພະຍາດທີ່ເກີດຂຶ້ນໃໝ່ ແລະ ກັບຄືນມາໃໝ່ ແລະ ການປະເມີນອັດຕາການເກີດຂອງພະຍາດຊຶມເຊື້ອຢູ່ເຂດຫ່າງໄກສອກຫຼີກ (ii) ເພື່ອຊອກຫາຫຼັກຖານ (ຂອງຄົນ ແລະ ຂໍ້ມູນພາຫະນຳເຊື້ອ) ແລະ ວິທີການຫຼຸດຜ່ອນການຕິດເຊື້ອ.

ໂຄງການນີ້ມີສອງອົງປະກອບ: ຄົນ ແລະ ຊີວະວິທະຍາຂອງພາຫະນຳເຊື້ອ ທີ່ມີຄວາມສຳພັນ ແລະ ສົ່ງເສີມເຊິ່ງກັນ ແລະກັນ.

ອົງປະກອບຂອງຄົນ

ໃນການສຶກສາຄັ້ງນີ້ ມີອາສາສະໝັກ 202 ຄົນຈາກ 8 ບ້ານ ເຊິ່ງມີອາຍຸລະຫວ່າງ 05-75 ປີ (ອັດຕາສ່ວນ ເພດຊາຍ/ຍິງ 1.7). ປະຊາກອນທີ່ເຂົ້າຮ່ວມການສຶກສາສ່ວນໃຫຍ່ເປັນໄວໜຸ່ມຊາຍ, ທີ່ເຮັດກະສິກຳ ແລະ ເຮັດກິດຈະກຳປ່າໄມ້, ດຳລົງຊີວິດໃກ້ສິດກັບສອງປະເພດຄື: ສັດປ່າ, ສັດລ້ຽງ ແລະ ມີການສຳພັດກັບແມງໄມ້ຕີນຂໍ້ສູງ.

ເຖິງແມ່ນວ່າຈະພົບຍຸງກົ້ນຊີ *Alnopheles* ຢູ່ໃນເຂດນີ້ ແຕ່ກໍບໍ່ພົບການຕິດເຊື້ອກາຝາກໄຂ້ຍຸງ *Plasmodium* ໃນອາສາສະມັກທີ່ມີສຸຂະພາບແຂງແຮງເຫຼົ່ານີ້, ອັດຕາການຕິດເຊື້ອພະຍາດກາຝາກໂຕກົມມີເຖິງ 80.2% (95% CI 73.1-87.2) ເຊິ່ງຄວາມຮຸນແຮງຂອງການຕິດເຊື້ອມີຕ່ຳ. ແຕກຕ່າງຈາກການສຶກສາອື່ນໆໃນແຂວງຄຳມ່ວນ, ອັດຕາການຕິດເຊື້ອໃບໄມ້ໃນຕັບ *Opistorchiasis viverrini* ແມ່ນຕ່ຳຫຼາຍ ອາດເປັນຍ້ອນອຸນຫະພູມໃນນ້ຳໃນບ້ານເຂດພູສູງເຢັນ ຫຼາຍ; ດັ່ງນັ້ນ ສະພາບທາງນິເວດວິທະຍາບໍ່ເອື້ອອຳນວຍໃຫ້ຫອຍຂະໜາດນ້ອຍທີ່ເປັນພາຫະນຳເຊື້ອ.

ຄວາມໜາແໜ້ນຂອງປະຊາຊົນໄດ້ສະແດງໃຫ້ເຫັນວ່າໃນອະດີດມີການຕິດເຊື້ອໄຂ້ແມງແດງສູງ Rickettsiae (IgG positive) ເຊິ່ງອັດຕາການຕິດເຊື້ອໂດຍລວມມີ 80.4% (95% CI 73.7-87.1) ໃນກຸ່ມໄຂ້ແມງແດງ 3 ສາຍພັນ Rickettsial serogroups (scrub typhus orientia group (STG), typhus group rickettsiae (TG) and spotted fever group rickettsia (SFG)) ຈາກການກວດຕົວຢ່າງເລືອດ 138 ຕົວຢ່າງ. ຄວາມຊຸກຊຸມຂອງທາດກາຍຕ້ານ ສະເພາະຂອງ STG ແມ່ນຫຼາຍ ແລະ ມີສູງເຖິງ 77.5% (95% CI 70.5-84.6), ຕິດຕາມມາດ້ວຍ 13.8% (95% CI 7.9-19.6) ຂອງທາດກາຍຕ້ານ SFG ແລະ 6.5% (95% CI 2.3-10.7) ຂອງທາດກາຍຕ້ານ TG. ອັດຕາການຕິດເຊື້ອພົບສູງໃນທຸກກຸ່ມອາຍຸທີ່ພົບການຕິດເຊື້ອຂອງພະຍາດເຫຼົ່ານີ້ກ່ອນໜ້າ ແລະ ກັບມາເປັນຄືນ. ທາດກາຍຕ້ານ IgG ຕ້ານຕໍ່ເຊື້ອ Ehrlichia chaffensis ແລະ *Anaplasma phagocytophilum* ບໍ່ໄດ້ຖືກກວດພົບໃນຕົວຢ່າງເຫຼົ່ານັ້ນ. ຂໍ້ມູນເບື້ອງຕົ້ນທີ່ໄດ້ຈາກການເຮັດ ELISA ຊີ້ໃຫ້ເຫັນວ່າປະຊາກອນທີ່ເຂົ້າຮ່ວມການສຶກສາຄັ້ງນີ້ ພົບການຕິດເຊື້ອໄວຣັສຫຼາກຫຼາຍຊະນິດ ທີ່ມີພາຫະເປັນໂຕນຳເຊື້ອ. ເຊັ່ນ ພົບການຕິດເຊື້ອ flaviviruses ສູງໃນປະຊາກອນຜູ້ເຂົ້າຮ່ວມການສຶກສາ ມີເຖິງ 69.1% (95% CI 62.0-75.0),

