ກະຊວງ ສາທາລະນະສຸກ
Ministry of Health

ສະຖາບັນ ປັດສະເຕີ ລາວ
INSTITUT PASTEUR DU LAOS

Institut Pasteur du Laos

Activities Report 2014

Réseau International des Instituts Pasteur

Summary

Mandate

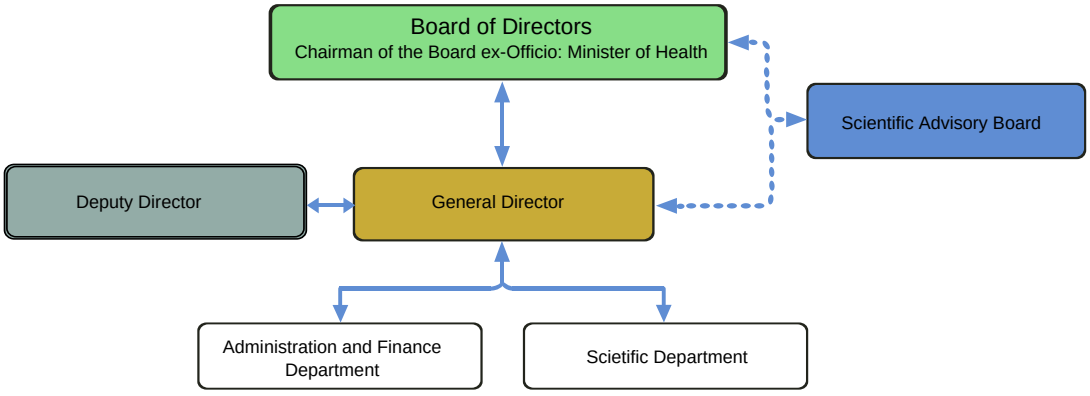
Institut Pasteur du Laos (IPL) is a Lao National Institution created by Prime Ministerial Decree in November 2007 IPL is the result of a long term and joint decision between Lao Ministry of Health and Institut Pasteur Paris which commits to stay 16 years before retroceding the full management of IPL.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

Pr. Dr. Eksavang VONGVICHIT, (Chairman), Minister of Health, Lao PDR.
Dr. Ponmek DALALOY (Honorary Chairman of the Board), Former Minister of Health, Lao PDR.
Pr. Dr. Bountiem PHISSAMAY, Minister to the Government Office, President of National University Council, Lao PDR.
Mrs. Khampheng PHOLSENA, Minister to the Government Office, President of the National Commission for the Advancement of Women, President of the National Commission for Mothers and Children, Lao PDR.
Pr. Dr. Didier SICARD, Honorary President of the National Ethic Committee of France, France.
Dr. Marc JOUAN, Secretary-General of the Institut Pasteur International Network, France.

Actual composition of Scientific Advisory Board:

Prof. Felix Rey, Institut Pasteur, Paris.
Prof. Olivier LORTHOLARY, Necker Hospital, Paris.
Dr. Samlane POMPIDA, Former Director National Center for Malariology, Parasitology and Medical Entomology, Ministry of Health, Lao PDR.

Letter from

Dr. Paul BREY

Director

During the past year, IP Laos has considerably consolidated its laboratory staff as well as its laboratory and training infrastructure. As of September 2014 IP Laos has a total staff of 53 persons of whom 43 are Laotian. In line our original plan, established in 2007, IP Laos is now fully staffed and functional with all laboratories occupied. In addition, an exceptional donation on the part of the Henry M. Jackson Foundation with support from US Department of Defense Global Emerging Infections System (GEIS), allowed IP Laos to fully equip its dedicated BSL1 & BSL2 training laboratories. The training laboratories provide a unique facility in Lao PDR and will contribute to the training of Lao medical doctors and Ministry of Health staff from the capital and the provinces.

In line with its National Institute status, IP Laos continues to work hand in hand with the Lao Ministry of Health providing evidence-based data to the Lao MOH on a weekly basis. To facilitate this communication Dr. Darouny Phonekeo was officially appointed as Deputy Director of IP Laos by the Minister of Health. Dr. Darouny's presence facilitates the communication and information transfer between IP Laos and the Ministry. Following the serious dengue epidemic of 2013, the Government of Lao PDR and other stakeholders such as the World Health Organization have recognized the Arbovirus and Emerging Viruses Lab of IP Laos for their excellence and tireless efforts in the area of dengue, surveillance, diagnostics and serotyping.

