ກະຊວງ ສາທາລະນະສຸກ
Ministry of Health

ສະຖາບັນ ປັດສະເຕີ ລາວ
INSTITUT PASTEUR DU LAOS

Institut Pasteur du Laos

Activity Report 2021-2022

Réseau International des Instituts Pasteur

Summary

Mandate

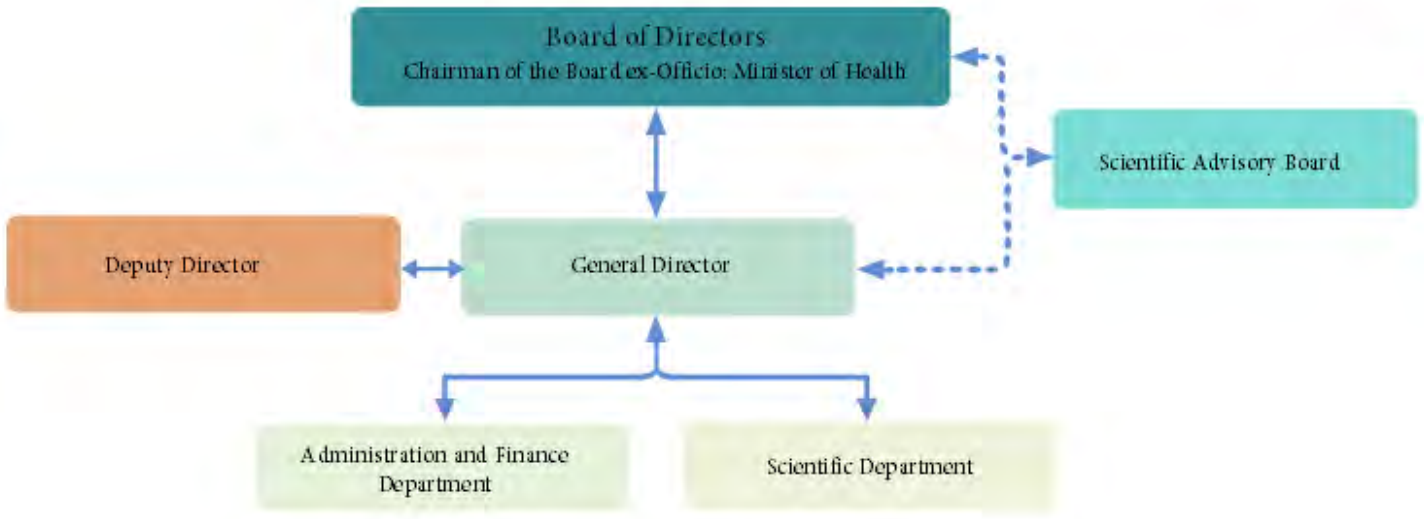
Institut Pasteur du Laos (IPL) is a Lao National Institution created by Prime Ministerial Decree in November 2007 IPL is the result of a long term and joint decision between Lao Ministry of Health and Institut Pasteur Paris which commits to stay 16 years before retroceding the full management of IPL. 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. Bounfeng PHOUMAMALYSITH (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:

Pr. Dr. Félix REY. Institut Pasteur, Paris, France.
Pr. Dr. Olivier LORTHOLARY. Necker Hospital, Paris, France.
Pr. Dr. JIMBA Masamine, The University of Tokyo, Japan.

Letter From

Dr. Paul BREY

Director

The past year has been one of the most difficult in recorded history world-wide due to the Covid-19 pandemic. Covid-19 has caused so much death and destruction that we have not seen the likes of which since the Great Influenza pandemic of 1918 and before that the Black Death that decimated nearly half the population of Europe in the 14th century.

In 2020, and early 2021, we thought that the Lao PDR would be spared from the scourge because the numbers of Covid-19 were so low. In fact, some neighboring countries suspected that the medical laboratory infrastructure in Laos was incapable of conducting Covid-19 diagnostics… This of course was totally untrue and unjustified as the National Laboratory for Laboratory and Epidemiology (NCLE) and Institut Pasteur du Laos (IPL) were operational for SARS-CoV-2 detection from human samples as early as January 2020. The reason Covid numbers were low is because the Lao Government and Ministry of Health put into place a series of very stringent control measures from early 2020 restricting travel, promoting mask wearing and social distancing closing certain entertainment venues. In the summer of 2020, a consortium of Lao research partners led by scientists from IPL carried out a country-wide SARS-CoV-2 serological survey study to determine the extent and spread of Covid-19 throughout the country in high-risk populations like health care workers, guano collectors, wildlife sellers, as well as the general public. Our study, recently published in Lancet Regional Health Western Pacific (Lancet Reg Health West Pac. 2021 Aug;13:100197. doi: 10.1016/j.lanwpc.2021.100197. Epub 2021 Jul) showed that Covid-19 had not circulated in the high-risk or general populations in 2020 and that the prevention measures put into place to mitigate the circulation of the early variants were successful in keeping the number of Covid-19 cases extremely low and limited to imported cases. When other countries around the world were going through severe transmission, Laos was living almost normally. Then around mid-April of 2021, during the Buddhist New Year festival, several illegal cross-border travelers brought the Delta variant of Covid-19 into Laos. Concomitantly the Delta variant surged through Thailand provoking a mass exodus of Lao migrant workers back into Laos making obligatory 14-day quarantine and Covid testing extremely difficult. Spillover of the Delta variant into the Lao population through community transmission, as was predicted by IPL, occurred given the intense infectivity of the Delta variant compared to the original Wuhan variant or even the Alpha variant that IPL also detected.

Concomitant to the Covid-19 serology study, IPL embarked on a study of the origins of SARS-CoV-2 in wild animals -mainly bats. For years IPL has been engaged in pathogen discovery in arthropods and most recently from bat ectoparasites, such as bat flies. In previous annual reports we have stated that we have found a new Reovirus in Laotian bat flies, as well as an Orthobunyavirus - Wolkberg originally discovered in fruit bats in South Africa.

These bat fly studies allowed us establish collaborations with the Department of Environmental Science at the Lao National University. Hence, when SARS-CoV-2 emerged in China in December 2020 and its closest Sarsbecovirus relative RaTG-13 was isolated from a *Rhinolophus affinis* bat in Yunnan province near the Lao border, we immediately started to search for funds to carry out an investigation to see if we could find SARS-CoV-2-like viruses and other alpha and beta coronaviruses in bats from Laos. With funding from the Institut Pasteur Paris Covid Task Force we continued our collaboration with Prof Marc Eloit of the Pathogen Discovery Laboratory at IP Paris. With our colleagues form the Lao National University several hundreds of bats were captured sampled (saliva and anal swab and blood sample) and released back into nature. Collections were made in four locations (2 locations in Fueng and Meth Districts of Vientiane province – 2 locations in Oudomsay province) Five Sarsbecoviruses close to SARS-CoV-2 were found in anal swabs, some of which were clearly close relatives to SARS-CoV-2 in certain portions of their genomes. An important finding was that the genomes of the Sarsbecoviruses result from mosaicism or recombinations of certain fragments of related Sarsbecoviruses. Our findings are summarized below as indicated in our manuscript summitted to the journal Nature.

"The animal reservoir of SARS-CoV-2 is unknown despite reports of various SARS-CoV-2-related viruses in Asian Rhinolophus bats [1,2,3,4], including the closest virus from R. affinis, RaTG13 [5,6]. Several studies have suggested the involvement of pangolin coronaviruses in SARS-CoV-2 emergence[7–9]. SARS-CoV-2 presents a mosaic genome, to which different progenitors contribute. The spike sequence determines the binding affinity and accessibility of its receptor-binding domain (RBD) to the cellular angiotensin-converting enzyme 2 (ACE2) receptor and is responsible for host range[10–12]. SARS-CoV-2 progenitor bat viruses genetically close to SARS-CoV-2 and able to enter human cells through a human ACE2 pathway have not yet been identified, though they would be key in understanding the origin of the epidemics. Here we show that such viruses indeed circulate in cave bats living in the limestone karstic terrain in North Laos, within the Indochinese peninsula. We found that the RBDs of these viruses differ from that of SARS-CoV-2 by only one or two residues, bind as efficiently to the hACE2 protein as the SARS-CoV-2 Wuhan strain isolated in early human cases, and mediate hACE2-dependent entry into human cells, which is inhibited by antibodies neutralizing SARS-CoV-2. None of these bat viruses harbors a furin cleavage site in the spike. Our findings therefore indicate that bat-borne SARS-CoV-2-like viruses potentially infectious for humans circulate in Rhinolophus spp. in the Indochinese peninsula." Nature mansucript under review.

In addition to our research efforts on the origins of SARS-CoV-2 research in 2021, IPL has been highly implicated in the public health activities in and around Covid-19, as a second line laboratory for SARS-CoV-2 RT-PCR testing (and genomic sequencing of SARS-CoV-2 positive samples. IPL is the only laboratory in the Lao PDR with the capacity to conduct genomic sequencing of the SARS-CoV-2 viruses and hence is the only lab that can identify variants of public health interest and concern by detecting mutations in the spike protein gene.

As I write these lines there are over 50,000 confirmed cases of Covid-19 throughout the country with the epicenter of the epidemic in Vientiane Capital. Nearly one hundred deaths have been registered most of fatalities had comorbidities such as old age, hypertension and/or diabetes. Approximately 50% of the Lao population has been fully vaccinated with the hope to achieve 70% by the end of 2021. Booster vaccination is also to start among healthcare workers in January 2022. The Lao government has not yet indicated when the country will reopen as Thailand and Vietnam are now opening.

It is interesting to note that Dr. Ponmek Dalaloy in 2004 had the foresight to establish Institut Pasteur du Laos as a high a high technology institute capable of training young Lao scientists to be able to detect and mitigate deadly emerging viruses in the Lao PDR. IPL's research this year exemplifies that after 10 years of activity IPL has truly fulfilled its mandate!

Scientific Activities 2021-2022

Arbovirus and Emerging viral diseases Laboratory

Head of Laboratory: Dr. Vincent LACOSTE, PhD

Scientist
Dr. Somphavanh SOMLOR, MD
Dr. Elodie CALVEZ, PhD

Junior Scientists
Dr. Thonglakhone XAYBOUNSOU, MD

Research Engineer
Mrs. Somsanith CHONEPHETSARATH

Technicians
Phaithong BOUNMANY
Souksakhone VIENGPHOUTHONG
Sitsana KEOSENHOM, Lab & Quality Agent

Trainees
Kedkeo INTAVONG
Longthor VACHOUAXIONG
Tomma KEOVIENGSAIY

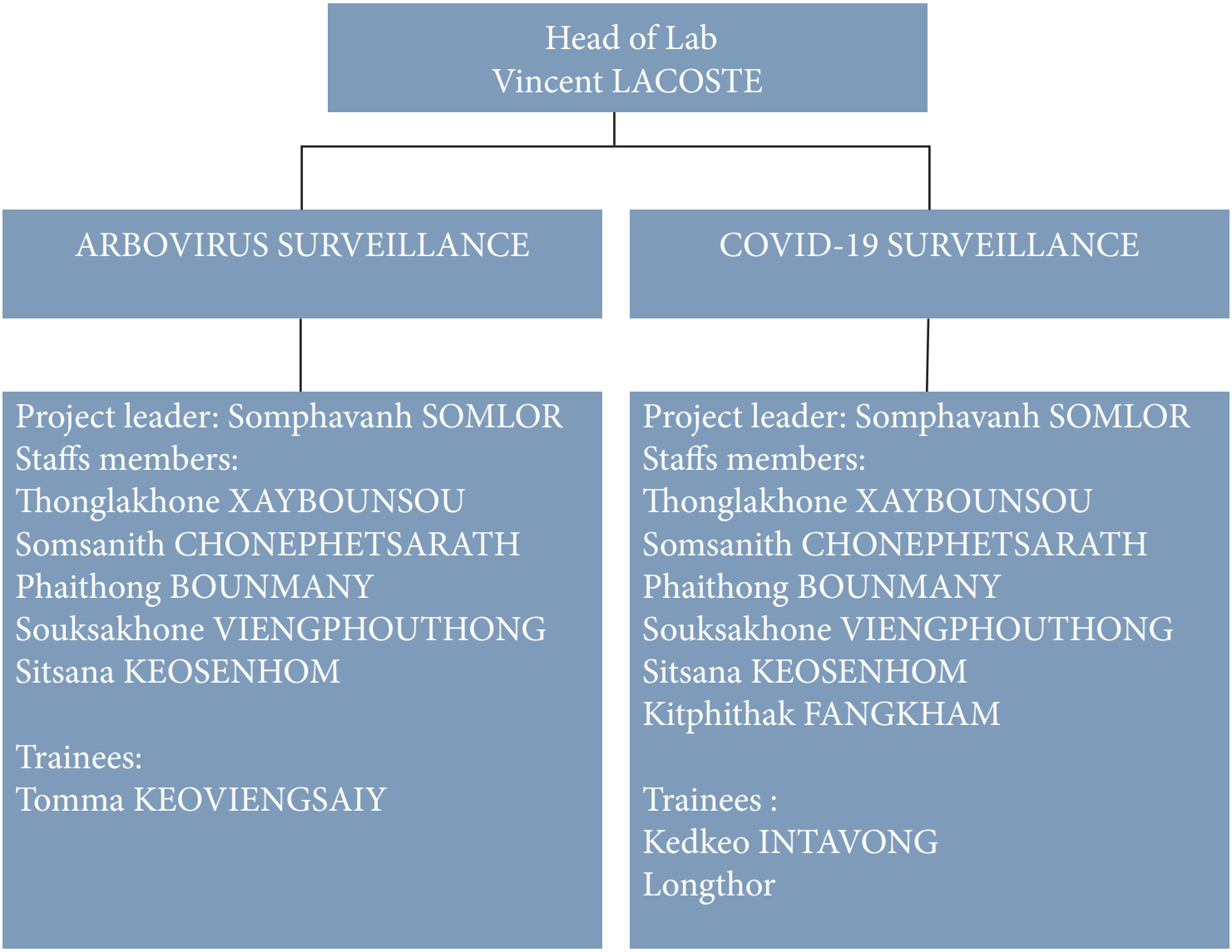
Projects

- Arbovirus Surveillance in Lao PDR
- LHSS COVID-19 activities

Executive summary

Since 2012, the Arbovirus & Emerging Viral Diseases (A&EVD) laboratory has combined field studies and public health activities on vector-borne viral diseases. The A&EVD team is also involved in training and educational activities to help technicians, students and scientists acquire theoretical and technical skills in virology. These activities are a main mission of the Institut Pasteur du Laos. They enable the laboratory to improve knowledge about the viral diseases studied and to become an important partner for national health authorities by providing recommendations for arbovirus diagnosis, control and prevention, as well as training to strengthen the health system.

Since March 2020, the A&EVD team has been requested by Lao MOH to participate in the capacity of Lao PDR to diagnose the COVID-19. Since then, the A&EVD team has been a frontline laboratory for SARS-CoV-2 diagnosis. In addition, the A&EVD team has developed the sequencing technique and is the only laboratory to investigate COVID-19 variants in Lao PDR.



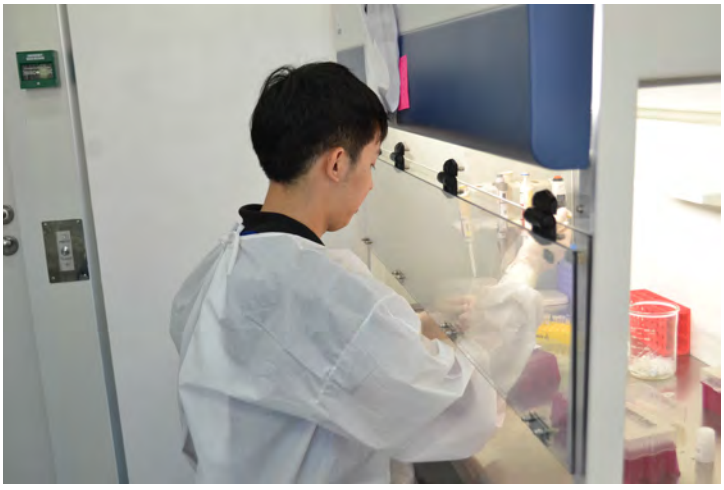
ສະຫຼຸບການປະຕິບັດວຽກງານ

ຕັ້ງແຕ່ປີ2012ເປັນຕົ້ນມາ,ຫ້ອງວິເຄາະArbovirus&Emerging Viral Diseases (A&EVD) ໄດ້ປະສົມປະສານການສຶກສາ ທັງພາກສະໜາມ ແລະ ວຽກງານທາງດ້ານສາທາລະນະສຸກສາດ ກ່ຽວກັບບັນດາເຊື້ອ

ພະຍາດຈຸລະໂລກທີ່ມີແມງໄມ້ເປັນພາຫະນຳເຊື້ອ. ນອກຈາກນັ້ນ, ທີມງານຫ້ອງວິເຄາະ A&EVD ຍັງມີສ່ວນຮ່ວມໃນການ ເຝິກອົບຮົມທາງດ້ານທິດສະດີ ແລະ ທາງດ້ານ ເຕັກນິກຕ່າງໆກ່ຽວກັບ ຈຸລະໂລກວິທະຍາໃຫ້ແກ່ ບັນດາແພດວິເຄາະ, ນັກສຶກສາ ແລະ ນັກວິທະຍາສາດ, ເຊິ່ງວຽກງານ ເຫຼົ່ານີ້ ແມ່ນ ໜຶ່ງໃນພາລະບົດບາດ ຂອງສະຖາບັນປັດສະເຕີລາວ. ຜູ້ທີ່ຜ່ານການເຝິກອົບຮົມເຫຼົ່ານີ້ , ແມ່ນມີຄວາມ ເຂົ້າໃຈຫຼາຍຂຶ້ນກ່ຽວກັບບັນດາພະຍາດຈຸລະໂລກ ຕ່າງໆ ແລະ ພວກເຂົາໄດ້ກາຍເປັນບຸກຄົນສຳຄັນໃນການໃຫ້ຄຳແນະ ນຳ ໃນການບ່ຽງມະຕິ, ການຄວບຄຸມ, ການປ້ອງກັນ ກ່ຽວກັບບັນດາ ເຊື້ອພະຍາດຈຸລະໂລກທີ່ມີແມງໄມ້ເປັນພາຫະນຳເຊື້ອ ແລະ ຕະຫຼອດ ຮອດການເຝິກອົບຮົມເພື່ອເສີມສ້າງຄວາມເຂັ້ມແຂງໃຫ້ແກ່ ລະບົບ ສາທາລະນະສຸກ.

ຍ້ອນເຫດຜົນນີ້ ນັບແຕ່ເດືອນມີນາ 2020 ເປັນຕົ້ນມາ, ທີມງານ A&EVD ໄດ້ຮັບມອບໝາຍຈາກກະຊວງສາທາ ລະນະສຸກໃຫ້ມີສ່ວນຮ່ວມໃນການຕ້ານເຊື້ອ COVID-19 ທີ່ມີການລະບາດໃນທົ່ວໂລກ. ນັບແຕ່ນັ້ນເປັນຕົ້ນມາ,ທີມງານ A&EVD ໄດ້ເປັນໜຶ່ງ ໃນຫ້ອງວິເຄາະໃນການບ່ຽງມະຕິ ພະຍາດ SARS-CoV-2 ໃຫ້ແກ່ປະເທດ. ນອກຈາກນີ້ທີມງານ A&EVD

Arbovirus surveillance in Lao PDR



Project Coordinator: Dr. Somphavanh Somlor

Staff members: Dr Thonglakhone Xaybounsou, Phaithong Bounmany, Sitsana Keosenhom, Souksakhone Viengphouthong, Somsanith Chonephetsarath

Funding:
supported by the Agence Française du Développement (AFD) through the Ecomore2 project and by the Defense Threat Reduction Agency through arboshield.

Background

Since 2012, the A&EVD laboratory has been providing the Lao Ministry of Health with information on circulation of arboviruses within Lao PDR, particularly on dengue and chikungunya transmission. Since 2012, the A&EVD laboratory has established a surveillance network for arboviruses in Vientiane capital.

In 2015, at the request of the Ministry of Health, surveillance was extended to the 2 southern provinces of Laos (Saravan and Attapeu). Since 2018, it has been extended to 7 more provinces (Champassack, Savannakhet, Luang-Prabang, Xiengkhuang, Phongsaly, Oudomxay and Vientiane province) as a result of the Arboshield project implemented together with the Lao Army Health Service. In 2020, 8248 dengue fever cases were reported nationwide, including 13 fatal cases. Of these, 4 were reported in Vientiane Capital. As of the end of October 2021, 1870 suspected cases have been investigated at IPL. 79.3% were from our hospitals network in Vientiane Capital. Of these, 56.3% were confirmed as Dengue fever (Figure 2). In 2020, all three serotypes (DENV-1, DENV-2 and DENV-4) were circulating in Lao PDR, with DENV-2 predominating. Since the end of 2020, the number of DENV1 increased and in 2021, DENV1 is the main serotyping circulating.

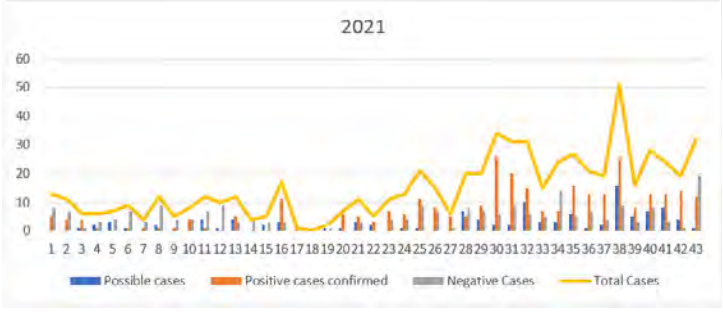
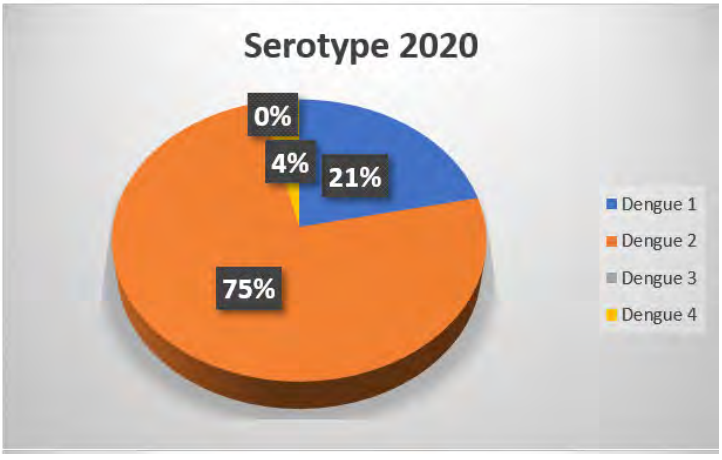


Figure1. Dengue surveillance data of dengue virus 2021



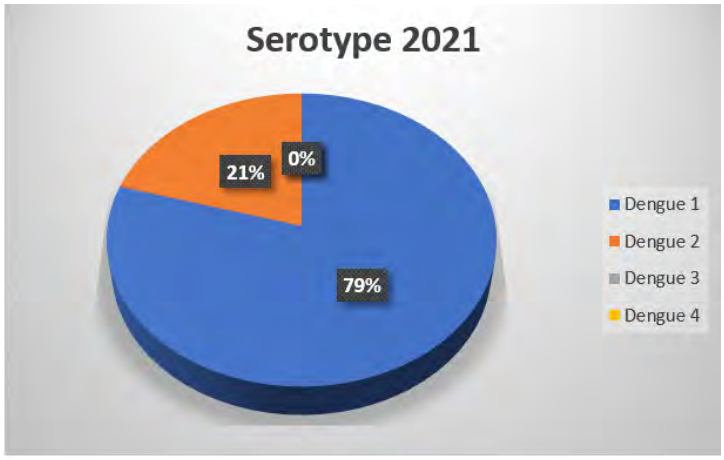


Figure 2: Dengue serotype distribution in 2020 and 2021

Differential diagnosis

The algorithm used to investigate dengue suspected cases includes a differential diagnostic approach. Chikungunya has been identified as having high impact in the Lao general population, especially in the southern provinces of Laos. As the diagnostic capacity is still limited and some doctors are unaware of this virus, passive surveillance of this virus is the most important key for early detection of introduction and/or circulation in the country.

In July 2021, many patients with unknown fever and arthralgia were detected in Attapeu province and samples were sent to IPL for diagnosis. By the end of October 2021, 27.9% (17/69) were diagnosed as infected with Chikungunya virus. The A&EVD continued to characterize the viral isolates at the genetic level.

As in July 2020, the chikungunya virus was also found in Bolikhamxay and Savannakhet provinces. These results indicate that the virus has spread to several provinces in southern Laos and that it is the most important virus for the differential diagnosis of dengue fever in Lao PDR.

LHSS COVID-19 Project

Project coordinator: Dr. Vincent LACOSTE and Dr. Somphavanh SOMLOR

Staff members: Dr Thonglakhone XAYBOUNSOU, Phaithong BOUNMANY, Sitsana KEOSENHOM, Souksakhone VIENGPHOUTHONG, Somsanith CHONEPHETSARATH, Kitphithak FANGKHAM

Trainees: Kedkeo INTAVONG and Longthor

Funding : LHSS under USAID, Luxembourg and French Embassy.

Background

Soon after the emergence of the Severe Acute Respiratory Syndrome - Coronavirus 2 (SARS-CoV2) virus in the city of Wuhan, China at the end of 2019, which led to the COVID-19 (Coronavirus Disease 19) pandemic, the Institut Pasteur du Laos (IPL) was requested by the Ministry of Health (MOH) to participate in the national response to the pandemic. Since then, IPL has been one of the frontline laboratories for the diagnosis of SARS-CoV2 in the country.

To strengthen diagnostic and training capacities in Laos as part of the COVID-19 response, LHSS initiated the Lao Pasteur COVID-19 activity, which started on October 1, 2020. LHSS engaged IPL as a subcontractor to perform the key tasks in this activity. The activity had three main objectives:

- Perform the molecular diagnosis of SARS-CoV2 using RT-PCR testing
- Generate SARS-CoV2 sequences to identify variants
- Train laboratory technicians on COVID19 diagnosis

RT-PCR TESTING OF SARS-COV2

Objectives and overview

The main objective of the diagnostic testing was for LHSS to improve the government's diagnostic capacity for detection of SARS-CoV2. LHSS, through its subcontractor IPL, aimed to increase the number of samples tested by RT-PCR within the country through systematic testing of a portion of travellers entering the country, suspected local cases and close contacts, with a target of approximately 13,250 tests by the end of September. Samples were mostly nasopharyngeal, though at times naso + oropharyngeal.

Results

Initially, IPL screened samples obtained from World Food Program flights. However, these flights were infrequent – a maximum of one per week - and IPL did not obtain sufficient samples to meet expected activity targets. Therefore, to increase the volume of activity, IPL reached an agreement with the director of the National Center for Laboratory and Epidemiology (NCLE) to also provide testing for passengers arriving at the Wattai International Airport, Vientiane, on two flights per week from Kunming, China. This allowed IPL to quickly achieve and exceed the goals set forth in the work plan of the project (Figure 3). In fact, this enabled IPL to make up for the delay accumulated (in terms of number of tests) during the first two months of the project and to reach (and subsequently exceed) the expected numbers during week 10 (Figure 4).

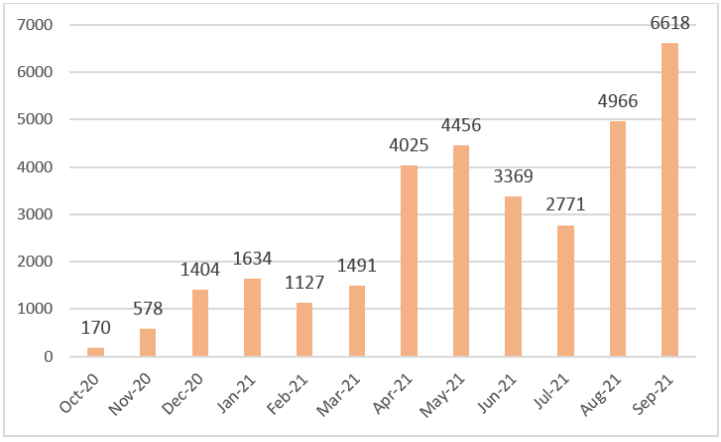


Figure 3. Monthly SARS-CoV2 RT-PCR diagnostic activity.

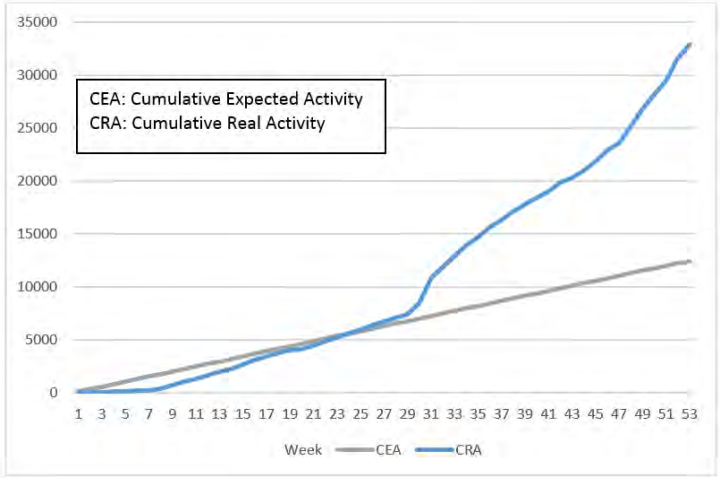


Figure 4. Evolution of the diagnostic activity (RTqPCR SARS-CoV2) per week (week 1 corresponding to the start of the project, i.e. the week of October 1). In blue is the expected volume of activity, in blue the actual volume of activity.

In early April 2021, more than a year after the start of the pandemic, IPL had identified only one positive case (from April 2020). As of September 2021, IPL had identified a total of 340 positive cases out of the 32,458 tests carried out since the start of the LHSS activity, which began in October 1, 2020.

RT-PCR as part of EPI-Surveillance

All RT-PCR results were shared with NCLE and DCDC on a daily basis; Excel files with test results were also shared each quarter with USAID. NCLE consolidated testing data from all laboratories and reported aggregate data to the MOH. This active surveillance enabled the MOH to track the epidemiologic situation, providing information to track close contacts to limit the spread of the virus. In addition, this information was used for public health decision making.

Key challenges and lessons learned

With the entry of the virus into the country in mid-April 2021 at the time of Pi Mai Lao, followed by the entry of the Delta variant in mid-June, our diagnostic activity increased dramatically during the latter half of the activity - with more than 3,900 tests on average per month since April. By the end of August, IPL had carried out a total of 32,468 diagnostic tests, thus doubling the number of tests expected by the end of the activity. To deal with this huge increase in activity, IPL had to adapt to handle such a large volume of samples in a given day. In addition, to reinforce the team, IPL engaged other institute staff in COVID-19 diagnostics. This significant increase in activity had consequences on the management of reagents and consumables and therefore on the dedicated budget, requiring IPL to obtain supplemental funding for reagents and consumables. The large volume also had consequences for the supply and delivery time of these reagents and consumables as stocks were running out around the world. Though we were able to obtain the needed products in time for testing, the large volume necessitated more frequent and larger volume orders.

Covid-19 sequencing

Objectives and overview

The main objective of the genomic sequencing task was to perform the sequencing of positive cases to follow the evolution of the SARS-CoV2 strains and identify the entry and spread of variants.

IPL signed a material transfer agreement with NCLE to obtain SARS-CoV2 RT-PCR positive samples from them, and to perform genomic sequencing on specific portions of the genome. IPL also conducted sequencing on some positive samples identified at IPL.

Results

IPL received the first samples for sequencing from NCLE in March 2021. Since then, IPL attempted to sequence a total of 237 samples, and obtained sequences for a total of 196 samples. For the remaining 41 samples, the laboratory was unable to obtain any amplification product, due to a too low viral load (Ct> 30). IPL investigated alternative approaches for sequencing and procured equipment to conduct next generation sequencing (which works even with lower viral loads), but did not obtain all equipment in time for this activity.

Regarding the 196 sequences obtained, four cases were infected with the "wild" strain of Wuhan in March, April and May at the origin of the pandemic, 81 were infected with the Alpha variant (English variant), 110 by the Delta variant (Indian variant), and finally one by a variant of interest, VOI B.1.621, initially identified in Colombia (Table 1). The Alpha variant is the one that entered at the turn of the Lao New Year in April 2021, which then spread throughout the country before being replaced by the Delta variant detected in June and responsible for the huge epidemic wave observed since then.

Table 1. Sequencing activities and results.

Month	Samples	Results
March	5	2 wild-type «Wuhan » 3 non-sequenced
April	14	1 wild-type «Wuhan » 11 Alpha variants 2 non-sequenced
May	43	1 wild-type «Wuhan » 22 Alpha variants 20 non-sequenced
June	30	25 Alpha variants 3 Delta variants 2 non-sequenced
July	60	16 Alpha variants 37 Delta variants 1 VOI B.1.621 6 non-sequenced
August	43	37 Delta variants 3 Alpha variants 3 non-sequenced
Sep	42	4 Alpha variants 33 Delta variants 5 non-sequenced
Total	237	4 wild-type «Wuhan » 81 Alpha variants 110 Delta variants 1 VOI B.1.621 41 non-sequenced

Genomic sequencing as part of epi-surveillance

All sequencing results were shared with the NCLE, WHO and all partner laboratories immediately, by email first and then via a database accessible virtually by various partners. On August 20, IPL submitted "prototypic" sequences (18 sequences) representative of the various variants identified in Laos on the GISAID database (https://www.gisaid.org/). Sequencing results allowed identification of the circulating strains present in Laos. These results have important implications for the MOH, to support their efforts to track the spread and to reduce the COVID-19 burden. This is in part because different variants present different potential for spreading, and can inform establishment of different mitigation strategies depending on the situation (opening of quarantine centers, closing schools, restaurant, borders, restrictions of movements between provinces, districts, etc).

Thus, the identification of variants was invaluable to the COVID-19 response in Laos.

Key challenges and lessons learned

Lao PDR has a very limited capacity for genomic sequencing. Indeed, only the Institut Pasteur du Laos is able to do this type of analysis, with only a few people trained to analyse and interpret the sequence results. The sequencing results generated at IPL provided important data to the MOH which was used to decide which strategies to apply to limit the spread of COVID-19 in the country.

The sequencing capabilities acquired during this activity by certain technicians and scientists at IPL have strengthened their technical capacity and can be useful in the analysis of other pathogens. This could be useful in the future to rapidly respond to new emerging or re-emerging diseases.

Lastly, to circumvent the problem of sequencing samples for which the viral load was low (Ct> 30), IPL pursued an alternative - the MinION technology from the Oxford Nanopore Technologies company, which makes it possible to generate sequences even from low viral load samples. Unfortunately, the reagents and consumables were received only in late July and therefore were not used for the sequencing conducted for this activity.

Training objective & approach

The overarching objective of the activity and of the training was for LHSS to enhance the government's diagnostic capacity for detection of SARS-CoV2. LHSS, through the subcontractor IPL, aimed to increase the number of samples tested by RT-PCR by training local laboratory technicians in the necessary diagnostic techniques. The goal of the training performed by IPL was to train four laboratory technicians in bio-safety, bio-security, extraction, reagent preparation, RT-PCR techniques, and all steps of COVID-19 diagnostics (which are also applicable for the other viral diseases).

The training was initially designed to last for six months, with some initial theoretical training followed by hands-on practical training. Due to challenges faced in recruiting laboratory technicians, the initial two trainees stayed on for the entire year, and therefore the training ultimately involved an initial 3 months of theoretical training followed by 9 months of practical training.

Selection of trainees

In selecting the initial two candidates for training, IPL consulted with the faculty of Medical Technology at the University of Health Sciences to consider expectations for the training and possible candidates. A list of interested students was provided to IPL by a professor at the University. IPL clearly explained to the candidates the criteria for selection, which would involve a fair and non-biased assessment to identify the best candidates. IPL administered a brief oral interview and written test to all interested candidates; the top two performers (in terms of test scores as well as suitability for the training) were selected for training.

End of training skills assessment

When the formal training concluded, the two trainees were assessed on their theoretical understanding with a post-test. The two trainees obtained post-test scores of 54/60 and 58/60 (see Annex II). Both trainees followed the training closely, and participated actively in teamwork. At the end of the training they were able to perform RT-PCR independently, work in BSL3 and even analyse the sequence results. Their skills were assessed in an end-of-training skills assessment, in which both trainees demonstrated their mastery of the necessary skills. When the need arose for training laboratory technicians in the military, the two IPL laboratory technicians assisted in training these technicians on RT-PCR, therefore further strengthening local capacity.

Challenges and lessons learned

With the COVID-19 lockdown and Universities shutting their doors, IPL was unable to identify two additional qualified candidates to recruit for the second round of training.

Therefore, instead of four, IPL was able to train only two trainees. However, these two trainees provided much-needed support to IPL in conducting a large number of tests, especially when cases skyrocketed in April 2021 and IPL was faced with conducting dozens of tests per day. Therefore, the trainees were able to support IPL in conducting additional tests to support the government's diagnostic capacity. The training was also an ideal opportunity for the trainees to apply their new skills on the job as they applied key concepts and also learned how to transfer what they learned to others.

Trainees were selected in part based on their interest and career goals, to ensure that they were suitable for the training and intended to continue their work on the implementation of RT-PCR to assist the country in the response to COVID-19 and other viral outbreaks in the future. This training was very effective in increasing the capacity of human resources as participants improved their leadership skills and confidence in working with dangerous viral diseases, as well as their ability to explain and share their knowledge with others and work well with the team and medical staff in the hospital network. In fact, both trainees have also provided support to train military laboratory technicians in performing RT-PCR.