ການຕິດເຊື້ອໄວຣັສເຫຍື່ອຫຸ້ມສະໝອງອັກເສບຍີປຸ່ນ (JEV) 28.3% (95% CI 21.8-34.7) ແລະ 4.2 % (95% CI 1.3-7.0) ພົບທາດການຕ້ານຂອງໄວຣັສໄຂ້ຍຸງລາຍ (DENV). ເຖິງຢ່າງໃດກໍ່ຕາມປະຕິກິລິຍາຂ້າມຜ່ານຂອງ Anti-flavivius ມີເຖິງ 30%. ຄວາມຈິງແລ້ວການຕີລາຄາຄວາມລະອຽດທາງ ດ້ານ ເຊຣອມ ຄືດັ່ງທີ່ເຮົາຮູ້ມັນມີຄວາມລະອຽດອ່ອນ ເນື່ອງຈາກ ພູມຄຸ້ມກັນປະຕິກິລິຍາລະຫວ່າງ ແອນຕິເຈນ ແລະ ແອນຕິເຈນ ໃນພູມຄຸ້ມກັນຂອງແຕ່ລະເຊື້ອ flavivius ມີຄວາມຊັບຊ້ອນ ແລະ ຕ້ອງໃຊ້ແຮງງານໃນການເຮັດການກວດ Plaque reduction neutralization tests (PRNT). ອີກວິທີໜຶ່ງກໍຄືການເຮັດ ELISA, the DEITAa (Discriminant ELISA for Typing Arbovirus antibodies), ໄດ້ເຮັດໃນໂຄງການນີ້ ແລະ ກຳລັງເຮັດການປະເມີນ ເພື່ອປັບປຸງຄວາມສາມາດໃນການຈຳແນກ ແລະ ການຕອບສະໜອງ ລະບົບພູມຄຸ້ມກັນຕ້ານຕໍ່ໄວຣັສ. ຜົນເບື້ອງຕົ້ນກວດພົບ PRNT ໂດຍການນຳໃຊ້ລະບົບ DEITAa ທີ່ເປັນໄປໄດ້ ເພື່ອຈຳແນກ ແອນຕິເຈນ flaviviruses (arbovirus).

ອົງປະກອບທາງດ້ານຊີວະວິທະຍາຂອງພາຫະນຳເຊື້ອ

ການສຶກສາຄັ້ງນີ້ໄດ້ອະທິບາຍລັກສະນະຄວາມຫຼາກຫຼາຍແລະຄວາມ ແຕກຕ່າງຂອງແມງໄມ້ຕົນຂໍ້ ທີ່ເປັນພາຫະນຳເຊື້ອ ແລະ ປະເມີນ ຄວາມສ່ຽງຂອງມະນຸດຕໍ່ການຕິດເຊື້ອ ທີ່ຮູ້ຈັກ ແລະ ບໍ່ທັນຮູ້ຈັກ ໂດຍການເກັບຕົວຢ່າງຍຸງ ແລະ ຮົ້ນໂດຍໃຊ້ຄົນເປັນເຫຍື່ອລໍ້ຈັບ (HDN) ແລະ ໄຈ້ແຍກພວກມັນເພື່ອຊອກຫາເຊື້ອ. ຍຸງຈະນວນ 51 ຊະນິດໄດ້ຖືກໄຈ້ແຍກຈາກ 9 ຕະກູນຄື: *Aedes, Anopheles, Armigeres, Culex, Heizmania, Malaya, Mansonia, Tripteroides*, and *Uranotaenia*. ແລະ 4 ຊະນິດແມ່ນມາຈາກຕະກູນ ຮົ້ນ Sergentomyia, ເຫັບ 8 ຊະນິດ ຈາກ 5 ຕະກູນ ແລະ ໝັດ 2 ຊະນິດ ຈາກ 2 ຕະກູນ ໄດ້ຖືກໄຈ້ແຍກ . ຍຸງທີ່ເປັນພາຫະນຳເຊື້ອທີ່ພົບ ໃນການສຶກສາສານີ້ມີຄື: *Ae. albopictus* [1], ຍຸງທີເປັນພາຫະນຳເຊື້ອພະຍາດໄຂ້ເຫຍື້ອຫຸ້ມ ສະໝອງອັກເສບ JEV vector *Cx. vishnui* s.l. [2] ແລະ ຍຸງ ທີ່ເປັນພາຫະນຳເຊື້ອພະຍາດຂາຊ້າງ the *lymphatic filariasis* vector *Ar. kesseli* [3].