During the year, IP Laos also welcomed Dr. Shigeyuki Kano and his team from the National Center for Global Health and Medicine (NCGM) Tokyo, who set up the Lao Japan Lab focusing on Malaria drug resistance and trematode infestations with the support of the Japan International Cooperation Agency (JICA) and Japan Science and Technology Agency. The Lao-Lux lab continues to contribute important finding on vaccine preventable diseases and has carried out several projects with the Lao Blood Bank, Ministry of Health and Luxemburg Development to improve disease surveillance, better monitor vaccination coverage and efficacy. The IP Laos Medical Entomology Lab continues its ongoing programs with the US Naval Medical Research Center Asia (NMRC-A), the French Ministry of Foreign Affaires and French Development Agency (AFD – ECOMORE) to identify medically important arthropod vectors, in specific biotopes (rubber plantations, caves, remote villages, primary forests, etc.), as well as their status to insecticide resistance.

These data will allow the Lao MOH to improve vector control strategies.
In addition, a special effort was made to reinforce our ties with the Lao Military Medical Department (LMMD) of the Lao Ministry of Defense. A Memorandum of Understanding (MOU) was signed on 27 May 2014 to allow LMMD staff to carry out joint research projects and to train in IP Laos laboratories.

With regard to training, IP Laos conducted three training courses: A Regional Chikungunya Workshop, a workshop on Taxonomy and Systematics of medically important arthropods for MOH staff from the central and provincial level and a Biosafety and Biosecurity Workshop for MOH staff at the central and provincial level.

IP Laos's junior scientists, working together with their foreign mentors, are becoming more and more involved in the Institutes research programs and are now taking the responsibility to manage these programs. After three years of Scientific Activity, IP Laos is becoming a center of excellence for research and training of Lao scientists/medical doctors in the area of infectious and vector borne diseases.

Finally, the Lao Ministry of Health and WHO have nominated IP Laos as the front-line laboratory for diagnostic of suspected Ebola cases in Lao PDR. The Ebola public health crisis has prompted IP Laos to upgrade the BSL2+ lab to a BSL3 status with the incorporation of a glove box and autoclave inside the security laboratory. Dr. Marc Grandadam, Head of the Arbovirus and Emerging Viruses Laboratory is working closely with the Lao MOH and WHO to put into place a failsafe 24 hour Ebola diagnostic platform. IP Laos has also participated in the real-time Ebola simulation exercises with the Lao Ministry of Health and other institutions, such as Wattay Airport, Mittapab Hospital and the National Center for Laboratory and Epidemiology (NCLE).

A decade ago, the idea of the creation of an Institut Pasteur du Laos emerged with the SARS and Avian Influenza H5N1 epidemics. At that time, the Lao Ministry of Health rallied the creation of IP Laos to provide Lao PDR with a research and training facility to deal with highly pathogenic emerging diseases threatening the country and the region. Today, IP Laos stands ready to fulfill this important role and to work with the Ministry of Health and other stakeholders to mitigate the arrival and spread of emerging infectious diseases.

Biography

Dr. Darouny Phonekeo

Deputy Director

Dr. Darouny Phonekeo graduated from University of Medicine in 1986 (12th promotion) as Medical Doctor in General Medicine. Right after graduation, she was appointed to work in National Institute of Health and Epidemiology (NIHE) which was, at that time, composed of Public Health Laboratory Unit, Epidemiology unit, Expended Program of Immunization (EPI), Water supply and environment sanitation, and Blood Bank. She started her first carrier in Bacteriology Laboratory with on the job training. She participated in many short and long courses in different areas of Laboratory such as:

Entomology, introduced by an expert from Pasteur Institute from Noumea, New Caledonia.

Food quality, introduced by NIH, Thailand

HIV, Poliomyelitis and Dengue lab diagnosis training courses in Japan.