Recommendations

Based on experience from this activity, LHSS and IPL share the following recommendations for the GOL to consider:

- Improve coordination between the reference laboratories and the provincial laboratories for a full standardization of the pre-analytic steps: collection of samples, storage of samples, packing and transportation of biological hazardous samples
- Train/sensitize medical staff on different aspects of sample collection, transport and analysis, so that they are aware of the procedures and can provide necessary support
- Apply standard operating procedures in all partner labs, to have comparative results
- Coordinate between the reference laboratories and the provincial laboratories in the selection of samples for the performance of genomic sequencing, so that the sampling is representative of diverse geographical areas
- Share feedback on the quality of samples with partner laboratories who are sharing samples, so that they can address any issues with sample quality accordingly
- Train additional laboratory technicians in the provinces as well as students at the university level in conducting RT-PCR
- Provide practical training on RT-PCR to all laboratory technology students
- Continue to build the capacity of other laboratory technicians for current and future health emergencies – by developing their capacity in molecular biology techniques (from extraction of nucleic acids to amplification for detection and sequence analysis)

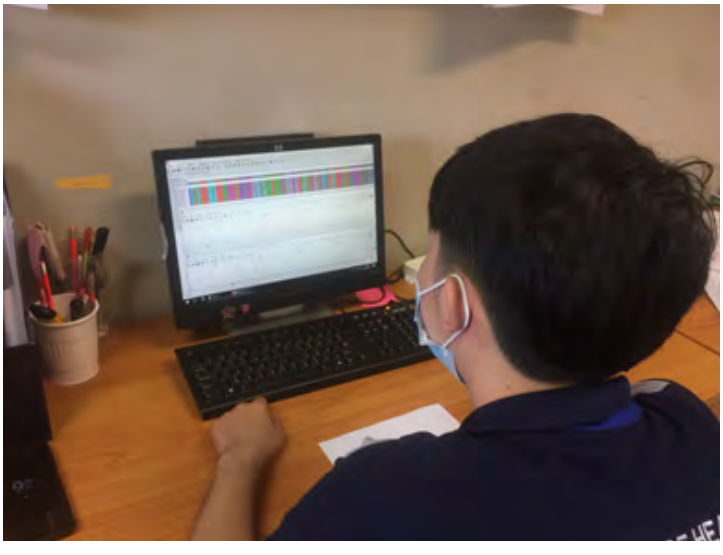
LHSS and IPL also suggest some longer-term strategies to strengthen future responses to SARS-CoV2 and also other pathogens:

- In the molecular biology curriculum at the University, establish a course with theoretical and practical sessions to train a new generation of technicians and scientists in PCR, sequence analysis and phylogeny -- from the sample collection to the interpretation of results.
- Establish a central laboratory where samples can be sent by partners for sequences to be generated. This lab should have different types of equipment for Sanger and next generation sequencing, for public health activities (COVID-19 and other pathogens), where every suspect sample can be tested and sequenced.

Conclusion

The implementation of this activity has enabled LHSS and IPL to make a significant contribution to the national effort to combat the COVID-19 pandemic by performing RT-PCR tests and to COVID-19 response strategies by generating viral sequences that allowed for the identification of variants. The project has also helped IPL to introduce new technologies to the country (next generation sequencing), which will be used in future activities.

Train the trainee on sample collection.



The trainee helps the team to analyse sequence results

The trainee passes on the knowledge to the military trainee.

Publication

1. Calvez E, Vetsaphong P, Somlor S, et al. First probable case of congenital Zika syndrome in Lao People's Democratic Republic. Int J Infect Dis. 2021;105:595-597. doi:10.1016/j.ijid.2021.03.019

Medical Entomology & Biology of Disease Vectors Laboratory

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.

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Vaekey VUNGKYLY

Projects

- Tick Map 4 project: Mapping of Vectors and Reservoir Hosts in Lao PDR
- Origins, natural reservoirs and Interspecies transmission of SARS-CoV-2 and other SARS-like CoVs (ONRITS)
- Assessing the risk of insecticide resistance in the tiger mosquito: A predictive approach combining experimental selection and molecular markers (TigeRisk2)

Executive summary

Like 2020, the ongoing Covid-19 pandemic this year (2021) has been greatly affected our research efforts in Medical Entomology and Vector-borne disease laboratory due to many lockdowns and mobilization of the Entomology staff to participate in Covid-19 diagnostics with the virology team.

In 2021, we continued our collaboration with the US Navy Medical Research Center Asia (NMRC-A) in Singapore on Tick Map Four. This program is an extension of previous Tick Map projects that started in 2014.

In order to identify common and emerging vector-borne pathogens in Laos, NAMRU-2 Singapore (SG) has established a study to assess the distribution and infection potential of vectors since 2014. Between 2020 and 2021, we continued to conduct tick and other ectoparasite collections in Oudomxay province in the northern part of Laos. The vectors were morphologically identified and pooled. Then nucleic acids were extracted and screened for pan-phlebovirus and pan-flavivirus respectively by conventional nested RT-PCR at IP-Laos; *Rickettsia* spp. were detected by qPCR at LOMWRU and arboviral pathogens were detecting by Next Generation Sequencing at IP-Paris.

A total of 582 ticks and other ectoparasites were collected during the study. No tick samples were positive by pan-phlebovirus and pan-flavivirus screening. However, results from NGS of two big pools of *Dermacentor* and *Haemaphysalis* ticks found 4 viral families known to infect vertebrates including *Rhabdoviridae* (Yongjia tick virus 2), Reoviridae (Rotavirus), *Flaviviridae* (Jingmen tick virus), and Unclassified *Riboviria* (Bole tick virus 4). 121/192 pools were positive for pan-rickettsia for Rickettsia spp. (17kDa by qPCR). However, none of them was positive for *R. typhi/Coxiella* spp. Our partner, LOMWRU, will continue to try to identify the Rickettsia species. Furthermore, another partner at IP-Paris will continue to perform deep sequencing on the samples collected in the course of this study.

From 2021-2022, we will continue our tick study/surveillance in Laos for both ticks and associated pathogens. Future research studies are needed to determine the pathogenicity of these viruses to livestock and humans in Laos.

With the advent of Covid-19 we set out to investigate the origins of SARS-CoV-2 and SARS-CoV-2-like viruses in karstic areas of Vientiane and Oudomxay provinces.

Since the discovery of severe acute respiratory syndrome-associated coronavirus (SARS-CoV) in Chinese horseshoe bats (*Rhinolophus sinicus*) in Hong Kong in 2005, a plethora of molecular epidemiological studies have been carried out in bats and animal species around the world to detect other animal reservoirs of this highly pathogenic CoV (Wong et al., 2019). Interestingly, of the >30 CoVs found in bats, a majority have been found in various species of horseshoe bats (Rhinolophus spp.) from China. A few others have been found in other bat genera such as, *Aselliscus, Chaerephon,* and *Hipposideros.*

Given the fact that two SARS Coronaviruses have emerged and cause important human loss of life in the past seventeen years (SARS and COVID-19), the scientific community postulates that other SARS Coronaviruses are presently circulating in Rhinolophus and other bat populations and could spill-over into human populations like SARS and COVID-19; hence, the surveillance and identification of these novel putative coronaviruses is necessary to mitigate a new future pandemic.

A better scientific understanding of the origin, natural history and dispersal of SARS-CoV-2 and SARS-like-CoVs in their natural environment, as well as the mechanisms leading to their interactions and interspecies jumping: Bat-Bat; Bat-Animal and Bat-Human are paramount to mitigate future spillover events that result in devastating epidemics like the one we are witnessing today with SARS-CoV-2 in China and around the world.

In 2020 and 2021 a total of 645 bats were captured from 4 collection sites and 608 saliva, 539 anal/feces, 157 urine swabs and 246 blood samples were collected. In addition, ectoparasites were collected from 74 bats.

Subsequent Pancoronavirus screening, a total of 539 extracted products of bat anal swabs/faces were screened at IP-Laos by targeting the RNA-dependent RNA polymerase gene. . A total of 24 individuals from 10 bat species were positive. BLAST analysis of obtained nucleotide sequences from Sanger sequencing identified Alphacoronavirus: *Decacovirus*, *Pedacovirus*, and *Rhinacovirus* subgenera; and Betacoronavirus: *Nobecovirus* and *Sarbecovirus* subgenera. Sequences of the *Sarbecovirus* subgenus were all identified from Rhinolophus individuals belonging to three different species i.e., *R. malayanus*, *R. marshalli*, and *R. pusillus.*

For rodents, a total of 115 extracted products of bat anal swabs/faces were screened at IP-Laos by targeting the RNA-dependent RNA polymerase gene using a pan-coronavirus RT nested PCR approach. A total of 6 individuals from 4 rodent species were positive. BLAST analysis of obtained sequences from Sanger sequencing identified Betacoronavirus sequences of the Embecovirus subgenus. Six sequences of the Embecovirus subgenus were identified from 2 *Berylmys berdmorei*, 2 *Mus* spp., 1 *Niviventer* cf. *fulvescens*, and 1 *Rattus* sp.

Our study showed that 25 coronaviruses were detected from 10 bat species (out of 539 samples tested), of which 3/5 sarbecoviruses with full-length sequences were closely related to SARS-CoV-2 > 96%. Preliminary results showed that Laotian bat-sarbecovirus RBD was able to bind to human ACE2 through modeling and binding experiments, and Pseudoviruses expressing BANAL-20-236 spike could enter into cells expressing human ACE2.

Further experiments such as investigating whether Pseudoviruses expressing BANAL-20-236 spike can directly entry into human cells, and pathogenicity of these viruses are warranted. Other questions will need also to be addressed such as whether the bat-exposed local populations have been infected by one of these viruses in Laos, whether such infections were associated with any symptoms, and whether exposure can confer immunity against subsequent SARS-CoV-2 infection.

Our preliminary results showed that there exist other bat sarbecoviruses that seem to have the same potential for infecting humans in Laos. People with close contact with bats seem to be at risk of being exposed, so they will need some level of personal protection if they have to contact with the bats.

Finally, we continued to explore the field of insecticide resistance, which poses a conundrum between environmental safety and reduction of vectors and hence the diseases they transmit.

Targeting adult mosquitoes with pyrethroid insecticides (PYR) such as deltamethrin constitutes the first line of defense to limit vector-borne disease outbreaks. However, mosquitoes have evolved effective resistance mechanisms to resists insecticides. PYR resistance is widespread in *Ae. aegypti* and reduces the efficiency of mosquito control in several tropical regions including French overseas territories. Recently resistance has also been reported in populations of the tiger mosquito *Ae. albopictus* located in various continents including southern Europe and La Réunion island. Considering its expanding distribution and its growing importance in arbovirus transmission, the rise of PYR resistance in this species represents a major public health concern.

In the context of the lack of new insecticides available for public health, managing insecticide resistance is vital until novel control tools are implemented. However, this requires understanding resistance mechanisms and tracking them efficiently in natural populations. Multiple insecticide resistance markers have been identified in *Ae. aegypti* but barely none are yet available in the tiger mosquito.

In this context, the TigeRisk2 project will break through scientific and technological barriers for characterizing novel DNA markers of PYR resistance in the tiger mosquito Ae. albopictus and track them in natural mosquito populations in order to evaluate the risk of resistance emergence in this species. For achieving this, the consortium will gather 5 complementary teams and combine field mosquito collections with long-term laboratory experiments and state-of-the-art molecular technologies.

ບົດສະຫຼຸບການປະຕິບັດວຽກງານ

ເຊັ່ນດຽວກັນກັບປີ 2020, ເນື່ອງຈາກສະພາບການລະບາດຂອງພະຍາດໂຄວິ-19 ທີ່ກຳລັງລະບາດໃນປີນີ້ (2021) ໄດ້ສົ່ງຜົນກະທົບຢ່າງຫລວງຫລາຍ ຕໍ່ຄວາມພະຍາຍາມດ້ານວຽກງານການຄົ້ນຄ້ວາຂອງຫ້ອງວິເຄາະ ຂອງພວກເຮົາ ຍ້ອນວ່າ ມີການປິດເມືອງຫລາຍຄັ້ງ ແລະ ການລະດົມພະນັກງານຫ້ອງວິເຄາະຂອງພວກເຮົາເຂົ້າຊ່ວຍວຽກງານການປ້ອງມະຕິພະຍາດໂຄວິດ-19 ຮ່ວມກັບທີມງານໄວຣັສ.

ໃນປີ 2021, ພວກເຮົາໄດ້ສືບຕໍ່ຮ່ວມມືກັບ US Navy Medical Research Center Asia ຢູ່ສິງກະໂປ ເພື່ອສືບຕໍ່ການຄົ້ນຄ້ວາກ່ຽວກັບພະຍາດທີ່ເກີດຈາກເຫັບ ໃນໂຄງການ Tick Map 4. ໂຄງການນີ້ແມ່ນການສືບຕໍ່ຂອງໂຄງການສຶກສາກ່ຽວກັບເຫັບ ແລະ ພະຍາດທີ່ເກີດຈາກເຫັບ ທີ່ໄດ້ເລີ່ມມາແຕ່ປີ 2014.

ເພື່ອຊອກຫາເຊື້ອພະຍາດທີ່ມັກພົບ ແລະ ເຊື້ອອື່ນໆທີ່ອາດຈະເກີດຂຶ້ນໃຫມ່ທີ່ມີແມງໄມ້ເປັນພາຫະໃນລາວ, NAMRU-2 Singapore (SG) ໄດ້ຈັດຕັ້ງປະຕິບັດການສຶກສາເພື່ອປະເມີນການແຜ່ກະຈາຍ ແລະ ຄວາມເປັນໄປໄດ້ຂອງການຕິດເຊື້ອຂອງແມງໄມ້ພາຫະ (vector) ຕັ້ງແຕ່ປີ 2014. ໃນລະຫວ່າງປີ 2020 ຫາ 2021, ພວກເຮົາໄດ້ສືບຕໍ່ດຳເນີນການເກັບຕົວຢ່າງເຫັບ ແລະ ແມງໄມ້ກາຝາກພາຍນອກອື່ນໆ (ectoparasite) ອື່ນໆ ໃນແຂວງອຸດົມໄຊ ໃນພາກເໜືອຂອງລາວ. ເຫັບໄດ້ຖືກຈຳແນກໂດຍໃຊ້ຮູບຮ່າງລັກສະນະພາຍນອກແລະລວມເຂົ້າກັນ. ຈາກນັ້ນ, ສານກຳມະພັນ (DANA/RNA) ໄດ້ຖືກສະກັດ ແລະ ນຳໄປກວດຫາເຊື້ອໄວຣັສໃນກຸ່ມຂອງphleboviruses ແລະflaviviruses ໂດຍໃຊ້ເຕັກນິກ RT-PCR ຢູ່ສະຖາບັນປັດສະເຕີລາວ. ສ່ວນເຊື້ອ ໄຂ້ແມງແດງ (Rickettsia spp.) ແມ່ນ ໄດ້ກວດຫາເຊື້ອໂດຍເຕັກນິກ qPCR ຢູ່ LOMWRU ແລະເຊື້ອໄວຣັສທີ່ມີແມງໄມ້ເປັນພາຫະອື່ນໆ ແມ່ນໄດ້ສົ່ງໄປຊອກຫາເຊື້ອດ້ວຍເຕັກນິກ Next Generation Sequencing ຢູ່ສະຖາບັນປັດສະເຕີ ປາຣີ.

ຈາກການເກັບຕົວຢ່າງ ເຫັບ ແລະ ແມງໄມ້ກາຝາກພາຍນອກອື່ນໆ ຈຳນວນທັງຫມົດ 582 ໂຕ ແມ່ນບໍ່ພົບເຊື້ອໄວຣັສ ໃນກຸ່ມ phleboviruses ແລະ flaviviruses ດ້ວຍການກວດເຕັກນິກ RT-PCR. ເຖິງຢ່າງໃດກໍ່ຕາມ, ການກວດຫາເຊື້ອໂດຍການຖອດລະຫັນກຳມະພັນທັງຫມົດ (NGS) ຂອງຕົວຢ່າງທີ່ລວມເຂົ້າກັນ ຂອງເຫັບໃນ ສະກຸນ Dermacentor ແລະ Haemaphysalis ພົບວ່າ ມີເຊື້ອໄວຣັສ 4 ຕະກູນ ທີ່ສາມາດຕິດໃສ່ສັດທີ່ມີກະດູກສັນຫລັງໄດ້ໃນເຫັບຄື: *Rhabdoviridae* (Yongjia tick virus 2), *Reoviridae* (Rotavirus), Flaviviridae (Jingmen tick virus), and Unclassified *Riboviria* (Bole tick virus 4). ຈາກການຊອກຫາເຊື້ອ *Rickettsia* spp. ພົບວ່າ 121/192 ຕົວຢ່າງແມ່ນ ພົບເຊື້ອໃດນຶ່ງ ເຊິ່ງກວດໂດຍເຕັກນິກ qPCR ຂອງ17kDa ຢີນ. ເຖິງຢ່າງໃດກໍ່ຕາມ, ຕົວຢ່າງທີ່ກວດຍັງບໍ່ພົບເຊື້ອທີ່ກໍ່ໃຫ້ເກີດພະຍາດໃນຄົນເຊັ່ນ: *R. typhi/Coxiella* spp. ຄູ່ຮ່ວມງານ LOMWRU ຂອງພວກເຮົາຈະສືບຕໍ່ໃນການຈຳແນກຊະນິດຂອງເຊື້ອດັ່ງກ່າວທີ່ພົບໃນເຫັບ. ເຊັ່ນດຽວກັນນັ້ນ, ຄູ່ຮ່ວມງານຂອງພວກເຮົາຢູ່ ສະຖາບັນປັສເຕີ ປາຣີ ກໍ່ຈະກວດແລະວິເຄາະຫາເຊື້ອໄວຣັສດ້ວຍເຕັກນິກ NGS. ໃນປີ 2021-2022 ທີມງານຂອງພວກເຮົາຍັງຈະໄດ້ຈະສືບຕໍ່ສຶກສາ/ເຝົ້າລະວັງກ່ຽວກັບເຫັບ ແລະ ເຊື້ອທີ່ອາດ ກໍ່ໃຫ້ເກີດພະຍາດຈາກເຫັບ. ນອກນີ້, ການສຶກສາອື່ນໆ ເຊັ່ນ ຄວາມຮຸນແຮງຂອງເຊື້ອໄວຣັສທີ່ພົບນຳເຫັບ ຕໍ່ສຸຂະພາບຄົນ ແລະ ສັດ ແມ່ນມີຄວາມສຳຄັນ ແລະ ຕ້ອງໄດ້ມີການສຶກສາຕື່ມ.

ເນື່ອງຈາກການເຂົ້າມາຂອງພະຍາດໂຄວິດ-19, ພວກເຮົາໄດ້ເລີ່ມສຳຫຼວດຕົ້ນກຳເນີດຂອງພະຍາດໄວຣັສ SARS-CoV-2 ແລະ ເຊື້ອທີ່ຄ້າຍຄື SARS-CoV-2 ໃນພື້ນທີ່ເຂດພູຫີນປູນຂອງແຂວງວຽງຈັນ ແລະ ແຂວງອຸດົມໄຊ.

ເລີ່ມຕັ້ງແຕ່ມີການຄົ້ນພົບ ເຊື້ອໄວຣັສໂຄໂລນາ ທີ່ກ່ຽວຂ້ອງກັບການອັກເສບລະບົບທາງເດີນຫາຍໃຈຮຸນແຮງ (SARS-CoV) ຢູ່ໃນເຈຍມຸງກຸດຈີນ (*Rhinolophus sinicus*) ໃນຮ່ອງກົງ ປີ 2005, ໄດ້ເຮັດໃຫ້ມີການສຶກສາກ່ຽວກັບລະບາດວິທະຍາທາງ ດ້ານໂມເລກຸນຢ່າງຫຼວງຫຼາຍໃນເຈຍ ແລະ ສັດຊະນິດອື່ນໆທົ່ວໂລກເພື່ອຊອກຫາແຫຼ່ງເກັບເຊື້ອຂອງເຊື້ອໄວຣັສໂຄໂລນາ ທີ່ຮຸນ ແຮງດັ່ງກ່າວນີ້ (Wong et al., 2019). ເປັນທີ່ນ່າສົນໃຈຫຼາຍ, ເຊື້ອໄວຣັສໂຄໂລນາຫຼາຍກວ່າ 30 ຊະນິດ ແມ່ນຖືກຄົ້ນພົບໃນເຈຍ, ໂດຍສ່ວນໃຫຍ່ແລ້ວແມ່ນພົບໃນກຸ່ມເຈຍມຸງກຸດ ຕະກຸນ *Rhinolophus* ໃນປະເທດຈີນ. ສ່ວນໜ້ອຍແມ່ນພົບໃນເຈຍຕະກຸນອື່ນເຊັ່ນ: *Aselliscus*, *Chaerephon* ແລະ *Hipposideros*.

ເປັນທີ່ຮັບຮູ້ກັນດີແລ້ວວ່າ ເຊື້ອໄວຣັສໂຄໂລນາ ໄດ້ມີການເກີດຂຶ້ນໃຫມ່ ແລະ ເຮັດໃຫ້ເກີດມີການສູນເສຍຊີວິດຂອງມະນຸດ ໃນໄລຍະ 17 ປີທີ່ຜ່ານມາ (SARS and COVID-19), ບັນດານັກວິທະຍາສາດ ຈຶ່ງໄດ້ຕັ້ງສົມມຸດຖານວ່າ ເຊື້ອພະຍາດໂຄໂຣນາອາດກຳລັງຫມູນວຽນຢູ່ໃນກຸ່ມປະຊາກອນຂອງເຈຍຕະກຸນ *Rhinolophus* ແລະ ກຸ່ມເຈຍຊະນິດອື່ນໆ ແລະ ອາດສາມາດແຜ່ລະບາດໄປສູ່ປະຊາກອນມະນຸດໄດ້ຄືກັນກັບ SARS and COVID-19, ດັ່ງນັ້ນ ການເຝົ້າລະວັງ ແລະ ການຈຳແນກເຊື້ອພະຍາດໂຄໂລນາຊະນິດໃໝ່ທີ່ອາດກໍ່ໃຫ້ເກີດພະຍາດ ຈຶ່ງມີຄວາມສຳຄັນ ແລະ ຈຳເປັນທີ່ສຸດ ເພື່ອຫຼຸດຜ່ອນການແຜ່ລະບາດຂອງເຊື້ອພະຍາດດັ່ງກ່າວໃນອະນາຄົດ.

ເພື່ອໃຫ້ມີຄວາມເຂົ້າໃຈ ທາງດ້ານວິທະຍາສາດ ທີ່ດີຂຶ້ນກ່ຽວກັບ ແຫຼ່ງທີ່ມາ, ການໝູນວຽນຂອງເຊື້ອໃນທຳມະຊາດ ແລະ ການແຈກຢາຍຂອງໄວຣັສ SARS-CoV-2 ແລະ SARS-like-CoVs ໃນສະພາບແວດລ້ອມທາງທຳມະຊາດ ຕະຫຼອດຮອດກົນໄກການນຳໄປສູ່ການປະຕິສຳພັນ ແລະ ການພັດທະນາຂ້າມສາຍພັນ ຈາກເຈຍສູ່ເຈຍ, ຈາກເຈຍສູ່ສັດຊະນິດອື່ນໆ ແລະ ຈາກເຈຍສູ່ຄົນ ແມ່ນມີຄວາມສຳຄັນທີ່ສຸດ ທີ່ຈະຊ່ວຍຫຼຸດຜ່ອນການແຜ່ກະຈາຍຂອງ ເຊື້ອເຂົ້າມາຫາຄົນໃນອະນາຄົດ ເຊິ່ງສິ່ງຜົນໃຫ້ເກີດການລະບາດທີ່ ຮ້າຍແຮງ ຄືກັບສິ່ງທີ່ເກີດຂຶ້ນໃນປັດຈຸບັນນີ້ຂອງພະຍາດ SARS-CoV-2 ໃນປະເທດຈີນ ແລະ ທົ່ວໂລກ.

ໃນປີ 2020 ແລະ 2021 ໄດ້ມີການຈັບເຈຍທັງໝົດ 645 ໂຕ ຈາກ 4 ສະຖານທີ່ ເພື່ອເກັບຕົວຢ່າງນ້ຳລາຍ 608 ຕົວຢ່າງ, ຕົວຢ່າງຈາກຮູທະອາກ ຫຼື ອາຈົມ 539 ຕົວຢ່າງ, ຢ່ຽວ 157 ຕົວຢ່າງ ແລະ ເລືອດ 246 ຕົວຢ່າງ. ນອກຈາກນີ້ ຍັງໄດ້ເກັບຕົວຢ່າງແມງໄມ້ ກາຝາກພາຍນອກຈາກເຈຍ 74 ໂຕ. ຕົວຢ່າງດັ່ງກ່າວແມ່ນຖືກນຳມາ ຊອກຫາເຊື້ອໃນຄອບຄົວໂຄໂລນາໄວຣັສ (Pancoronavirus), ຕົວຢ່າງຈາກຮູທະອາກ ຫຼື ອາຈົມ ທັງໝົດ 539 ຕົວຢ່າງໄດ້ຖືກສະກັດເອົາສານກຳມະພັນ ແລະ ກວດຊອກຫາເຊື້ອຢູ່ທີ່ ສະຖາບັນ ປັດສະເຕີ ລາວ ໂດຍນຳໃຊ້ເຕັກນິກ RT-PCR ຂອງຢີນ RNA-dependent RNA polymerase gene. ມີທັງໝົດ 24 ຕົວຢ່າງ ຈາກເຈຍ 10 ຊະນິດ ແມ່ນພົບເຊື້ອ. ຈາກການວິເຄາະລຳດັບຂອງສາຍ ກຳມະພັນທີ່ໄດ້ມາຈາກຕົວຢ່າງທີ່ພົບເຊື້ອ ໂດຍການນຳໃຊ້ເຕັກນິກ Sanger ພົບວ່າ ມີໄວຣັສໂຄໂລນາຕະກູນ Alphacoronavirus ເຊິ່ງປະກອບມີຕະກູນຍ່ອຍຄື: *Decacovirus*, *Pedacovirus*, ແລະ *Rhinacovirus* ແລະ ຕະກູນ *Betacoronavirus* ມີຕະກູນຍ່ອຍຄື: *Nobecovirus* ແລະ *Sarbecovirus*. ໂດຍຕະກູນຍ່ອຍຂອງເຊື້ອ *Sarbecovirus* ທັງໝົດນີ້ແມ່ນໄດ້ມາຈາກເຈຍຕະກູນ *Rhinolophus* ເຊິ່ງມີ 3 ຊະນິດຄື: *R. malayanus*, *R. marshalli* ແລະ *R. pusillus*.

ສຳລັບກຸ່ມສັດເລັ້ມແຫ້ນໄດ້ມີການກວດວິເຄາະທັງໝົດ 115 ຕົວຢ່າງທີ່ເກັບຈາກຈາກຮູທະອາກ ຫຼື ອາຈົມທີ່ໄດ້ຖືກສະກັດເອົາ ສານກຳມະພັນ ເພື່ອຊອກຫາເຊື້ອໃນຕະກູນໂຄໂລນາ ຢູ່ສະຖາບັນ ປັດສະເຕີ ລາວ ເຊັ່ນດຽວກັນ ໂດຍການໃຊ້ເຕັກນິກ RT-PCR ຂອງຢີນ RNA-dependent RNA polymerase gene. ມີທັງໝົດ 6 ຕົວຢ່າງ ຈາກໜູ 4 ຊະນິດ ແມ່ນພົບເຊື້ອ. ຈາກການ ວິເຄາະລຳດັບຂອງສາຍກຳມະພັນທີ່ໄດ້ມາຈາກຕົວຢ່າງທີ່ພົບ ເຊື້ອ ໂດຍການນຳໃຊ້ເຕັກນິກ Sanger ພົບວ່າ ມີໄວຣັສໂຄໂລນາ ຕະກູນດຽວຄື Betacoronavirus, ຕະກູນຍ່ອຍແມ່ນ Embecovirus. ທັງໝົດ 6 ຕະກູນຍ່ອຍຂອງ Embecovirus ທີ່ຈຳແນກໄດ້ ແມ່ນໄດ້ມາຈາກໜູໃນກຸ່ມ *Berylmys berdmorei* 2 ໂຕ, ກຸ່ມ *Mus* spp. 2 ໂຕ, *Niviventer cf. fulvescens* 1 ໂຕ ແລະ *Rattus* sp. 1 ໂຕ.

ຈາກການສຶກສາ ຂອງພວກເຮົາໄດ້ສະແດງໃຫ້ເຫັນວ່າ ມີ ເຊື້ອໂຄໂຣນ່າໄວຣັສ 25 ຊະນິດ ພົບນຳເຈຍ 10 ຊະນິດ (ຈາກການວິເຄາະທັງໝົດ 539 ຕົວຢ່າງ), ເຊິ່ງໃນນັ້ນ 3/5 ຂອງເຊື້ອ sarbecoviruses ທີ່ໄດ້ມີການສົ່ງໄປສຶກສາມີລຳດັບ ຄວາມຍາວຂອງສາຍກຳມະພັນທັງໝົດ ແມ່ນມີຄວາມຄ້າຍຄືກັບເຊື້ອ SARS-CoV-2 > 96%. ໂດຍຜົນໄດ້ຮັບເບື້ອງຕົ້ນສະ ແດງໃຫ້ເຫັນວ່າໜາມບ່ອນຈັບ ຂອງເຊື້ອ sarbecovirus RBD ຈາກເຈຍໃນລາວ ສາມາດຈັບກັບເຄື່ອງຮັບ ACE2 ຂອງມະນຸດໄດ້ ໂດຍຜ່ານການສ້າງແບບຈຳລອງ ແລະ ທົດລອງການຈັບກັນທາງດ້ານໂມເລກຸນ, ແລະ ເຊື້ອ Pseudoviruses ທີ່ຕິດໜາມບ່ອນຈັບຂອງເຊື້ອທີ່ພົບນຳເຈຍຢູ່ ລາວຈາກຕົວ ຢ່າງ BANAL-20-236 ແມ່ນສາມາດຜ່ານເຂົ້າໄປໃນ ຈຸລັງທີ່ມີເຄື່ອງ ຮັບ ACE2 ຂອງມະນຸດໄດ້.

ການທົດລອງເພີ່ມເຕີມອື່ນໆ ເຊັ່ນການທົດລອງ ຄືນວ່າ Pseudoviruses ທີ່ຕິດໜາມ ບ່ອນຈັບຂອງເຊື້ອທີ່ພົບນຳເຈຍຈາກ ຕົວຢ່າງ BANAL-20-236 ນັ້ນສາມາດຜ່ານເຂົ້າໄປໃນຈຸລັງຂອງ ມະນຸດໂດຍກົງແທ້ ຫຼື ບໍ ແລະ ມີຄວາມຈຳເປັນທີ່ຕ້ອງໄດ້ສຶກສາຕໍ່ວ່າ ເຊື້ອທີ່ພົບມີຄວາມຮຸນແຮງ ແລະ ສາມາດກໍ່ໃຫ້ເກີດພະຍາດໄດ້ຫຼືບໍ່. ນອກນັ້ນ ຍັງມີຄຳຖາມອື່ນໆ ຍັງຈະຕ້ອງໄດ້ສຶກສາຕື່ມ ເຊັ່ນວ່າ: ປະ ຊາກອນທ້ອງຖິ່ນທີ່ສຳຜັດກັບເຈຍເຄີຍໄດ້ຕິດເຊື້ອໄວຣັສຊະນິດໃດ ໜຶ່ງທີ່ພົບນີ້ ຫຼື ບໍ່, ແລະການຕິດເຊື້ອດັ່ງກ່າວມີການສະແດງອາການຫຼື ບໍ່ ແລະ ການສຳຜັດນັ້ນສາມາດສ້າງພູມຄຸ້ມກັນຕໍ່ກັບເຊື້ອ SARS-CoV-2 ໄດ້ ຫຼື ບໍ່.

ຈາກຜົນການວິໄຈເບື້ອງຕົ້ນຂອງພວກເຮົາໄດ້ສະແດງໃຫ້ເຫັນວ່າ ຍັງມີເຊື້ອໄວຣັສ sarbecoviruses ຢູ່ນຳເຈຍຊະນິດອື່ນໆ ທີ່ມີທ່າ ອຽງມີໂອກາດທີ່ຈະແຜ່ເຊື້ອສູ່ຄົນໃນລາວ. ສ່ວນຄົນທີ່ມີການພົວພັນ ແລະ ສຳຜັດໃກ້ຊິດກັບເຈຍ ອາດຈະມີຄວາມສ່ຽງສູງຕໍ່ການສຳ ຜັດກັບເຊື້ອພະຍາດ, ດັ່ງນັ້ນ ຜູ້ທີ່ຈຳເປັນຕ້ອງສຳຜັດກັບເຈຍ ຄວນມີການປ້ອງກັນໃນລະດັບໃດໜຶ່ງ.

ສຸດທ້າຍນີ້, ພວກເຮົາຍັງໄດ້ສືບຕໍ່ຄົ້ນຄ້ວາດ້ານການຕ້ານຢາຂ້າແມງ ໄມ້ຂອງຍຸງລາຍທີ່ເປັນພາຫະ, ທີ່ມີຄວາມຫຍຸ້ງຍາກລະຫວ່າງການ ຮັກສາສິ່ງແວດລ້ອມ ແລະ ການຫຼຸດຜ່ອນຈຳນວນປະຊາກອນຍຸງພາ ຫະນຳເຊື້ອ ແລະ ຫຼຸດຜ່ອນເຊື້ອພະຍາດທີ່ມີຍຸງເປັນພາຫະ.

ການກຳນົດເປົ້າໝາຍຍຸງຕົວແກ່ດ້ວຍການໃຊ້ຢາຂ້າແມງໄມ້ໃນກຸ່ມ pyrethroid (PYR) ເຊັ່ນ deltamethrin ປະກອບເປັນມາດ ຕາການການໃຊ້ຢາຂ້າແມງໄມ້ຂັ້ນທຳອິດເພື່ອຈຳກັດການລະບາດຂອງ ພະຍາດທີ່ເກີດຈາກພາຫະນຳເຊື້ອ. ເຖິງຢ່າງໃດກໍ່ຕາມ, ຍຸງໄດ້ພັດ ທະ ນາກົນໄກການຕ້ານຕໍ່ຢາທີ່ມີປະສິດທິພາບເພື່ອຕ້ານກັບຢາຂ້າແມງ ໄມ້. ການຕ້ານຕໍ່ໃນກຸ່ມ PYR ແມ່ນພົບຢ່າງກ້ວາງຂວາງ ໃນຍຸງລາຍ *Ae. aegypti* ແລະ ໄດ້ຫຼຸດຜ່ອນປະສິດທິພາບຂອງການຄວບຄຸມ ຍຸງ ລາຍໃນຫຼາຍພາກພື້ນ ເຂດຮ້ອນ ເຊິ່ງລວມເອົາທັງອາເມຣິກ

ຂອງຝຣັ່ງທີ່ຢູ່ບ່ອນອື່ນ. ເມື່ອບໍ່ດົນມານີ້, ຍັງມີລາຍງານການຕ້ານຕໍ່ຢາຢູ່ໃນປະຊາກອນຍຸງລາຍ *Ae. albopictus* ທີ່ມີຢູ່ໃນທະວີບຕ່າງໆລວມທັງພາກໃຕ້ຂອງເອີຣົບ ແລະ ເກາະ La Reunion. ເມື່ອພິຈາລະນາຈາກການແຈກຢາຍທີ່ເພີ່ມຂຶ້ນ ຮ່ວມກັບ ຄວາມສຳຄັນທີ່ເພີ່ມຂຶ້ນໃນການສົ່ງຕໍ່ເຊື້ອພະຍາດ arbovirus, ດັ່ງນັ້ນ ການເພີ່ມຂຶ້ນຂອງການຕ້ານຕໍ່ຢາ PYR ໃນຍຸງຊະນິດນີ້ຈຶ່ງກາຍເປັນບັນຫາທາງດ້ານສາທາລະນະສຸກ.

ໃນສະພາບທີ່ບໍ່ມີການຄົ້ນພົບຢາຂ້າແມງໄມ້ຊະນິດໃໝ່ສຳລັບວຽກງານສາທາລະນະສຸກ, ການບໍລິຫານຈັດການກັບການຕ້ານຕໍ່ຢາຂ້າແມງໄມ້ຈຶ່ງມີຄວາມສຳຄັນ ແລະ ຈຳເປັນ ຈົນ ກ່ວາຈະມີການນຳໃຊ້ມາດຕາການການຄວບຄຸມ ແບບໃໝ່. ເຖິງຢ່າງໃດ ກໍຕາມ, ມັນຮຽກຮ້ອງໃຫ້ມີຄວາມເຂົ້າໃຈຕໍ່ ກົນໄກການຕ້ານຢາ ແລະ ມີການຕິດຕາມເຝົ້າລະວັງຢ່າງມີປະສິດທິພາບ ຂອງປະຊາກອນຍຸງທີ່ມີໃນທຳມະຊາດ.ລັກສະນະທາງດ້ານກຳມະພັນຫຼາຍລັກຊະນະ ແມ່ນໄດ້ຖືກຈຳແນກໃນຍຸງລາຍ *Ae. aegypti*, ແຕ່ຍັງບໍ່ທັນມີການຈຳແນກລັກສະນະທາງດ້ານກຳມະພັນໃດໃນຍຸງລາຍ *Ae. Albopictus*.

ໃນສະພາບການນີ້, ໂຄງການ TigeRisk2 ຈະທຳລາຍຂໍ້ຈຳກັດທາງວິທະຍາສາດ ແລະ ເຕັກໂນໂລຢີໃນການ ຈຳແນກລັກສະນະທີ່ໃຫມ່ຕ່າງໆຂອງກຳມະພັນDNAຂອງການຕ້ານຕໍ່ຢາກຸ່ມPYRຂອງຍຸງລາຍ *Ae. albopictus* ແລະ ໃຊ້ໃນການຕິດຕາມການຕ້ານຕໍ່ຢາໃນປະຊາກອນຍຸງໃນທຳມະຊາດເພື່ອປະເມີນຄວາມສ່ຽງ ຕໍ່ການເກີດການຕ້ານຕໍ່ຢາດັ່ງກ່າວໃນຍຸງລາຍຊະນິດນີ້. ເພື່ອໃຫ້ບັນລຸເປົ້າໝາຍດັ່ງກ່າວ, ສະມາຄົມດັ່ງກ່າວຈະເຕົ້າໂຮມ 5 ທີມງານຮ່ວມກັນ ແລະ ສົມທົບກັບການເກັບຍຸງໃນພາກສະໜາມ, ຕາມດ້ວຍການທົດລອງໃນຫ້ອງວິເຄາະທີ່ໃຊ້ໄລຍະເວລາຍາວ ແລະ ເຕັກໂນໂລຊີທາງດ້ານໂມເລກຸນທີ່ທັນສະໄໝ.