ການຊອກຫາ Arboviral ສຳລັບ alpha, flavi ແລະ phleboviruses ໃນຍຸງ, ເຫັບ ແລະ ຮົ້ນ ແມ່ນໃຊ້ເຕັກນິກ pan-flavi, pan-alpha ແລະ ເຕັກນິກ pan-phlebo RT-PCR, PCR ໃຊ້ຊຸດກວດ Dengue univeral ແລະ JEV group primers ແລະ ການເຮັດ Sequencing ແມ່ນບໍ່ພົບ ໄວຣັດຕ່າງໆ ໂຕເຫັບໄດ້ຖືກນຳມາກວດເພື່ອຊອກຫາເຊື້ອ *Rickettsia spp, Neorickettsia sennetsu*, *Ehrilichia spp*, *Anaplasma spp Coxiella burnetii* ແລະ *Leptospira spp* ດ້ວຍວິທີເຮັດ PCR ເຊິ່ງແຕກຕ່າງຈາກການສຶກສາທີ່ໄດ້ເຮັດ ຜ່ານມາ ໃນເຂດດຽວກັນ ເຊື້ອພະຍາດດັ່ງກ່າວບໍ່ໄດ້ຖືກກວດພົບ. ເຖິງຢ່າງໃດກໍຕາມ, *Rickettsia spp* ໄດ້ຖືກກວດພົບຢູ່ໃນໂຕໝັດ ທີ່ເກັບໄດ້ໃນໄລຍະເຮັດການສຶກສາ ເຊິ່ງມັນເຊື່ອມໂຍງກັບຜົນກວດ ທາງເຊຣອມວິທະຍາຂອງທາດການຕ້ານ TG ໃນຕົວຢ່າງເລືອດຂອງ ຜູ້ເຂົ້າຮ່ວມການສຶກສາ.

ຈຸດປະສົງ ເພື່ອໃຈ້ແຍກການຕິດເຊື້ອທີ່ບໍ່ຮູ້ສາເຫດ ເພື່ອອະທິບາຍ Virome ໃນໂຕຍຸງ ແລະ ຮົ້ນ ໂດຍການຈັດລຳດັບແບບຢັ່ງເລິກ ເຊິ່ງຖືກດຳເນີນການຢູ່ ສະຖາບັນ ປັດສະເຕີ ປາຣີ IPP. ບັນດາໄວຣັສ ທີ່ມີຍຸງເປັນພາຫະ ຈຳນວນ 1,471 ແລະ ໂຕຮົ້ນເປັນພາຫະ ຈຳນວນ 55 ໄດ້ມີການຈັດລຳດັບ ແລະ ວິເຄາະ. ບັນດາຄອບຄົວໄວຣັສທີ່ຖືກແຍກ ຫຼາຍທີ່ສຸດທີ່ພົບໄວຣັສ RNA ແມ່ນຈຳພວກທີ່ເຮັດໃຫ້ ພືດ ແລະ ສັດທີ່ບໍ່ມີກະດູກສັນຫຼັງມີການຕິດ ເຊື້ອ. ໄວຣັສ ທີສາມາດເຮັດໃຫ້ສັດມີກະດູກສັນຫຼັງຕິດເຊື້ອນັ້ນ ແມ່ນໄວຣັສ *Flaviviridae, Picornaviridae, Astroviridae, Hepeviridae, Phenuiviridae, Peribunyaviridae, Orthomyxoviridae, Rhabdoviridae* ແລະ ຕະກູນ Reoviridae. ໄວຣັສສ່ວນໃຫຍ່ ມີລັກສະນະທາງສາຍເລືອດຂຶ້ນ ກັບຕະກູນ arboviruses ມີ *Phenuiviridae* /phlebovirus L ທີມີຄວາມສົມບູນຂອງ phlebovirus ຊະນິດໃໝ່ທີກວດພົບໃນ *Culex vishnui* ໄດ້ປະກົດຂຶ້ນ.

ການວິເຄາະ Phylogenetic ໄດ້ຖືກເຮັດຢູ່ຮາກຖານຂອງ clade ທີ່ເກີດຈາກ arbo-phlebiviruses ໃນຍຸງ ແລະ ຮົ້ນ. ການວິເຄາະ ເພີ່ມເຕີມຈະດຳເນີນການໃນອານາຄົດ.

ORFs ຂອງໄວຣັສທີ່ເປັນພາຫະນຳເຊື້ອ Zoonotic ໄວຣັສເຫັບ, ໄວຣັສ Jingmen tick virus-like virus (JMTV) ທີ່ໄດ້ໃຈ້ ແຍກໄວ້ກ່ອນໜ້ານີ້ຈາກໂຕເຫັບທີເກັບໄດ້ໃນເຂດດັ່ງກ່າວໄດ້ເຮັດ ການຈັດລຳດັບຢ່າງເລິກເຊິ່ງ ແລະ ຈົບລົງໂດຍການເຮັດ PCR-Sanger. ການສະແດງຢູ່ພາຍໃນ JMTV ໄດ້ຖືກກວດຢູ່ໃນຕົວ ຢ່າງເລືອກຂອງຜູເຂົ້າຮ່ວມການສຶກສາ ໃນໂຄງການ BiolaoPlus ໂດຍໃຊ້ລະບົບ Luciferase ImmunoPrecipitation Systhem (LIPS), ການກວດທາງເຊຣອມວິທະຍາຂອງ ທາດ ການຕ້ານໂດຍການໄຫຼດ້ວຍຄວາມໄວສູງເພື່ອຕ້ານຕໍ່ໂປຕອິນທາງ ພາຍນອກຂອງໄວຣັສ ທີ່ຜ່ານມາຍັງບໍ່ກວດພົບ. ໄວຣັສເປົ້າໝາຍອື່ນໆ ຈະສະແດງ ແລະ ນຳໃຊ້ເປັນແອນຕເຈນສຳລັບ LIPS.

Metrology

Install a metrology laboratory at Institut Pasteur du Laos, to do the pipette service calibration & preventive maintenance.

Background

A pipette is used to precisely measure and transfer small volumes of liquid in the laboratory in experiments that demand high accuracy. However, the sources of error in pipette measurements could arise from device failure or due to misuse by the operator. Any discrepancy in volumes dispensed may affect the outcomes and reproducibility of an experiment such as qPCR results. It is, therefore, necessary to check pipette calibration every few months to ensure accuracy by dispensing right volumes. This is why pipette calibration is considered a fundamental part of good laboratory practice (GLP). Regular pipette preventive maintenance and calibration service can significantly reduce the costs also, risks and liabilities associated with out-of-calibration pipettes, sure, most pipettes work... but are they accurate?