In 1998, the NIHE was split into different institutions such as: Center for Laboratory and Epidemiology (NCLE), Center for HIV and AIDS Control (CHAS), Center for water Supply and Sanitation, EPI and Blood Bank. She remained in Laboratory with Serology-Virology Division (NCLE).

Dr. Darouny was involved in teaching Laboratory technician students on Bacteriology and later on Immunology topics in Medical Technology Faculty under University of Medical Sciences.

From 1999 to end of 2001, she did a master degree course in IFMT (Institut Francophonie pour la Médicine Tropicale) (1st Promotion) and received Diplôme d'Etude et de Recherche Approfondis (DERA).

In 2007, she was nominated to be Deputy Chief of Laboratory Division, responsible for Serology and Virology Unit.

Experience:

With support of WHO/US CDC, she Initiated, coordinated and managed the Influenza Like Illness (ILI) and SARI (Severe Acute Respiratory Infection) Laboratory Surveillance in NCLE. This surveillance is comprised of 8 hospital networks through the country since 2006. In 2010, NIC (National Influenza Center) was accredited by WHO to NCLE. Dengue Laboratory Surveillance was added to this ILI and SARI surveillance network later (2012).

She participated to develop an Avian Influenza Strategic Plan for Lao PDR and collaborate closely with the Surveillance unit of NCLE for investigation of AI suspected cases.

Participated to Biosafety and Biosecurity activities (Training, encouraged application in Laboratory hospitals of each province through the country…)

Participated to establish Laboratory Network in the country.

Participated to develop Health Laboratory Policy (completed in 2013).

In 2009, she was assigned by Ministry of Health to be involved in the construction of Institut Pasteur building and follow up its construction until completion (beginning 2011).

Since 2012, she was nominated by Minister of MoH to be Deputy Director of Pasteur Institute, part time and later full time.

Scientific Activities 2014

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

Arbovirus infections are a persistent public health threat for many countries worldwide. However, their impact may vary depending on climatic conditions, herd immunity and the existence of sustainable prevention programs. Lao PDR faced a major dengue outbreak in 2013 during which a co-circulation of chikungunya virus was confirmed. Surveillance remained a major activity of the arbovirus and emerging viral disease laboratory in 2014. The clinical network was kept active and the setting up of new collaborative projects offered the opportunity to interact with new partners. Research programs allowed young Lao staff members to acquire their first experience of project management, student mentoring and the process of communicating with health authorities.

In addition to our laboratory staff, we have also invited trainees (Lao nationals) from other institutions to conduct their Master degree internships, as well as other students to participate in a course on biosafety.

Head of Laboratory: **Marc GRANDADAM, PhD**

Junior Scientists:
Malayvanh LAO, MD
Sompavanh SOMLOR, MD
Kouxiong SAYTENG, MD

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

Projects

- Dengue surveillance in Vientiane city
- Japanese encephalitis
- Follow up of chikungunya patients
- Investigation of arbovirus in ticks (TIBO project)
- Response to health alert
- Education activities

Dengue surveillance in Vientiane city

Project coordinator: Malayvanh Lao
Member of staff: Phaithong Bounmany, Sitsana Keosenhom

Background

In 2013, a dengue outbreak was characterized by a precocious circulation of the dengue 3 virus. In late 2013, dengue 4 was detected in Vientiane Capital, whereas in the past only sporadic cases have been detected in villages located in suburban areas. However, particularly high temperatures and a late beginning of the rainy season were probably the main factors that hampered early dengue 4 circulation and avoided a new outbreak in the capital city. At the end of September 2014, only 110 samples from suspected cases collected in Vientiane Capital hospitals were investigated compared to 1957 samples at the same time in 2013 (Table I). Among those samples, 40 tested positive for a direct marker (RT-PCR and/or Ns1 antigen) and were inoculated to C6/36 cells. A total of 30 strains were obtained. Epidemiologic data revealed that seven of the confirmed cases were imported into Laos by tourists coming from Thailand (n=6) and Indonesia (n=1). Sequence analyses are ongoing to determine the degree of similarity between autochthonous and imported dengue 4 cases.