Tick Map 4 project: Mapping of Vectors and Reservoir Hosts in Lao PDR

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Partners

- The Lao-Oxford University-Mahosot Hospital-Wellcome Trust Research Unit (LOMWRU)
- The Pathogen Discovery Laboratory, IP-Paris-France

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Summary

In order to identify common and emerging vector-borne pathogens in Laos, NAMRU-2 Singapore (SG) has established a study to assess the distribution and infection potential of vectors since 2014. Between 2020 and 2021, we continued to conduct tick and other ectoparasite collections in Oudomxay province in the northern part of Laos. The vectors were morphologically identified and pooled. Then nucleic acids were extracted and screened for pan-phlebovirus and pan-flavivirus respectively by conventional nested RT-PCR at IP-Laos; *Rickettsia* spp. were detected by qPCR at LOMWRU and arboviral pathogens were detecting by Next Generation Sequencing at IP-Paris.

A total of 582 ticks and other ectoparasites were collected during the study. No tick samples were positive by pan-phlebovirus and pan-flavivirus screening. However, results from NGS of two big pools of *Dermacentor* and *Haemaphysalis* ticks found 4 viral families known to infect vertebrates including *Rhabdoviridae* (Yongjia tick virus 2), *Reoviridae* (Rotavirus), *Flaviviridae* (Jingmen tick virus), and Unclassified *Riboviria* (Bole tick virus 4). 121/192 pools were positive for pan-rickettsia for *Rickettsia* spp. (17kDa by qPCR). However, none of them was positive for *R. typhi/Coxiella* spp. Our partner, LOMWRU, will continue to try to identify the Rickettsia species. Furthermore, another partner at IP-Paris will continue to perform deep sequencing on the samples collected in the course of this study. From 2021-2022, we will continue our tick study/surveillance in Laos for both ticks and associated pathogens. Future research studies are needed to determine the pathogenicity of these viruses to livestock and humans in Laos.

Background

Vector-borne diseases constitute a significant infectious disease risk for local populations. In Laos, definitive diagnosis is often not available for vector-borne illnesses, so the infectious diseases which are a threat to populations are not well-defined. In order to identify common and emerging vector-borne pathogens in Laos, NAMRU-2 Singapore (SG) has established a study to assess the distribution and infection potential of vectors (including ticks and associated arthropods). In this study, tick and associated arthropod vectors were surveyed from the environment and their associated hosts to provide biological specimens for diagnostic purposes. The samples were transported to the Institut du Pasteur (IPL) laboratory in Vientiane, where a wide range of diagnostic tests can be performed to identify both the vector and pathogens with which they may be infected.

Objectives

- Survey and modern identification (ID) of indigenous tick and associated arthropod species
- Collection ID and extraction of vector DNA for submission to and development of regional repository. This will provide a valuable resource for downstream modern molecular analysis and genotyping.
- Building of local capacities and competencies.

Methodology

Field sites and times

We selected three sites from two villages (Cut and Hauyxang) of Xay district, Oudomxay province (Cut: loc.1: 20.706578°N, 102.113547°E; loc.2: 20.741500°N, 102.107226°E; and Hauyxang: loc.3: 20.763224°N; 102.124142°E) for our surveys. Our first field mission of 9 days was conducted between 11 and 19 February, 2021 and the second mission between 25 July and 4 August, 2021.

Field collection procedure

Tick dragging/flagging: Tick dragnets were swept/dragged along the forest ground at approximately 1–2 m intervals before being examined for ticks. Ticks were removed from the sheets using forceps, then transferred to 1.5 ml labeled cryotubes, and stored -20°C. Our dragnet collecting was carried out in all three sites.

Small Mammal Trapping: rodent traps were set for three nights in Cut village (site 2) and in Hauyxang village (site 3). In both study sites, 50 traps (baited with bananas, sticky rice, or dried fish/meat) were placed in the format of a transect according to the topography. All rodents were released after checking for ectoparasites.

Additional tick collection was carried out by examining domestic animals (cows) in site 2 (Cut village) and in site 3 (Huayxang village). The animal owners were asked to help to examine their animals. Once ticks attached on animals were found, they were collected by direct removal with forceps.
All ectoparasite samples were stored in -20°C in the field and transported to Vientiane laboratory (IPL) using dry ice.

Laboratory work

Sample identification and preparation

Ticks were identified under microscopes and grouped in cool conditions (on ice packs) by using reference determination from Dr. Richard G. Robbins of the US Armed Forces Pest Management Board (AFPMB), together with related references from Southeast Asia, Japan, Korea, the Ryukyu Islands (Yamaguti, Tipton et al. 1972), L. E. Robinson keys for genus Amblyomma (Nuttall, Cooper et al.), and keys from Thailand (Tanskull and Inlao 1989) for adult Haemaphysalis ticks. As there are no morphological identification keys available for pre-imago forms, all larval and nymph stages were grouped into genus. After tick identification and pooling, all information was registered with the Pathogen Asset Control System (PACS) software and all tick samples were stored at −80°C in IP-Laos, Vientiane Capital, for further analysis.

Chigger mites from rodents were mounted on slides using PVA mounting medium. Mite samples were identified using a compound microscope to genus level by referring to the published taxonomic key of Nadchatram & Dohany 1974.

Sample preparation and RNA/DNA extraction

Specimens were placed in 1.5 ml vials containing 1 ml of 1X cold Phosphate Buffered Saline (PBS) and Lysing Matrix A zirconium beads (MP Biomedicals). Tick pools were homogenized for 10 min at a vibration frequency of 25/s in a TissueLyser II system (Qiagen). After grinding, beads and tissues were spun down by centrifugation for 5 min at 3000 rpm. To obtain total nucleic acid (both DNA and RNA) for bacterial and viral detection by polymerase chain reaction (PCR), 100 µl of each pool was extracted and purified by using NucleoSpin® 8 Virus extraction kit following the manufacturer's protocol. The remaining 400 µl of each pool was stored at –80°C for future pathogen isolation.

Arboviral screening at IPL

Phleboviruses and flaviviruses were screened by conventional nested RT-PCR as previously described (Sanchez-Seco, Rosario et al. 2003; Sanchez-Seco, Rosario et al. 2005).

Bacterial screening

The bacterial screening was carried out in collaboration with LOMWRU, based at Mahosot Hospital, Vientiane. To investigate the occurrence of Rickettsia spp. in ticks, a molecular screening approach targeting the 17kDa gene was taken (Jiang et al. 2004). The presence of *Anaplasma* spp. and Coxiella was also investigated.

Next generation Sequencing at IP-Paris

A total 203 pools were sent to IPP for deep sequencing. Our partner will select 26 pools for NGS.

Results

Number, species abundance and composition of ticks and other ectoparasites

A total of 582 samples of ticks and other ectoparasites were collected and pooled into 339 pools, of which 477 samples were collected from animals and 105 samples from dragging method. Five species of ticks were collected from cows and rats whereas 8 tick species were collected from vegetation by dragging. Laelapidae and Trombiculidae were collected only from rats (Table 1 below for more detail).

Table 1: Number of ticks and other ectoparasites collected from different methods by districts.

Coll. Method	Source	Family	Genus	Species	Village		
					Huaysang	Kat	Total
Animal	Cows	Ixodidae	*Amblyomma*	*A. sp*	10	1	11
			Rhipicephalus	*Rh. heamaphysoloides*	13		13
				Rh. microplus	120	176	296
				Rh. sanguineus		1	1
	Rats	Ixodidae	*Ixodes*	*I.* sp.		2	2
		Laelapidae	*Echinolaelaps*			26	26
		Trombiculidae				128	128
		Total			**143**	**334**	**477**
Dragging	Vegetations	Ixodidae	*Amblyomma*	*A. testudinarium*	4	23	27
			Dermacentor	*D. auratus*		1	1
				D. bellulus		2	2
				D. sp.		13	13
			Haemaphysalis	*H. hystricis*		13	13
				H. quadriaculata		2	2
				H. spp.		35	35
			Ixodes	*I.* sp.		12	12
		Total			**4**	**101**	**105**
		Total			**147**	**435**	**582**

Preliminary results on identification of pathogens associated with ticks

Arboviral screening

A total of 194 pools (266 tick samples) and 253 pools (328 tick samples) were screened for pan-phlebovirus and pan-flavivirus, respectively by conventional nested RT-PCR. None of them was positive (Table 2 and Table 3).

Table 2: Results of Pan-Phlebovirus by RT-PCR

Species	No. of pools	No.of samples	Pan-Phebo results	
			Negative	Positive
A. sp	4	11	4	0
A. testudinarium	6	24	6	0
D. bellulus	1	1	1	0
D. sp	6	13	6	0
H. hystricis	8	8	8	0
H. quadriaculata	2	2	2	0
H. sp	10	33	10	0
I. sp	4	12	4	0
Rh. heamaphysoloides	13	13	13	0
Rh. microplus	140	149	140	0
	194	**266**	**194**	**0**

Table 3: Results of Pan-Flavivirus by RT-PCR

Species	No. of pools	No.of samples	Pan-Flavi results	
			Negative	Positive
A. sp	4	11	4	0
A. testudinarium	7	27	7	0
D. auratus	1	1	1	0
D. bellulus	2	2	2	0
D. sp	6	13	6	0
H. hystricis	13	13	13	0
H. quadriaculata	2	2	2	0
H. sp	11	35	11	0
I. sp	6	14	6	0
Rh. heamaphysoloides	13	13	13	0
Rh. microplus	187	196	187	0
Rh. sanguineus	1	1	1	0
	253	**328**	**253**	**0**

Bacterial screening

To date, a total of 192 pools containing 296 ectoparasites have been screened for the presence of *Rickettsia* spp. at LOMWRU. A total of 121 pools were positive for *Rickettsia* spp. (17kDa) with the highest number of pools positive among *Rhipicephalus* ticks (Table 4). None of the positive pools (0/100) were positive for *R. typhi/ Coxiella* spp. The team will continue to try to identify the Rickettsia species.

Table 4: Rickettsial screening from ticks

Source	Sample Stage	Genus	Species	Total No. of pools	Total No. of samples	Rickettsia spp. (17kDa) qPCR Positive No. of Pools (No. of samples)
Cows	**Adult**	*Amblyomma*	*A. testudinarium*	1	1	0
		Rhipicephalus	*Rh. heamaphysoloides*	13	13	12 (12)
			Rh. microplus	139	139	105 (105)
	Nymph	*Amblyomma*	*A. testudinarium*	3	10	0
		Rhipicephalus	*Rh. microplus*	1	10	1 (10)
		Total				
Rats	**Larvae**	Trombiculidae		7	62	1 (10)
	Nymph	*Ixodes*	*I. spp.*	2	2	0
		Total				
Vegetations	**Adult**	*Haemaphysalis*	*H. hystricis*	7	7	0
			H. quadriaculata	2	2	0
	Larvae	*Dermacentor*	*D. spp.*	1	2	0
		Haemaphysalis	*H. spp.*	1	1	0
	Nymph	*Amblyomma*	*A. testudinarium*	4	19	1 (5)
		Dermacentor	*D. spp.*	3	6	0
		Haemaphysalis	*H. spp.*	6	18	1 (2)
		Ixodes	*I. spp.*	2	4	0
		Total				
Grand Total				**192**	**296**	**121 (144)**

Next generation Sequencing at IP-Paris

To date, deep sequencing has been performed on two pools of *Dermacentor* (16 ticks) and *Haemaphysalis* (10 ticks) and other remaining pools have now finished Library preparation as follows:

Library preparation and sequencing by Illumina Nextseq 500

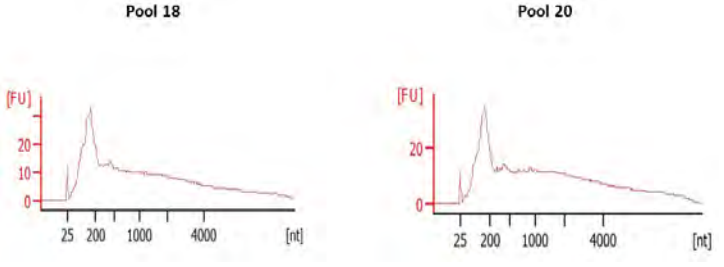
2 pools of ticks were selected because of the likeliness of pan phlebo test positivity: Pool 18 (with 16 ticks) and pool 20 (with 10 ticks) (Table 5).

Total RNA extracts were quantified using Qubit RNA High sensitivity assay and quality was checked with an Agilent BioAnalyzer RNA (Table 5 & Figure 1).

Table 5: two pools of ticks selected for first NGS

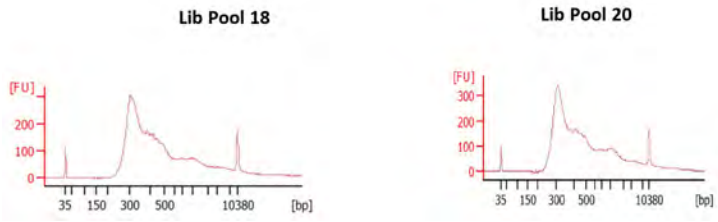
Pool no	Tick Genus	Nb of individuals /pool	RNA (ng/µL)	Library DNA (ng/µL)	Nb of raw reads
18	*Dermacentor*	16	4.8	44.8	97 060 057
20	*Haemaphysalis*	10	4	59.6	51 390 369

Figure 1: RNA profiles analyzed onto an Agilent BioAnalyzer



The 2 pools of RNA extracts were used as input for library preparation using the SMARTer Stranded Total RNA-seq kit v3-Pico input mammalian. Quality controls of the libraries comprised a quantification by Qubit DNA High sensitivity assay and a characterization of size profiles by BioAnalyzer DNA High Sensitivity chips (Table 5 & Figure 2).

Figure 2: DNA profiles analyzed onto an Agilent BioAnalyzer



Sequencing was then carried out on an Illumina NextSeq 500 sequencer in a single-read 1 x 150 bp.

Bioinformatic Analysis

Total raw reads were processed with an in-house bioinformatics pipeline (Microseek) comprising quality check and trimming reads normalization, *de novo* assembly using Megahit tool and ORF prediction of contigs and singletons.

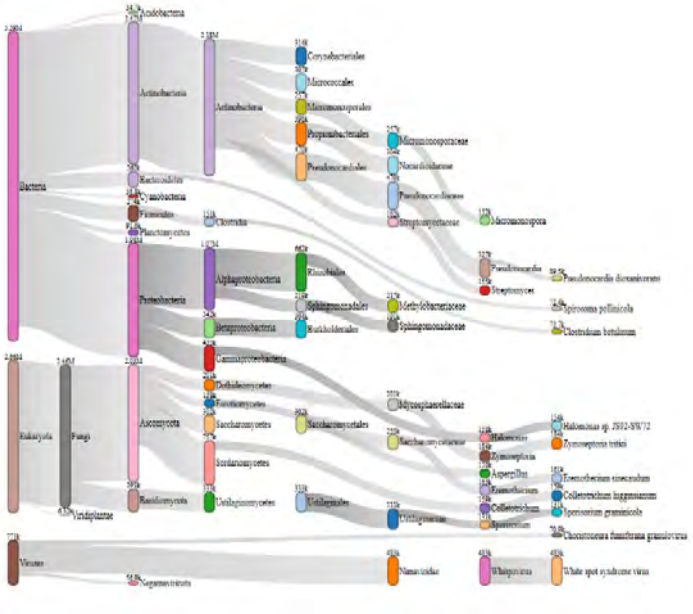
A BLAST-based similarity search was performed for all contigs and singletons against the comprehensive and curated protein Reference Viral database (RVDB-prot) followed by a BlastP-based verification of the accuracy of the viral taxonomic assignation against the whole protein NCBI/nr database. A final BLASTN-based verification was performed against NCBI/nt to confirm that no better hit was obtained with non-coding sequences present in NCBI/nt. The quantification of abundance of each viral taxon was first estimated by summing the length (in amino-acids) of all sequences (contigs and singletons) being associated to this taxon instead of summing the raw number of sequences, to take into account the identification of long viral contigs.

Results from NGS

a. Microbiome data

The Sankey diagrams based on Kraken2 analyses revealed that pool 18 and pool 20 microbiomes are largely represented by bacteria while viruses represent a small minority (Figure 3).

Pool 18



Pool 20

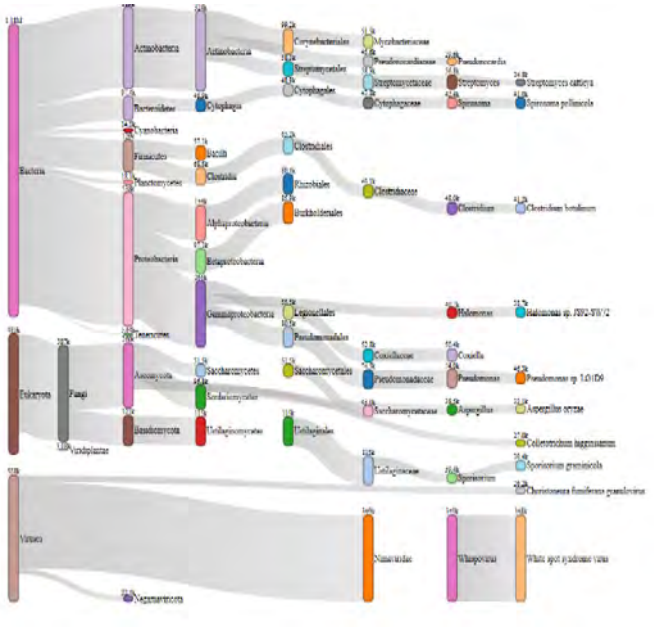


Figure 3: Representation of tick microbiomes using Sankey diagrams based on Kraken2 analyses

b. Virome data

Sequences of many viruses were identified in Pool 18 and most of them usually infect plants and invertebrates (Table 6). In contrast only sequences of three viruses were identified in pool 20, of which 2 were known to infect vertebrates.

Table 6: Viral sequences identified in ticks using Microseek

Pool n°	Order	Family	Genus	Best hit	Principal host	Abondance (in aa)	AA identity (%)
18	*Caudovirales*	*Podoviridae*	*Unclassified Podoviridae*	Xanthomonas phage RiverRider	Bacteria	150	82
		Autographiviridae		Acinetobacter phage Aristophanes		147	57
		Siphoviridae	*Fromanvirus*	**Mycobacterium phage MyraDee**		432	79
			Unclassified siphoviridae	Siphovirus conting 89		291	46-62
	Unclassified Riboviria			**Bole tick virus 4**	**Invertebrates/vertebrates**	**992**	**60**
				Riboviria sp.		447	64-72
				Changjiang crawfish virus 1	Invertebrates	273	98-100
	Durnavirales	*Partitiviridae*	*Unclassified Partitiviridae*	**Grape vine partivirus**	Plants/Fungi	102	100
				Plasmopara viticola lesion associated Partitivirus 2	Fungi	138	87
	Amarillovirales	***Flaviviridae***	***Unclassified Flaviviridae***	**Unclassified *Flaviviridae***	**Vertebrates**	**75**	**96**
	Tolivirales	*Tombusviridae*	*Unclassified Tombusviridae*	**Rice Tombus-like virus 3**		435	63-93
	Mononegavirales	***Rhabdoviridae***	***Curiovirus***	**Rochambeau curiovirus**	**Invertebrates/vertebrates**	**405**	**57**
			Unclassified Rhabdoviridae	**American dog tick rhabdovirus-2**	**Invertebrates/vertebrates**	**117**	**64**

	Cryppavirales	*Mitoviridae*	*Mitovirus*	Sclerotinia sclerotiorum mitovirus 32	Fungi	81	74
	Ourlivirales	*Boutourmiaviridae*	*Ourmiavirus*	Bremia lactucae associated ourmia-like virus 1	Plants	144	77
				Plasmopara viticola lesion associated ourmia-like virus 15		294	98
			Unclassified Boutourmiaviridae	Erysiphales ourmia-like virus 2		414	84-93
	Wolframvirales	*Narnaviridae*	*Unclassified Narnaviridae*	Botryosphaeria dothidea narnavirus 3	Fungi	129	76
20	***Mononegavirales***	***Rhabdoviridae***	***Ledantevirus***	**Yongjia tick virus 2**	**Invertebrates/vertebrates**	**20 865**	**95-100**
	Caudovirales	*Siphoviridae*	*Fromanvirus*	Mycobacterium phage Xena	Bacteria	93	77
				Unclassified *Fromanvirus*		213	82
	Reovirales	***Reoviridae***	***Sedoreovirinae***	***Rotavirus***	**Vertebrate**	**294**	**100**

c. Mapping of relevant viruses

Rhabdoviridae family

Different genera were identified depending on the pool of tick. In pool 20, approximatively 70% of amino-acid sequences that belonged to the *Rhabdoviridae* were assigned to Yongjia tick virus 2 with 95-100% amino-acid identity. Seventy three percent of genome of the Yongjia tick virus 2 was covered to reference sequence (Figure 4).

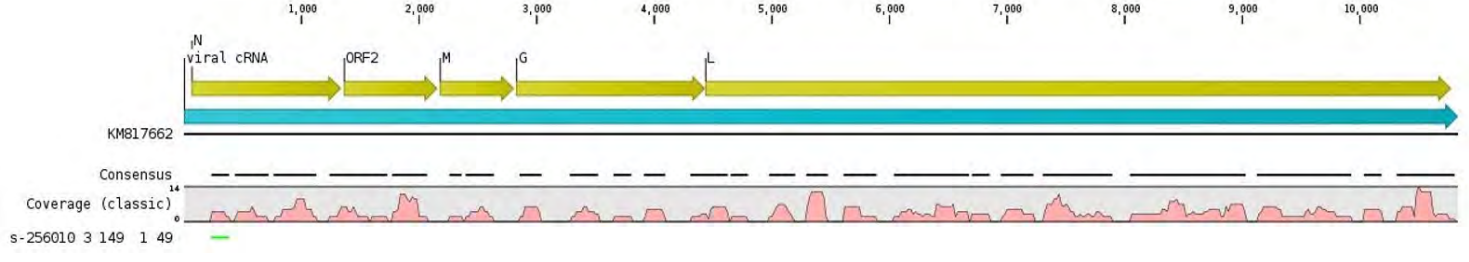


Figure 4: Mapping of Yongjia tick virus 2 from Pool 20

In pool 18, 78% of amino-acid belonging to *Rhabdoviridae* were assigned to Rochambeau virus and 22% were assigned to American dog tick rhabdovirus 2. The latter derive from unclassified rhabdoviruses. It is important to note that these sequences represent a low percentage of amino-acid identity (57% and 64% respectively) and only one read was identified for each best hit. Therefore, we were not able to map these sequences onto the corresponding reference sequences.

Reoviridae family

Reoviridae family were identified in Pool 20. Only 2 reads were obtained and 100% of these reads were assigned to *Rotavirus*. They mapped onto a small fragment in the VP6 segment of the virus.

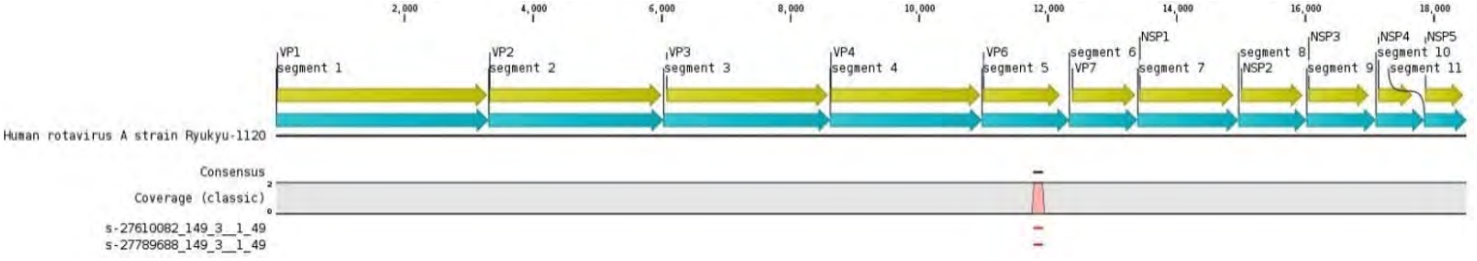


Figure 5: Mapping of *Rotavirus* from Pool 20

laviviridae family

The Flaviviridae family is observed in Pool 18. Only a few sequences were identified and assigned to *Jingmen tick virus*. These sequences were mapped onto segment 1 coding for the viral polymerase and segment 4 coding for the capsid protein.

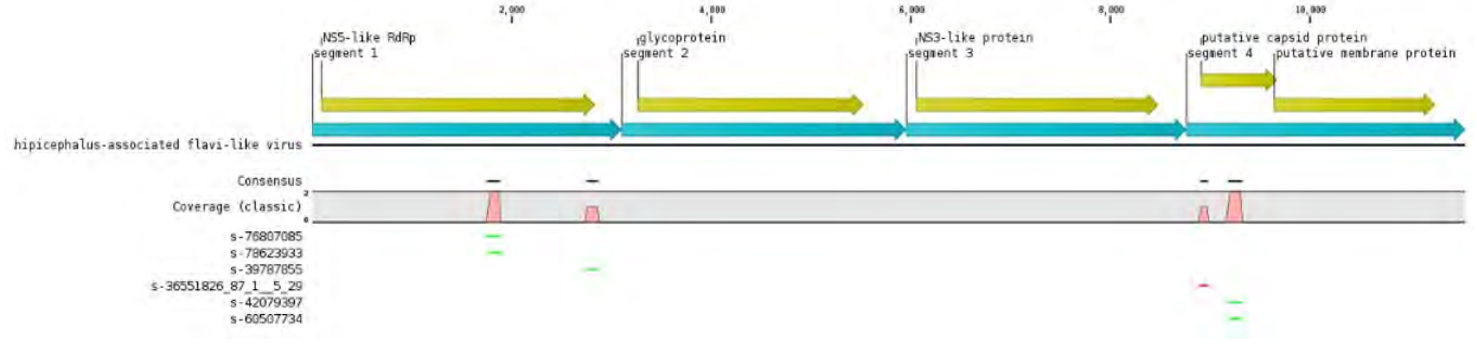


Figure 6: Mapping of *Flaviviridae* family from Pool 20

Unclassified *Riboviria*

Within unclassified *Riboviria*, sequences assigned to Bole tick virus 4 were identified. A stop codon was observed in the middle of the consensus sequence, but it was covered only by 2 reads.

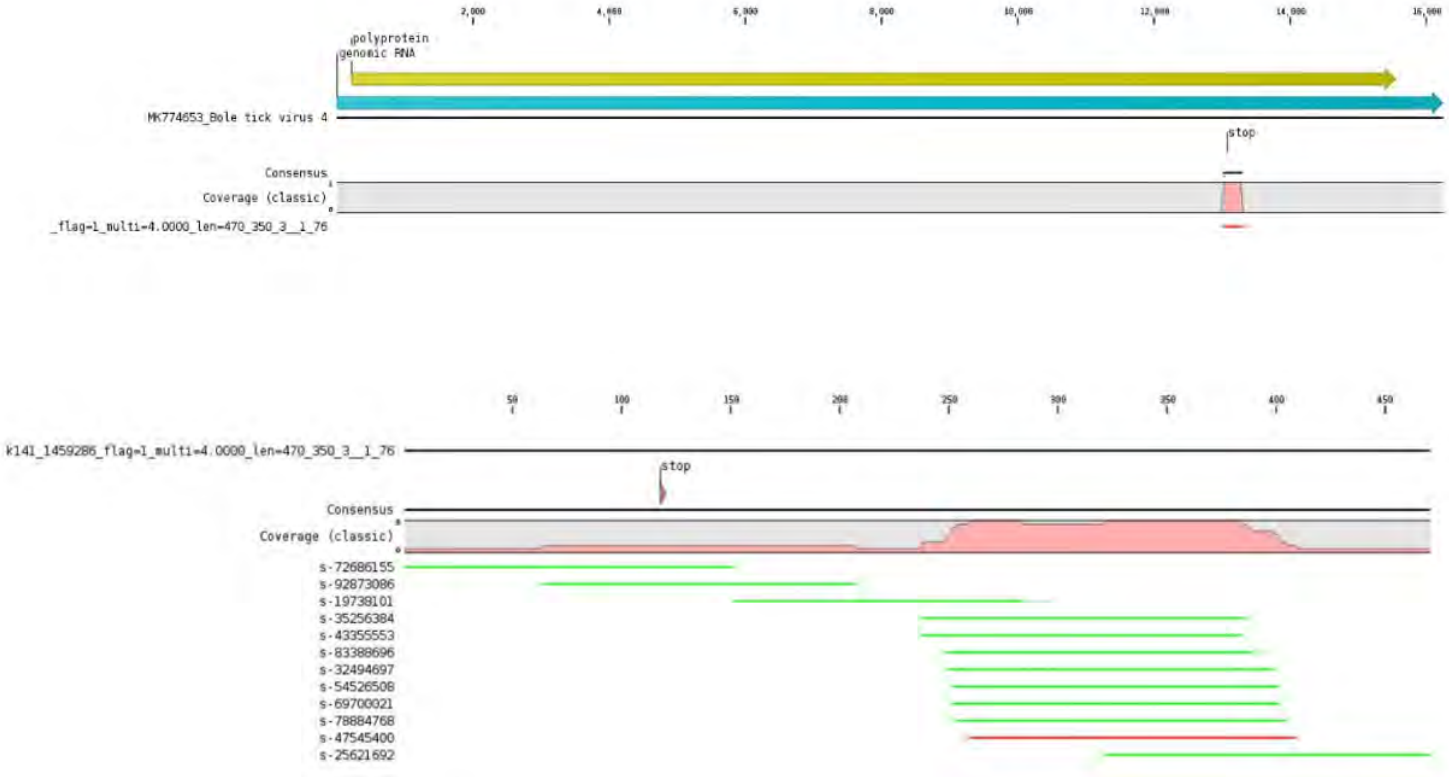


Figure 7: Unclassified *Riboviria* from Pool 20

a. Phylogenetic analysis of Yongjia tick virus 2-like from Laos

Phylogenetic analysis performed on the RNA-dependent RNA polymerase amino-acid sequence placed Yongjia tick virus 2-like identified in Pool 20 in the Ledantevirus genus, including Yongjia tick virus 2 previously identified in Haemaphysalis hystricis ticks from China. Interestingly, viruses close to Yongjia tick virus 2 were previously identified in vertebrates: Nkolbisson virus in bats and human cases, Nishimuro virus and Fukuoka virus in ungulates and Barur virus in rodents (Evolution of Genome Size and Complexity in the *Rhabdoviridae*; Walker P)

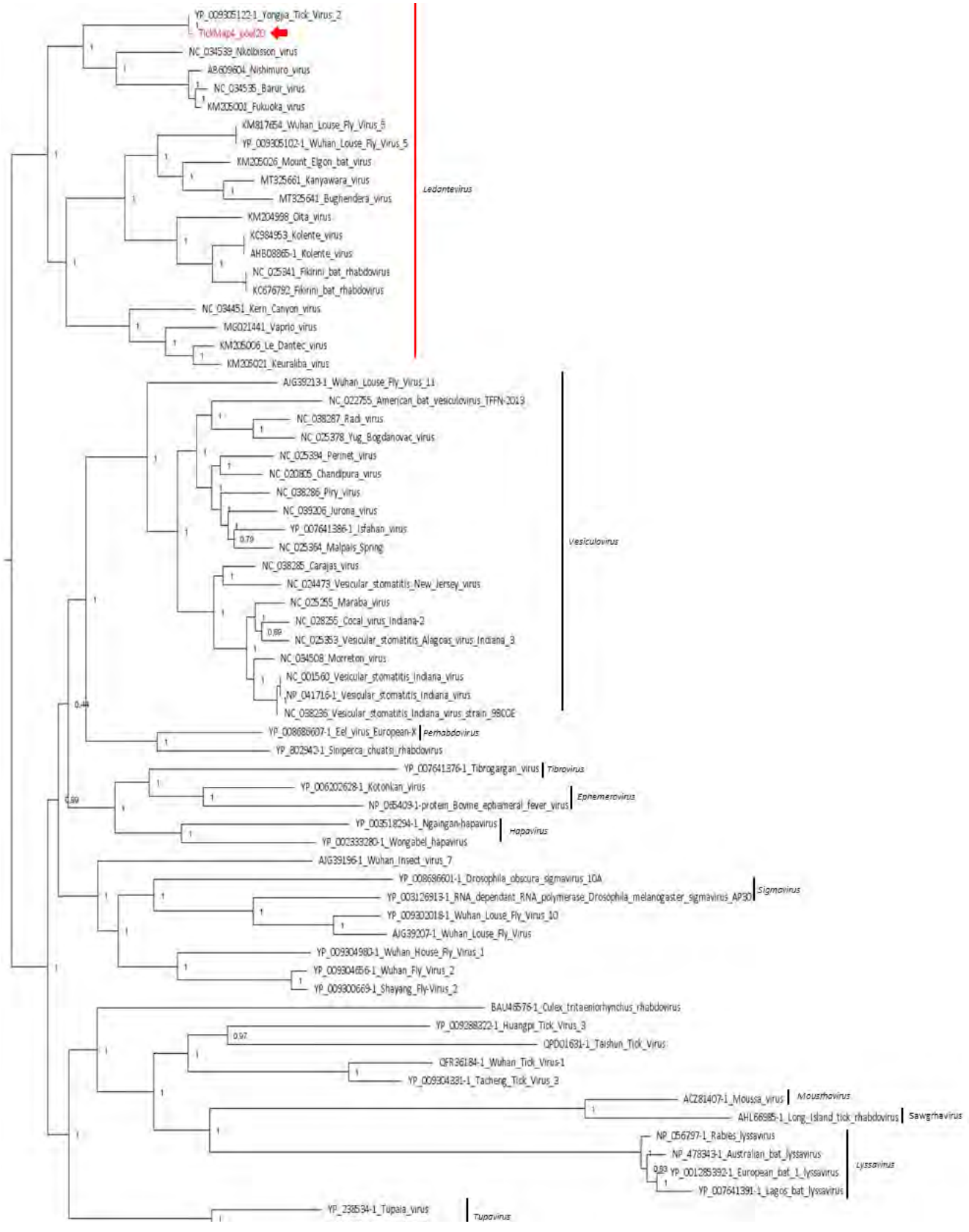


Figure 7: Phylogenetic Tree of Yongjia tick virus 2-like from

Problems Identified during our project and Follow-up Actions

- The pandemic of COVID-19 is the only challenge that we have faced so far. However, we have finished all of our field collections on time as planned.
- All samples were stored at between -20°C and -80°C for future pathogen study.
- Our partner, LOMWRU, will continue to identify the Rickettsia species.
- Our partner at IP-Paris will continue to work on deep sequencing
- We will continue the tick study/surveillance in Laos for both ticks and associated pathogens in 2021-2022.
- Future research studies are needed to determine the pathogenicity of these viruses to livestock and humans in Laos.

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Origins, natural reservoirs and Interspecies transmission of SARS-CoV-2 and other SARS-like CoVs (ONRITS)

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Outside partners:
- National Institute of Hygiene & Epidemiology-Vietnam
- The Pathogen Discovery Laboratory, IP-Paris-France

Funded by the « URGENCE COVID-19 » fundraising campaign of Institut Pasteur, Institut Pasteur du Laos, the United Kingdom Foreign and Commonwealth Office as well as Luxembourg Development special grant.

Period of Project: 2020-2021

Summary

Since the discovery of severe acute respiratory syndrome-associated coronavirus (SARS-CoV) in Chinese horseshoe bats (*Rhinolophus sinicus*) in Hong Kong in 2005, a plethora of molecular epidemiological studies have been carried out in bats and animal species around the world to detect other animal reservoirs of this highly pathogenic CoV (Wong et al., 2019). Interestingly, of the >30 CoVs found in bats, a majority have been found in various species of horseshoe bats (*Rhinolophus* spp.) from China. A few others have been found in other bat genera such as, *Aselliscus*, *Chaerephon*, and *Hipposideros.*

Given the fact that two SARS Coronaviruses have emerged and cause important human loss of life in the past seventeen years (SARS and COVID-19), the scientific community postulates that other SARS Coronaviruses are presently circulating in Rhinolophus and other bat populations and could spill-over into human populations like SARS and COVID-19; hence, the surveillance and identification of these novel putative coronaviruses is necessary to mitigate a new future pandemic.

A better scientific understanding of the origin, natural history and dispersal of SARS-CoV-2 and SARS-like-CoVs in their natural environment, as well as the mechanisms leading to their interactions and interspecies jumping: Bat-Bat; Bat-Animal and Bat-Human are paramount to mitigate future spillover events that result in devastating epidemics like the one we are witnessing today with SARS-CoV-2 in China and around the world.

Objectives

The specific objectives of this project are to:

- Assess the presence of SARS-CoV2 and related viruses in Laos and Vietnam wildlife (bats and other mammal communities at the interface with them);
- Search for other SARS-like CoVs using New Generation Sequencing (NGS)-based metagenomic analysis of selected samples;
- Detect past beta-coronavirus infections using specifically developed antibody tests for SARS-CoV2 and novel closely-related viruses.

Methodology

The field collection was conducted in Laos by teams from the Institut Pasteur du Laos (IP-Laos), and in Vietnam by the National Institute of Hygiene & Epidemiology (NIHE)-Vietnam, in collaboration with the Pathogen Discovery Laboratory at the Institut Pasteur (IP-Paris) to determine the presence of SARS-CoV-2 and to identify its natural host using Next Generation Sequencing (NGS), virus isolation, etc.

Site selection and time for field sampling in Laos

Between July 2020 and January 2021, our team conducted field collection from 4 sites within 2 provinces (Table 1): Fueng and Meth Districts, Vientiane Province, and in Namor and Xay Districts, Oudomxay Province. Our field mission was authorized and facilitated by the Ministry of Agriculture and Forestry of Laos.

Table 1: selection sites for our field collection between 2020-2021

Province	District	Village	Locality	Latitude	Longitude
Vientiane Province	Fueng	Na Ang	Pha Tong	18° 32.879'N	101° 58.938'E
			Tham Nam cave	18° 32.914'N	101° 58.459'E
			Tham Pha	18° 34.018'N	101° 58.338'E
		Noon Hin Hae	Pha Nouk Kok	18° 31.743'N	101° 58.648'E
		Pha Louang	Tham Pha Louang	18° 30.495'N	101° 57.421'E
	Meth	Had Yao	Pha Keo Phong Mon	18° 54.426'N	101° 58.789'E
			Pha Khee Chear (Keo Tao)	18° 54.898'N	101° 59.048'E
		Houay Pa Mak	Pha Mum	18° 53.717'N	101° 56.957'E
			Pha Mum, Keo Tao	18° 54.787'N	101° 58.292'E
			Pha Yao, Keo Tao	18° 55.386'N	101° 57.723'E
Oudomxay Province	Na Mor	Na Thong	Nam Ook Hoo Cave	20° 52.396'N	101° 46.973'E
				20° 51.907'N	101° 46.926'E
	Xay	Chom Ong	Chom Ong Cave	20° 43.071'N	101° 45.811'E
			Houay Om Loung	20° 43.111'N	101° 46.577'E
			Houay Pak Veuk	20° 41.647'N	101° 47.179'E
			Pa Noo Khom	20° 44.143'N	101° 46.770'E

Training on biosafety prior field bat collection

The training prior to field work was organized once for 6 participants who joined field work collection. The one-day training session was organized on 24 June 2020 at IP-Laos. The aim was to introduce participants to our SOPs and to the transmission risks of infectious agents that may arise from bats/rodents and how to identify, assess, and mitigate the risks, as well as to make participants better understand methods that could minimize the risks, and to identify and practice the use of PPE suitable for the field work.