That is why we installed a metrology laboratory at Institute Pasteur du Laos. We started by doing a training for the involved staff at IPL. The training was provided by staff from Institute Pasteur Cambodia.

They are doing this procedure in Cambodia since many years already and where able to inform us about the needed equipment, environment, documentation and proceeds for the pipette calibration.

By getting this knowledge we where able to install the metrology lab under the rules of the ISO Standard 8655

Picture: Mr. Vongphachanh Ounenalath and Mrs. Phoutsana Khamsuvat calibrating pipettes in the metrology laboratory.

They are in charge of the pipette calibration and maintenance service.

They acquired the required knowledge through training from Institut Pasteur of Cambodia' technicians.
They also have been trained and know about the importance of a proper documentation regarding to these duties.

The IPL metrology lab with a ultra-precise balance and a special antivibration bench

Principles of Pipette Calibration

At 20°C temperature and one atmosphere of pressure, water density stays constant at 1 g/mL. The volume of water can be determined by weighing dispensed water. Temperature, atmospheric pressure, and humidity may influence the accuracy of measurements. These factors are combined to generate the Z factor, applied in the calculation of the volume of water, and compared with the theoretical volume, which finally determines the accuracy of the pipette.

Steps Involved in Pipette Calibration

- Take distilled water in a beaker and record its temperature. Also, gather your pipette and the correct tips based on both the small and large volumes that the pipette can dispense.
- Place a weigh boat on a balance that can accurately weigh in the microgram range, and set it to zero after closing the balance door.
- Pre-rinse the tip by aspirating and dispensing the set volume three times and push fully to remove any remaining liquid.
- Aspirate the calibration volume without bubble formation and dispense the liquid slowly into the weigh boat. Then, record the weight on the balance and repeat the process ten times.
- Calculate the dispensed volume by using the equation V = W x Z where W is the weight of the water, Z is the Z factor, and V is the calculated volume of dispensed water. Next, determine the mean value from ten trials.
- Finally, calculate accuracy by using the equation A = 100 x Vavg/V0, where A is the accuracy of the pipette, Vavg is the average calculated volume and V0 is the theoretical volume you tried to dispense. If the accuracy value lies in the 99-101% range, the pipette is considered normal and calibrated.

All the proceeds are installed now at Institut Pasteur du Laos and we have a proper documentation also.

The next step in the very near future will be that we get a external Audit about our proceeds and documentation. That will ensure that we do it to 100% under the rules of the ISO standart for pipette calibration

IPL publications 2019

M. Motoki, D. Fonseca, Ian W. Sutherland, J. Hertz, PT Brey and S. Marcombe. Population genetic structure of *Aedes albopictus* from east and South-East Asia. December 2019. Parasites & Vectors December 2019, 12:477.

Motoki, M. T., K. Vongphayloth, L. M. Rueda, E. F. Miot, A. Hiscox, J. C. Hertz and P. T. Brey. "New Records and Updated Checklist of Mosquitoes (Diptera: Culicidae) from Lao People's Democratic Republic, with Special Emphasis on Adult and Larval Surveillance in Khammuane Province." Journal of vector ecology : journal of the Society for Vector Ecology 44, no. 1 (Jun, 2019): 76-88.10.1111/jvec.12331

Depaquit, J., K. Vongphayloth, P. Siriyasatien, R. Polseela, A. Phumee, M. Loyer, A. Vol, G. Varlot, N. Rahola, P. T. Brey, I. W. Sutherland, J. C. Hertz, F. Gay and N. Leger. "On the True Identity of Sergentomyia Gemmea and Description of a Closely Related Species: Se. Raynali N. Sp." Medical and veterinary entomology 33, no. 4 (Dec, 2019): 521-529.10.1111/mve.12393

Marcombe S, Fustec B, Cattel J, Chonephetsarath S, Thammavong P, et al. Distribution of insecticide resistance and mechanisms involved in the arbovirus vector *Aedes aegypti* in Laos and implication for vector control. December 2019. Accepted in Plos Neglected Tropical Diseases.

Marcombe S, …, Brey PT and Jones A. Malaria and Dengue mosquito vectors from Lao PDR show a lack of the rdl mutant allele responsible for cyclodiene insecticide resistance. 2019. Accepted in Journal of Medical Entomology.

Temmam, S., K. Vongphayloth, J. C. Hertz, I. Sutherland, B. Douangboubpha, M. Grandadam, T. Bigot, P. T. Brey and M. Eloit. "Six Nearly Complete Genome Segments of a Novel Reovirus Identified in Laotian Batflies." Microbiology resource announcements 8, no. 46 (Nov 14, 2019).10.1128/MRA.00733-19

Temmam, S., T. Bigot, D. Chretien, M. Gondard, P. Perot, V. Pommelet, E. Dufour, S. Petres, E. Devillers, T. Hoem, V. Pinarello, V. Hul, K. Vongphayloth, J. C. Hertz, I. Loiseau, M. Dumarest, V. Duong, M. Vayssier-Taussat, M. Grandadam, E. Albina, P. Dussart, S. Moutailler, J. Cappelle, P. T. Brey and M. Eloit. "Insights into the Host Range, Genetic Diversity, and Geographical Distribution of Jingmenviruses." mSphere 4, no. 6 (Nov 6, 2019).10.1128/mSphere.00645-19

Seroprotection at different levels of the health care system after routine vaccination with DTPw-HepB-Hib in Lao PDR. Hefele L, Syphan S, Xayavong D, Homsana A, Kleine D, Chanthavilay P, Nouanthong P, Xaydalasouk K, Phathammavong O, Billamay S, Xeuatvongsa A, Reinharz D, Muller CP, Black AP. Clin Infect Dis. 2019 Feb 19.