Among the patients investigated, two fatal cases were recorded but only one could be formally associated with a dengue infection.

The syndromic surveillance carried out at a national level in 2014 recorded a total of 1561 suspected dengue cases. The laboratory in charge of the samples' analysis does not perform lab testing as long as the number of weekly cases is below the "historical threshold". Thus, the etiologies at the origin of these dengue-like syndromes remain undetermined.

Assessment of the vector population was systematically conducted around confirmed dengue cases. Patients were contacted for an interview and home visit. This strategy was implemented in early 2014 to evaluate the risk of a major dengue 4 epidemic in Vientiane Capital. Low densities of vector population were generally recorded in and around the houses of the confirmed cases. For some patients, high risks of exposure to mosquito bites were evidenced at their workplaces or in schools. Virologic investigations performed on mosquitoes caught in the neighborhood of a DENv-4 case allowed the isolation of a dengue virus serotype 3.

Hospitals	Cases	PCR +/- NS1Ag	NS1Ag +	IgM/ IgG+	Dengue serotypes				
					D1	D2	D3	D4	ND
Sethathirath hosp.	29	2	0	0	0	0	1	1	0
Children's hosp.	47	3	0	0	0	0	0	3	0
Military hosp 103	8	0	1	0	0	0	0	0	0
Police hosp. 5	1	0	0	0	0	0	0	0	0
Maysa French medical centre	11	8	2	0	1	2	2	2	1
Mittaphab hosp.	13	0	0	0	0	0	0	0	0
Mahosot hosp.	1	0	0	0	0	0	0	0	0
Total	110	13	3	0	1	2	3	6	1

ND: not determined

Table I: Dengue surveillance results from 1 January 2014 to 29 September 2014

Retrospective investigation of dengue virus

Sequencing data of dengue 3 strains isolated in 2012-2013 from patients residing in Vientiane Capital evidenced the co-circulation of two genotypes. The earlier strains of dengue virus serotype 3 isolated in 2012 belonged to genotype II. In late November 2012, dengue virus related to genotype III was identified in two districts of the city. As the number of confirmed cases exceeded 50% of the suspected dengue cases (up to 80% at the height of the epidemic), dengue typing was only performed on a subset of confirmed cases and systematic sequencing was abandoned. To further characterize the dynamic of dengue genotypes in Vientiane Capital, a rapid approach for discriminating dengue serotype 3 genotypes II and III has been set up. Restriction maps of *env* gene fragments targeted by the typing real-time RT-PCR were established using preliminary sequence data. This comparison allowed the identification of a unique restriction site that specifically cut within the fragments obtained derivate from genotype II isolates but not within those obtained from the genotype III virus (see Figure n°1).

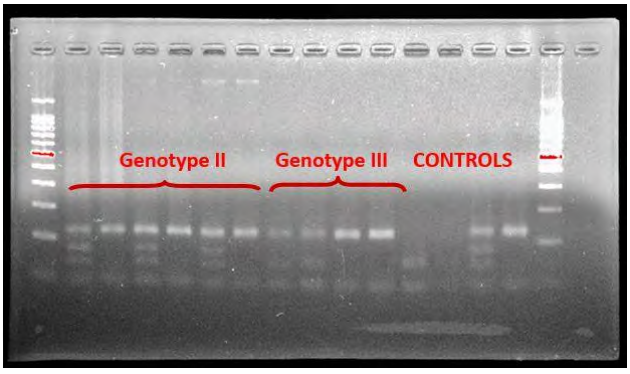


Figure n°1: RLFP Discrimination of dengue serotype 3 genotypes II and III

Up to now, 121 isolates have been tested by this approach of which 71% belonged to genotype II. This retrospective typing will help in understanding the chronology of the spread of the two genotypes during the 2013 epidemic.