Figure 1: Biosafety training at IP-Laos prior field investigation

Field collection and identification procedure

Bats

Bats were collected using four-bank harp traps (Francis, 1989) (Figure 2) and mist nets. Harp traps were set across natural trails and over small streams in the forest understorey, and at the entrance of caves in relatively concealed conditions. Mist nets were also set at similar places to harp traps, but were more often used in open spaces. Both harp traps and mist nets were set before sunset; harp nets were left overnight. Captured bats were held individually in cloth bags. Species, sex, age (adult or juvenile), and reproductive condition (pregnant or lactating) were determined in the field. Species were identified following Francis (2008), Csorba et al. (2003), and Corbet and Hill (1992). Adults or juveniles were identified by the presence of unfused epiphyses of the phalanges and metacarpal joints (Brunet-Rossinni and Wilkinson, 2009). The reproductive status of female bats was determined by examining the nipples (Racey, 2009). Some external characteristics, including forearm length (FA), were measured using calipers and body mass (W) was taken using a Pesola spring balance. Most bats were marked with wing bands for individual identification and were released at the capture point within 12 hours. For each species, two or three specimens were retained to confirm identification. Bats were euthanized using chloroform. Specimens were fixed in 95% ethanol in the field and were transferred to 70% ethanol when they were brought back to the laboratory. All specimens were registered and catalogued in the Zoological Collection of the Faculty of Environmental Sciences, National University of Laos, Lao PDR.

Figure 2. Harp trap setting

Rodents

Rodent habitats were identified, especially at entrance of caves and peri karstic areas. Small wire live-traps (14x14x30 cm in WxHxL), Sherman traps, or other similar devices specially fitted for small mammals were set for rodent collection. Between 50 and 100 traps per night were placed in the format of either grid or transect according to the topography, and each trapping session lasted from 6-8 nights. Traps were baited with appropriate baits (e.g., bananas, sticky rice, or dried fish) and checked every morning.

For each species of rodent, two or three specimens were retained to confirm identification. Rodents were euthanized using chloroform. Specimens were fixed in 95% ethanol in the field and were transferred to 70% ethanol when they are brought back to the laboratory. All of specimens were registered and catalogued in the Zoological Collection of the Faculty of Environmental Sciences, National University of Laos, Lao PDR.

Types of biological samples from animals

The following basic set of samples were collected, if possible, from each bat and other animals:

- Oral-pharyngeal swab: 1 aliquot
- Feces (fresh fecal sample) or rectal swab: 1/2 aliquots
- Blood (serum; red blood cell/white blood cell pellet): 2 aliquots
- Urine (free catch method or urogenital swab): 1/2 aliquot
- Ectoparasites : 1 aliquot

Laboratory procedure

Extraction

Total nucleic acids (DNA/RNA) were extracted from bio-samples, especially for bat and rodent anal swabs by using Nuclo Spin®8 Virus (MACHEREY-NAGAL, Germany).

Lab techniques and assay procedure for Pan-corona screening at IP-Laos

cDNA was synthesized using Maxima H minus first strand cDNA synthesis kit (Thermo Scientific) with random hexamers following the manufacturer's instructions. Pan-corona PCR targeting the RNA-dependent RNA Polymerase gene was used for screening of coronaviruses as previously described by Chu et al. 2011. PCR products of the expected size were directly sequenced on both strands by Sanger sequencing using the nested PCR primers. The sequences obtained were confirmed by similarity analysis using the NCBI BLASTn search (http://www.ncbi.nlm.nih.gov/BLAST).

NGS at IP-Paris

Total nucleic acids (DNA/RNA) for both positive and negative samples and aliquot of anal swabs that were positive following pan-corona PCR at IP-Laos were sent to IP-Paris for in-depth analysis.

Preliminary results

Biological sampling

A total of 645 bats were captured from 4 collection sites and 608 saliva, 539 anal/feces, 157 urine swabs and 246 blood samples were collected. In addition, ectoparasites were collected from 74 bats (Table 2).

Table 2: Total number of bats captured and biological samples

Field #	Province/District	Total capture	Sample type				
			Saliva swab	Anal/feces swab	Urine swab	Blood sample	Ectoparasite
	Oudomxay Province						
Field # 3	Na mor	13	12	12	5	12	
	Xai	135	134	102	37	34	3 bats
Subtotal		**145**	**146**	**114**	**42**	**46**	**3** bats
	Vientiane Province						
Field # 1	Feuang	335	308	285	72	156	38 bats
Field # 2	Maed	165	154	140	43	44	33 bats
Subtotal		**500**	**462**	**425**	**115**	**200**	**71** bats
Total		**645**	**608**	**539**	**157**	**246**	**74** bats

For rodents and other animals, a total of 117 were trapped/sampled from markets and 114 saliva, 115 anal/feces, and 64 urine swabs were collected. In addition, ectoparasites were collected from 36 rodents (Table 3).

Table 3: Total number of rodents and other animals captured/from markets and biological samples

Province	Total capture	Saliva swab	Anal/Feces swab	Urine swab	Ectoparasite
Oudomxay	82	80	81	45	23 Rodents
Vientiane Prov.	35	34	34	19	13 Rodents
Total	**117**	**114**	**115**	**64**	**36 Rodents**

Pan-corona screening at IP-Laos

A total of 539 extracted products of bat anal swabs/faces were screened at IP-Laos by targeting the RNA-dependent RNA polymerase gene. A total of 24 individuals from 10 bat species were positive (Table 4). BLAST analysis of obtained nucleotide sequences from Sanger sequencing identified Alphacoronavirus: *Decacovirus, Pedacovirus,* and *Rhinacovirus* subgenera; and Betacoronavirus: *Nobecovirus* and *Sarbecovirus* subgenera. Sequences of the *Sarbecovirus* subgenus were all identified from Rhinolophus individuals belonging to three different species i.e., *R. malayanus*, *R. marshalli*, and *R. pusillus* (Table 4).

Table 4: Results from pan-coronavirus RT nested PCR and Sanger sequencing for bat anal swabs/feces at IP-Laos. Blast search based on nucleotide sequences.

Anal Swab ID	Bat Genus	Bat Species	Genus	Subgenus	District	Village	Locality
BANAL-20-27	*Rhinolophus*	*pusillus*	co-infection *Alpha/Betacorona virus*	unclassified *Decacovius/ Sarbecovirus*	Feuang (Site 1)	Nonhinhae	Pha Nok Kok
BANAL-20-51	*Hipposideros*	*pomona*	*Alphacoronavirus*	*Decacovirus*	Feuang (Site 1)	Nonhinhae	Pha Nok Kok
BANAL-20-52	*Rhinolophus*	*malayanus*	*Betacoronavirus*	*Sarbecovirus*	Feuang (Site 1)	Nonhinhae	Pha Nok Kok
BANAL-20-103	*Rhinolophus*	*pusillus*	*Betacoronavirus*	*Sarbecovirus*	Feuang (Site 1)	Na Ang	Tham Pha
BANAL-20-115	*Hipposideros*	*pomona*	*Alphacoronavirus*	*Decacovirus*	Feuang (Site 1)	Pha Luang	Tham Pha Luang
BANAL-20-116	*Rhinolophus*	*malayanus*	*Betacoronavirus*	*Sarbecovirus*	Feuang (Site 1)	Pha Luang	Tham Pha Luang
BANAL-20-178	*Hipposideros*	*pomona*	*Alphacoronavirus*	*Decacovirus*	Feuang (Site 1)	Pha Luang	Tham Pha Luang
BANAL-20-191	*Chaerephon*	*plicatus*	*Alphacoronavirus*	*Pedacovirus*	Feuang (Site 1)	Pha Luang	Tham Pha Luang
BANAL-20-197	*Chaerephon*	*plicatus*	*Alphacoronavirus*	*Pedacovirus*	Feuang (Site 1)	Pha Luang	Tham Pha Luang
BANAL-20-212	*Chaerephon*	*plicatus*	*Alphacoronavirus*	*Pedacovirus*	Feuang (Site 1)	Pha Luang	Tham Pha Luang
BANAL-20-213	*Chaerephon*	*plicatus*	*Alphacoronavirus*	*Pedacovirus*	Feuang (Site 1)	Pha Luang	Tham Pha Luang
BANAL-20-236	*Rhinolophus*	*marshalli*	*Betacoronavirus*	*Sarbecovirus*	Feuang (Site 1)	Na Ang	Pha Tong
BANAL-20-242	*Rhinolophus*	*malayanus*	*Betacoronavirus*	*Sarbecovirus*	Feuang (Site 1)	Na Ang	Pha Tong
BANAL-20-247	*Rhinolophus*	*malayanus*	*Betacoronavirus*	*Sarbecovirus*	Feuang (Site 1)	Na Ang	Pha Tong
BANAL-20-251	*Hipposideros*	*khaokhouayensis*	*Alphacoronavirus*	*Decacovirus*	Feuang (Site 1)	Na Ang	Pha Tong
BANAL-20-273	*Rhinolophus*	*pusillus*	*Alphacoronavirus*	unclassified *Decacovirus*	Feuang (Site 1)	Na Ang	Pha Tong
BANAL-20-289	*Rhinolophus*	*affinis*	*Alphacoronavirus*	*Rhinacovirus*	Feuang (Site 1)	Na Ang	Pha Tong
BANAL-20-290	*Eonycteris*	*spelaea*	*Betacoronavirus*	*Nobecovirus*	Maed (Site 2)	Had Yao	Keo tao + Had Yao
BANAL-20-390	*Eonycteris*	*spelaea*	*Betacoronavirus*	*Nobecovirus*	Maed (Site 2)	Huay Pa Mak	Keo Tao
BANAL-20-395	*Eonycteris*	*spelaea*	*Betacoronavirus*	*Nobecovirus*	Maed (Site 2)	Huay Pa Mak	Keo Tao
BANAL-20-398	*Eonycteris*	*spelaea*	*Betacoronavirus*	*Nobecovirus*	Maed (Site 2)	Huay Pa Mak	Keo Tao
BANAL-20-403	*Eonycteris*	*spelaea*	*Betacoronavirus*	*Nobecovirus*	Maed (Site 2)	Huay Pa Mak	Keo Tao
BANAL-20-416	*Cynopterus*	*sphinx*	*Betacoronavirus*	*Nobecovirus*	Maed (Site 2)	Huay Pa Mak	Pha Moum
BANAL-20-497	*Myotis*	sp.	*Alphacoronavirus*	*Pedacovirus*	Xay (Site 3)	Chom-Ong	Near Chom-Ong Cave

For rodents, a total of 115 extracted products of bat anal swabs/faces were screened at IP-Laos by targeting the RNA-dependent RNA polymerase gene using a pan-coronavirus RT nested PCR approach. A total of 6 individuals from 4 rodent species were positive (Table 5). BLAST analysis of obtained sequences from Sanger sequencing identified Betacoronavirus sequences of the Embecovirus subgenus. Six sequences of the Embecovirus subgenus were identified from 2 *Berylmys berdmorei,* 2 *Mus* spp., 1 *Niviventer* cf. *fulvescens*, and 1 *Rattus* sp. (Table 5).

Table 5: Results from pan-coronavirus RT nested PCR and Sanger sequencing for rodent anal swabs/feces at IP-Laos. Blast search based on nucleotide sequences.

Rat ID	Species	Genus	Subgenus	District	Village
Rat_Anal_52	*Niviventer* cf. *fulvescens*	*Betacoronavirus*	*Embecovirus*	Na Mor	Na Tong
Rat_Anal_79	*Rattus* sp.	*Betacoronavirus*	*Embecovirus*	Na Mor	Na Tong
Rat_Anal_102	*Berylmys berdmorei*	*Betacoronavirus*	*Embecovirus*	Xai	Chom-Ong
Rat_Anal_105	*Berylmys berdmorei*	*Betacoronavirus*	*Embecovirus*	Xai	Chom-Ong
Rat_Anal_116	*Mus* sp.	*Betacoronavirus*	*Embecovirus*	Xai	Chom-Ong
Rat_Anal_123	*Mus* sp.	*Betacoronavirus*	*Embecovirus*	Xai	Chom-Ong

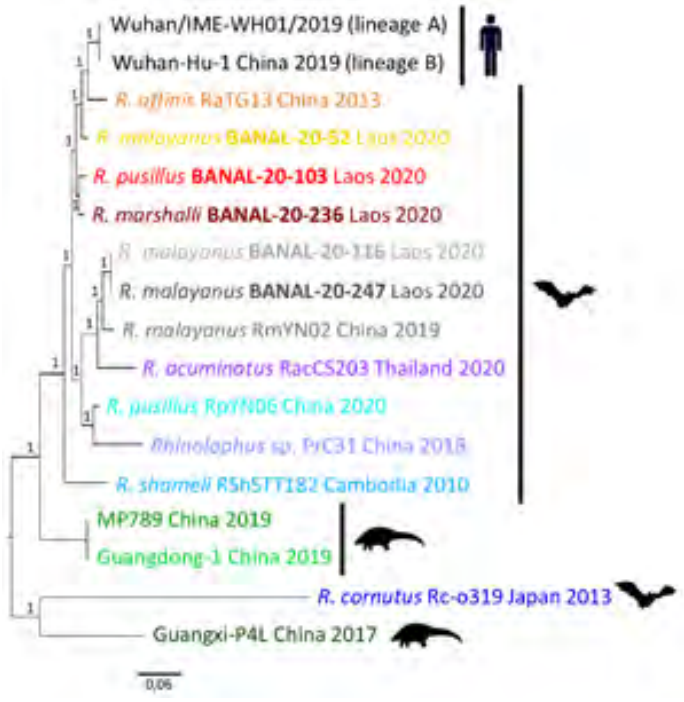
NGS preliminary results from bat anal swabs/feces samples at IP-Paris

The subgenus of Sarbecoviruses was selected as priority for complete genome sequencing as SARS-CoV-2 belongs to this genus. A total of 5 complete genome sequences were obtained by NGS from seven Sarbecovirus positives by pan-coronavirus PCR. BLAST analysis of obtained complete sequences showed that these 5 sequences from three bat species – *Rhinolophus malayanus, R. marshalli*, and *R. pusillus* had identity >96% to SARS-CoV-2 (Table 6).
Phylogenetic analyses of the complete genome sequences of human SARS-CoV-2, and other bat and pangolin sarbecoviruses showed that the 3 Laotian bat coronaviruses: *R. malayanus* BANAL-20-52, *R. pusillus* BANAL-20-103, and *R. marshalli* BANAL-20-236 were closely related to *R. affinis* RaTG13 coronaviruses and human SARS-CoV-2, while 2 other: *R. malayanus* BANAL-20-116 and BANAL-20-247 coronaviruses were grouped to a sister clade with other bat coronaviruses (RmYN02, RacCS203, RpYN06, and PrC31) from different *Rhinolophus* species (Figure 3). Like other sarbecoviruses found so far in animals from the Asian Peninsular region, all Laotian bat sarbecoviruses found here had an absence of the furin cleavage site.

Table 6: Results from complete genome sequencing at IP-Paris. Blast search based on nucleotide sequences.

No	Host species	Sample ID	% Identity	Accession No.	Classification
1	*Rhinolophus malayanus*	Banal-20-52	96.83	OK436642.1	Human SARS-CoV-2
2	*Rhinolophus pusillus*	Banal-20-103	96.75	MZ070553.1	Human SARS-CoV-2
3	*Rhinolophus malayanus*	Banal-20-116	97.09	MT614556.1	Human SARS-CoV-2
4	*Rhinolophus marshalli*	Banal-20-236	96.51	OK085085.1	Human SARS-CoV-2
5	*Rhinolophus malayanus*	Banal-20-247	97.60	LC542976.1	Human SARS-CoV-2

Figure 3. Phylogenetic analyses of the complete genome sequences of Laotian bat coronaviruses, human SARS-CoV-2, and other bat and pangolin sarbecoviruses



Other preliminary results were obtained from IP-Paris teams investigating molecular dynamics simulations of the binding of Laotian bat-sarbecovirus RBD to human ACE2 complexes and Pseudoviruses expressing BANAL-20-236 spike entry into cells express human ACE2. Laotian bat-sarbecovirus RBD were able to bind to human ACE2 and Pseudoviruses expressing BANAL-20-236 spike could entry into cells express human ACE2.

Conclusion/Ongoing activities/Perspective

Our study showed that 25 coronaviruses were detected from 10 bat species (out of 539 samples tested), of which 3/5 sarbecoviruses with full-length sequences were closely related to SARS-CoV-2 > 96%. Preliminary results showed that Laotian bat-sarbecovirus RBD was able to bind to human ACE2 through modeling and binding experiments, and Pseudoviruses expressing BANAL-20-236 spike could enter into cells expressing human ACE2.

Further experiments such as investigating whether Pseudoviruses expressing BANAL-20-236 spike can directly entry into human cells, and pathogenicity of these viruses are warranted. Other questions will need also to be addressed such as whether the bat-exposed local populations have been infected by one of these viruses in Laos, whether such infections were associated with any symptoms, and whether exposure can confer immunity against subsequent SARS-CoV-2 infection.

Our preliminary results showed that there exist other bat sarbecoviruses that seem to have the same potential for infecting humans in Laos. People with close contact with bats seem to be at risk of being exposed, so they will need some level of personal protection if they have to contact with the bats.

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TigeRisk2

Assessing the risk of insecticide resistance in the tiger mosquito: A predictive approach combining experimental selection and molecular markers

Project coordinator: JP David (CNRS, France), Sebastien Marcombe (IPL)
Staff members: Phoutmany Thammavong, Phonsavanh. Luangamath, Somphat. Nilaxay and Vaeky Vungkyly

Context and Objectives

By transmitting several arboviral diseases (dengue, Chikungunya, Zika, …) *Aedes* mosquitoes affect public health worldwide including French overseas territories. Since its invasion by the tiger mosquito *Aedes albopictus*, France metropolitan is now at risk of arbovirus transmission.

Targeting adult mosquitoes with pyrethroid insecticides (PYR) such as deltamethrin constitutes the first line of defense to limit outbreaks. However, mosquitoes have evolved resistance mechanisms to resists insecticides.

PYR resistance is widespread in *Ae. aegypti* and reduces the efficiency of mosquito control in several tropical regions including French overseas territories. Recently resistance has also been reported in populations of the tiger mosquito *Ae. albopictus* located in various continents including southern Europe and La Réunion island. Considering its expanding distribution and its growing importance in arbovirus transmission, the rise of PYR resistance in this species represents a major public health concern.

In the context of the lack of new insecticides available for public health, managing insecticide resistance is vital until novel control tools are implemented. However, this requires understanding resistance mechanisms and tracking them efficiently in natural populations. Multiple insecticide resistance markers have been identified in *Ae. aegypti* but barely none are yet available in the tiger mosquito.

In this context, the TigeRisk2 project will break through scientific and technological barriers for characterizing novel DNA markers of PYR resistance in the tiger mosquito *Ae. albopictus* and track them in natural mosquito populations in order to evaluate the risk of resistance emergence in this species. For achieving this, the consortium will gather 5 complementary teams and combine field mosquito collections with long-term laboratory experiments and state-of-the-art molecular technologies.

These objectives will be tackled by combining the expertise of two research teams highly recognized in the field of insecticide resistance in mosquitoes (CNRS-LECA Grenoble and ISEM Montpellier) together with three partners having a high expertise in mosquito collections and surveillance (EID-RA, IPL and ARSOI). By positioning itself upstream the emergence of insecticide resistance in the tiger mosquito, the TigeRisk2 project will ensure the delivery of useful tools for the detection and the management of resistance in this invasive mosquito species.

Methods

Mosquito collections locations and dates

In Laos, during the rainy season 2020, *Aedes* sp. larvae and pupae were collected in 4 provinces including 6 different districts of in more than 40 villages (Figure 1). All the collection sites were geo-referenced. In Cambodia, other collections were made in 2 provinces in September 2020 in Phnom Penh and Kampot. As *Ae. albopictus* lives in more rural or semi-rural areas or in the forest, for the collections, we selected locations surrounding the city or villages rural areas and/or close to forests. Larvae were collected in several types of breeding habitats such as buckets, flower pots, household utensils and other containers but most of the times in abandoned tires. After collection, the biological material were brought back to the laboratory at the Institut Pasteur du Laos for rearing and identification. Approximately 20,000 *Aedes* sp. larvae were collected from all the study sites. Table 1 shows the list of populations reared at the laboratory and used for the bioassays. In 2021, mosquitoes were collected in the same areas as 2020 but only in two provinces (Vientiane capital and V. province) due the shutdown of provincial boundaries implemented against COVID-19. Similarly, in Cambodia, it was not possible to collect mosquitoes.

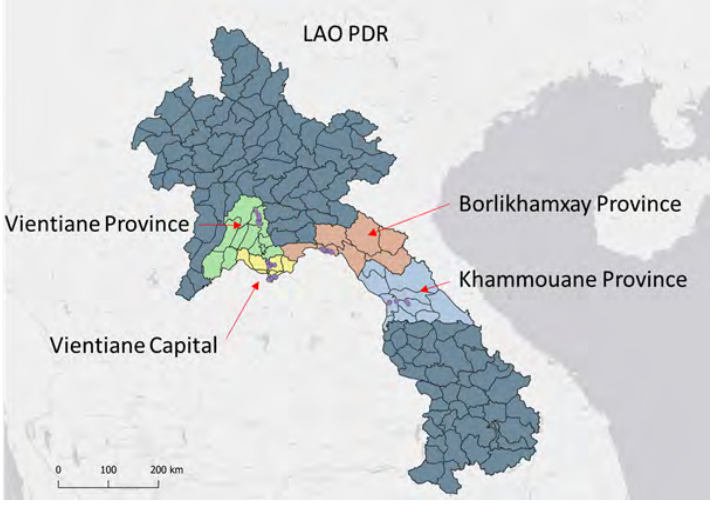

Figure 1. Locations of mosquito collections in Lao PDR, 2020/2021.

Morphological mosquito identification

For all the mosquito populations collected, larvae and pupae were reared until adult (F0 generation). After adult identification, the mosquitoes obtained were separated by species. There was a great proportion of *Ae. aegypti* and *Armigeres* sp. found in the collection samples. Only *Aedes albopictus* were kept for breeding. Females mosquitoes were then blood fed using the Hemotek membrane feeding system and the eggs obtained were kept for the adult bioassays and the composite population composition. For each populations, 30 males and 30 females were kept at -20°C in silica gel.

We received eggs from Cambodia in October 2020 but unfortunately, after emergence most of the individuals were identified as *Ae. aegypti* mosquitoes resulting in a very low number of *Ae. albopictus*. This number (<200) was not enough to implement both the bioassays and the composite population. We chose to use the *Ae. albopictus* mosquitoes for the composite population.

Susceptibility bioassays

For the bioassays, all the *Ae. albopictus* individuals from a single province were mixed together to obtain a sufficient number of mosquito to implement the bioassays and the creation of the composite population. Adult bioassays were run against the four different mosquito populations from Laos using filter papers provided by LECA, treated with dose of deltamethrin at 0.015% and 0.03%. Mortality resulting from tarsal contact with treated filter papers was measured using WHO test kits against adult mosquitoes of the different populations. Six batches of 25 non-blood-fed females (3–5 days of age, F1) 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.

Composite population (CP)

The *Aedes albopictus* composite population was established at the end of the year 2020 using the mosquito populations for each geographic areas sampled from Laos and Cambodia.

At least 1,000 founding individuals from each natural population (except Cambodia) were used to maximize the genetic diversity of composite populations before initiating the selection process for resistant alleles of pyrethroids. For each population, male and female mosquitoes (500 individuals) were separated into different cages just after emergence and were mixed at the same time in a bigger cage when they were about 3-5 days old. After mating, the female were blood-fed. The next generation was then use for the selection.

Selections

For the selection, male and female of the CP were separated just after emergence and were exposed to dose-response bioassays to evaluate the Lethal Dose for 75% (LD 75) with deltamethrin. However, looking at the difficulty encountered to breed *Ae. albopictus* compared to *Ae. aegypti* in our laboratory (low fecundity, fertility and low blood-feeding rates), we decided to use the LD50 to ensure to avoid the loss of the CP during the process. The CP was then selected with deltamethrin separately on male and female to generate a resistant strain. The survivors (>500) male and female were mixed for mating and bred until the next generation. Selection was made at every generations. In parallel, the CP was maintained without selection to be used as a reference for molecular analysis. Resistance levels of the 2 strains, selected (CP-R) and non-selected (CP-Ns) were implemented every 2 selections. For both strains, 30 males and 30 females were kept in freezer.

Results

Insecticide resistance status of the field collected population in Laos

Results are presented in Table 2 and Figure 2. The mortalities of the different *Ae. albopictus* populations from Laos varied from 82 to 98% and 85 to 99% at the dose of 0.015 and 0.03% respectively. The results with the 0.03% WHO diagnostic dose indicate that the VTVV population was resistant to deltamethrin and the other populations presented reduced susceptibility to this pyrethroid insecticide (suspected resistance).

Table2. Insecticide resistance bioassay on adult *Ae. albopictus* from Laos

Province	District	Strain	*Aedes* sp.	Insecticide	Dose	n	% Mortality
Vientiane Province	Vangvieng	VTVV	*Ae. albopictus*	Deltamethrin	0.015%	146	82
					0.03%	146	89
Vientiane Capital	Xaythany/Hatxayfong	VTC	*Ae. albopictus*	Deltamethrin	0.015%	150	98
					0.03%	149	98
Khammouane	Mahaxay/Thakek	KHM	*Ae. albopictus*	Deltamethrin	0.015%	150	85
					0.03%	147	91
Bolikhamxay	Paksan	BLX	*Ae. albopictus*	Deltamethrin	0.015%	146	98
					0.03%	150	99
Control strain		USDA	*Ae. aegypti*	Deltamethrin	0.015%	250	100
					0.03%	250	100

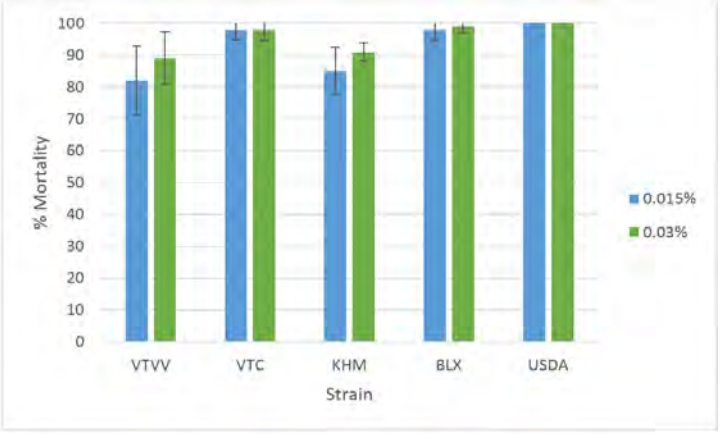

Figure 2. Mortality and SE of *Ae. albopictus* populations against deltamethrin at different doses (0.015% and 0.03%), USDA, *Ae. aegypti* control susceptible strain.

Mortalities measured during selection of the Composite population

Four selection tests were implemented during the reporting period. For each selections, females of the CP were exposed to the LD50 times determined during the preliminary tests (20 to 23min) but only showed 26 to 29% mortality. Males of the Laos CP showed mortality with an exposure time of 15min between 43 to 60%. Figure 3 shows the mortality of the CP at each selection tests for male and female adults. The results showed that the exposure times should be increased for the female to select more pyrethroid resistant alleles.

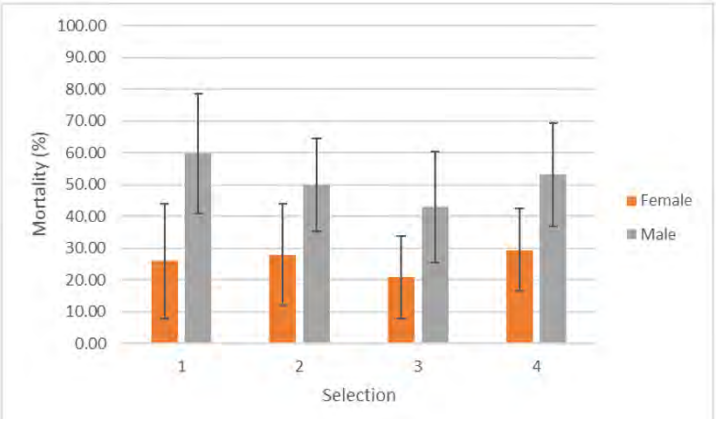

Figure 3. Mortality (%) observed during selection with deltamethrin (0.015%) of the female and male mosquitoes of the CP of Laos with Standard Errors

Insecticide resistance status of the Composite Population of Laos, CP-R and CP-Ns

The results are presented in the table 4. Female mosquitoes were exposed to the different insecticides during 1 hour.

Insecticide	Name	Selection / Generation	% Mortality	SD	SE	N
Deltamethrin 0.03%	CP-R	S1	97	7.7	1.4	150
Deltamethrin 0.03%	CP-R	S3	91	8.3	1.3	149
Deltamethrin 0.03%	CP-Ns	G1	89	8.25	1.62	100
Deltamethrin 0.03%	CP-Ns	G3	75	6.81	1.2	123
Permethrin 0.25%	CP-R	S1	91	6.4	1.03	122
Permethrin 0.25%	CP-R	S3	83	12	2.2	125
Permethrin 0.25%	CP-Ns	G1	100	0	0	100
Permethrin 0.25%	CP-Ns	G3	88	6.2	0.95	150

CP-R: selection; CP-NS: no selection

Bioassays on the CP-R and CP-Ns against the different insecticides are presented in Figure 4. The mortality of the CP-R selection 3 against deltamethrin (0.03%) is lower than CP-R after the first selection. For both selection stages mortality is lower with CP-Ns.

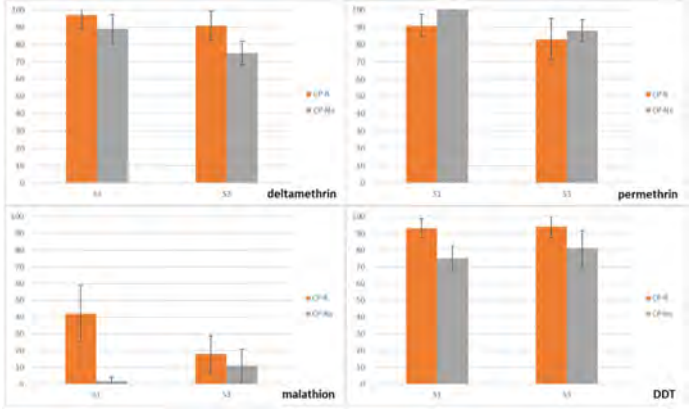

Figure 4. Mortality (%) of the CP-R and CP-Ns after deltamethrin selection tests.

Next step

The collections, insecticide resistance tests and selections are completed. The meta-population composed in the laboratory and the dead and alive mosquitoes resulting from the bioassay will be send to France at the CNRS. They will be used to combine dose-response bioassays and with the next-generation sequencing to identifying DNA markers of pyrethroid resistance. The results will help to design high-throughput diagnostic tests for the best resistance markers in the tiger mosquito *Ae. albopictus* to track them in natural mosquito populations in order to evaluate the risk of resistance emergence in this species.

Vaccine Preventable Diseases Laboratory

Head of Laboratory:
Dr. Antony Black, PhD

Scientists:
Dr. Phonethipsavanh Nouanthong, PhD
Dr. Siriphone Virachith, MD, PhD

Junior Scientists:
Dr. Vilaysone Khounvisith, MD

Technicians:
Latdavone Khenkha
Bounta Vongphachanh
Nouna Innoula

Projects

- Low seroprevalence of COVID-19 in Lao PDR, late 2020
- Age-stratified seroprevalence of vaccine-preventable infectious disease in Saravan, Southern, Lao PDR
- Detection of Hepatitis C in the general population in Saravan Province, Lao PDR
- An age-stratified serosurvey against purified *Salmonella enterica* serovar Typhi antigens in the Lao PDR
- Luxembourg-Laos Partnership for Research and Capacity Building in Infectious Disease Surveillance – PaReCIDS; a summary of activities from 2016 to 2021 and future plans.

Executive summary

The Vaccine-preventable disease (VPD) laboratory has a remit to build capacity for investigations of human and animal infectious diseases that are of relevance for Lao PDR. Our activities are in collaboration with the Clinical and Applied Virology group at the Luxembourg Institute of Health (LIH), Luxembourg, headed by Judith Hübschen. We work closely with local partners and focus on the epidemiology and seroprevalence of vaccine-preventable infectious diseases, as well as animal and zoonotic diseases. As such, we can provide stakeholders in public and animal health with estimates of the burden of infections, promote outbreak control and vaccination programmes, and propose measures to optimize national health strategies. Our evidence-based results and recommendations are communicated to stakeholders and partners in the form of written and oral reports and policy briefs. In this year's report, we show data from several completed studies.

The laboratory continues to support the Virology team at IPL for the SARS-CoV-2 testing and also took the lead on a collaborative study to determine the seroprevalence of antibodies against SARS-CoV-2 in Lao PDR in 2020. In collaboration with Pasteur Paris, University of Health Sciences, Lao Tropical and Public Health Institute and the National Centre for Laboratory and Epidemiology, we found no evidence for significant circulation of SARS-CoV-2 in Lao PDR during or before September 2020. This likely results from decisive measures taken by the government early in the pandemic, social behaviour, and low population density. High anti-N antibodies/low anti-S antibodies in bat/wildlife contacts may indicate exposure to cross-reactive animal coronaviruses with threat of emerging novel viruses. These data show the importance of control measures but also demonstrated the vulnerability of the Lao population to SARS-CoV-2 outbreaks. The results and recommendations were communicated directly with the Lao Ministry of Health and have been published as a manuscript.

We completed the analysis and reporting of a serostudy for vaccine-preventable infectious diseases in Saravan Province, in the south of Lao PDR. We found that hepatitis B exposure and chronic infection were high in adults and that diphtheria and tetanus seroprevalence were low, indicating poor vaccine coverage. Measles serology indicated an immunity gap, especially at young ages and we concluded that routine vaccination in Saravan needs strengthening, particularly infants and vulnerable groups. We also investigated the prevalence of hepatitis C virus (HCV) infection in the participants. The seroprevalence of HCV was very high, especially in participants from Samuoi district. PCR and genotyping of the virus did not suggest any large scale transmission events and further studies are recommended to determine the source and risk factors of infection. These important public health issues in Saravan were reported to the provincial authorities, the Lao Department of Communicable Disease Control, WHO and others and have been accepted for publication as two separate manuscripts.

Lastly, we report a study done in collaboration with University of Cambridge, UK and others, looking for anti-typhoid antibodies in different Lao populations. Typhoid is a vaccine-preventable infectious disease that occurs in areas with poor sanitation. Typhoid vaccination is not routine in Lao PDR but has taken place during outbreak situations. Our data show evidence for high rates of infection in young children. We reported the data to the Lao Ministry of Health and have submitted the data for publication.

In September 2021, Vilaysone Khounvisith from the laboratory began her PhD, registered with the Swiss Tropical and Public Health Institute in Basel. This collaboration between Switzerland, IPL, LIH and the Lao Tropical Public and Health Institute (LTPHI) will allow Vilaysone to receive further training in biostatistics and epidemiology and other courses, whilst carrying out her project looking at the relationship between water, sanitation and hygiene levels and infectious diseases in Lao PDR.

In collaboration with the LTPHI, we supervised two Masters students who completed their dissertations on hepatitis B virus in Lao healthcare workers and tetanus/diphtheria seroprevalence in Lao adolescents. These studies will be detailed in a future report.

From September we were joined by Lard Salivanh, a Lao military healthcare worker, who will train on research skills with our lab for 6-9 months in the framework of the Arboshield program. Overseas visitors to the laboratory were limited this year due to COVID-19 related travel restrictions.

In her joint capacity as a scientist from the laboratory and a member and Executive Secretary of the Nation Immunization Technical Advisory Group, Dr. Phonethipsavanh Nouanthong supported the National Immunization Program in several activities. These included; providing technical support, new vaccine review, protocol development and training for trainers at national and subnational levels; joining supportive supervision during the COVID-19 vaccination roll out; helping the National Center Laboratory and Epidemiology to review the COVID-19 epidemic burden and vaccine access; assessing vaccination effectiveness / modelling in collaboration with University of Health Science, Lao PDR.

Publications

Virachith S, Pommelet V, Calvez E, Khounvisith V, Sayasone S, Kounnavong S, Mayxay M, Xangsayarath P, Temmam S, Eloit M, Escriou N, Rose T, Vongphayloth K, Hübschen JM, Lacoste V, Somlor S, Phonekeo D, Brey PT, Black AP. *Low seroprevalence of COVID-19 in Lao PDR, late 2020. Lancet Reg Health West Pac.* 2021 Aug;13:100197.

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Xaydalasouk K, Sayasinh K, Hübschen J, Khounvisith V, Keomany S, Muller C, Black AP. *Age-stratified seroprevalence of vaccine-preventable infectious disease in Saravan, Southern Lao People's Democratic Republic.* International Journal of Infectious Diseases. April 2021.

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Pollack E, Kunlaya K, Keokhamphoui C, Souksakhone C, Chanthavilay P, Sayasone S, Black AP, Nouanthong P. *Suboptimal knowledge of hepatitis B infection and concerns regarding HBV vaccination among blood donors in Lao People's Democratic Republic (PDR).* Lao Medical Journal, 2021.

Khampanisong P, Pauly M, Nouanthong P, Vickers MA, Virachith S, Xaydalasouk K, Black AP, Muller CP, Hübschen JM. *Waning of Maternal Antibodies against Measles Suggests A Large Window of Susceptibility in Infants in Lao People's Democratic Republic*. Pathogens, 2021.