Ong KIC, Iwagami M, Araki H, Khattignavong P, Soundala P, Keomalaphet S, Prasayasith P, Lorpachan L, Xangsayalath P, Pongvongsa T, Hongvanthong B, Brey PT, Kano S, Jimba M. Prevalence of G6PD Viangchan variant in malaria endemic areas in Lao PDR: an implication for malaria elimination by 2030, Malaria Journal, 18: 75, 2019.

Vilay P, Nonaka D, Senamonty P, Lao M, Iwagami M, Kobayashi J, Hernandez PM, Phrasisombath K, Kounnavong S, Hongvanthong B, Brey PT, Kano S. Malaria prevalence, knowledge, perception, preventive and treatment behaviour among military in Champasack and Attapeu provinces, Lao PDR: a mixed methods study, Tropical Medicine and Health, 47: 11, 2019.

Pongvongsa T, Nonaka D, Iwagami M, Soundala P, Khattignavong P, Xangsayarath P, Nishimoto F, Kobayashi J, Hongvanthong B, Brey P, Kano S. Malaria among foreign migrant workers in Savannakhet province, Lao PDR: a cross-sectional study, Tropical Medicine and Health, 47: 10, 2019.

Knowledge, attitudes, and practices regarding vaccination among healthcare workers in Lao PDR. Sengchaleun V, Khampanisong P, Aye-Soukhathammavong P, Reinharz D, Black AP. Southeast Asian J Trop Med Public Health, March 2019.

Varicella zoster and fever rash surveillance in Lao People's Democratic Republic
Nouanthong P, Hübschen JM, Billamay S, Mongkhoune S, Vilivong K, Khounvisith V, Sinner R, Grandadam M, Phonekeo D, Black AP, Muller CP. BMC Infect Dis. 2019 May 8;19(1):392.

Cross-species transmission of poultry pathogens in backyard farms: ducks as carriers of chicken viruses. Pauly M, Snoeck CJ, Phoutana V, Keosengthong A, Sausy A, Khenkha L, Nouanthong P, Samountry B, Jutavijittum P, Vilivong K, Hübschen JM, Black AP, Pommasichan S, Muller CP. Avian Pathol. 2019 Jun 14.

High prevalence of helminth infections in mother-child pairs from three central provinces of Lao People's Democratic Republic. Pauly M, Sayasinh K, Muller CP, Sayasone S, Black AP. Parasite Epidemiology and Control. 2019

Seroprevalence of anti-tetanus antibodies in mothers and cord blood and associated factors in health-care settings in Lao People's Democratic Republic. Ounnavong P, Chanthavilay P, Khampanisong P, Reinharz D, Muller CP, Black AP. Vaccine. 2019

Temmam S, Vongphayloth K, Hertz J, Sutherland I, Douangboubpha B, Grandadam M, Thomas Bigot T, Brey P, Eloit M. Six nearly-complete genome segments of a novel reovirus identified in Laotian batflies. 2019. Microbiology Resource Announcements (in press)

Nouanthong P, Hübschen JM, Billamay S, Mongkhoune S, Vilivong K, Khounvisith V, Sinner R, Grandadam M, Phonekeo D, Black AP, Muller CP. Varicella zoster and fever rash surveillance in Lao People's Democratic Republic. BMC Infect Dis. 2019 May 8;19(1):392. doi: 10.1186/s12879-019-3990-7.

Apanaskevich, D. A., A. Chaloemthanetphong, K. Vongphayloth, A. Ahantarig, M. A. Apanaskevich, P. T. Brey, J. C. Hertz, K. Lakeomany, I. W. Sutherland and W. Trinachartvanit. "Description of a New Species of Dermacentor Koch, 1844 (*Acari: Ixodidae*) from Laos and Thailand." Systematic parasitology 96, no. 6 (Jul, 2019): 475-484.10.1007/s11230-019-09861-z

Matthew T Robinson, Khamsing Vongphayloth, Jeffrey C Hertz, Paul Brey and Paul N Newton. "Tick-transmitted human infections in Asia." Microbiology Australia, (Published online: 24 October 2018)

Marcombe S, Tangena JAA, Thammavong P, Chonephetsarath, Sompong B, Xayteng K, Grandadam M, Sutherland IW, Lindsay SW, Brey PT. Bionomics and insecticide resistance of the arboviral vector *Aedes albopictus* in northern Lao PDR. 2018. PLoS ONE 13(10): e0206387.

Marcombe S., Khounsombat K., Hertz J, Sutherland I, and Brey PT. Alternative insecticides for larval control of the dengue vector *Aedes aegypti i*n Lao PDR: insecticide resistance and semi-field trial study. 2018. Parasites & Vectors 2018, 11:616.

Bionomics and insecticide resistance of the arboviral vector Aedes albopictus in northern Lao PDR.
Tangena JA, Marcombe S, Thammavong P, Chonephetsarath S, Somphong B, Sayteng K, Grandadam M, Sutherland IW, Lindsay SW, Brey PT. PLoS One. 2018 Oct 25;13(10):e0206387. doi: 10.1371/journal.pone.0206387. eCollection 2018.

First Record of *Aedes* (*Stegomyia*) malayensis Colless (Diptera: Culicidae) in the Lao People's Democratic Republic, Based on Morphological Diagnosis and Molecular Analysis.

Motoki MT, Miot EF, Rueda LM, Vongphayloth K, Phommavanh N, Lakeomany K, Debboun M, Hertz JC, Brey PT. US Army Med Dep J. 2018 Jan-Jun;(1-18):1-7.

Iwagami M, Tangpukdee N, Wilairatana P, Krudsood S, Dao LD, Nakazawa S, Sinuon M, Socheat D, Yasuoka J, Jimba M, Watanabe H, Kobayashi J, Toma H, Vanisaveth V, Hongvanthong B, Brey PT, Kano S. *Pfcrt* genotypes and related microsatellite DNA polymorphisms on *Plasmodium falciparum* differed among populations in the Greater Mekong Subregion. Parasitology International. 2018 Dec;67(6):816-823. doi: 10.1016/j.parint.2018.08.008. Epub 2018 Aug 28.

Iwagami M, Nakatsu M, Khattignavong P, Soundala P, Keomalaphet S, Lorpachan L, Xangsayalath P, Matsumoto-Takahashi E, Pommelet V, Hongvanthong B, Brey PT, Kano S. Heterogeneous distribution of *K13* mutations in *Plasmodium falciparum* in Lao PDR. Malaria Journal, 17: 483, 2018.

Takahashi E, Nonaka D, Iwagami M, Vilay P, Chanthakoumane K, Kobayashi J, Pongvongsa T, Kounnavong S, Hongvanthong B, Brey PT, Kano S. Patients' adherence to artemisinin-based combination therapy and healthcare workers' perception and practice in Savannakhet province, Lao PDR, Tropical Medicine and Health, 46: 44, 2018.

Vincent JP, Komaki-Yasuda K, Iwagami M, Kawai S, Kano S. Combination of PURE-DNA extraction and LAMP-DNA amplification methods for accurate malaria diagnosis on dried blood spots, Malaria Journal, 17: 373, 2018.

Iwagami M, Nakatsu M, Khattignavong P, Soundala P, Lorphachan L, Keomalaphet S, Xangsayalath P, Kawai S, Hongvanthong B, Brey PT, Kano S. First case of human infection with *Plasmodium knowlesi* in Lao PDR, PLOS Neglected Tropical Diseases, 12(3): e0006244, 2018.

Araki H, Ken Ong KIC, Lorphachan L, Soundala P, Iwagami M, Shibanuma A, Hongvanthong B, Brey PT, Kano S, Jimba M. Mothers' *Opisthorchis viverrini* infection status and raw fish dish consumption in Lao People's Democratic Republic: determinants of child infection status, Tropical Medicine and Health, 46: 29, 2018.

Hypoglycemic Toxins and Enteroviruses as Causes of Outbreaks of Acute Encephalitis-Like Syndrome in Children, Bac Giang Province, Northern Vietnam. Phan NT, Gouilh MA, Paireau J, Phuong L, Cheval J, Ngu ND, Hébert C, Nguyen TH, Lortholary O, Tondeur L, Manuguerra JC, Barouki R, Sander J, Janzen N, Nguyen HT, Brey PT, Fontanet A, Eloit M. Emerg Infect Dis. 2018 Aug;24(8):1435-1443. doi: 10.3201/eid2408.171004.

New Locality Records of Ixodes granulatus and Ixodes vespertilionis (Acari: Ixodidae) From Tree-Shrews (*Scandentia: Tupaiidae*) and Bats (*Chiroptera: Hipposideridae*) in Laos. Vongphayloth K, Douangboubpha B, Sanamxay D, Xayaphet V, Robbins RG, Apanaskevich DA, Sutherland IW, Brey PT.J Med Entomol. 2018 Jun 28;55(4):1035-1039. doi: 10.1093/jme/tjy019.

The Genus Dermacentor (Acari: Ixodidae) in Laos: A Review and Update of Species Records. Vongphayloth K, Hertz JC, Lakeomany K, Apanaskevich DA, Robbins RG, Sutherland IW, Brey PT. J Med Entomol. 2018 Jun 28;55(4):1047-1050. doi: 10.1093/jme/tjy041.

Re-evaluate yellow fever risk in Asia-Pacific region. Brey PT, Fontenille D, Tang H.Nature. 2018 Feb 1;554(7690):31. doi: 10.1038/d41586-018-01305-w. No abstract available.

Immunity levels to poliovirus in Lao children and adults before the vaccine-derived polio outbreak: a retrospective study. Pauly M, Black AP, Khampanisong P, Nouanthong P, Hübschen JM, Nanthavong N, Sayasinh K, Jutavijittum P, Samountry B, Xeuatvongsa A, Diedrich S, Muller CP. PLoS One. 2018 May 15;13(5):e0197370. doi: 10.1371/journal.pone.0197370

Evidence of increased hepatitis E virus exposure in Lao villagers with contact to ruminants. Tritz SE, Khounvisith V, Pommasichan S, Ninnasopha K, Keosengthong A, Phoutana V, Camoin M, Hübschen JM, Black AP, Muller CP, Snoeck CJ, Pauly M. Zoonoses Public Health. 2018 Jun 10. doi: 10.1111/zph.12483.

Seroprevalence and risk factors of hepatitis B and C virus infections in female workers of Lao garment factories. Xaydalasouk K, Strobel M, Buisson Y, Black AP, Muller CP. PLoS One. 2018 Jul 16;13(7):e0199919. doi: 10.1371/journal.pone.0199919

High circulation of hepatitis E virus in pigs professionals exposed to pigs in Laos. Khounvisith V, Tritz S, Khenkha L, Phoutana V, Keosengthong A, Pommasichan S, Nouanthong P, Hübschen JM, Snoeck CJ, Reinharz D, Muller CP, Black AP, Pauly M. Zoonoses Public Health. 2018 Dec;65(8):1020-1026. doi: 10.1111/zph.12520.