Meetings and presentations

European Scientific Conference on Applied Infectious Disease Epidemiology, online 16 November 2021 "Analyses of blood donor samples from eight provinces in Lao PDR suggest considerable variation concerning HBV exposure and carriage" Poster presentation by Lisa Hefele

Global health: viruses, liver and cancers 2021, online 18-24 July 2021. Attended by Siriphone Virachith

Japan Collaborative Group for Lao Health Science and Medicine, annual meeting. Online 7th November, 2021. "The COVID-19 outbreak in Laos" Oral presentation by Siriphone Virachith.

Vilaysone Khounvisith from Vaccine Preventable Disease Laboratory, with fellow students from Swiss Tropical Public Health Institute.

Training given by Vaccine-preventable disease Lab staff

On-the-Job Training at IPL for Military personnel under the "Arboshield plus" project (DTRA): Biosafety and Biosecurity; General Biology; Basic immunology

Supervision of two Masters students from LTPHI;

- Sonephet Vantava "Tetanus and diphtheria seroprotection in adolescents from Bolhikhamxay and Vientiane Capital"

- Khanxayaphone Phakhounthong "Hepatitis B virus exposure, seroprotection and current infection in Lao Healthcare workers"

Supervision of Master student from Vu University;

- Trude Dekker "Trends and factors determining hepatitis B birth dose and pentavalent vaccination coverage over time"

Sonephet Vantava (LTPHI student) and Bounta Vongphachanh (Vaccine Preventable Disease Laboratory technician)

ສະຫຼຸບການປະຕິບັດວຽກງານ

ຫ້ອງທົດລອງພະຍາດທີ່ປ້ອງກັນດ້ວຍວັກຊີນ ແມ່ນໄດ້ສ້າງຄວາມອາດສາມາດ ໃນການກວດຫາພະຍາດຕິດເຊື້ອໃນຄົນ ແລະ ສັດທີ່ມີຢູ່ໃນ ສປປ ລາວ. ວຽກຂອງພວກເຮົາ ແມ່ນໄດ້ມີການຮ່ວມມືກັບຫນ່ວຍງານໄວຣັສວິທະຍາ, ທີ່ສະຖາບັນສາທາລະນະສຸກ, ປະເທດລັກຊໍາເບີກ, ນໍາໂດຍ ດຣ ຈູດິດ ເຮັບສເກັນ. ພວກ ເຮົາ ຍັງໄດ້ປະຕິບັດວຽກງານຢ່າງແຫນ້ນແຟ້ນ ຮ່ວມກັບ ຄູ່ຮ່ວມມື ທ້ອງຖິ່ນ ແລະ ເນັ້ນໃສ່ ໃນດ້ານການລະບາດວິທະຍາ ແລະ ອັດຕາຊຸກຊຸມທາງເຊຣອມວິທະຍາ ຂອງພັນດາພະຍາດທີ່ປ້ອງກັນ ດ້ວຍວັກຊີນ, ພ້ອມກັນນັ້ນ ກໍຍັງມີພະຍາດໃນສັດ ແລະ ພະຍາດທີ່ ສົ່ງຕໍ່ຈາກສັດສູ່ຄົນນໍາອີກດ້ວຍ. ຕົວຢ່າງເຊັ່ນ, ພວກເຮົາໄດ້ສະຫນອງ ຂໍ້ມູນໃຫ້ກັບ ພາກສ່ວນສາທາລະນະສຸກ ແລະ ພາກສ່ວນລ້ຽງສັດ ເພື່ອປະເມີນ ບັນຫາຂອງພະຍາດຊຶມເຊື້ອ, ການໃຫ້ຄໍາແນະນໍາໃນ ການຄວບຄຸມການລະບາດ ແລະ ການໃຫ້ວັກຊີນ, ແລະ ຍັງສະເຫນີ ນະໂຍບາຍທີ່ແທດເຫມາະ ທາງດ້ານສາທາລະນະສຸກນຕື່ມອີກ ດ້ວຍ. ຜົນຂອງການຄົ້ນຄວ້າ ແລະ ຄໍາແນະນໍາຕ່າງໆ ໄດ້ຖືກເອົາມາປຶກ ສາຫາລືກັນລະຫວ່າງພາກສ່ວນທີ່ກ່ຽວຂ້ອງ ແລະ ຄູ່ຮ່ວມພັດທະນາ ໃນຮູບແບບບົດລາຍງານແບບຂຽນ ແລະ ການບັນລະຍາຍ ແລະ ບົດນະໂຍບາຍແບບຄັດຫຍໍ້. ບົດລາຍງານຂອງປີນີ້, ພວກເຮົາໄດ້ມີຂໍ້ ມູນທີ່ຫລາກຫລາຍຈາກການສຶກສາທີ່ໄດ້ສໍາເລັດໄປແລ້ວ.

ຫ້ອງທົດລອງພວກເຮົາຍັງໄດ້ຊ່ວຍທີມຫ້ອງທົດລອງໄວຣັສວິທະຍາ ທີ່ສະຖາບັນປັດສະເຕີລາວສໍາລັບການກວດຫາ ບໍ່ງມະຕິ SARS-CoV-2 (ໂຄວິດ-19) ແລະ ຍັງໄດ້ເປັນຜູ້ເຮັດການສຶກສາເພື່ອຊອກຫາອັດຕາຊຸກຊຸມຂອງທາດພາຍຕ້ານຕໍ່ກັບ SARS-CoV-2 (ໂຄວິດ-19) ໃນ ສປປ ລາວ ຊ່ວງປີ 2020. ໂດຍການຮ່ວມມືກັບ ສະຖາບັນປັດສະເຕີ ປາຣີ, ມະຫາວິທະຍາໄລ ວິທະຍາສາດ ສຸຂະພາບ, ສະຖາບັນສາທາລະນະສຸກສາດ ແລະ ການແພດເຂດຮ້ອນ ແລະ ສູນວິເຄາະ ແລະ ລະບາດວິທະຍາແຫ່ງຊາດ, ພວກເຮົາ ບໍ່ພົບເຫັນການແຜ່ລະບາດຂອງ SARS-CoV-2 (ໂຄວິດ-19) ໃນສປປ ລາວໃນຊ່ວງກ່ອນເດືອນ ກັນຍາ ປີ 2020. ຜົນທີ່ພົບນີ້ ໄດ້ມາຈາກມາດຕະການການຕັດສິນໃຈທັນທ່ວງທີຂອງລັດຖະບານ ສປປ ລາວ ຕໍ່ກັບການລະບາດໃນທົ່ວໂລກ, ວັດທະນະທໍາສັງຄົມ, ແລະ ອັດຕາຄວາມຫນາແຫນ້ນຂອງປະຊາກອນທີ່ຫນ້ອຍ. ທາດພາຍຕ້ານຕໍ່ໂປຣຕີນເອັນ (anti-N antibodies) ທີ່ສູງ/ ທາດພາຍຕ້ານຕໍ່ໂປຣຕີນເອັສ (anti-S antibodies) ທີ່ຕໍ່ໍ ໃນຜູ້ທີ່ເຄີຍສໍາພັດກັບ ເຈຍ/ສັດປ່າ ອາດຊີ້ໃຫ້ເຫັນເຖິງ cross-reactive (ປະຕິກິລິຍາຂ້າມກັນລະຫວ່າງ ທາດພາຍຕ້ານ-ເຊື້ອພະຍາດ) ຂອງເຊື້ອ ໃນຕະກູນໂຄໂລນາໃນສັດ ພ້ອມກັບການ ເກີດໄພຂົ່ມຂູ່ໃນການເກີດເຊື້ອໄວຣັສຊະນິດໃຫມ່ໄດ້. ຂໍ້ມູນດັ່ງກ່າວ ຍັງສະແດງໃຫ້ເຫັນເຖິງຄວາມຈໍາເປັນຂອງມາດຕະການຄວບຄຸມ ແຕ່ກໍຍັງສະແດງໃຫ້ເຫັນເຖິງ ຄວາມອ່ອນໄຫວຕໍ່ກັບການລະບາດ ຂອງພະຍາດໃນປະຊາກອນລາວອີກດ້ວຍ. ຜົນຂອງການສຶກສາ ແລະຂໍ້ແນະນໍາໄດ້ມີການປຶກສາຫາລືໂດຍກົງກັບ ກະຊວງສາທາລະ ນະສຸກ ແລະ ໄດ້ຕີພິມເປັນອາລະສານສາກົນນໍາຕື່ມ.

ພວກເຮົາໄດ້ສໍາເລັດການວິເຄາະ ແລະ ລາຍງານຜົນຂອງການ ສຶກສາບັນດາພະຍາດທີ່ປ້ອງກັນດ້ວຍວັກຊີນທີ່ແຂວງສາລະ ວັນ, ທາງພາກໃຕ້ຂອງ ສປປ ລາວ. ພວກເຮົາພົບວ່າ ການສໍາພັດ ກັບເຊື້ອໄວຣັສຕັບອັກເສບຊີ ແລະ ການຕິດເຊື້ອແບບຊໍາເຮື້ອ ແມ່ນມີສູງໃນຜູ້ໃຫຍ່ ແລະ ອັດຕາຊຸກຊຸມທາດພາຍຕ້ານຂອງພະຍາດ ຄໍຕີບ ແລະ ບາດທະຍັກ ກໍພົບວ່າຫນ້ອຍ, ເຊິ່ງນັ້ນກໍຊີ້ໃຫ້ເຫັນເຖິງ ອັດຕາປົກຄຸມວັກຊີນທີ່ຍັງຕໍ່ໍ. ການສຶກສາທາງເຊຣັມວິທະຍາຂອງ ພະຍາດຫມາກແດງໃຫຍ່ ພົບວ່າມີຄວາມແຕກຕ່າງກັນໃນດ້ານພູມ ຄຸ້ມກັນ, ໂດຍສະເພາະໃນຄົນໄວຫນຸ່ມ ແລະ ເຮົາຍັງໄດ້ເນັ້ນຫນັກ ຄວາມຈໍາເປັນໃນການໃຫ້ວັກຊີນຢ່າງສະຫມໍ່ໍາສະເຫມີນໍາອີກ, ໂດຍສະເພາະ ໃນກຸ່ມເດັກ ແລະ ກຸ່ມທີ່ມີຄວາມສ່ຽງ. ພວກເຮົາຍັງ ໄດ້ຊອກຫາອັດຕາຊຸກຊຸມຂອງການຕິດເຊື້ອໄວຣັສຕັບອັກເສບຊີ ໃນຜູ້ເຂົ້າຮ່ວມການສຶກສາ. ອັດຕາຊຸກຊຸມຂອງໄວຣັສຕັບອັກເສບຊີ ແມ່ນສູງ, ໂດຍສະເພາະໃນຜູ້ເຂົ້າຮ່ວມທີ່ມາຈາກເມືອງສະມ້ວຍ.

PCR ແລະ genotyping ຂອງໄວຣັສ ແມ່ນບໍ່ໄດ້ເຫັນການ ແຜ່ລະບາດທີ່ກວ້າງດີເທົ່າທີ່ຄວນ ແລະ ຈໍາເປັນຕ້ອງໄດ້ມີ ການສຶກສາເພີ່ມເຕີມໃນຕໍ່ຫນ້າ ເພື່ອຊອກຫາແຫລ່ງ ແລະ ປັດໄຈສ່ຽງຂອງການຕິດເຊື້ອ. ບັນຫາສໍາຄັນທາງດ້ານສາທາລະນະສຸກ ທີ່ແຂວງສາລະວັນນີ້ ແມ່ນໄດ້ຖືກລາຍງານໃຫ້ກັບສາທາລະນະສຸກ ແຂວງ, ກົມຄວບຄຸມພະຍາດຕິດຕໍ່, ອົງການອະນາໄມໂລກ ແລະ ຄູ່ຮ່ວມງານອື່ນໆ, ນອກນີ້ຍັງໄດ້ຕີພິມລົງໃນອາລະສານສາກົນໄດ້ ສອງສະບັບ.

ສຸດທ້າຍແລ້ວ, ພວກເຮົາໄດ້ລາຍງານການສຶກສາທີ່ໄດ້ເຮັດ ຮ່ວມກັບ ມະຫາວິທະຍາໄລ ແຄັມບຣິຈ, ປະເທດອັງກິດ ແລະ ຄູ່ຮ່ວມງານອື່ນໆ, ເພື່ອເບິ່ງທາດພາຍຕ້ານຕໍ່ກັບພະຍາດໄຂ້ຫໍລະພິດ ໃນປະຊາກອນລາວ. ພະຍາດໄຂ້ຫໍລະພິດ ແມ່ນຫນຶ່ງໃນພະຍາດທີ່ ປ້ອງກັນດ້ວຍວັກຊີນ ທີ່ເກີດໃນບ່ອນທີ່ມີສຸຂະອານະໄມທີ່ບໍ່ສະ ອາດ. ວັກຊີນປ້ອງກັນຫໍລະພິດຍັງບໍ່ໄດ້ຖືກເຂົ້າມາໃນຕາຕະລາງສັກ ຢາກັນພະຍາດ ໃນ ສປປ ລາວ ແຕ່ໄດ້ຖືກນໍາໃຊ້ໃນຊ່ວງທີ່ມີການ ລະບາດ. ຂໍ້ມູນຂອງພວກເຮົາພົບວ່າ ອັດຕາການຕິດເຊື້ອແມ່ນສູງ ໃນກຸ່ມເດັກນ້ອຍ. ພວກເຮົາໄດ້ລາຍງານຜົນໃຫ້ກັບທາງກະຊວງສາ ທາ ລະນະສຸກ ແລະ ໄດ້ຕີພິມລົງໃນອາລະສານສາກົນ.

ເດືອນກັນຍາ 2021, ດຣ ວິໄລສອນ ຂຸນວິສິດ ໄດ້ໄປຮຽນປະລິນຍາເອກ ທີ່ Swiss Tropical and Public Health Institute ໃນ Basel ປະເທດ ສະວິສເຊີແລນ ການຮ່ວມມືລະຫວ່າງສະຖາບັນປັດສະເຕີ ແລະ ສະຖາບັນສາທາລະນະສຸກສາດ ຈະໃຫ້ ວິໄລສອນ ໄດ້ຮັບການຝຶກ ອົບຮົມເພີ່ມເຕີມທາງດ້ານສະຖິຕິ ແລະ ລະບາດວິທະຍາ ແລະ ວິຊາອື່ນໆ ແລະ ຫົວຂໍ້ວິໄຈແມ່ນຈະໄດ້ສຶກສາຄວາມສໍາພັນລະຫວ່າງ

ນ້ຳສະອາດ ແລະ ລະດັບຄວາມສະອາດ ແລະ ການຕິດເຊື້ອພະຍາດໃນ ສປປ ລາວ ນອກຈາກນີ້ການຮ່ວມມືກັບສະຖາບັນສາທາລະນະສຸກສາດ (the Lao Tropical and Public Health Institute; LTPHI), ພວກເຮົາຍັງໄດ້ເປັນທີ່ປຶກສາໃຫ້ກັບນັກສຶກສາປະລິນຍາໂທສອງຄົນ ແລະ ໄດ້ສຳເລັດຫົວຂໍ້ວິໄຈກ່ຽວກັບ ພະຍາດໄວຣັສຕັບອັກເສບບີ ໃນພະນັກງານແພດ ແລະ ການ ສຶກສາ ທາງດ້ານເຊຣອມວິທະຍາພະຍາດຄໍຕີບບາດທະຍັກ ໃນໄວໜຸ່ມລາວ ລາຍລະອຽດຂອງການສຶກສານີ້ຈະໄດ້ລາຍງານຕໍ່ໄປນັບແຕ່ເດືອນກັນຍານີ້ ທ່ານ ຫລາດ ສາລິວັນ ພະນັກງານແພດທະຫານ ຈະມາຝຶກອົບຮົມທັກສະທາງດ້ານວິໄຈເປັນເວລາ 6-9 ເດືອນ ພາຍໃຕ້ໂຄງການ ອາໂບຊິວ ປີນີ້ມີຂໍ້ຈຳກັດ ເນື່ອງຈາກການລະບາດຂອງໂຄວິດ ເຮັດ ໃຫ້ ຈຳກັດຊ່ຽວຊານຕ່າງປະເທດທີ່ຈະມາຢ້ຽມຢາມຖອດຖອນບົດ ຮຽນ ການສ້າງຂີດຄວາມສາມາດຮ່ວມຈາກນັກວິທະຍາສາດຈາກແລບ ດຣ ພອນທິບສະຫວັນ ນວນທອງ ໄດ້ຮັບຜິດຊອບຫນ້າທີ່ເປັນເລ ຂາ ທິການຂອງຄະນະກຳມະການທີ່ປຶກສາວັກຊີນແຫ່ງຊາດ (Nation ImmunizationTechnicalAdvisoryGroup;NITAG)ແລະ ຊ່ວຍວຽກງານສັກວັກຊີນແຫ່ງຊາດ ໂດຍສະເພາະການນຳໃຊ້ວັກຊີນ ທາງດ້ານວິຊາການ ສ້າງ ແລະ ພັດທະນາທົບທວນຄູ່ມື ການຝຶກອົບ ຮົມແກ່ພະນັກງານຝຶກອົບຮົມລະດັບຊາດ ລະດັບແຂວງ ແລະ ຮ່ວມ ວຽກງານຕິດຕາມໃຫ້ຄຳແນະນຳ ແລະ ປະເມີນຜົນ ການຈັດຕັ້ງປະ ຕິບັດວຽກງານການໃຫ້ວັກຊີນໂຄວິດ ແລະ ຊ່ວຍ ສຸມວິເຄາະ ແລະ ລະບາດວິທະຍາແຫ່ງຊາດ ໃນການທົບທວນການລະບາດພະຍາດ ແລະ ພ້ອມທັງຮ່ວມມືກັບມະຫາວິທະຍາໄລ ວິທະຍາສາດ ສຸຂະພາບ ໃນການສ້າງຮູບແບບຈຳລອງໃນການປະເມີນປະສິດທິພາບຂອງການ ນຳໃຊ້ວັກຊີນ ແລະ ການເຂົ້າເຖິງວັກຊີນອີກດ້ວຍ.

ການປະຊຸມ ແລະ ການນຳສະເໜີ

ກອງປະຊຸມວິທະຍາສາດ ຂອງບັນດາປະເທດ ເອີຣົບ ກ່ຽວກັບລະບາດວິທະຍາ ຂອງພະຍາດຊຶມເຊື້ອ *European Scientific Conference on Applied Infectious Disease Epidemiology*, ການນຳສະເໜີແບບ ອອນໄລ, ວັນທີ16 ເດືອນພະຈິກ 2021

ການວິເຄາະຕົວຢ່າງຈາກຜູ້ບໍລິຈາກເລືອດໃນ 8 ແຂວງໃນ ສປປ ລາວ, ເຊິ່ງການສຶກສານີ້ໄດ້ສະແດງໃຫ້ເຫັນການປ່ຽນ ແປງຂອງຜູ້ ທີ່ສຳພັດກັບພະຍາດຕັບອັກເສບ ບີ (HBV) ແລະ ຜູ້ທີ່ຕິດເຊື້ອ ການນຳສະເໜີແບບໂປສເຕີ, ໂດຍ ນາງ ລີຊ່າ ເຮເຟ່ເລ້ *Global health: viruses, liver and cancers 2021*, ການນຳສະເໜີແບບ ອອນໄລ *18-24* ກໍລະກົດ *2021*

ການເຝິກອົບຮົມ ໂດຍ ພະນັກງານຈາກ ຫ້ອງທົດລອງ ພະຍາດທີ່ປ້ອງກັນດ້ວຍວັກຊີນ

ການເຝິກອົບຮົມໃຫ້ພະນັກງານທະຫານພາຍໃຕ້ໂຄງການ Arboshield plus (DTRA): ຄວາມປອດໄພ ແລະ ຄວາມຫມັ້ນ ຄົງທາງດ້ານຊີວະພາບ, ຊີວະວິທະຍາທົ່ວໄປ, ພື້ນຖານ ຂອງລະບົບ ພູມຄຸ້ມກັນວິທະຍາ.

ເປັນທີ່ປຶກສາ ໃຫ້ກັບນັກຮຽນປະລິນຍາໂທຈາກ ສະຖາບັນ ສາທາລະ ນະສຸກສາດ ແລະ ການແພດເຂດຮ້ອນ

ດຣ ສອນເພັດ ວັນຕະວາ Tetanus and diphtheria seroprotection in adolescents from Bolhikhamxay and Vientiane Capital - ການສຶກສາພູມຄຸ້ມກັນ ພະຍາດຄໍຕີບ ແລະ ບາທະຍັກ ໃນປະຊາກອນໄວໜຸ່ມຈາກແຂວງບໍລິຄຳໄຊ ແລະ ນະຄອນຫລວງວຽງຈັນ

ດຣ ຂັນໄຊຍະພອນ ພະກຸນທອງ Hepatitis B virus exposure, seroprotection and current infection in Lao Healthcare workers - ການເຄີຍສຳພັດ, ພູມຄຸ້ມກັນ ແລະ ການຕິດເຊື້ອ ໄວຣັສຕັບອັກເສບບີ ໃນພະນັກງານແພດຫມໍ

ເປັນທີ່ປຶກສາ ໃຫ້ນັກສຶກສາປະລິນຍາ ໂທ ຈາກມະຫາວິທະຍາໄລ ອູ Trude Dekker Trends and factors determining hepatitis B birth dose and pentavalent vaccination coverage over time

Low seroprevalence of COVID-19 in Lao PDR, late 2020

Blood sample collection from participants in community-based cohort, village office, Luangprabang province

Project Coordinator: Siriphone Virachith, Vilaysone Khounvisith, Antony Black

Other IPL laboratories: Virginie Pommelet, Elodie Calvez, Khamsing Vongphayloth, Vincent Lacoste, Somphavanh Somlor, Darouny Phonekeo, Paul T. Brey

Collaborating institutions: Lao Tropical Public Health Institute, Lao University of Health Sciences, National Centre for Laboratory and Epidemiology, Institut Pasteur, Paris, Luxembourg Institute of Health

Communications: report to collaborators, written report to International Pasteur Network, manuscript accepted for publication, online presentation to Pasteur COVID Scientific Meeting

Background

The Lao National COVID-19 task force was established in February 2020, approximately 3 months after the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; the causative agent of COVID-19) in Wuhan, China. In the middle of March 2020, the task force implemented restrictions such as school closure (until May 2020) and a lockdown was implemented the 20th of March until the 3rd of May. Domestic flights were cancelled until May 15, and a restriction on most international flights was started.

From January 2020, testing for COVID-19, as recommended by WHO guidelines, was conducted on nasopharyngeal and oropharyngeal swabs using real-time reverse transcription polymerase chain reaction (RT-PCR). This testing was done on suspected cases – those with symptoms including fever and at least one of the following: cough, runny nose, sore throat, shortness of breath or difficulty to breath - and their contacts. Travelers into and out of Lao PDR were also tested by the same technique and a 14-day quarantine was imposed on all arrivals.

Although the first case of COVID-19 in Lao PDR was confirmed early - on March 24, 2020 - there were less than 40 SARS-CoV-2 positive confirmed cases in total in 2020 and no recorded COVID-19-related deaths. This low number contrasted with those of several southeast Asian countries and was among the lowest proportionally worldwide.

The low number of confirmed COVID-19 cases in 2020 could largely be due to the strict control measures set in place by the Lao Government. Another reason could be the relatively low population density limiting the spread of the virus or the young age structure of the population; 51% are under 24 years old and less than 7% over 60. This young population may allow mild or asymptomatic infections (approximately 80% of COVID-19 cases) to go undetected. This is particularly relevant in the context of countries, such as Lao PDR, where routine disease surveillance is challenging.

Healthcare workers are a particular risk group who may come into contact with COVID-19 patients. Importantly, the seroprevalence of anti-SARS-CoV-2 antibodies within the healthcare settings can offer insight into the effectiveness of infection prevention and control measures. Another potential risk group for infection are individuals in close contact with wildlife including bats, one of the natural reservoirs of coronaviruses, possibly including SARS-CoV-2 or its progenitor. The large diversity of bat species in Lao PDR and the geographical closeness of the Hubei province in China where SARS-CoV-2 was first detected suggest that such viruses could naturally circulate in Lao PDR.

In this context, the seroprevalence of antibodies against SARS-CoV-2 before vaccine introduction is a crucial indicator of virus exposure and can guide prevention measures. Recent studies have demonstrated an heterogeneous anti-SARS-CoV-2 seroprevalence depending on the location and serological studies can indicate the circulation of SARS-CoV-2 in the absence of detection by routine surveillance.

Thus, the aim of this study was to determine the seroprevalence of anti-SARS-CoV-2 antibodies in the general population, healthcare workers, and individuals with close contact to bats/wildlife in Lao PDR.

Methods

Population selection and recruitment

In this study, three cross-sectional seroprevalence surveys were conducted in parallel from August to September 2020: a population-based survey and two sub-surveys focusing on two high-risk groups: healthcare workers and guano-collectors, wildlife traders and people in close contacts with bats.

For the population-based survey and the healthcare workers survey, five provinces were selected: Vientiane Capital, two provinces in the North-Luang-Prabang, Oudomxay and two provinces in the South -Savanakhet and Champassak (Figure 1).

Figure 1. Map of Lao PDR showing study locations.

Participants of the population-based survey were selected using a multistage cluster sampling design to select households in the five provinces. In each province, districts were randomly selected and considered as strata. In each district, villages were then randomly selected using probability-proportional-to-size (PPS) sampling method using official lists of villages based on the population data from the 2015 census. A fixed number of households were then randomly selected in each village and in each household; all inhabitants aged five and above were invited to participate. In Vientiane Capital, four districts were randomly selected, then nine villages were selected using the PPS method and six households were randomly selected. In the four other provinces, three districts were selected per province; six villages and five households of each village were selected (see sample size calculation).

In Vientiane Capital, the survey focusing on healthcare workers was conducted in four central hospitals and in two district hospitals.

In the four other provinces, only healthcare workers working in the provincial hospitals were included. All staff assigned to dedicated COVID-19 teams were invited to participate -including clinical and non-clinical staff (e.g administrative staff, drivers, cleaners etc). In central and provincial hospitals, nurses, doctors and lab-technicians working in emergency departments (ER), outpatient departments (OPD), intensive care units (ICU), infectious disease departments, inpatient departments (IPD) and paediatric departments were randomly selected maintaining the nurses to doctors ratio in each department. In the two district hospitals, staff working in the OPD and ER were randomly selected.

For the wildlife contacts, four villages in Feung District, Vientiane province, were visited in September 2020. The villages were selected as they are locations where the profession of bat guano collecting - for selling as fertilizer – is common. Villagers who had contact with bats or other wildlife were informed about the study by the head of their village and invited to participate.

5mL of whole blood was collected into dry collection tubes and allowed to clot. Serum was separated by centrifugation and stored at 4°C for a maximum of seven days until storage at -80°C.

Sample size calculation

For the high-risk groups, we aimed to recruit around 900 healthcare workers in the three regions and 100 bat/wildlife contacts in Vientiane Province.
In order to establish the cut-off values and validate the anti-N and anti-S ELISAs, 265 pre-COVID-19 samples were tested. These samples were collected as part of a separate hospital-based serostudy in the South of Lao PDR in 2018 (ethics approval number NECHR018/2017). In addition, serum from 15 confirmed SARS-CoV-2 patients from Lao PDR, collected between four and nine weeks after diagnosis and ten longitudinal samples from one patient (diagnosed in France and living in Lao PDR) were used after consent was obtained.

Antibody detection

SARS-CoV-2-specific anti-nucleocapsid (N) and anti-spike (S) antibodies were detected by ELISA. IgM/IgG rapid tests were done according to manufacturer's instructions. These tests are reported by the manufacturer to have sensitivity and specificity of 100% and approximately 99%, respectively.

Ethics approval

The study protocol was approved by all participating institutes and by the Lao National Ethics Committee for Health Research (NECHR) (Ref #052/2020). Oral and written informed consent was obtained from people aged 15 years and older. Parental consent was taken for children aged between 5 and 11 years, and assent from children aged between 12 and 14 years in addition to parental consent, before the survey.

Results

Participants

A total of 3173 participants were recruited between the 12th of August and the 25th of September 2020. This consisted of participants from the general population (n=2433; mean age 42.6years (range 5-55) and healthcare workers (n=666; mean age 36.8 years (range 20-65)) from two Northern, one Central and two Southern provinces (Table 1 and Figure 1). Seventy-four serum samples were collected from bat/wildlife contacts; 12 bat guano collectors, five wildlife animal vendors and 57 bat contacts, catchers, or bat guano collectors' families in Vientiane Province (mean age 43.5years (range 17-86)).

1593 individuals were unavailable for the study due to reasons such as "working" or "at school" and were therefore replaced by other randomized participants from the same location. Only two healthcare workers and six people from the community-based cohort refused to participate in the study due to fear of needles and unwillingness to answer questions.

Table 1. Participant demographics

	Community-based n (%)	Healthcare workers n (%)	Bat/wildlife contacts n (%)
Age			
≤ 18 years	233 (9.6)	-	5 (6.8)
19-40 years	849 (34.9)	448 (67.3)	30 (40.5)
41-60 years	1000 (40.1)	217 (32.6)	28 (37.8)
> 60 years	351 (14.4)	1 (0.1)	11 (14.9)
Sex			
Female	1414 (58.1)	518 (77.8)	39 (52.7)
Male	1019 (41.9)	148 (22.2)	35 (47.3)
Province			
Vientiane Capital	746 (30.7)	410 (61.6)	
Luangprabang	465 (19.1)	47 (9.2)	
Oudomxay	396 (16.3)	31 (4.6)	
Savanakhet	412 (16.9)	106 (15.9)	
Champassak	414 (17.0)	72 (10.8)	
Vientiane Province	-	-	74 (100)
Travel outside of laos since December 2019? *	101 (4.1)	42 (6.3)	4 (5.4)
Thailand	87 (3.6)	29 (4.4)	3 (4.1)
Other**	11 (0.5)	12 (1.8)	1 (1.4)
Total	**2433**	**666**	**74**

*Missing country of travel for 4 participants

**China, Vietnam, Korea

Anti-N and anti-S antibody serology

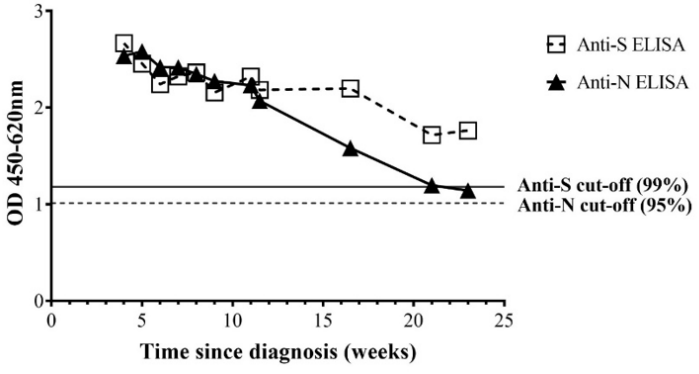
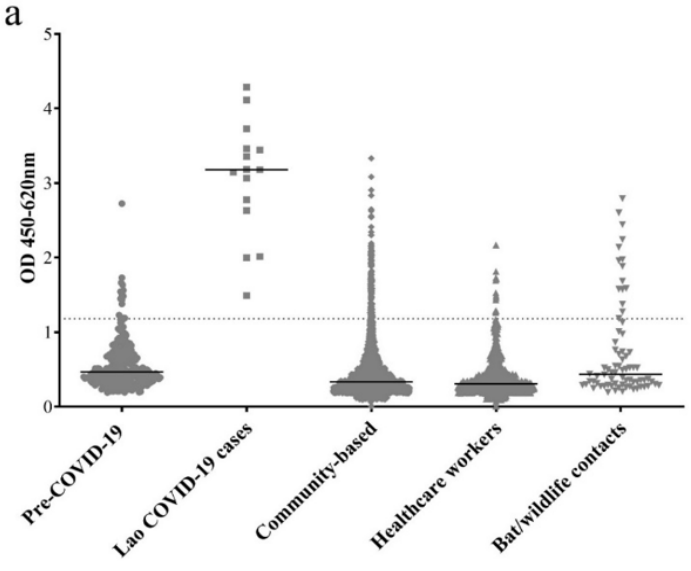


Figure 2. Longitudinal anti-N and anti-S antibody serology. Data represent mean of duplicate results from a COVID-19 positive individual (diagnosed in France, end of August 2020).

Cut-offs of 95% and 99% were set for defining anti-N and S antibody seropositivity, using pre-COVID-19 samples taken from individuals in the South of Lao PDR in 2018. Thus, 14/265 (5.3% [3.1-8.7%]) pre-COVID-19 samples were positive for anti-N antibodies. Three pre-COVID-19 samples were positive for anti-S antibodies (1.1% [0.3-3.5%]). No pre-COVID-19 samples were double-positive for anti-N and anti-S antibodies and 3 double-negative samples (1.1%) were anti-SARS-CoV-2 IgM positive (1.1%) and one IgG positive (0.4%) by rapid test. Longitudinal samples from the French confirmed case with mild symptoms showed double-positive N and S antibody seropositivity and were rapid test positive until at least 21 weeks post diagnosis despite a decline in ELISA OD (Figure 2). Samples from confirmed COVID-19 cases identified by the national COVID-19 surveillance in Lao PDR (n=15) were all double-positive for anti N- and S-antibodies and rapid test positive (Figure 3).



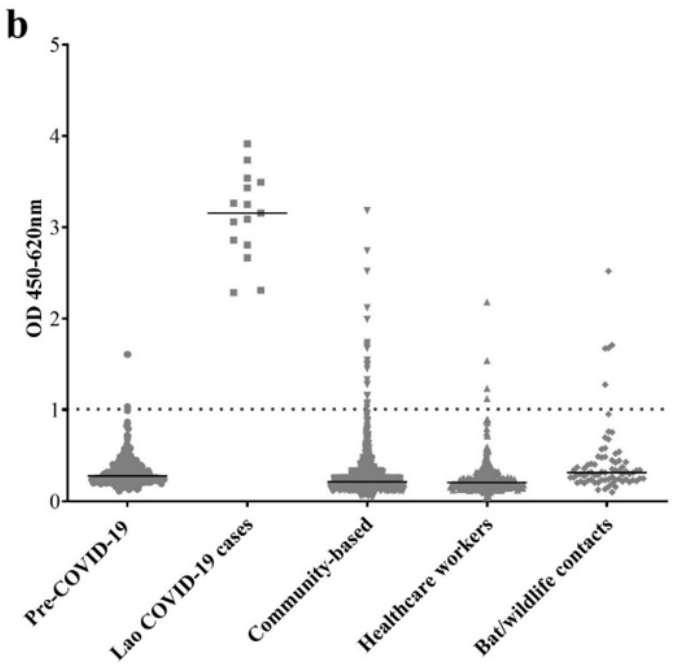


Figure 3. a. Anti-N antibodies b. Anti-S antibodies. Data show average of duplicate OD values for each sample. The dotted line represents the OD of 95% cut-off for anti-N antibodies and 99% cut-off for anti-S antibodies, established from the pre-COVID-19 samples. The solid lines represent the median OD.

In the participant samples from 2020, anti-N antibodies were detected in only 131/2433 (5.4% [4.5-6.3%]) of the general population, 13/666 (2.0% [1.3-3.3%]) of the healthcare workers but 15/74 (20.3% [12.6-31.0%]) of the bat/wildlife contacts.

Only 3/159 (1.9% [0.6-5.7%]) of the anti-N antibody positive samples were positive by rapid test (one IgM single positive and two IgM/IgG double positive). These samples were all anti-S antibody negative and therefore they were not considered as true "COVID-19 cases". Due to limited reagents, anti-S antibody ELISAs were done on a smaller selection of participants (n=1417), including all anti-N antibody positive participants and all bat/wildlife contacts plus a randomly selected sample from the general population and healthcare workers. Anti-S antibodies were detected in only 19/1061 (1.8% [1.2-2.8%]) of the general population, 4/282 (1.4% [0.3-3.7%]) of the healthcare workers and 5/74 (6.8% [2.8-15.3%]) of the bat/wildlife contacts. Only two out of all 2020 participant samples (2/3173; 0.1%) were double-positive for both anti-N and anti-S antibodies (Figure 4). These two samples were from the general population and were negative by anti-SARS-CoV-2 IgM/IgG rapid test and therefore also considered unlikely to be true "COVID-19 cases".

Only one of the anti-S antibody positive samples from the 2020 participants was rapid test positive (IgG). This participant was a bat/wildlife contact (anti-N antibody negative) aged 74 years. He had worked previously as a guano collector in caves. Two members of his family tested negative for anti-N and anti-S antibodies although his daughter had travelled to Thailand five months previously.

Within the general population, anti-N antibody positive status increased with age from 3.9% before 18 years to 8.8% above 61 years (p<0.0001, using score test for trend of odds). No other parameters were associated with anti-N or S antibody status in any cohort.

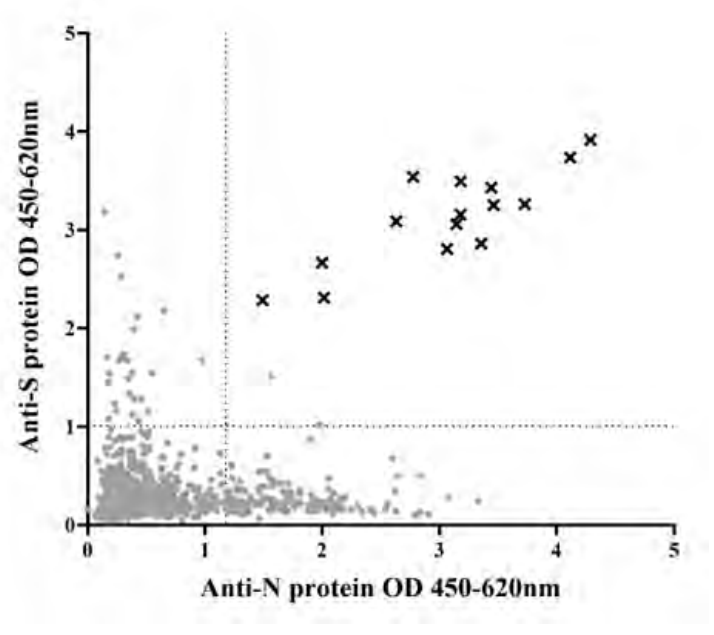


Figure 4. Relationship between anti-N and anti-S antibody amounts. Points show mean OD of individual samples from the general population, healthcare workers, and bat/wildlife contacts. Crosses represent mean OD of 15 confirmed SARS-CoV-2 cases, identified by the Lao national surveillance. Dotted lines represent 95% cut-off value for anti-N antibodies and 99% cut-off for anti-S antibodies.