Etiology of viral respiratory infections in Northern Lao People's Democratic Republic. Snoeck CJ, Ponghsavath V, Luetteke N, Kaufmann S, Sausy A, Samountry B, Jutavijittum P, Weber B, Muller CP. J Med Virol. 2018 Oct;90(10):1553-1558. doi: 10.1002/jmv.25237

Elliott F. Miot, Elodie Calvez, Fabien Aubry, Stéphanie Dabo, Marc Grandadam, Sébastien Marcombe, Catherine Oke, James G. Logan, Paul T. Brey, Louis Lambrechts. Potential of the sylvatic mosquito *Aedes malayensis* to act as an arbovirus bridge vector in a forested area of the Nakai district, Laos". Parasites & Vectors. (submitted)

Tangena JA, Marcombe S, Thammavong P, Chonephetsarath S, Somphong B, Sayteng K, Grandadam M, Sutherland IW, Lindsay SW, Brey PT. Bionomics and insecticide resistance of the arboviral vector *Aedes albopictus* in northern Lao PDR. PLoS One. 2018 Oct 25;13(10):e0206387. doi: 10.1371

They visited Institut Pasteur du Laos!

24 October, 2019: Mr. Alain Mérieux and his delegation from the Fondation Mérieux

19 September, 2019: Mr. Trevor Smith and his delegation from the Canadian WMD Treat Reduction Program

22 August, 2019: Study tour from Ritsumeikan University, Tokyo, Japan

27August 2019: Study tour from the Tokyo Medical and Dental University, Tokyo, Japan

09 September, 2019: H.E. Mme Paulette Lenert, Minister of Development Cooperation and Humanitarian Affairs and Minister of Consumer Protection of Luxembourg and his delegation

13 September, 2019: Visit of Vysnova

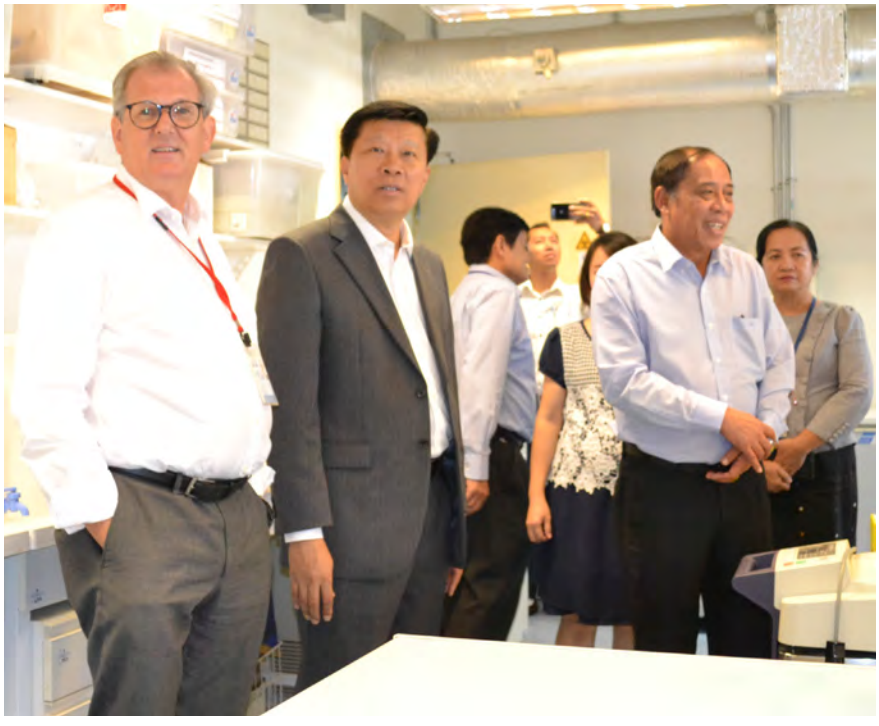
16 March, 2019: Dr. Shigeyuki Kano received Ichiro Miyazaki Award in Nagasaki, Japan

23 August, 2019: Study tour from University of the Ryukyus, Okinawa, Japan

11 September, 2019: Visit of Rear Admiral Louis C. Tripoli, Command Surgeon, United States Indo-Pacific Command, Hawaii

12 March, 2019: Study tour from Keio University

14 March, 2019: Dr. Moritoshi Iwagami received Professor Tsutomu Takeuchi International Memorial Award, the Ohyama Health Foundation in Tokyo, Japan



23 August, 2019: Lao Deputy Minister of Health Dr. Phouthone Muangpak

12 March, 2019: Temasek Foundation International Scale Programme, Singapore

10 April, 2019: Dr. Rosanna Peel and Dr. Debi Boeras London School of Tropical Medicine and Hygiene UK

11 February, 2019: US Department of Defense, Global Emerging Infections System USA

06 February, 2019: H.E. Dominic Goh, Ambassador of Singapore

12 March, 2019: Dr. Elizabeth Ashley and Dr. Andrew Simpson The Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit (LOMWRU)

23 January, 2019: Dr. Jean-Paul Moatti, Director General and his delegation from IRD, France

06 December, 2018: Dr. Robert Redfield, Director General and his delegation from US CDC, Atlanta GA USA

Teaching/Training

04 November, 2019: Vector Borne Disease Surveillance Training, Arboshield Project, Opening Ceremony.

12 June, 2019: Four militaries from Institute of Disease Prevention of Army accomplished one year of Laboratory training in IPL(Arboshield project)