Discussion

In this study, performed in Lao PDR from August until September 2020, the seroprevalence of anti-SARS-CoV-2 antibodies in the general population and healthcare workers was extremely low. These data likely reflect the low incidence of COVID-19 within the country and are in agreement with the few confirmed cases to date and low reported rates of hospitalisation of patients with respiratory illnesses in Lao PDR.

Why did Lao PDR remain largely free of COVID-19 in 2020, in contrast to many other countries? The answer is likely a combination of several factors. Firstly, the Government of Lao PDR implemented a robust control of visitors from outside the country, starting in March 2020. This included greatly restricting the number of commercial flights, and the requirement of all visitors to have proof of a negative SARS-CoV-2 RT-PCR test within the previous 72 hours, a negative test upon arrival, and to undertake a 14-day quarantine upon arrival. Even before such measures, Lao PDR had a relatively small number of international arrivals and, additionally, the land border point-of-entries are mostly connected to low-density areas of neighbouring countries. In addition, the rapid and decisive response of the Government of Lao PDR and other countries in the Lower Mekong region perhaps reflects a greater preparedness for pandemic outbreaks, born out of experience with SARS-CoV-1 in 2002-2003 and several avian influenza outbreaks in recent years.

An association between low population density and reduced COVID-19 incidence has been shown in some studies but not others. Thus, even though the population is not evenly distributed throughout the country, the low population density in Lao PDR -approximately 32 people per km2 - may have played some role in limiting the spread of COVID-19 to date. In addition, as younger people are more likely to be asymptomatic, the young age structure of the population may have reduced the number of symptomatic cases and disease-associated mortality. The lack of a mass-transit system could be another factor.

We cannot discount that some cultural or societal factors played a role, such as a low number of people travelling within the country. Physical contact may be lower in Lao PDR, for example, shaking hands is not common and hand-holding in public is rare, while wearing of face-masks is often practiced. Lastly, it has been speculated that climate may have some role in the spread of SAR-CoV-2, with spread being limited in warmer and more humid environments. However, this is unlikely to have made a significant impact in Lao PDR, given the high rates of infection in countries with similar climates.

N and S proteins from SARS-CoV-2 have been shown to be highly immunogenic, with anti-S antibodies being more specific. In our study, pre-COVID-19 controls from 2018 had anti-N and anti-S antibody seroprevalence of 5.3% and 1.1%, respectively, with none seropositive for both although three (1.1%) were positive by IgM rapid test. Seroprevalence studies in France observed similar pre-COVID-19 levels of anti-SARS-CoV-2, likely representing cross-reaction with non-SARS-CoV-2 coronaviruses. In the 2020 study participants, we found that there were only 159/3173 (5.0%) seropositive for anti-N antibodies and only 28/1417 (2.0%) were seropositive for anti-S antibodies. Only two participants (0.1%) were double-seropositive for anti-S and -N antibodies. These participants were negative by IgM/IgG rapid test and likely represent false positives as the prevalence was similar to the pre-COVID-19 controls. Some of the "single-positives" may reflect true cross-reactivity with other unknown or seasonal alpha- and beta- coronaviruses such as HKU1, NL63, OC43 and 229E. Notably, it is thought that most pre-existing cross-reactive antibodies do not confer protection against SARS-CoV-2 (38). Although a prior infection with SARS-CoV-1, the causative agent of SARS, may confer cross-reactivity to SARS-CoV-2 due to neutralizing activity of antibodies against the highly homologous Receptor Binding Domain, such instances are probably insignificant in Lao PDR. It is nevertheless possible that exposure to other coronaviruses occurs in Lao PDR given the frequent trade in bush meat and low biosafety awareness.

Indeed, it has been shown that bats and rodents from Lao PDR and bordering countries can be infected with a diverse array of alpha- and beta- coronaviruses including SARS-CoV-2–like viruses. The participants in our study who had frequent contact with bats or other wildlife had high anti-N antibody seroprevalence. Also, one bat/wildlife contact whose daughter had been to Thailand five months previously was anti-S antibody positive, anti-N antibody negative and rapid test (IgG) positive. This could be due to exposure to coronaviruses from wildlife although we have no control group from the same location, and anti-S antibodies are thought to be less cross-reactive than anti-N antibodies. Neutralisation assays were not performed on these samples and it remains possible that there was a localized COVID-19 outbreak in this population. Therefore, in addition to coronavirus detection and typing among the bats and other wildlife populations, these particular populations deserve further studies.

Anti-SARS-CoV-2 antibodies in mild cases are thought to be lost rapidly – up to 20% of such cases in neighbouring Thailand were shown to be seronegative within two weeks after onset of symptoms. It is therefore possible that some of the participants in the current study were infected with SARS-CoV-2 and did not seroconvert or had lost antibodies by the time of the study. However, similar to other studies, longitudinal samples from one patient who was infected in late August 2020 and presented only mild symptoms were seropositive for anti-N antibody until at least week 21 after diagnosis and longer for anti-S antibodies.

The main limitation of this study was that only five of the 18 Lao provinces were purposively selected. However, these provinces were selected due to a perceived high risk of SARS-CoV-2 introduction (international flight arrivals into Luang Prabang and Vientiane Capital, large numbers of Chinese migrant workers in Oudomxay, and migrant workers returning to Lao PDR from Thailand in Champassack and Savannakhet). Nevertheless, it is possible that we missed areas where SARS-CoV-2 virus entered e.g. from neighbouring countries in the North.

Other limitations included the non-representative nature of the age structure of the sampled population. Lastly, clarification regarding the cross-reactive nature of the few anti-N and anti-S antibodies detected in participants and pre-COVID-19 samples could be determined by neutralisation assays, which were not available to us at the time of the study. Despite these caveats, we believe that it is highly unlikely that the results would change in any meaningful way given the sample size and the three different tests used.

In summary, our data indicate that Lao PDR remained largely free of COVID-19, at least until late 2020. The Government of Lao PDR should be commended on the swift and decisive action taken to reduce the risk of SARS-CoV-2 introduction into the country. Nevertheless, Lao PDR remains at risk of virus introduction, either from neighbouring countries or further afield. The borders cannot remain closed indefinitely and therefore other measures need to be strengthened such as active surveillance, vaccination of at-risk groups and vaccination status checks at points of entry. With the rapid development and opening up of the country, such as the imminent launch of the high-speed rail system connecting China, Lao PDR and Thailand, this is as pertinent as ever. Lastly, the high seroprevalence of anti-N antibodies in bat/wildlife contacts in Lao PDR is a stark reminder that the threat of emergence of future pandemics is real and that steps need to be taken to mitigate and prepare for them.

Age-stratified seroprevalence of vaccine-preventable infectious disease in Saravan, Southern Lao People's Democratic Republic

VPD staff working at IPL

Project Coordinator: Kinnaly Xaydalasouk, Vilaysone Khounvisith, Antony Black

Collaborating institutions: Luxembourg Institute of Health, Saravan provincial and district hospitals.

Publications and communications: written report to provincial and district health office, Lao DCDC and WHO. Manuscript accepted for publication

Background

Routine infant vaccination within Lao PDR was formalized in the 1970s with the introduction of the Expanded Programme of Immunisation (EPI). Initially, this consisted of six vaccines but it was gradually expanded and now routine childhood vaccination includes; BCG and hepatitis B virus (HBV) vaccine at birth; pentavalent vaccine (Diphtheria-Tetanus-Pertussis-Hepatitis B-Haemophilus influenzae b; DTP-HepB-Hib), Polio vaccine, Pneumococcal vaccine at 6, 10 and 14 weeks; measles/rubella combined vaccine at 9-11 and 12-18 months.

Adult vaccination focuses on diphtheria/tetanus vaccination of pregnant women and women of childbearing age, in addition to various supplementary immunization activities (SIA) e.g. against polio, measles and rubella. Despite the comprehensive vaccination programme, outbreaks of vaccine-preventable diseases (VPD) continue to occur, such as polio, measles, diphtheria and pertussis. Outbreaks near to the borders with neighboring countries such as Vietnam have also raised fear of cross-border spread of disease.

Vaccination coverage of the HBV birth dose also remains low due to the high number of unattended home-births. As mother-to-child HBV transmission is believed to be the main route of infection in Lao PDR, HBV birth dose is key to reducing the burden of chronic infection, which is as high as 9% in adult males, and the consequent high levels of cirrhosis and liver cancer. Disease outbreaks often affect particular subgroups of the Lao population or remote regions of the country where vaccination coverage and immunogenicity are challenging.

Saravan province is a largely rural province situated in the south of Lao PDR. The provincial population was 396,942 in 2015 with eight districts, two of which border with Vietnam to the West and two bordering Thailand to the East. The main languages are Lao-Tai and Mone-Khmer and there are 14 main ethnic groups. Vaccination coverage is low, for example only 44.4% received HBV birth dose vaccination in 2017 (ranking 4th lowest out of 18 Lao provinces) and only 10.4% of women received at least two doses of tetanus vaccine during their last pregnancy (2nd lowest nationwide).

Due to its large rural population, ethnic diversity and poor health indicators and its borders with two neighboring countries, we aimed to determine the exposure and susceptibility of the population to VPD in Saravan province.

Methods

Participants

Participants aged 5 and over were recruited whilst attending the provincial hospital in Saravan town and 7 district hospitals for unrelated reasons (Khongxedone, Lakhonepheng, Toomlarn, Vapy, Lao Ngam, Samoui and Taoiy). Sample size was chosen according to feasibility. Thus, a maximum target size of 2500 male and female participants was chosen.

Both in-patients and out-patients were asked by the healthcare staff to participate in the study and sign the informed consent after having the study explained and reading the information sheet. In the case of participants unable to read Lao language, the healthcare workers would read the consent form and for participants unable to sign, a finger-print would be taken with the signature of a witness. The parents or guardians gave consent for children less than 18 years old.

After collecting demographic information (age, sex, home-town, place of birth, religion, ethnicity, occupation and marital status) the participants donated 5 mL of blood. HBsAg rapid testing was done by the participating hospital staff and results/counselling was given to the participants. The remaining blood was allowed to clot and serum separated by centrifugation. Serum was stored at -20°C at the hospital and then sent to Institut Pasteur du Laos where it was stored at -80°C until use. Antibodies were assessed by ELISA.

Ethics approval for the study was provided by the Lao National Ethics Committee for Health Research (reference 018/NECHR/2017)

Results

Population characteristics

Only two of the selected participants refused to participate due to fear of needles and blood and fear of stigmatisation depending on the blood test result. Therefore, a total of 2463 participants were enrolled. The median age was 28 years (range 5 to 90) and 57.0% were females. 40.2% of participants were from Lao Ngam district and 31.0%, 11.7% and 10.3% were from Saravan, Taoiy and Samoui districts, respectively. Less than 10% of the participants were recruited from the remaining districts. 59.8% of participants were married and the main occupation was farming (60.8%). There were 10 ethnic minority groups represented in the study with 52.9% following animism, and 46.5% Buddhism. Almost half of participants did not know their place of birth, however 46.7% stated that they had been born at home (Table 1). One serum sample had only enough volume to test for HBV markers, and therefore all other markers had a denominator of n=2462.

Table 1. Demographics of study population (or their parents)

Variable		n (%)
Sex	Male	1058 (43.0)
	Female	1405 (57.0)
Age	Median (range)	28 years (5 to 90)
District	Lao Ngam	990 (40.2)
	Saravan	764 (31.0)
	Taoiy	289 (11.7)
	Samoui	254 (10.3)
	Toomlarn	118 (4.8)
	Vapy	31 (1.3)
	Khongxedone	9 (0.4)
	Lakhonepheng	8 (0.3)
Ethnicity	Taoiy	356 (14.5)
	Lao	350 (14.2)
	Laven	324 (13.2)
	Suay	300 (12.2)
	Pako	279 (11.3)
	Katang	252 (10.2)
	Phouthai	168 (6.8)
	Katou	162 (6.6)
	Other (Alux/ Gnae/Kadeau/Tang)	155 (6.3)
	Inh	117 (4.8)
Place of birth	No information	1213 (49.2)
	Home	1151 (46.7)
	Hospital	99 (4.0)
Marital status	Married	1472 (59.8)
	Single	938 (38.1)
	Other (widow/divorced)	53 (2.2)
Occupation	Farmer	1498 (60.8)
	Student	692 (28.1)
	Government staff	161 (6.5)
	Unemployed	45 (1.8)
	Unknown	32 (1.3)
	Business person	22 (0.9)
	Other (monk, teacher, retired, soldier)	13 (0.5)
Belief	Animist	1304 (52.9)
	Buddhist	1145 (46.5)
	Christian	14 (0.6)

Serology

Hepatitis B virus (HBV)

Overall past exposure to HBV (anti-HBc positive) was 817/2463 (33.2%) (Table 2, Figure 1). After multivariate analysis, exposure increased significantly with age; those aged 5-10 years had 1.5% anti-HBc seropositivity compared to 62.4% in those more than 50 years old (OR 72.0 [24.5-211.7], p<0.0001). Males had significantly higher exposure than females (37.4% and 30.0%; OR 1.7 [1.3-2.0], p<0.0001). Ethnicity had only a small effect on exposure; Katou group had slightly higher exposure compared with the Inh group who had lowest exposure (OR 2.6 [1.3-5.2], p=0.003).

The prevalence of chronic (or acute) infection, as defined by HBsAg detection by ELISA, was 3.8% overall (Table 2, Figure 1). Prevalence was 0% in those 5-10 years old, increased until the age of about 20 and then stabilised at around 5%. Males had a significantly higher HBsAg prevalence (5.7%) than females (2.4%; OR 2.5 [1.6-3.9], p<0.0001).
The serological profile for vaccination (anti-HBs positive, anti-HBc negative) was only 9.9% overall and highest in those aged 5-10 years old (39.5%) (Table 2). None of the hepatitis B markers were associated with district or place of birth.

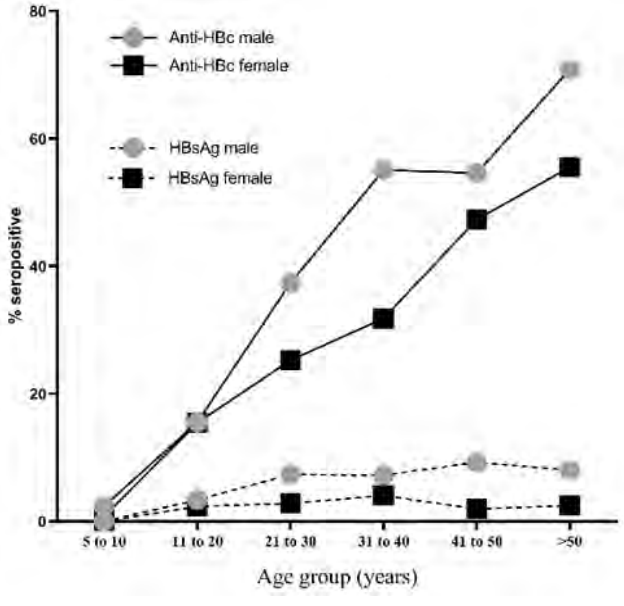


Figure 1. Age and sex-stratified HBV exposure and HBsAg seroprevalence

Table 2. Hepatitis B serological markers. Data show results from ELISA testing. All anti-HBs positive samples or anti-HBc negative samples were assumed to be HBsAg negative

Serological markers			No. samples (% of age group)						
Anti-HBs	Anti-HBc	HBsAg	5-10	11-20	21-30	31-40	41-50	>50	Total
-	-	-	152 (58.9)	467 (73.1)	310 (65.3)	182 (54.2)	145 (46.3)	146 (33.0)	1402 (56.9)
+	-	-	102 (39.5)	73 (11.4)	22 (4.6)	15 (4.5)	12 (3.8)	20 (4.5)	244 (9.9)
+	+	-	2 (0.8)	54 (8.5)	77 (16.2)	65 (19.3)	59 (18.8)	131 (29.6)	388 (15.8)
-	+	-	2 (0.8)	27 (4.2)	44 (9.3)	56 (16.7)	83 (26.5)	123 (27.8)	335 (13.6)
-	+	+	0 (0)	18 (2.8)	22 (4.6)	18 (5.4)	14 (4.5)	22 (5.0)	94 (3.8)

Tetanus and diphtheria

Overall 42.4% had "insufficient immunity" against tetanus and 11.3% had "low immunity". Sufficient immunity (titres >0.5 IU/ml) was detected in only 46.3%, with 14.2%, 27.9% and 4.2% who needed future booster doses in 2-5, 5-10 and 10 years. After multivariate analysis, sex had a large impact on protection; males (14.5%) were significantly less protected against tetanus than females (70.2%; OR 0.05 [0.04-0.07], p<0.0001). Overall levels of protection were highest in the age group 31-40 years (61.9%), compared to 5-10 year olds (40.7%; OR 2.9 [1.9-4.4], p<0.0001). This was mainly due to the increased titres and protection in older women. The level of protection in women over 20 years of age was 78.5%. Ethnicity, district, occupation and religion were not associated with level of tetanus protection (Figure 2).

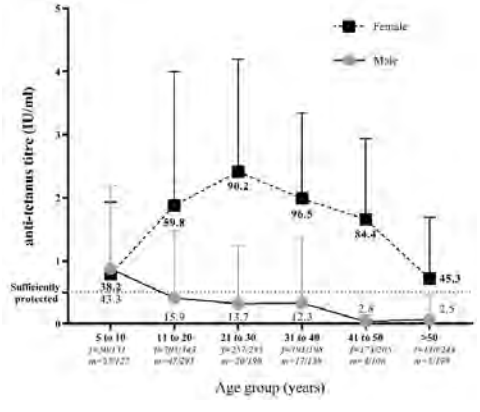


Figure 2. Age and sex-stratified average titres for anti-tetanus antibodies. Numbers represent percentage of protected males (standard font) and females (bold font).

Only 3.3% of participants overall had "no protection" against diphtheria and 56.2% had "uncertain protection". "Immunization protection" was present in 33.1% and only 7.3% had "long term protection". The highest protective levels were found in women aged 11-20 (53.6%). After multivariate analysis, seroprevalence of protective antibodies (titres > 0.1 IU/ml) was slightly lower in males (37.0%) than in females (43.0%; OR 0.7 [0.6-0.9], p=0.001). No other factors were associated with diphtheria protection (Figure 3).

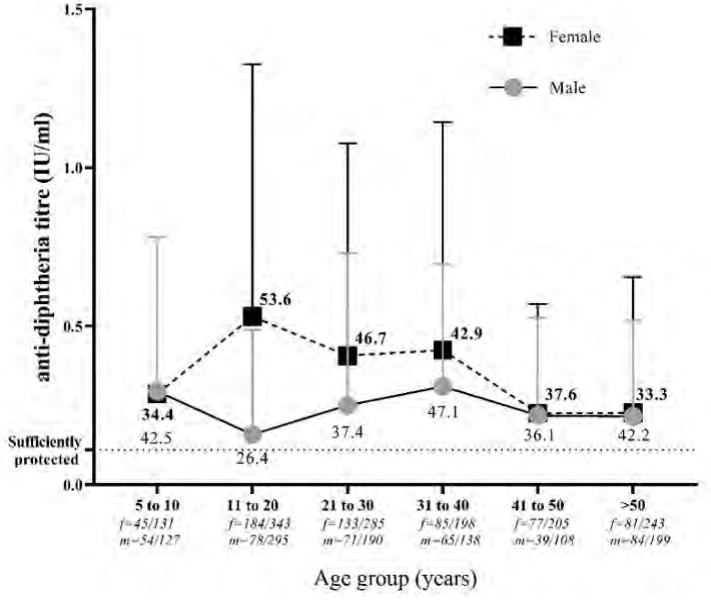


Figure 3. Age and sex-stratified average titres for anti-diphtheria antibodies. Numbers represent percentage of protected males (standard font) and females (bold font).

Measles and rubella

Most participants had immunity against measles (1808/2462; 73.4%) and 6.6% were borderline. Measles seroprevalence increased significantly with age from only 16.7% in the 5-10 year olds to 97.7% in those over 50 years (OR 145.9 [65.8-323.5], p<0.0001). Males had slightly lower prevalence of protective anti-measles antibodies (68.4%) than females (77.2%) at all ages (OR 0.6 [0.5-0.8], p<0.001).

There was a significant difference in anti-measles seroprevalence between some of the ethnic groups; namely, the Lao Tai group had lower seroprevalence (69.1%) than the Inh group (93.1% OR 0.2 [0.08-0.5], p=0.001). There was no significant association of protective anti-measles seroprevalence with any of the other parameters (Figure 4).

Anti-rubella IgG seroprevalence was high from an early age with 93.0% seroprotected overall and 1.5% with borderline results. Seroprevalence of protective antibodies varied by district, the lowest being in Khongsedone (77.8%), which was significantly different from the highest prevalence in Toomlarn (98.3%; OR 21.5 [2.5-182.9], p=0.005). There was no association of anti-rubella seroprevalence with ethnicity, sex or any other parameter (Figure 4).

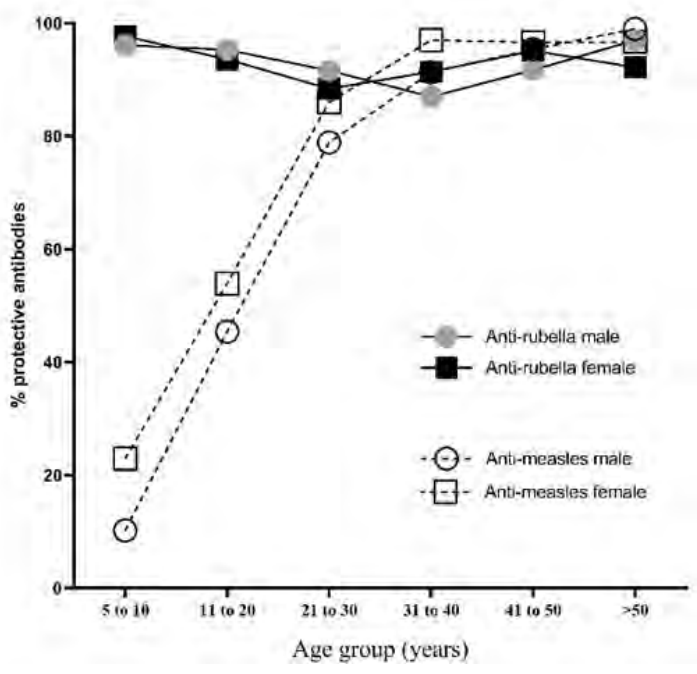


Figure 4. Sex and age-stratified measles and rubella seropositives (excluding borderline results)

Discussion

In order to reduce the high burden of HBV infection in Lao PDR, the vaccine was introduced at 6, 10 and 14 weeks of age between 2002 and 2004 onwards and was complemented by the birth dose in 2003/2004. In the current study in Saravan Province, in Southern Lao PDR, we show that HBV exposure (anti-HBc positive) increased significantly with age – from only 1.5% in those aged 5-10, to more than 60% in those older than 50. HBsAg prevalence also increased with age from 0% in the 5-10 year olds, to 2.8% in 11-20 year olds, and then stayed about 5% in those older than 20. The low level in the youngest age group indicates a positive impact of the introduction of the routine HBV infant vaccine and is in agreement with our previous data. Conversely, high levels of exposure and HBsAg in adults born before vaccine introduction are comparable to other studies of Lao adults from central Lao PDR but lower than those in the North of the country. As in previous studies by our group and others in Lao PDR and elsewhere, males had a higher prevalence of HBsAg (5.7%) and exposure (37.4%) than females (2.4% and 30.0%, respectively). This disparity started from a young age, and in other settings has been suggested to be a result of a difference in sex hormones. Despite this sex difference, HBsAg seroprevalence in women aged 20-50 was high at 2.9%. This indicates a significant risk of onward transmission via mother-to-child infection, which is thought to be the main route of infection in Lao PDR. Given that most chronic infections have been shown to be established during birth and the perinatal period, vaccination with the birth dose of HBV vaccine needs to be maintained in Saravan province. The vaccine serological profile (anti-HBs positive/anti-HBc negative) was only 39.5% in children 5-10 years old. According to the Lao Social Indicator study II (LSIS II), vaccination coverage with HBV birth dose in Saravan province was only 44.4% and only 60.2% of infants received all three DTP-HepB-HiB doses in 2017. Therefore the low percentage of people with vaccination serological profile that we see here and previously in central Lao PDR probably reflects low vaccination coverage and/or low vaccine immunogenicity and antibody waning.

The Katou group had 49.4% HBV exposure overall, which was significantly higher than the reference group (Inh) after multivariate analysis. This may reflect differences in vaccination coverage or risk practices although there were no significant differences in vaccination serology or HBsAg prevalence. Unfortunately, further analysis was not possible due to small numbers of Katou participants, therefore this warrants further investigations.

A positive impact of the Lao national immunisation programme was reflected in the elimination of maternal and neonatal tetanus in 2014. In the current study, more women were protected than males (70.2% and 14.5%, respectively) due to tetanus vaccination of all women aged between 15 and 49 years before and during pregnancy. The WHO recommends that at least 80% of women receive 2 or more doses of tetanus vaccine during pregnancy to maintain maternal neonatal tetanus elimination. Nevertheless, more than 20% of women in child-bearing age remain susceptible to tetanus. This corresponds to our earlier findings of low levels of tetanus vaccination in Savannakhet province in Lao PDR, challenging tetanus elimination, particularly in remote areas. We also found that males and children were particularly susceptible, suggesting the need to improve routine infant vaccination coverage and to monitor disease incidence in men.

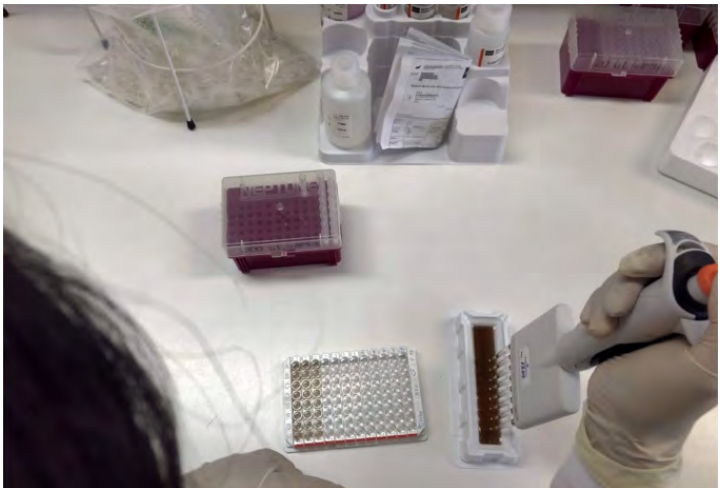
We detected a low level of protection against diphtheria in all age groups. Only 40.5% overall had antibody titres associated with sufficient immunity (> 0.1IU/ml). Although susceptibility was high in all age groups, this is particularly concerning in young children, who have highest risk of severe disease. Since the introduction of 3 doses of diphtheria-containing vaccine in infants more than 40 years ago, there has been a significant reduction in diphtheria outbreaks. Nevertheless, cases still occur, for example, there were 73 nationwide in 2019, emphasizing the need for further strengthening of routine vaccination coverage and vaccine management. The small but significant difference between seroprevalence in males (37.0%) compared with females (43.0%) is most likely due to the introduction of diphtheria vaccine to women of child-bearing age in the form of diphtheria-tetanus (dT) nationwide in 2012.

Seroprevalence of protective measles antibodies was less than 20% in 5-10 years old children, and only 50% in those 11-20 years old. This high susceptibility of the younger population is surprising, especially since Lao PDR introduced measles vaccine for children age 9-11 months in 1984, and a second dose in 2017 for children aged 12-18 months. The significant increase of measles seroprevalence in the older age groups probably indicates cumulative wild-type exposure with age or possibly inclusion in SIA such as that in 2011 which covered children aged 9 months to 19 years (approximately 9-30 years at the time of the study). Several recent measles outbreaks in Lao PDR, including 1119 reported cases in 2019, have likely resulted from low vaccination coverage and compromised vaccine immunogenicity. Interestingly, seroprevalence was particularly high in the Inh ethnic group (93.1%) perhaps reflecting wild-type virus circulation. There were only 117 participants from this ethnicity, and we could find no data on measles cases.

In contrast to measles, prevalence of protective anti-rubella antibodies was high in all age groups in our study. Given the lower measles seroprevalence in children and our recent data showing wild-type rubella circulation in Lao children, it is likely that the high rubella virus seroprevalence in children in Saravan is due to wild-type infection as well as vaccination. The different seroprevalences between districts may indeed reflect local outbreaks of the disease or different vaccine coverage. The high seroprevalence in adults is mostly due to natural virus infection, as the rubella containing M/R vaccine was only added to the childhood immunization schedule in 2011/12, although there have been subsequent SIA, which may partially account for some of the seropositivity in the age groups up to 30 years of age. In women aged over 20, the rubella seroprevalence was high (91.5%), indicating that the risk of infection during pregnancy and consequent Congenital Rubella Syndrome is low in this setting.

Overall, our data show that the routine childhood vaccination in Saravan needs to be strengthened. Regarding measles and rubella, further investigations are needed into the apparent low immunogenicity of the measles component of the vaccine. Indications of disparate vaccine coverage and/or disease exposure between districts and ethnicities also warrant further investigation. A booster dose of DT containing vaccine is suggested worldwide by WHO at the age of 12-23 months and later. We recommend that Lao PDR review this policy for the DTP-HepB-Hib vaccine, as indicated by the low diphtheria, tetanus and HBV protection in our study. In addition, the coverage of the birth dose of HBV vaccine needs improving, in order to reduce the early life exposure to HBV e.g. mother-to-child transmission.

Detection of Hepatitis C in the general population in Saravan Province, Lao People's Democratic Republic (PDR)



Running an ELISA in the lab

Project Coordinator: Kinnaly Xaydalasouk, Vilaysone Khounvisith, Antony Black

Collaborating institutions: Saravan provincial and district hospitals, Luxembourg Institute of Health

Publications and communications: written report to the Lao Department of Communicable Disease Control, local and regional WHO, manuscript accepted for publication

Background

Hepatitis C virus acute infection progresses to chronic infection in approximately 75-85% of cases with a consequent high risk of liver complications, cirrhosis and liver cancer. Transmission of the virus is via blood, for example by using non-sterilised equipment during medical procedures, tattooing or intravenous drug use. Sexual transmission is rare and mother-to-child vertical transmission occurs in approximately 6% of infected pregnant women.

There have been few studies on hepatitis C virus prevalence in Lao People's Democratic Republic (PDR). The Lao Red Cross report that less than 1% of the blood donors are anti-HCV positive. Similarly, studies in Lao healthcare workers and female garment factory workers in Vientiane found approximately 1% were anti-HCV positive.

The aim of this research was to determine the exposure and susceptibility to hepatitis C virus in the population in Saravan province, southern Lao PDR.

Methods

755 participants aged 5 to 90 years were recruited from Saravan province. Recruitment took place via the provincial and district hospitals. Serum samples from the participants were tested for exposure to hepatitis C virus (ELISA for IgG antibodies) and for vaccination/exposure to other infectious diseases. The study was approved by the Lao National Research Ethics Committee (ref 005/2018 NECHR).

Results

Anti-hepatitis C virus IgG antibodies were detected in 11.7% (88/755) of participants. Males had a slightly higher proportion of positives (13.7%) than females (10.0%).

When stratified by age, the majority of anti-hepatitis C positive cases were adults, with 19.9% seroprevalence in those aged more than 30 years and 4.4% in those 30 years or younger.

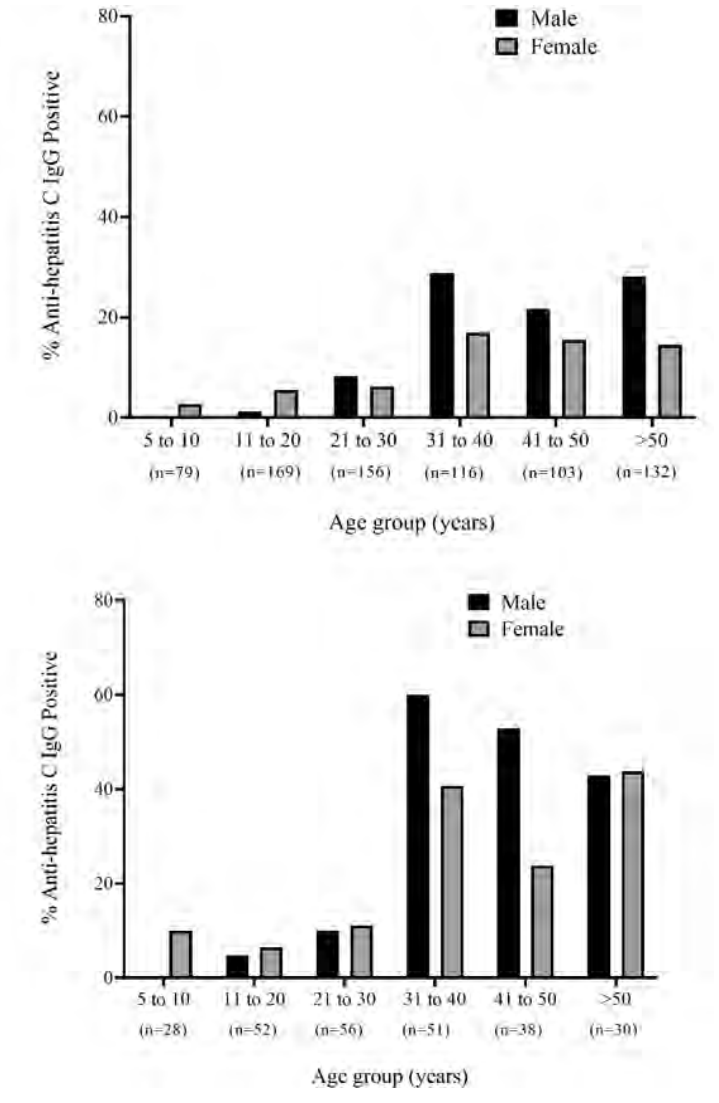
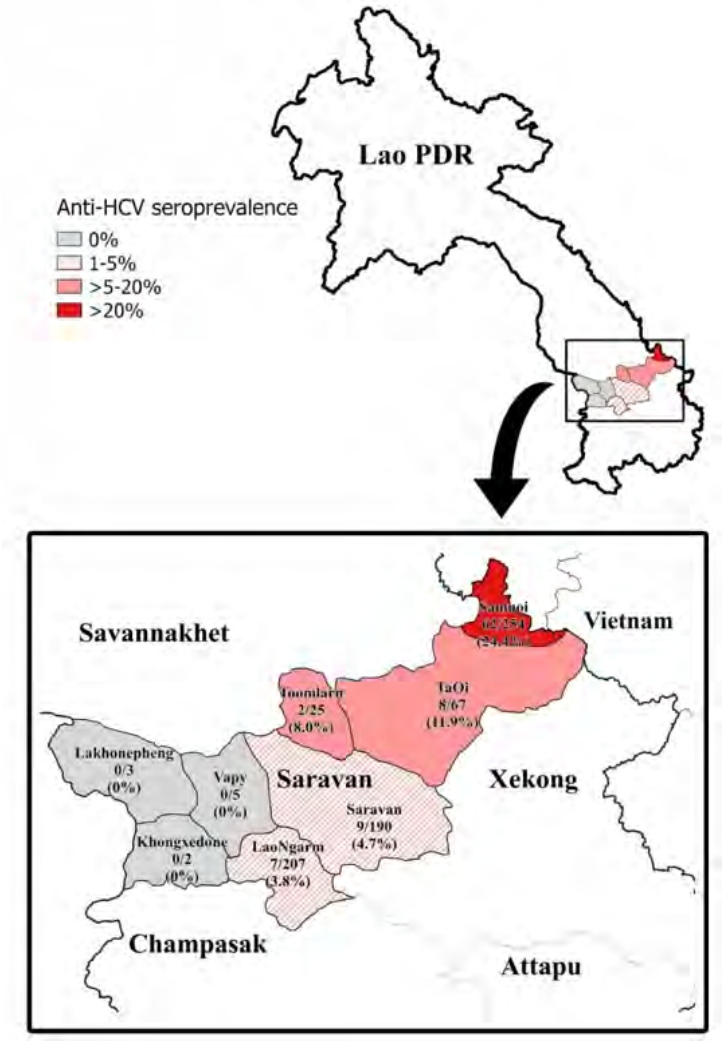


Figure 1. Anti-HCV seropositivity by age and sex in Saravan province (top graph) and Samuoi district (bottom graph). The numbers in brackets below the bars represent the total number of sera that were tested per age group

Seroprevalence was particularly high in Samuoi district, with 24.3% (62/255) overall and 43.7% (52/119) in those aged over 30 years. The prevalence in Samuoi was high whether they were attending the district hospital for reasons associated with hepatitis (acute hepatitis/fever/fever of unknown origin/hepatocellular carcinoma; 25.3%) or unrelated reasons (13.6%).

44/88 of the seropositive samples were sequenced and all found to belong to HCV genotype 6.



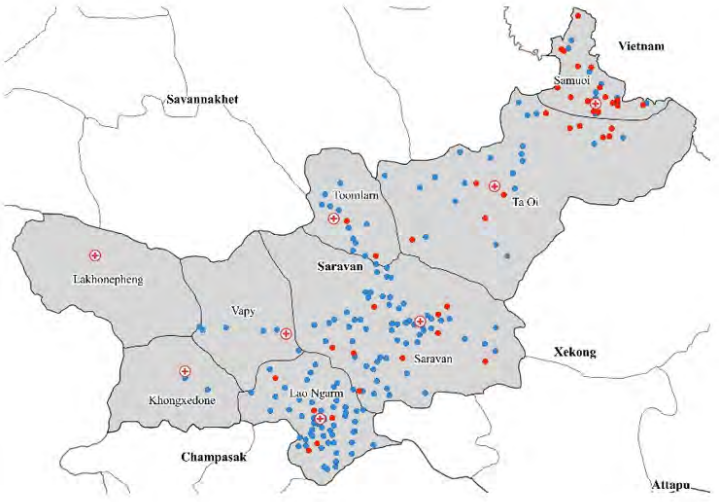


Figure 2. Map of Lao PDR showing location of Saravan province. Inset shows location of districts of Saravan province. Colours represent different seroprevalence levels (upper map).Location of villages with at least one anti-HCV seropositive participant (red dots) and villages where all participants were anti-HCV seronegative (blue dots; lower map)

Discussion

The high prevalence of exposure to hepatitis C virus in Saravan, particularly in adults from Samuoi province, is concerning and unprecedented. The high morbidity and mortality associated with chronic hepatitis C virus infection suggests that there will be a large proportion of the population with liver-related complications in the future.

In most countries worldwide, prevalence of hepatitis C infection is below 5%. Notably, in Egypt, mass anti-schistosome campaigns between the 1950s and 1980s facilitated the spread of hepatitis C by using contaminated medical equipment for administrating the intravenous medication. As a consequence, the prevalence of hepatitis C in Egyptian adults was more than 40% in 1996. In other countries with high incidence, the source of infection is also often iatrogenic.

In the current study in Saravan, it was not possible to determine the source of the hepatitis C virus infections, although the localised nature of the cases suggests an initial source of infection in the Samuoi area. The high prevalence in adults indicates either a) adults are at higher risk of infection or b) the adults were infected many years ago, during their childhood or early adulthood. Never-the-less, a substantial proportion of children are also infected (albeit to a much lesser degree), either by mother-to-child transmission or via the same route as the adults. Further studies are needed to investigate the source of infection and to assess the knowledge and practice related to HCV prevention.

Recommendations

At the time of the study, curative treatment for hepatitis C virus infection was not readily available in Lao PDR and it was not within the capacity of this study to test and treat participants for hepatitis C as indicated in our Ethics and informed consent documents. It was also not feasible to determine the source of infection e.g. by an in-depth case-control study. However, such approaches are warranted in the future, in order to mitigate the impact of this high burden in Saravan Province. Prevention strategies could be focused on Saravan province, in particular in Samuoi district. A follow-up study is planned in 2022.

VPD staff collecting blood from a participant in COVID-19 serostudy, Mitthaphab Hospital, Vientiane Capital

An age-stratified serosurvey against purified *Salmonella enterica serovar* Typhi antigens in the Lao People´s Democratic Republic

Project Coordinator: Lisa Hefele, Siriphone Virachith, Antony Black

Collaborating institutions: Wellcome Trust Major Overseas Programme, Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam, Luxembourg Institute of Health

Publications and communications: Presentation at the Typhoid vaccine stakeholder meeting, Vientiane (with MOH, WHO, GAVI); Manuscript submitted for publication.

Background

Typhoid fever is a systemic disease caused by the bacterium *Salmonella enterica*, subspecies enterica serovar Typhi (S. Typhi) and is transmitted through contaminated food or water. Blood culture is regarded as gold standard for the diagnosis of typhoid fever, but it is less sensitive than the more invasive bone marrow culture and requires the necessary laboratory capacities, which poses a challenge for many countries.

As an alternative, the serology-based Widal test is another method for the diagnosis of typhoid fever, although its utilization remains controversial. Due to the inadequate performance of the currently used diagnostic tests, the search for novel methods is on-going.

The investigation of serological markers for typhoid fever prevalence may aid overcoming diagnostic challenges and provide estimates of subclinical typhoid fever infections. A number of potential biomarkers have been proposed in previous research works. The antigens hemolysin E (HlyE) and cytolethal distending toxin subunit B homolog (CdtB) are expressed in S. Typhi and S. Paratyphi A, but only rarely in other Salmonella spp. Both antigens are considered to be immunogenic upon S. Typhi infection. Anti-HlyE IgG and anti-CdtB IgG have been previously suggested to be useful biomarkers in distinguishing typhoid fever cases from non-typhoid fever cases. Other studies focus on antibody responses against the immunogenic polysaccharide capsular Vi "virulence" antigen (anti-Vi) which presents the prime target for vaccine development. The Vi antigen is only present in S. Typhi, S. Dublin and S. Paratyphi C, but is absent from S. Paratyphi A and most of the gastroenteritis-causing serovars.

In the Lao PDR, tyhpoid fever is a notifiable disease. Cases of typhoid fever are reported to the National Center for Laboratory and Epidemiology in Vientiane. However, access to health care is still inadequate especially in remote areas and the capacity for blood culture testing in Lao PDR is limited to only three laboratories in the whole country. Currently, the requirements for an adequate typhoid fever surveillance are not given which hinders an assessment of disease burden in Lao PDR. The lack of data also impeded recent considerations of including a typhoid fever vaccine into the national immunization schedule. Therefore, typhoid fever vaccination is not part of the national immunization schedule in the Lao PDR.

In order to contribute to the understanding of typhoid fever epidemiology in Lao PDR, we conducted a serological, cross-sectional study using existing serum samples from different age groups and areas of Lao PDR. This study is the first serology based study in Lao PDR and provides initial insights into age-related exposure to S. Typhi and baseline antibody titers against the HlyE, CdtB and Vi antigen in the general population.

Methods

ELISAs were performed on 937 serum samples (317 children and 620 adults) from across Lao PDR to measure IgG antibody titers against Vi polysaccharide and the experimental protein antigens, CdtB and HlyE.

A commercial ELISA kit (Vacczyme, Binding site, UK) was used for determining the anti-Vi IgG antibody responses. Antibody concentrations were derived from the optical density (OD) data using a standardized curve-fitting 4-parameter logistic method. Any sample below the calculation limit of the assay was classified as "left-censored" for the purpose of analysis. In-house ELISAs were performed to determine anti-HlyE IgG and anti-CdtB IgG levels in the two stud populations.

Statistical analyses were performed in R. Non-parametric statistical tests were employed as appropriate. The relationship between age and antibody levels was assessed by fitting generalized additive models or an Akritas–Theil–Sen non-parametric regression estimation to the data.

Results

Study population

Sera from 317 children and 620 adults were inlcuded in the study. The majority of children in the child cohort were from Vientiane (249/317; 78.6%) and most (171/317; 53.9%) were female. The age of the children ranged from 0 to 15 years, with a median age of 8 years. The majority (373/620; 60.2%) of the adult participants were male and over a third (232/620; 37.4%) were students. The age of the adult participants ranged from 17 to 40 years (median 26 years).

The seroprevalence of anti–S. Typhi IgG antibodies

We measured IgG antibodies targeting HlyE, CdtB, and Vi antigen in serum from the 937 participants. Overall, the anti-Vi antibody titers ranged from 7.4 U/ml to 600 U/ml, the anti-HlyE IgG antibody titers ranged from 12.6 EU to 5163.2 EU, and the anti-CdtB IgG antibody titers ranged from 2.8 to 1466.1 EU (Table 2). Notably, 469/937 (50.1%) of the samples generated anti-Vi IgG titers that were below the calculation limit of 7.4 U/ml. The mean anti-HlyE, anti-CdtB, and anti-Vi IgG titers among all participants were 453.8 EU, 16.8 EU and 7.5 U/ml, respectively (Table 1).

Our data shows a clear distinction between children and adult typhoid fever serology (Figure 1). Median anti-HlyE IgG and anti-CdtB IgG titer were higher in children than in adults (p<0.0001, Wilcoxon test). Median anti-Vi IgG titer were higher in adults than in children (p<.0001, Wilcoxon test).

		N	N cens	Median	Mean	sd	Max	Min
anti-HlyE IgG (EU)	All data	937	0	234.32	453.83	672.37	5163.20	12.64
	Cohort 1: Children	317	0	351.74	734.59	980.75	5163.20	28.34
	Cohort 2: Adults	620	0	198.14	310.29	362.72	4520.40	12.64
anti-CdtB IgG (EU) [1]	All data	935	0	16.80	77.15	176.20	1466.10	2.75
	Cohort 1: Children	317	0	52.59	178.68	270.01	1466.10	3.73
	Cohort 2: Adults	618	0	12.87	25.07	40.57	470.18	2.75
Anti-Vi IgG (U/ml)								
All observations	All data	937	469	7.53	27.84	59.01	600.00	7.40
	Cohort 1: Children	317	218	3.02	10.34	23.15	204.60	7.42
	Cohort 2: Adults	620	251	11.32	36.71	69.00	600.00	7.40
Uncensored observations[2]	All data	468	0	24.49	52.78	75.71	600.00	7.40
	Cohort 1: Children	99	0	15.12	28.47	35.20	204.57	7.42
	Cohort 2: Adults	369	0	29.92	59.29	82.11	600.00	7.40

N = total number per group; N cens = number of observations below the calculation limit (censored values); sd = standard deviation

1Two participants whose samples were repeatedly below the calculation limit in the anti-CdtB IgG assay were excluded from the analysis

[2]Robust regression on order statistics were used to calculate summary statistics, due to the high number of observations below the limit of calculation

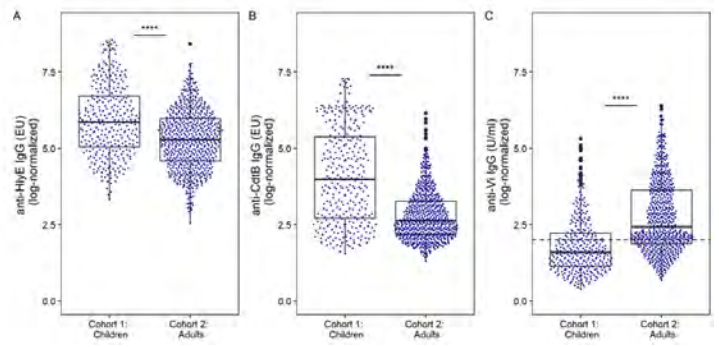


Figure 1. The distribution of anti–S. Typhi serum IgG titers in children and adults in Lao PDR

Each dot shows the antibody titer of an individual sample for (A) anti-HlyE IgG, (B) anti-CdtB IgG, and (C) anti-Vi IgG with an underlying boxplot. The dashed line in panel C represents the censoring limit, all data points below were treated as left-censored data. Differences between groups were assessed using Wilcoxon rank sum test followed by Dunn's post-hoc test with Bonferroni correction: ****p<0.0001.

The relationship between age and anti–S. Typhi serum IgG antibodies

In order to investigate the relationship between anti-HlyE IgG / anti-CdtB IgG and age we fitted generalized additive models. The models suggested highest antibody prevalences in children under 5 years of age and a decrease in antibody titers until the age of 20 years (Figure 2).

The anti-Vi IgG data was fitted as a function of age, which showed a positive relationship using Akritas–Theil–Sen non-parametric regression to account for the censored data; suggesting that anti-Vi IgG increases with age (Figure 2).

Trends in anti–S. Typhi serum IgG antibodies regarding sex, occupation, and location

There was no significant difference for any of the anti-S. Typhi antibodies between male and female children or adults. There were certain differences between occupation groups: Median anti-HlyE IgG titer were higher in office workers compared to students and compared to soldiers.

Median anti-CdtB IgG titers were higher in office workers compared to students and compared to soldiers. There were no significant differences in anti-Vi IgG titers between occupation groups.

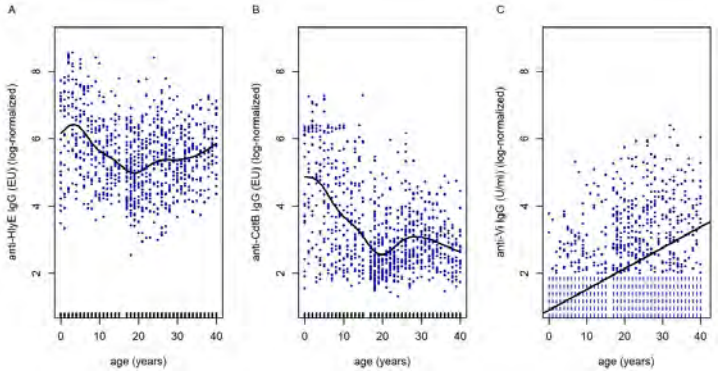


Figure 2. Results of generalized additive and linear models assessing anti–S. Typhi IgG antibody prevalence in children and adults in Lao PDR as a function of age. Non-linear smooths were fitted for age in the model for anti-HlyE IgG (A) and anti-CdtB IgG (B) data. The tick marks on the x-axis are observed data points. In panel C, the Akritas-Thiel-Sen regression line relating to the anti-Vi IgG titer data as function of age was plotted in order to account for the censored values (censored observations were plotted as vertical dashed lines).

Discussion

In this study, 50.1% of the participants had anti-Vi IgG antibody concentrations <7.4 U/ml with an estimated median titer of 7.5 U/ml. Median baseline concentrations were higher in adults as compared to children (11.3 vs 3.5 U/ml) and a positive relationship of anti-Vi IgG titer and age was observed. The median anti-Vi titer without those measurements below the calculation limit was 24.5 U/ml in general and 29.9 U/ml among adults, which was higher than previously reported median titers of 8.6 U/ml and 21 U/ml in healthy adults from Spain and Germany (Sanchez-Ramon et al., 2016, Evans et al., 2018).

In contrast to the anti-Vi IgG data, the median anti-HlyE IgG and anti-CdtB IgG titers were higher in children than adults. The non-linear trend line fitted to the HlyE IgG data suggested a peak in concentration in children below 5 years of age, followed by a decrease and before increasing again after the age of 20 years. The anti-CdtB IgG data followed largely the same projection; however, an increasing trend after the age of 30 years was not observed.

This study offered first insights into the age-related prevalence of three antibodies against S. Typhi. The prevalence of antibodies developed against the two protein antigens, anti-HlyE IgG and anti-CdtB IgG, indicates a high exposure in children. These findings are somewhat in contrast to a hospital based surveillance study in Vientiane which reported no incidents of typhoid fever in children over the course of two years (Chanthavilay et a., 2020).

The results of this study have recently been submitted for publication.

COVID-19 serostudy. Study team (Institut Pasteur du Laos staff and students from Lao Tropical Public Health Institute), and participants holding study information sheets, outside a health centre in Oudomxay province.

Luxembourg-Laos Partnership for Research and Capacity Building in Infectious Disease Surveillance – PaReCIDS; a summary of activities from 2016 to 2021 and future plans.

Since 2012, the Vaccine Preventable Disease laboratory (Lao Lux Lab) at Institut Pasteur du Laos has been supported by funds from the Luxembourg Ministry of Foreign Affairs under the "*Luxembourg-Laos Partnership for Research and Capacity Building in Infectious Disease Surveillance – PaReCIDS*" program in close collaboration with the Luxembourg Institute of Health. 2021 marked the end of the second 5-year cycle and the commencement of PaReCIDS III.

The remit of the PaReCIDS program is (i) to investigate the burden, the epidemiology and the aetiology of infectious diseases in humans and animals, (ii) to provide health officials with the scientific evidence required for their decisions to improve public and animal health, (iii) to evaluate and strengthen current disease prevention and control measures, including vaccination programs, (iv) to build qualitative and sustainable laboratory capacity and (v) to train the next generation of scientists, healthcare workers and medical doctors in public health research.

Figure 1. PaReCIDS cycle of activity (adapted from PaReCIDS III proposal).

During PaReCIDS II, the program supported between six and seven permanent Lao staff at Institut Pasteur du Laos at any one time, plus a number of visiting scientists and students. The research activities covered a number of important public health issues such as; hepatitis B virus infection in different population and risk groups (e.g. dentists, healthcare workers, women and young children); immunogenicity and timeliness of routine infant vaccination; vaccine knowledge and awareness among different populations, including healthcare workers; tetanus vaccination among women of childbearing age; incidence and prevalence of fever-rash diseases such as measles and rubella; incidence and prevalence of respiratory infections, including SARS-CoV-2; investigations of hepatitis C virus and liver disease.

The research activities resulted in 22 publications in peer-reviewed journals, 18 oral conference presentations and 22 conference poster presentations. Training of Lao staff was a central tenet, and notable achievements included the training of Vilaysone Khounvisith in molecular techniques in Luxembourg Institute of Health in 2019. Siriphone Virachith joined the laboratory during PaReCIDS II, after completion of her PhD in Japan. Dr. Virachith has been involved in many activities including co-supervision of students from Lao Tropical Public Health Institute,

leading a key study on COVID-19 serology, supervision of laboratory technicians, and securing independent funding from Institut Pasteur, Paris to study the role of endonucleases in hepatitis B virus infections. During PaReCIDS II, the laboratory members also trained staff and students from partner organisations, including Lao Military Healthcare workers and over 20 students from Lao Tropical and Public Health Institute, University of Health Sciences and others (Table 1).

Table 1. Activities during PaReCIDS I and II (adapted from PaReCIDS III proposal)

Communications and empowerment of lab staff

	Policy briefs	Scientific publications (with Lao staff as first author)	Conference posters (presented by Lao staff)	Oral presentations at (inter)national conferences (presented by Lao staff)	No. of training sessions offered to lab staff
PaReCIDS I		4 (1)	7 (4)	9 (4)	4
PaReCIDS II	6	22 (9)	22 (12)	18 (11)	20

Broadening capacity building

	No. of students trained in lab	Total duration of trainings (in months)	No. of lectures given (given by lab staff)
PaReCIDS I	5	40	13
PaReCIDS II	27	120	21 (5)

Strong partnerships

Ministry of Health, WHO, National blood bank, LuxDev, Lao Tropical and Public Health Institute, Children's hospital,...

Many of the studies had important implications for health policy. The evidence based-data were communicated to partners and policy makers – such as the WHO, the National Immunization Programme, the Lao Department of Disease control and the National Centre for Laboratory and Epidemiology - in the form of policy briefs and other written or oral communications. Phonethipsavanh Nouanthong from the VPD laboratory acts as Executive Secretary to the National Immunization Technical Advisory Group (NITAG), and as such, acts as an effective link between evidence based data and policy recommendations. Examples of health policy recommendations include; strengthening of routine infant vaccination; enhancing surveillance for fever rash diseases; improving hepatitis B vaccination for healthcare workers; booster vaccination doses for adolescents; raising awareness of vaccination among the general population.

Table 2. Examples of evidence-based recommendations made during PaReCIDS II (adapted from PaReCIDS III proposal)

Evidence-based recommendation	Communication	Outcome
Pentavalent booster recommendation	Policy brief to NITAG	Initiated discussion within Ministry of Health
Need to change algorithm for Tetanus vaccination for pregnant women	Reported directly to NITAG and WHO	Increased awareness among healthcare workers regarding timely vaccination
Need for improved communication, confirmatory testing and seropositive tracking system for repeated blood donors	Shared blood bank data directly with Lao Red Cross	Setting up of database to better track repeat donors. Follow-on studies initiated
Need to strengthen fever/rash and measles/rubella surveillance (including varicella as a reportable disease)	Reported results to the Children's Hospital, Vientiane, WHO and MoH	Increased awareness of hospital staff regarding fever/rash surveillance and reporting
Need for an occupational health programme for professionals	Communicated factory results with factories and also Lao Youth Union	Increased awareness of Hepatitis B exposure in this group
Need for improved water sanitation (Hepatitis A, E and typhoid studies)	Reported directly to NITAG, Ministry of Agriculture and WHO	Follow-up studies initiated
Strengthen reliability of vaccination records and vaccine management (e.g. pentavalent vaccine)	Reported directly to NITAG and WHO	Increased awareness of importance of accurate reporting in health facilities

In October 2021, an agreement was signed between IPL and Luxembourg Institute of Health for the continuation of the PaReCIDS activities until 2026. The focus of PaReCIDS III will remain on research and training in the field of infectious diseases and will build on and expand the areas of interest, including infectious diseases such as hepatitis A, B and C, diphtheria, tetanus, and SARS-CoV-2. The training and development of Lao staff and partners is continuing with the PhD studentship of Vilaysone Khounvisith, in collaboration with the Swiss Tropical Public Health Institute to investigate the relationship between water, sanitation and hygiene (WASH) levels and infectious disease. The training and development of staff within IPL and external partners will continue to be a key aim of PaReCIDS III, in addition to generation of evidence-based data in order to inform health policy. We acknowledge the generous support and clear vision of the Luxembourg Ministry of Foreign and European Affairs, which make our research possible.

Parasitology Laboratory

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 area of malaria and other parasitic diseases.

Head of Laboratory:
Dr. Shigeyuki KANO, MD, PhD

Scientist:
Dr. Moritoshi IWAGAMI, PhD, Laboratory Manager
Phonepadith KHATTIGNAVONG, MD

Junior Scientists:
Dr. Phoyphaylinh PRASAYASITH, MD
Dr. Sengdeuane KEOMALAPHET, MD
(Fulbright scholarship, Master course at Tulane University, USA, 2021-2023)

Technicians:
Pheovaly SOUNDALA
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Trainees:
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Projects

- Arboshield Project
- Antimalarial Drug Therapeutic Efficacy Study
- 5-Aminolevlinic acid (5-ALA) Asymptomatic Malaria Project
- SARS-CoV-2 LAMP Study

Executive summary

The Arboshield project is aim to improve capacity of surveillance, outbreak detection/response and diagnosis/treatment/prevention of vector-borne diseases, as well as biosafety/security, especially for the Lao Military sector. One role of the Lao-Japan Parasitology lab in this project is to improve malaria surveillance and diagnosis for the military sector, especially in military network hospitals through trainings and quality assessment. We conducted one year of on-the-job training of military staff in the Institute of Preventative Medicine and 103 hospital. As a quality assessment, we analyzed blood samples (n=20 in 2021) by malaria rapid diagnostic test (RDT, Malaria Ag P.f/P.v, Standard Diagnostics, Inc., The Republic of Korea) and PCR, collected from malaria-suspected patients in the military hospitals. Only one sample, from 103 hospital, Vientiane Capital was PCR positive for *Plasmodium vivax*. A malaria survey among the Lao Military personnel in Savannakhet province showed that only 0.17% (1/593) of the Military personnel were PCR positive for malaria. Surprisingly, 33% (96/289) of the Military personnel believed that malaria infection is caused by drinking dirty water in the forest.

An antimalarial drug therapeutic efficacy study was launched in November 2019 to evaluate drug efficacy by *in vitro* culture technique and molecular markers at Institut Pasteur du Cambodia (IPC). In this study, IPL, Parasitology Lab is responsible for sample collection and preservation in liquid nitrogen in a collaboration with the Center of Malariology, Parasitology and Entomology (CMPE), the Lao Ministry of Health and the local healthcare facilities. Live *Plasmodium falciparum* samples were collected from malaria patients who participated in this study in Savannakhet, Salavan, Sekong, Champasak and Attapeu provinces (87 samples were collected from Savannakhet, Salavan and Attapeu provinces, respectively, as of 1st November 2021). Currently, it is impossible to send the frozen *P. falciparum* samples to IPC because there are no flights between Lao PDR and Cambodia, and land border is also closed due to the pandemic of COVID-19. This study is financially supported by WHO Western Pacific Regional Office.

We have conducted a "5-aminolevlinic acid (5-ALA) asymptomatic malaria project" since October 2019. 5-ALA is a health food supplement produced by neopharma Japan Co. Ltd and is commercially available in Japan and other countries. Some studies showed that 5-ALA has an efficacy to kill or inhibit *Plasmodium* growth both *in vitro* and *in vivo*. The objective of this project is to evaluate the efficacy to kill or inhibit *Plasmodium* growth among asymptomatic *Plasmodium* carriers in malaria high endemic villages in Savannakhet province. Large-scale screening surveys for asymptomatic *Plasmodium* carriers were conducted in Nong and Sepon districts, Savannakhet province in October-November 2019 (n=2,716), December 2019 (n=1,353) and February 2020 (n=2,260). After malaria PCR analysis, we identified 66 eligible candidates for this study. However, 2 of 66 eligible candidates were not included. The supplement administration was initiated in February (n=40), March (n=4) and June (n=20) 2020. Follow-up of the study participants was finished except Month 14 of group 3. HbA1c data were also recorded. Collected blood samples were sent to National Center for Global Health and Medicine (NCGM), Japan and are being analyzed by PCR. If this supplement is effective for asymptomatic *Plasmodium* carriers to clear the parasites, this will be an effective tool to eliminate asymptomatic *Plasmodium* carriers in the endemic areas.

We conducted a performance evaluation of a RT-LAMP kit for SARS-CoV-2 (Loopamp™ SARS-CoV-2 Detection Kit, Eiken Chemical, Co., Ltd., Japan) by comparing it with the performance of RT-PCR for SARS-CoV-2. A total of 302 clinical samples were used for this study. 237 samples were collected by IPL while 65 samples were collected by National Center for Laboratory and Epidemiology (NCLE). Sensitivity, specificity, and positive and negative predictive values were calculated using the data of RT-PCR as a reference. Sensitivity and specificity of the LAMP test were 93.2% and 99.1%, respectively. Positive and negative predictive values of the LAMP kit were 97.6% and 97.2%, respectively. The sensitivity of the LAMP kit was slightly lower than that of the RT-PCR. However, we found that the LAMP kit is simple and quick, and all the reagents can be stored at refrigerator (2-8°C). Thus, the LAMP kit can be used at a resource limited setting such as small clinics and quarantine centers.

ສະຫຼຸບການປະຕິບັດວຽກງານ

ໂຄງການອາກໂບຊິ ອ (The Arboshield project) ແມ່ນແນ ໃສ່ການສ້າງຄວາມເຂັ້ມແຂງຂອງລະບົບເຝົ້າລະວັງ, ການຄົ້ນພົບ/ ຕອບໂຕ້ພະຍາດລະບາດ, ແລະ ການປ້ອງມະຕິ/ປິ່ນປົວ/ປ້ອງກັນພະຍາດທີ່ ມີແມງໄມ້ຕີນຂໍ້ເປັນພາຫະນຳເຊື້ອ; ແລະ ກວມໄປເຖິງຄວາມ ປອດໄພ-ໝັ້ນ ຄົງທາງຊີວະພາບໃນຂະແໜງການກອງທັບ. ໜຶ່ງໃນບົດ ບາດທີ່ສຳຄັນ ຂອງຫ້ອງວິເຄາະແມ່ກາຝາກວິທະຍາໃນໂຄງການນີ້ແມ່ນສ້າງຄວາມເຂັ້ມ ແຂງໃນການເຝົ້າລະວັງ ແລະ ປ້ອງມະຕິພະຍາດໄຂ້ມາລາເຣຍໃຫ້ແກ່ບັນດາ ໂຮງໝໍທະຫານ ທີ່ເປັນເຄືອຂ່າຍຂອງໂຄງການ, ໂດຍຜ່ານການຝຶກອົບຮົມ ແລະ ປະເມີນຄຸນນະພາບ. ຫ້ອງວິເຄາະພວກເຮົາໄດ້ຈັດຕັ້ງການຝຶກ ອົບຮົມກັບທີ່ ໄລຍະເວລາ 01 ປີໃຫ້ແກ່ພະນັກງານແພດທະຫານຈາກ ສະຖາບັນອະນາໄມ ກັນພະຍາດ ແລະ ສົ່ງເສີມສຸຂະພາບກອງທັບ ແລະ ໂຮງໝໍສູນກາງກອງທັບ 103. ສຳລັບປະເມີນຄຸນນະພາບ ກໍ່ຄືການກວດຕົວຢ່າງເລືອດ (ປີ 2021, 20 ຕົວຢ່າງ) ດ້ວຍເຄື່ອງມືການກວດແບບຮູ້ຜົນໄວ (RDT, Malaria Ag P.f/P.v, Standard Diagnostics, Inc., The Republic of Korea) ແລະ ດ້ວນເຕັກນິກ PCR, ເຊິ່ງຕົວຢ່າງ ແມ່ນ ໄດ້ຈາກຄົນເຈັບທີ່ມີອາການສົງ ໄສພະຍາດໄຂ້ມາລາເຣຍຈາກບັນດາໂຮງໝໍທະຫານດັ່ງກ່າວ. ມີ 01 ຕົວຢ່າງ ຈາກໂຮງໝໍສູນກາງກອງທັບ 103, ພົບເຊື້ອໄຂ້ມາລາເຣຍຊະນິດ ວີວັກ (*Plasmodium vivax*). ມີການສຳຫຼວດໜຶ່ງກ່ຽວກັບພະຍາດ ໄຂ້ມາ ລາເຣຍນຳພະ ນັກງານທະຫານທີ່ແຂວງສະຫວັນນະເຂດ ພົບວ່າ 0.17% (1/593) ຂອງທະຫານ ມີເຊື້ອມາລາເຣຍ, ແຕ່ເປັນທີ່ໜ້າຕົກໃຈຄື 33% (96/289) ຂອງທະຫານ ເຊື່ອວ່າພະຍາດໄຂ້ມາລາເຣຍແມ່ນເກີດ ຈາກການດື່ມນ້ຳທີ່ບໍ່ສະອາດຢູ່ປ່າ.

ການສຶກສາປະສິດທິພາບ ຂອງຢາຕ້ານມາລາເຣຍ ໄດ້ລິເລີ່ມໃນເດືອນພະ ຈິກປີ 2019, ເພື່ອປະເມີນປະສິດທິພາບຂອງ ຢາດ້ວຍເຕັກນິກການ ປູກເຊື້ອ ທີ່ມີຊີວິດໃນຫ້ອງທົດລອງ (*invitro*) ແລະ ການກວດຊອກ ເຄື່ອງໝາຍທາງໂມເລກຸນ ທີ່ສະຖາບັນປັດສະເຕີກຳປູເຈຍ (IPC). ເຊິ່ງການ ສຶກສານີ້,ສະຖາບັນປັດສະເຕີ ລາວ ກໍ່ຄືຫ້ອງວິເຄາະແມ່ກາຝາກວິທະຍາ ແມ່ນຮັບຜິດຊອບເກັບຮັກສາຕົວຢ່າງໃນ liquid nitrogen, ໂດຍມີການຮ່ວມມືຢ່າງໃກ້ຊິດ ກັບສູນໄຂ້ຍຸງ ແມ່ກາຝາກ ແລະ ແມງໄມ້, ກະຊວງສາທາລະນະສຸກ ນັບແຕ່ຂັ້ນສູນກາງຈົນຮອດຂັ້ນ ທ້ອງຖິ່ນ. ຕົວຢ່າງເຊື້ອ *Plasmodium falciparum* ທີ່ມີຊີວິດ ໄດ້ຖືກເກັບຈາກ ຄົນເຈັບທີ່ເປັນພະຍາດໄຂ້ມາລາເຣຍທີ່ໄດ້ເຂົ້າຮ່ວ ມການສຶກສານີ້, ຈາກແຂວງ ສະຫວັນນະເຂດ, ສາລະວັນ, ເຊກອງ, ຈຳປາສັກ ແລະ ອັດຕະປື (ມາຮອດວັນທີ 1 ເດືອນພະຈິກ ປີ 2021, ໄດ້ຮັບຕົວຢ່າງທັງໝົດ 87 ຕົວຢ່າງ). ໃນສະພາບປັດຈຸບັນພາຍໃຕ້ການ ລະບາດຂອງພະຍາດ COVID-19 , ພວກເຮົາບໍ່ສາມາດນຳສົ່ງຕົວຢ່າງ ເຊື້ອ *P. falciparum* ທີ່ແຊ່ແຂງ ໄປສະຖາບັນປັດສະເຕີກຳປູເຈຍໄດ້, ເນື່ອງຈາກບໍ່ມີສາຍການບິນລະ ຫວ່າງປະເທດ ສປປ ລາວ ແລະ ກຳປູເຈຍ ແລະ ຊາຍແດນທາງ ບົກລະຫວ່າງປະເທດກໍ່ປິດດຽວເຊັ່ນກັນ. ການສຶກສາດັ່ງກ່າວ ແມ່ນໄດ້ຮັບການສະໜັບສະໜູນຈາກອົງການອະນາໄມ ໂລກ ພາກພື້ນອາຊີປາຊີຟິກຕາເວັນຕົກ.

ນອກຈາກນີ້, ພວກເຮົາຍັງໄດ້ຈັດຕັ້ງປະຕິບັດໂຄງການ 5-ອາມິໂນ ເລວູລິ ນິກອາຊິດ (5-ALA) ໄຂ້ມາລາເຣຍທີ່ບໍ່ສະແດງອາການ ນັບແຕ່ເດືອນຕຸລາ 2019, 5-ALA ເປັນອາຫານເສີມ ທີ່ຜະລິດໂດຍບໍລິສັດ neopharma Japan Co. Ltd (ປະເທດຍີ່ປຸ່ນ), ເຊິ່ງມີການຄ້າຂາຍທີ່ປະເທດຍີ່ປຸ່ນ ແລະ ປະເທດອື່ນໆ. ມີບາງການສຶກ ສາພົບວ່າອາຫານເສີມດັ່ງກ່າວສາມາດຂ້າ ຫຼື ຢັບຢັ້ງການຂະຫຍາຍ ຕົວຂອງເຊື້ອມາລາເຣຍ (*Plasmodium*)ໄດ້ທັ້ງ *in vitro* and *in vivo*. ຈຸດປະສົງແມ່ນ ເພື່ອປະເມີນປະສິດທິພາບໃນການຂ້າ ຫຼື ຢັບຢັ້ງການຂະ ຫຍາຍຕົວຂອງເຊື້ອໃນຜູ້ທີ່ຕິດເຊື້ອທີ່ບໍ່ ສະແດງອາການ ໃນເຂດທີ່ມີອັດ ຕາຊຸກຊຸມຂອງພະຍາດໄຂ້ມາລາເຣຍສູງ ຢູ່ແຂວງສະຫວັນນະເຂດ. ພວກ ເຮົາໄດ້ເຮັດການສຳຫຼວດຂະໜາດໃຫຍ່03ຄັ້ງເພື່ອຊອກຫາຜູ້ທີ່ຕິດເຊື້ອແຕ່ ບໍ່ສະແດງອາການ ຢູ່ເມືອງນອງ ແລະ ເມືອງເຊໂປນ ແຂວງສະຫວັນນະເຂດ, ໃນເດືອນຕຸລາ-ພະຈິກ ປີ 2019 (n=2,716), ເດືອນທັນວາ ປີ 2019 (n=1,353) ແລະ ເດືອນກຸມພາ ປີ 2020 (n=2,260). ຫລັງຈາກການ ກວດຕົວຢ່າງດັ່ງກ່າວດ້ວຍເຕັກນິກ PCR, ເຮົາສາມາດຄັດເລືອກໄດ້ 64 ເຂົ້າໃນການສຶກສານີ້. ການຢາຍອາການເສີມໃຫ້ຜູ້ເຂົ້າຮ່ວມໄດ້ເລີ່ມ ໃນເດືອນ ກຸມພາ (n=40), ເດືອນມີນາ (n=4) ແລະ ເດືອນມີຖຸນາ (n=20) ປີ 2020. ໃນປັດຈຸບັນນີ້ ຂະບວນການການຕິດຕາມການ ກິນ ອາຫານເສີມຂອງຜູ້ເຂົ້າຮ່ວມໄດ້ສິ້ນສຸດລົງຍົກເວັ້ນກຸ່ມ 3 ເດືອນທີ 14, ຄຽງຄູ່ກັບການຕິດຕາມການກິນອາຫານເສີມແລ້ວ, ພວກເຮົາຍັງກວດ ແລະ ບັນທຶກ ຄ່ານ້ຳຕານໃນກະແສເລືອດອີກດ້ວຍ (HbA1c). ຕົວຢ່າງ ເລືອດ ທີ່ໄດ້ຈາກການຕິດຕາມແມ່ນໄດ້ສົ່ງກວດ PCR ທີ່ສູນສຸຂະພາບ ແລະ ການແພດແຫ່ງຊາດ (NCGM), ປະເທດຍີ່ປຸ່ນ. ຖ້າຫາກອາຫານເສີມນີ້ມີ ປະສິດທິພາບໃນການກຳຈັດ ເຊື້ອກາຝາກໃນຜູ້ທີ່ຕິດເຊື້ອ ປລາສໂມດຽມ ແຕ່ບໍ່ສະແດງອາການ, ອາຫານເສີມນີ້ຈະກາຍເປັນສິ່ງທີ່ມີປະສິດທິພາບ ໃນ ການລົບລ້າງກໍລະນີທີ່ຕິດເຊື້ອໄຂ້ມາລາເຣຍແຕ່ບໍ່ສະແດງອາການໃນເຂດທີ່ ມີການ ຊຸກຊຸມຂອງພະຍາດນີ້.

ພວກເຮົາໄດ້ດຳເນີນການປະເມີນເຕັກນິກ RT-LAMP ສຳລັບ SARS-CoV-2 (Loopamp™ SARS-CoV-2 Detection Kit, ບໍລິສັດ Eiken Chemical, Co., Ltd., ປະເທດຍີ່ປຸ່ນ) ດ້ວຍການສົມທຽບກັບເຕັກນິກ RT-PCR. ທັງໝົດ 302 ຕົວຢ່າງໄດ້ນຳ ໃຊ້ເຂົ້າການສຶກສານີ້, ໃນນັ້ນ 237 ຕົວຢ່າງໄດ້ຈາກສະຖາບັນປັດສະເຕີລາວ ແລະ 65 ຕົວຢ່າງແມ່ນໄດ້ຈາກສູນວິເຄາະ ແລະ ລະບາດວິທະຍາແຫ່ງຊາດ.

ຄວາມເມັ້ນຢຳ (Sensitivity), ຄວາມຈຳເພາະ (specificity), ແລະ ຄ່າຄາດຄະເນໃນທາງບວກ ແລະ ລົບ (positive and negative predictive values) ໄດ້ຖືກຄຳນວນ, ເຊິ່ງເອົາຂໍ້ມູນຈາກ RT-PCR ເປັນຕົວອ້າງອີງ. ຄວາມເມັ້ນຢຳ ແລະ ຄວາມຈຳເພາະຂອງ LAMP ແມ່ນ 93.2% ແລະ 99.1%, ຕາມລຳດັບ. ສ່ວນ ຄ່າຄາດຄະເນໃນທາງບວກ ແລະ ລົບ ຂອງ LAMP ແມ່ນ 97.6% ແລະ 97.2%, ຕາມລຳດັບ. ຄວາມເມັ້ນຢຳ ຂອງ LAMP ແມ່ນຕ່ຳກວ່າ RT-PCR ເລັກໜ້ອຍ. ແຕ່ເຖິງຢ່າງໃດກໍ່ຕາມ, ພວກເຮົາເຫັນວ່າການເຮັດເຕັກນິກ LAMP ແມ່ນງ່າຍ ສະດວກ ແລະ ໃຊ້ເວລາສັ້ນກວ່າ, ແລະ ການເກັບຮັກສາ ບັນດານ້ຳຢາຕ່າງໆສາມາດເກັບຮັກສາໄວ້ໄດ້ໃນຕູ້ເຢັນທີ່ມີອຸນຫະພູມ 2-8°C. ດັ່ງນັ້ນ, ເຕັກນິກ LAMP ສາມາດນຳໃຊ້ໄດ້ໃນສະຖານທີ່ ທີ່ ມີຄວາມຈຳກັດດ້ານສິ່ງອຳນວຍຄວາມສະດວກຢ່າງເຊັ່ນ ຫ້ອງກວດ ພະຍາດຂະໜາດນ້ອຍ ແລະ ບັນດາສູນກັກກັນຕ່າງໆ.