12 November, 2019: Four militaries training

23 October, 2019: Data management and GIS training for the pilot project of *Aedes* vector surveillance (PL and WMP)

26 March, 2019: Training for two students from National University of Laos

08 May, 2019: Kaptitude e-learning IPL staffs Biological and chemical Risk training

10 June, 2019: International Medical Entomology Course

10 December, 2019: In house English teaching by Kyle Tevlin teacher

Meetings

29 November, 2019: ECOMORE II, 2nd National Stakeholder meeting at Settha Palace Hotel, Vientiane Capital

27 August, 2019: 2nd Arthropod-borne diseases Surveillance Meeting at Settha Palace Hotel, Vientiane Capital (Arboshield project)

02 April, 2019: JICA/AMDED SATREPS Project Final Dissemination Meeting, Development of Innovative Research Technique in Genetic Epidemiology of Malaria and Other Parasitic Diseases in Lao PDR fo containment of their Expanding Endemicity

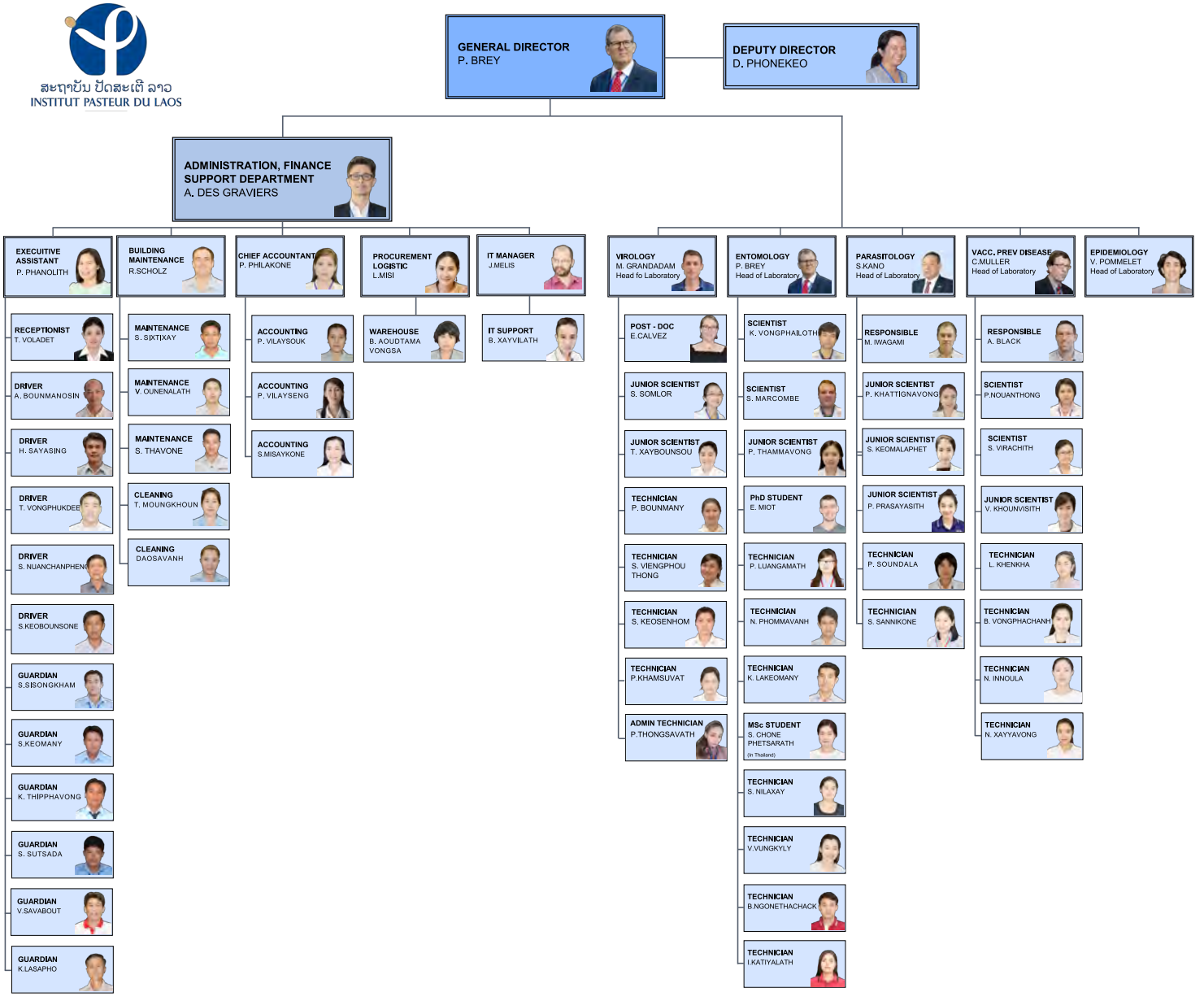
26 February, 2019: 1st Arthropod-borne diseases Surveillance Meeting at Settha Palace Hotel, Vientiane Capital (Arboshield project)

23 August, 2019: Field Epidemiology Training short course presentation meeting for militaries with the participation of DTRA, NCLE, WHO, US CDC, Institute of Diseases prevention and health promotion of Lao Army and IPL(Arboshield project)

07 March, 2019: ECOMORE2 Annual Meeting at Settha Palace Hotel, Vientiane Capital

30 January , 2019: Interim meeting of ECOMRE2 project, Alternative strategy for dengue vector control in Lao PDR

Main organigram



ສະຖາບັນ ປັດສະເຕີ ລາວ
INSTITUT PASTEUR DU LAOS

Institut Pasteur du Laos

Samsenthai Road, Ban Kao-Gnot, Sisattanak district,
P.O Box 3560, Vientiane, Lao PDR.
www.pasteur.la