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. Phoyphaylinh Prasayasith, Ms. Pheovaly Soundala, and Ms. Sonesimmaly Sannikone

Finance and administration:
Dr. Antoine des Graviers and Ms. Phouvanhnamalee Vilaysouk

Background and objectives

The military sector in Lao People's Democratic Republic (Lao PDR) covers a certain portion of the Lao population including their family. The Lao Ministry of Defense has a medical institute in Vientiane capital and several hospitals across the country. However, unfortunately, the Lao Ministry of Defense has limited support from donors to improve their capacity and facilities since most of the donors hesitate to support the military sector. On the other hand, several studies reported that soldiers are at high risk population for malaria infection because they patrol and stay in the forests where malaria and other vector-borne diseases are highly prevalent [1,2]. Moreover, our previous study found household clustering of asymptomatic malaria infections which means malaria is transmitted by *Anopheles* mosquito within a family [3]. These results suggested that even though malaria is not frequently transmitted in a village nowadays, malaria can be introduced by their family members who work and stay in the forests.

The objective of the Arboshield project is to strengthen the capacity of the medical sector in the Ministry of Defense and some of the selected civilian hospitals in the Ministry of Health, Lao PDR in the areas of vector-borne diseases, especially surveillance, outbreak detection/ response, diagnosis, treatment, prevention, as well as biosafety and security through training and improving their facilities. The roles of the Lao-Japan Parasitology lab in this project are giving a training course on malaria for the trainees from military sector and civil sector, and quality assessment of malaria diagnosis in the Lao Institute for Disease Prevention and six military provincial hospitals (Xieng Khouang, Luang Prabang, Vang Vieng, Vientiane Capital, Savannakhet, and Champasak), which participate in the Arboshield project.

Results of quality assessment for malaria diagnosis

Blood samples were collected from 20 malaria suspected patients in the military hospitals and transported to IPL in 2021. The samples were diagnosed by RDT and nested PCR for malaria. As of 1st November 2021, only one sample was PCR positive for *Plasmodium vivax* while all RDT were negative for malaria. These results showed that sensitivity of the malaria PCR is higher than that of the RDT. Thus, a highly-sensitive diagnostic method like PCR is necessary for accurate diagnosis for malaria case management and control in Lao PDR. It is noted that the number of malaria suspected cases in the military hospitals in the Arboshield project are decreasing from 2019 (n=320), 2020 (n=39) and 2021 (n=20). This may indicate improvement of malaria prevention and control among the military sector in Lao PDR.

Malaria survey for Lao military personnel in Savannakhet province

A malaria survey for Lao military personnel was conducted at the 4th Brigade Division, Savannakhet province from 17-24 June 2019. A total of 593 military personnel who had no signs and symptoms of malaria participated in this survey. An interview survey was performed using a structured questionnaire for the personnel (n=300) showing that3.7% (11/300) had not heard of malaria. For the remaining 289 participants, 63.7% (184/289) knew that malaria is transmitted by mosquito bite. However, 23.9% (69/289) answered that malaria infection occurs "by drinking dirty water", 9.3% (27/289) by "Drinking stream water in forests" and 3.1% (9/289) by "Cough or sneeze." All the participants (n=593) tested negative by malaria RDT. Dried blood samples were collected from the participants (n=593) on filter paper (FTA™ Classic Card, GE Healthcare Life Sciences, Whatman™, UK) for PCR analysis. Genomic DNA was extracted from all the filter papers by using QIAamp DNA Mini Kit (QIAGEN, Germany). Malaria screening by nested PCR was performed and only one sample was PCR positive for *P. vivax* (0.17%; 1/593).This PCR analysis was performed by the Arboshield trainee: Dr. Phouniloud HONGVANGTHONG under the guidance of the staff in the Lao-Japan Parasitology Lab.

Financial support

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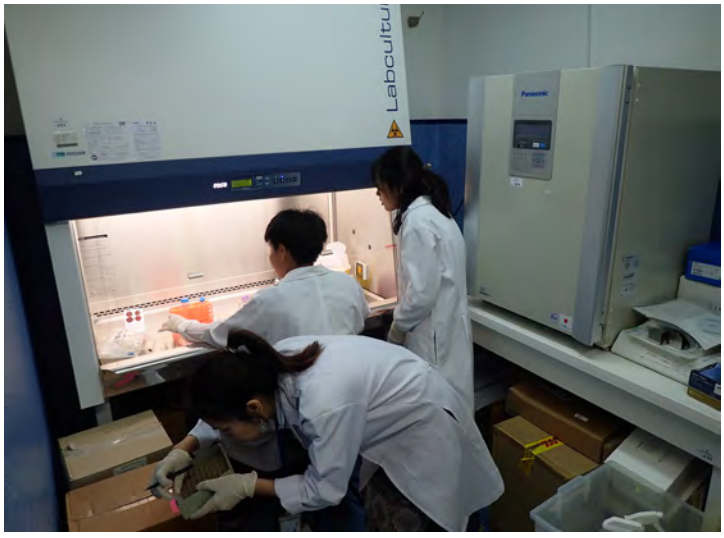
Antimalarial Drug Therapeutic Efficacy Study



Project Coordinator: Dr. Moritoshi IWAGAMI

Partners: Dr. Center of Malariology, Parasitology and Entomology (CMPE), Ministry of Health, Vientiane, Lao PDR

2019-2022
Savannakhet Provincial Health Department
Salavan Provincial Health Department
Champasak Provincial Health Department

2021-2022
Savannakhet Provincial Health Department
Sekong Provincial Health Department
Attapeu Provincial Health Department

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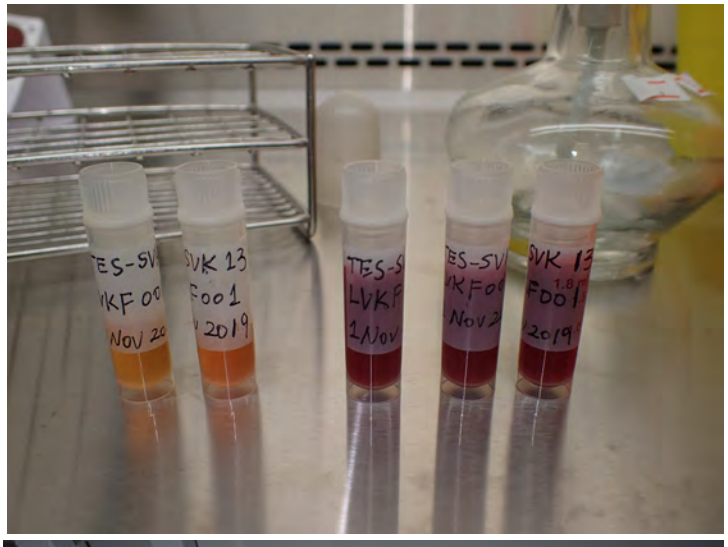
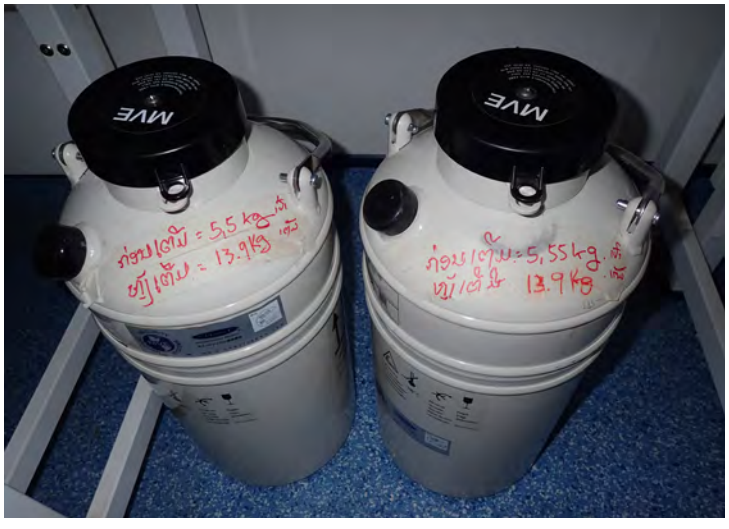
Finance and administration:
Dr. Antoine des Graviers and Ms. Phonesavanh Vilayseng

Background

Malaria, caused by protozoan parasite genus *Plasmodium*, has long been one of the most medically important parasitic diseases in the Greater Mekong Sub-region (GMS) including Lao PDR. Nevertheless, morbidity and mortality of malaria have significantly decreased in the GMS over the last decade due to extensive efforts by the governments, partners and international organizations, such as World Health Organization, Global Fund, Bill & Melinda Gates Foundation [1]. The Lao Ministry of Health adopted an ambitious goal to eliminate *Plasmodium falciparum* malaria by 2025 and all forms of human malaria by 2030. Artemisinin-based combination therapy (ACT) has been used for malaria treatment worldwide. In Lao PDR, Coartem (artemether-lumefantrine), one of the ACTs, has been used for the treatment of *P. falciparum* malaria since 2004. However, clinical artemisinin resistance (or delayed parasite clearance) in *P. falciparum* was first reported in Pailin, western part of Cambodia in 2009 [2] and gradually spread or emerged in the neighboring countries, which is a serious threat for malaria control and elimination in the GMS and globally.

Percentages of the *k13* mutations detected were 55.7% (660/1,185) in 2015, 44.6% (179/401) in 2016 and 23.9% (109/457) in 2017. The predominant mutation was C580Y, which was also predominant in Cambodia, followed by Y493H, R539T and P574L. The percentage of the mutations was higher in the two southernmost provinces, Champasak and Attapeu. In Phongsaly, all the *P. falciparum* samples (3/3) possessed the C580Y in 2017. This study suggested the percentages of the *k13* mutations seemed to be decreasing in Lao PDR. However, caution is needed because this tendency might be due to the relatively large number of *P. falciparum* samples from Savannakhet province with lower percentages of the mutation: 21.1% (35/166) in 2016 and 5.3% (14/266) in 2017. In addition, the mutation rate as high as 77.0% (47/61) was still observed in the southernmost province, Champasak in 2017. On the other hand, an antimalarial drug therapeutic efficacy study (TES) conducted by CMPE demonstrated that 10%-14% of cases were artemisinin resistant in 2013-2017 [1].





This TES result, together with the prevalence of the *k13* mutations suggested that the efficacy of Coartem is slightly decreasing and the partner drug, lumefantrine is the main actor to clear malaria parasites in the patients. However, no molecular marker has been identified for lumefantrine so far.

Objective

The objective of this study is to collect blood samples (living *Plasmodium falciparum*) from malaria patients in the field for laboratory analysis to identify and map resistant markers in the artemisinin partner drug, lumefantrine that is currently used in Lao PDR and many African countries for the first line treatment of malaria. The main role of the Lao-Japan Parasitology Lab, IPL in this study is to preserve the sample of *P. falciparum* in liquid nitrogen in collaboration with CMPE and the local healthcare facilities in the study sites. The laboratory analysis will be conducted at Institut Pasteur du Cambodia (IPC).

Methodology

The study sites of this project are at public hospitals (provincial hospitals and district hospitals) in malaria endemic areas in Savannakhet, Salavan and Champasak provinces in 2019-2020 and Savannakhet, Sekong and Attapeu provinces in 2021-2022. Live *P. falciparum* samples are collected from malaria patients who participated in this study. The collected samples (approximately 2mL of fresh blood sample in EDTA tube) are sent by public bus in a Thermos bottle with ice water to IPL and red blood cells are preserved in liquid nitrogen with freezing solution. The live *P. falciparum* samples will be sent to IPC with dry shippers (special liquid nitrogen tanks) at the end of the project by air or land. Ethical clearance for this study was obtained by CMPE.

Results and future plan

In 2019, a total of 50 samples (47 samples: Savannakhet; 3 samples: Salavan) was collected in this study and the red blood cells were stored in the liquid nitrogen tanks. In 2020, 29 samples (26 samples: Savannakhet; 3 samples: Salavan) were collected. In 2021, 8 samples were collected in Attapeu. Serum samples and the remaining red blood cells were preserved in a freezer (-30°C) and filter paper, respectively. Currently, it is impossible to send the frozen-live *P. falciparum* samples to IPC because no airline or courier company that accepts frozen sample is available between Lao PDR and Cambodia due to the COVID-19 pandemic. The land border is also currently closed.

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Financial support

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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 in Lao PDR:
Dr. Mayfong Mayxzy, University of Health Sciences (UHS), Lao PDR

Principal Investigator in Japan:
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Staff members in Parasitology Lab:
Dr. Moritoshi Iwagami, Dr. Phonepadith Kahttignavong, Dr. Sengdeuane Keomalaphet, Dr. Phoyphaylinh Prasayasith, Ms. Pheovaly Soundala, Ms. Sonesimmaly Sannikone, and Mr. Takashi Sekine

Background

Recently, the Lao Ministry of Health adopted a goal to achieve elimination of malaria by 2030. However, several studies demonstrated that there were asymptomatic *Plasmodium* carriers (hidden malaria, parasite reservoir) in the malaria endemic areas in Lao PDR [1-3]. Most of them were adult populations who had histories of malaria episodes, and were engaged in forest related occupations [1]. 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 asymptomatic *Plasmodium* infections cannot be detected by standard diagnostic methods that are available in the endemic areas (microscopy and RDT). The current malaria control and elimination strategy in Lao PDR targets only symptomatic malaria patients. Therefore, to accelerate elimination of malaria in Lao PDR, a new effective strategy for targeting asymptomatic *Plasmodium* carriers is urgently needed in the endemic areas. It was found in pre-clinical studies that sodium ferrous citrate (SFC) enhanced the *P. falciparum*-killing potency of 5-ALA and significantly inhibited the parasite growth both *in vitro* and *in vivo* [4,5]. These novel findings may lead us to develop a new functional health supplement containing antimalarial activity using 5-ALA. In addition, 5-ALA is being sold as health food supplement, which has the functional claim "5-ALA supports to bring higher fasting blood glucose levels closer to normal" in Japan [6,7]. In this study, we will evaluate acceptability, safety and efficacy of 5-ALA phosphate (PO4) with SFC and Zn supplements for treatment of asymptomatic *Plasmodium* carriers in malaria endemic villages, Nong and Sepon districts, Savannakhet province, Lao PDR for one year.

The efficacy of 5-ALA PO4 to control *Plasmodium* infection will be examined by reduction of *Plasmodium* DNA positivity rate by PCR, compared to that of a 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 give a benefit for the study participants. It is reported that type 2 diabetes increases risk for malaria infection [8]. Therefore, in addition to evaluating the efficacy of 5-ALA PO4 in *Plasmodium* infection control, we will also evaluate the level of HbA1c, which is one of markers of type 2 diabetes. Expected outcomes will contribute to malaria elimination as well as type 2 diabetes control in Lao PDR.

Objectives

- To assess influence of 5-ALA PO4, SFC and Zn on *Plasmodium* reduction (*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 ithout symptoms (as detected by PCR).

- To investigate the HbA1c level in asymptomatic malaria parasite carriers after daily administration of 5-ALA PO4, SFC and Zn.

Study period of the project

Two years (October 2019- March 2022)

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, IPL ethics review committee, 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 (age: 18-65 years-old) were enrolled into a screening process during which all inclusion/exclusion criteria, including laboratory assessments were checked for eligibility. Those individuals who have any signs and symptoms of malaria, malaria RDT positive, and pregnant ladies were excluded from the screening. PCR screening for detecting *Plasmodium* infection will be performed at IPL and NCGM. Eligible participants will be randomized into one of two arms: Arm 1 (Study group): 5-ALA PO4 25 mg/day + SFC 28.7 mg + zinc 10 mg (12 months), or Arm 2 (Placebo group): SFC 28.7 mg + zinc 10 mg (12 months) (Fig. 1). Clearance of *Plasmodium* DNA [Time Frame: 1, 2, 3, 6, 9 and 12 months] will be evaluated by malaria PCR using blood samples of the participants. HbA1c will be evaluated by a handy HbA1c monitoring device. Blood samples (maximum 800μL per sampling) will be collected using a lancet or syringe and needle, and preserved on filter paper. A follow up survey will be conducted 2 months after the end of the administration of 5-ALA PO4 or Placebo. In the follow up survey, blood samples will be collected and examined by PCR for checking *Plasmodium* DNA.

Screening of asymptomatic *Plasmodium* carriers

Three field surveys for screening of asymptomatic Plasmodium carriers were conducted in Nong district (20th October-14th November 2019), in Sepon district (15th-28th December 2019) and in Nong and Sepon districts (4th-24th February 2020), Savannakhet province (Fig. 2). All the surveys were conducted with a team that consisted of IPL, UHS, CMPE, Savannakhet Provincial Health Department, Nong and Sepon District Health Offices, and Health Centers in collaboration with village chiefs and village health volunteers. A total of 6,329 villagers participated in the screening surveys and 66 villagers (1.0%) were eligible (asymptomatic malaria carriers) for the 5-ALA supplement study, i.e. malaria RDT negative and malaria PCR positive. Malaria screening PCR analysis was performed using primer sets previously designed [10] at IPL and NCGM.

Administration of the supplement

To avoid time lag between the screening survey and the supplement administration, the 5-ALA PO4 supplement or placebo was administered to the eligible participants when the PCR analysis was finished for each survey. The supplement administration was initiated in February, March and June for group 1 (participants in the 1st screening survey), group 2 (participants in the 2nd screening survey) and group 3 (participants in the 3rd screening survey), respectively. In group 1, one eligible candidate refused to participate in the study for unknown reasons. In group 3, one eligible candidate was excluded from the study because, according to the local healthcare staff and village chief, the candidate was an illegal drug user and often disappeared from his village.

One tablet of the supplement or placebo was taken every day at any time by the study participant (subject) (Fig. 3). Adherence of the supplement or placebo is monitored by several levels of the local people. The Chief of village or village health volunteer monitors the administration daily by using a check sheet and counting remaining tablets. Health Center staff also monitor the administration and health condition of the participants weekly during the first three months, then once every two weeks on and after four months, by visiting the study participants. District Health Office staff also monitor Health Center staff every week by telephone or visiting the Health Centers. IPL staff monitor the district staff by telephone or a social networking service every week or once every two weeks.

The follow-up of the participants was conducted by the follow-up team (IPL, UHS, CMPE, Provincial Health Department staff, District Health Office staff, and Health Center staff) according to the follow-up schedule in Figure 1. In the follow-up, basic health check (body temperature, health consultation, pregnancy test), HbA1c test, and dried blood sample collection on filter paper (FTA™ Classic Card, GE Healthcare Life Sciences, Whatman™, UK) for PCR were conducted. HbA1c test was performed by using A1CNow® (PTS Diagnostics, USA).

Impact of the lockdown due to COVID-19 for this study

Due to the national lockdown in place to control the spread of COVID-19 in Lao PDR, the follow-up in April and May 2020 were conducted by the health center staff. Although it was prohibited to travel between provinces in the country in April due to the strict lockdown, IPL obtained a travel permission from the Lao Ministry of Health and an IPL driver, Mr. Hongnakhone XAYASING, managed to deliver the supplements and consumables to Nong District Health Office. . In fact, he could not reach the Nong District Health Office, but could reach a gate to Nong district. He handed over the supplements and consumables to health center staff at the gate and the health center staff delivered them to the Nong District Health Office. In fact, he could not reach the Nong District Health Office, but could reach a gate to Nong district. He handed over the supplements and consumables to health center staff at the gate and the health center staff delivered them to the Nong District Health Office.

Unfortunately, HbA1c tests were not able to be performed in April and May because it is difficult for the local healthcare staff to use the test kit which is highly sensitive to temperature (18ºC-28ºC). In addition, the test kit has to be stored in a refrigerator, which is not available in some health centers. The follow-up surveys in April and May 2021 were conducted by the local healthcare staff by sending the supplements, FTA cards and HbA1c test kits by IPL car (again, IPL staff were not able to go to the study sites due to the COVID-19 pandemic). The follow-up survey in August (Month 14 of the Group 3) has not yet been conducted due to the COVID-19 pandemic (as of 1st November 2021). The local healthcare staff are not available because they have to support hospitals and quarantine centers from August until the beginning of November 2021.

Financial support

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

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SARS-CoV-2 LAMP Study

Project coordinator: Dr. Moritoshi Iwagami

Staff members in Parasitology Lab:
Dr. Phonepadith Khattignavong, Dr. Sengdeuane Keomalaphet, Dr. Phoyphaylinh Prasayasith, Ms. Pheovaly Soundala, Ms. Sonesimmaly Sannikone

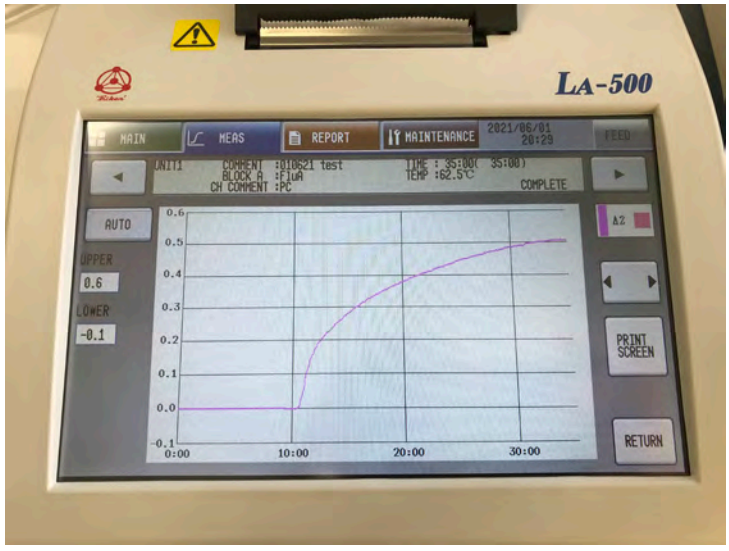
Staff members in Arbovirus & Emerging viral diseases laboratory Lab:
Dr. Vincent Lacoste
Dr. Somphavanh Somlor

Background

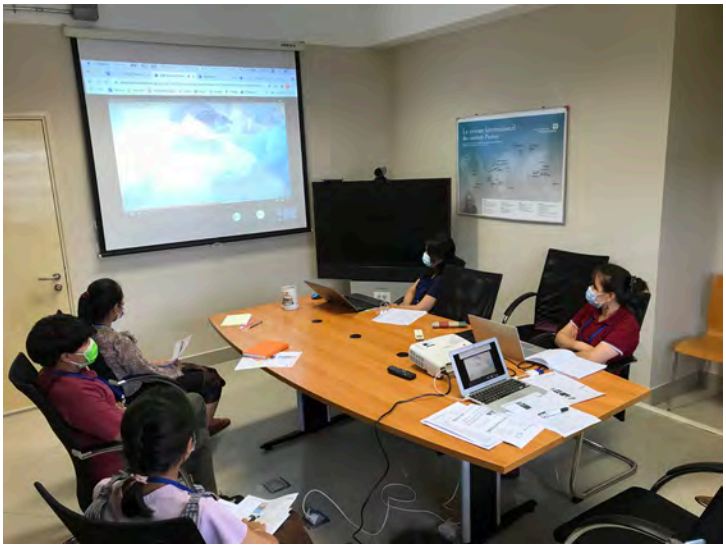
This is a collaborative study between the Parasitology Lab and Arbovirus & Emerging viral diseases laboratory Lab. Loop-mediated isothermal amplification (LAMP) is one of the nucleic acid amplification methods similar to PCR. We conducted a performance evaluation of a Reverse-transcription LAMP (RT-LAMP) kit for SARS-CoV-2 (Loopamp™ SARS-CoV-2 Detection Kit, Eiken Chemical, Co., Ltd., Japan) by comparing it with the performance of RT-PCR for SARS-CoV-2.

Methodology

A total of 302 clinical samples were used for this study. 237 samples were collected by IPL while 65 samples were collected by National Center for Laboratory and Epidemiology (NCLE) for the SARS-CoV-2 test. RNA was extracted from 150µL of the swab samples using NucleoSpin® RNA Virus (MACHEREY-NAGEL, Germany). The RT-qPCR was performed by Berlin method [1] while the RT-LAMP was performed according to the instructions of the RT-LAMP kit. Briefly, 15 µL primer mix and 10µL RNA template were added in the reaction tube with the kit and mixed well. Dried enzymes are applied inside of a cap of the reaction tubes. The reaction tube was incubated at 62.5°C for 35 min and then inactivated at 95°C for 2 min by using a Real-Time Turbidimeter LA-500 (Eiken Chemical Co. Ltd, Japan) (Fig. 4). Sensitivity, specificity, and positive and negative predictive values were calculated using the data of RT-PCR as a reference.



Results and discussion



Sensitivity and specificity of the RT-LAMP test were 93.2% and 99.1%, respectively (Table 1). Positive and negative predictive values of the RT-LAMP kit were 97.6% and 97.2%, respectively. The sensitivity of the RT-LAMP kit was slightly lower than that of the RT-PCR. However, we found that the RT-LAMP kit is simple and quick to perform. In addition, all the reagents including an RNA extraction kit can be stored at refrigerator (2-8°C). Thus, this RT-LAMP kit can be used at resource limited settings such as small clinics and quarantine centers.

Financial Support:

Japan International Cooperation Agency (JICA)

Reference

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Scientific communications

Oral presentation:

1. Moritoshi Iwagami, Masami Nakatsu, Sengdeuane Keomalaphet, Phonepadith Khattignavong, Pheovaly Soundala, Phoyphaylinh Prasayasith, Bouasy Hongvanthong, Viengxay Vanisaveth, Paul T. Brey, Shigeyuki Kano, Molecular surveillance of the distribution of artemisinin resistant *Pf* parasites in Laos during 2015-2017, The 90th Annual Meeting of the Japanese Society of Parasitology and the 32nd Annual Meeting of the Japanese Society of Clinical Parasitology, Nara, Japan, 15-17, April, 2021 (Online meeting)

2. Current situation and challenges of field work in Lao PDR under the COVID-19 pandemic: A report from Laos, Moritoshi Iwagami, The 80th East Branch Meeting of The Japanese Society of Parasitology, Dokkyo Medical University, Tochigi, Japan, 30th, October, 2021 (Online meeting)

Poster presentation:

1. Moritoshi Iwagamim, Masami Nakatsu, Phonepadith Khattignavong, Sengdeuane Keomalaphet, Pheovaly Soundala, Kanako Komaki-Yasuda1, Paul T. Brey, Shigeyuki Kano, Performance evaluation of malaria-LAMP tests using dried-blood samples of malaria patients in Laos in 2015, The 62nd Annual Meeting for the Japanese Society of Tropical Medicine, Tohoku University, Miyagi, Japan, 3-5, November, 2021 (Online meeting)

2. Moritoshi Iwagami, Masami Nakatsu, Phonepadith Khattignavong, Sengdeuane Keomalaphet, Pheovaly Soundala, Kanako Komaki-Yasuda, Paul T. Brey, Shigeyuki Kano, Sensitivity and specificity of LAMP tests for malaria diagnosis using dried-blood samples of suspected malaria patients in Lao PDR, Joint International Tropical Medicine Meeting 2021 (JITMM Virtual 2021), Bangkok, Thailand, 15-17 December 2021 (Online meeting)

Publication

Moritoshi Iwagami, Current Situation and Challenges of Parasitic Diseases in Lao PDR- Schistosomiasis, Opisthorchiasis, and others (in Japanese), Modern Media, 66(12): 375-388, 2020 (https://www.eiken.co.jp/uploads/modern_media/literature/P23-36.pdf)

Table:

Table 1. Summary of performance of the SARS-CoV-2 LAMP Kit

Index			95% CI	
Sensitivity	93.2	%	87.9	98.4
Specificity	99.1	%	97.8	100.0
Positive Predictive Value	97.6	%	94.4	100.0
Negative Predictive Value	97.2	%	95.1	99.4
Accuracy	97.4	%	95.5	99.2

Loopamp™ SARS-CoV-2 Detection Kit, Eiken Chemical Co., Ltd., Japan. Performance of the SARS-CoV-2 LAMP Kit was calculated based on the results of the RT-PCR.

Figure Legend:

Figure 1. Scheme of the 5-ALA asymptomatic malaria study

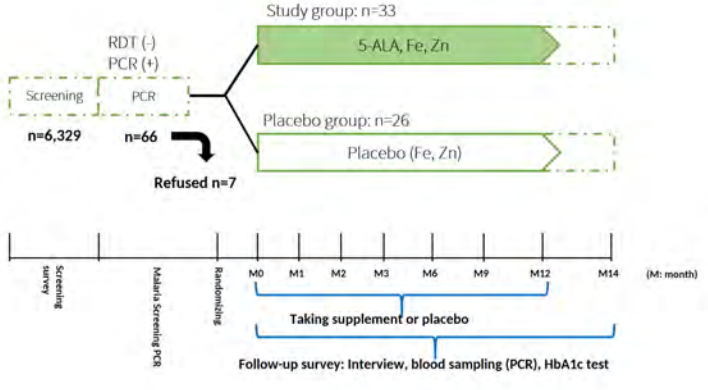
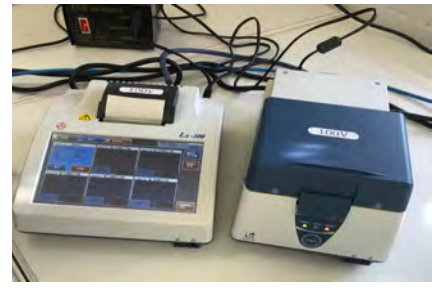


Figure 2. Study sites of the 5-ALA asymptomatic malaria study

Figure 3. 5-ALA supplement or Placebo

Appearance of 5-ALA supplement and Placebo are identical to each other.

Figure 4. Device for the LAMP test



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IPL publication 2021

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They visited Institut Pasteur du Laos!

25 October 2021: S. Exc. Madame CHHUOR Siv-Leng, Ambassadrice de France au Laos

04 March 2021: H.E. Mr. Paul Kelly, Ambassador of Australia and his delegation

04 March 2021: Field visited of biosafety and biosecurity at IPL

26 February 2021: Ambassador H.E. Ms. Ina Marciulionyte

Teaching/Training

08 November 2021: Opening Ceremony of 2nd Field Epidemiology Training for medical military personnel (3-week course, November 8-26, 2021).

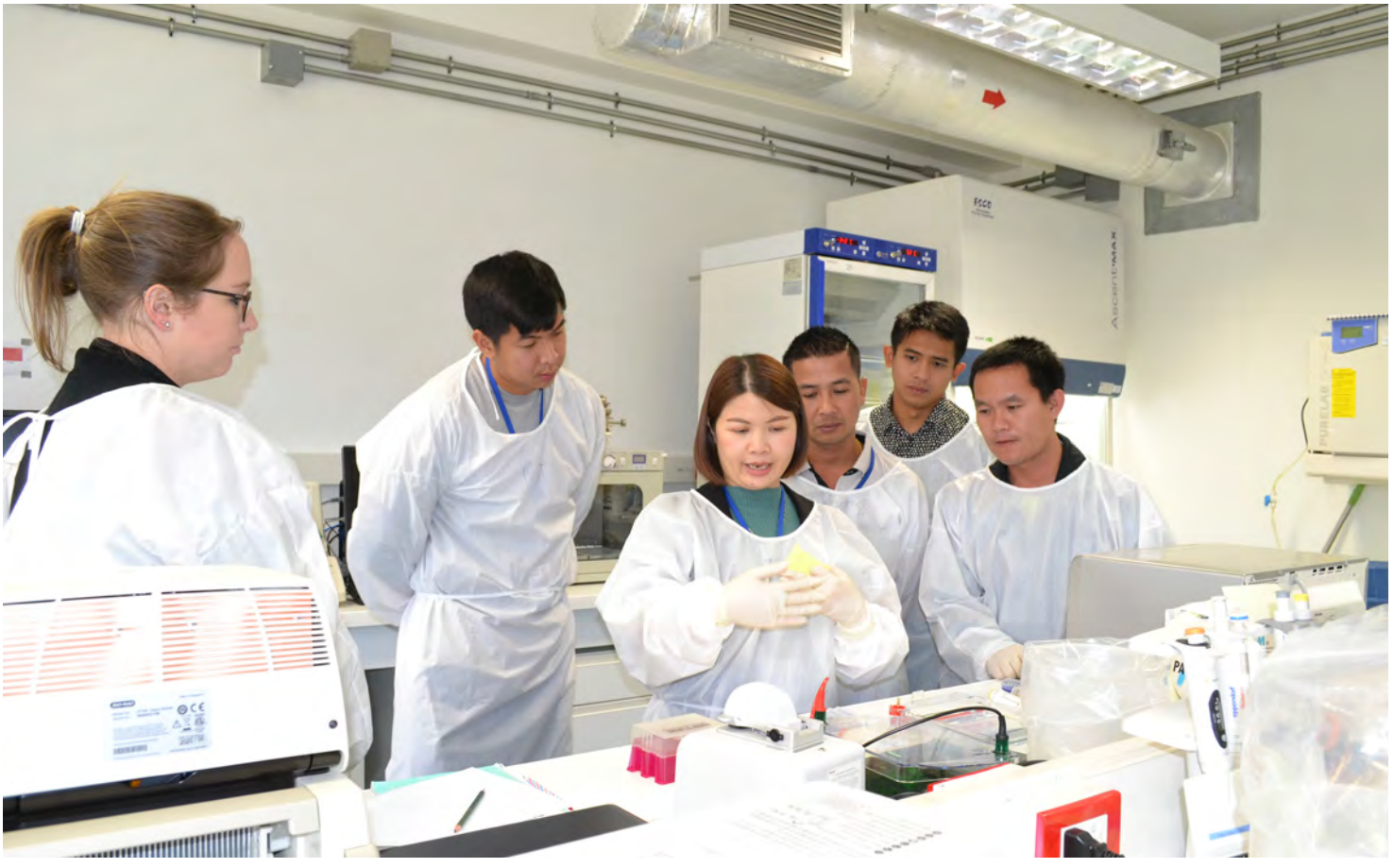
28 Sebtember 2021: Hospital Visiting, in order to assess and provide guidance on how to improve the laboratory facility and capacity.

01 October 2021: Offline and Online Lectures for the 5th military group of On-the-job Training.

24 February 2021: Certificate Handover ceremony of the 4th group of On-the-job Training.

03 February 2021: ELISA training for students of the University of Health Sciences

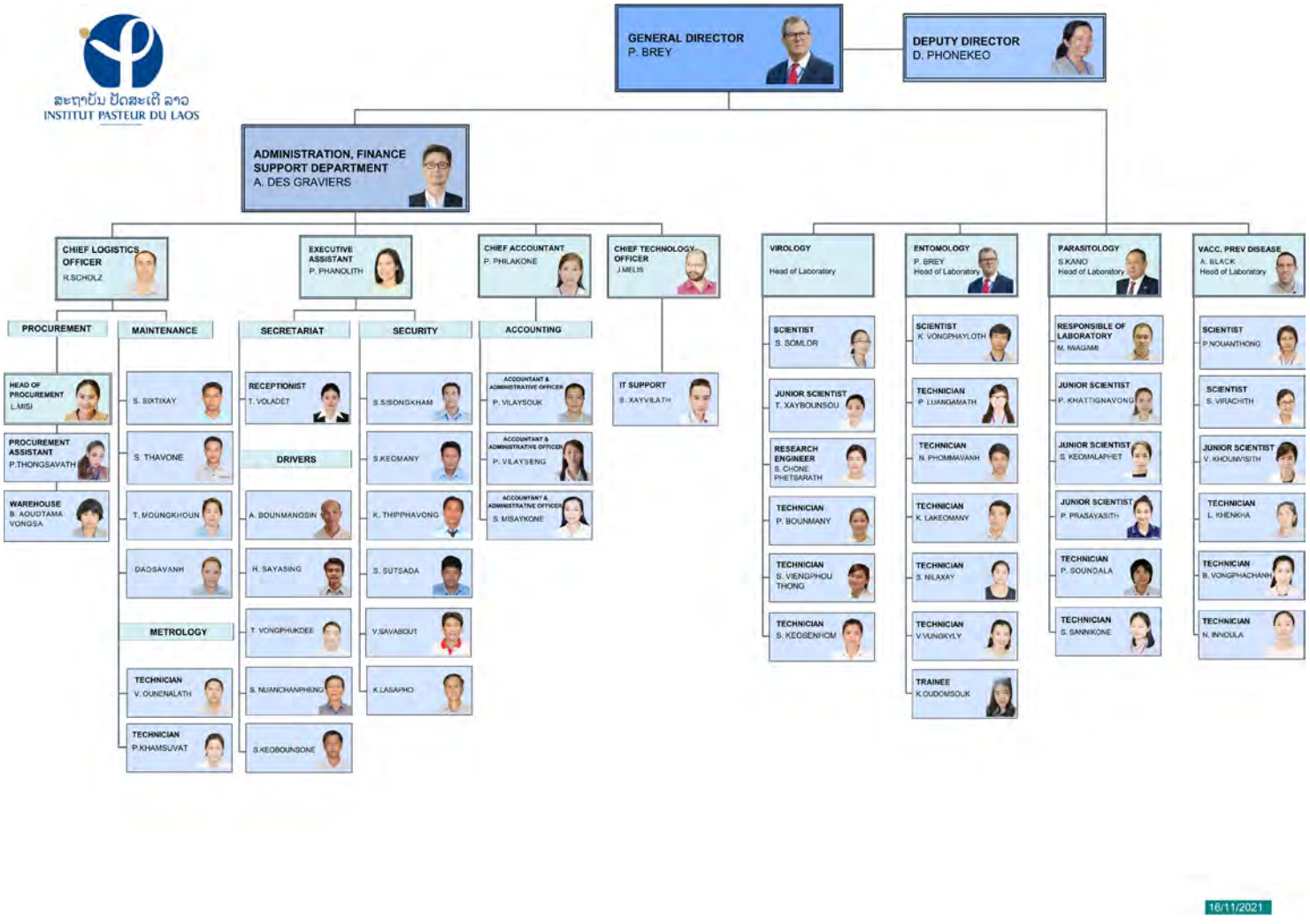
29 January 2021: Training PPE suit, Nasopharyngeal and throat swab and Fit test mask FFP3 for Sisattanak district hospital



19 January 2021: Lab technique demonstration to the 4th group of On-the-job Training (Lab technique and management).

14 January 2021: Certificate Handover ceremony of Refresher training on Biosafety and Biosecurity.

Main organigram



ສະຖາບັນ ປັດສະເຕີ ລາວ
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