Japanese Encephalitis Infection

Project coordinator: Kouxiong Sayteng
Member of staff: Souksakhone Viengphouthong

In 2013, a series of samples taken from pigs were tested for their reactivity to the Japanese encephalitis virus antigen. To complement this preliminary study, 258 human sera from subjects who had close contacts with pigs (farmers, slaughterhouse staff, etc) were collected in 74 villages distributed among 23 districts of 8 provinces (Louangnamtha, Louangprabang, Houaphan, Xiengkhouang, Vientiane, Borikhamxay, Saravanh and Champasak). These samples were analyzed by indirect ELISA tests for the presence of anti-JEV IgG. Positive reactions were detected in 210 (81%) of the samples tested. As cross-reactions exist between members of the flavivirus genus, further specific tests are required to determine the specificity of the antibodies. Combined with the study on pigs, these data will help in drawing up a first map of JEV in Laos.

Follow up of chikungunya patients

Project coordinator: Somphavanh Somlor
Member of staff: Chintana Lathaphasavang, S. Virachit

Background

A seroprevalence study was carried out by our IPL team in 2013 to determine the post-epidemic prevalence of Chikungunya virus in the Southern province of Champasak. A follow-up of participants was organized to evaluate the impact of post-chikungunya chronic arthralgia. Of the volunteers who were interviewed and retested in 2014, 56% were found positive for anti-chikungunya virus antibodies, confirming the high attack rate suggested by the seroprevalence study performed in 2013. Within the confirmed cases, 39,6% declared suffering recurrent arthralgia.

In collaboration with the Parasitology unit of IPL, the opportunity was taken to collect stool samples to update knowledge on helminthes carriage in Southern Laos. Prevalence of helminthes carriage recorded reached 80% with multiple species identified in 38%. Stool examination evidenced the presence of three different groups of helminthes: (i) Nematodes (39,6%), (ii) Cestodes (5,2%) and Trematodes (66,4%). A total of eight different species could be identified with two dominant species, Opisthorchis viverrini, Ankylostoma duodenal (29,5%). Up to 48% of the volunteers admitted using antiparasitic treatment, suggesting that the prevalence, abundance and diversity of worm species may be underestimated

Dengue and chikungunya virus surveillance in Southern provinces:

Dengue-like syndromes were reported in different Southern provinces of Laos in early 2014. The highest incidence rates were recorded in Salavan, Attapeu and Champasack. However, no lab investigations were performed to identify the etiologies. IPL offered its support to other institutions or provincial hospital to document these cases. The Lao-Oxford-Mahosot- Wellcome Trust Research Unit (LOMWRU) shared samples collected in the course of a chronic febrile syndrome project. Among the 4 samples that matched a dengue-chikungunya case definition, one was found positive for dengue NS1 antigenemia.

A total of 93 samples from patients hospitalized in the provincial hospitals of Champasak and Attapeu were analyzed by RT-PCR for real-time Chikungunya RT-PCR. Among these, 1 was found positive and the virus was isolated. The presence of anti-CHIKv IgG was shown in 33.6% of the patients. None of them was found positive for dengue.

Altogether, these results indicate that multiple etiologies, including different arboviruses, are the origin of dengue-like syndromes in Southern Lao provinces.

Seeking for arbovirus in ticks (TIBO project)

Project coordinator: Ian Sutherland and Paul Brey
Member of staff: Khamsing Vongphayloth

Background

Ticks species and pathogens transmitted by ticks are poorly documented in Lao PDR. The TIBO project aims to draw up a map of tick species distribution in Laos and explore the putative pathogens they may harbor. Tick identification has been performed in collaboration with the Walter Reed Army Institute for Research/Smithsonian Institute, Washington DC, USA (see medical entomology unit section). Arbovirus screening has been performed by IPL whereas bacterial investigations have been conducted by the Lao-Oxford-Mahosot Wellcome Trust Research Unit (LOMWRU).

A total of 6692 ticks, mainly larvae and nymphs, were collected in two different remote areas. After sorting the ticks by place of capture, stage of development and similarity of morphology, pools of up to 10 specimens were prepared. Specimens representative of the different species were saved for entomological identification. After grinding with specific zirconium bead, total nucleic acids were extracted from half of the samples and subjected to pan-alphavirus or pan-flavivirus RT-PCR. Of the 768 pools tested, 15 were found positive for pan-alphavirus sequences and 3 for pan-flavivirus sequences. Sequencing of amplicons is ongoing to attempt to determine the complex of the virus within the *flavivirus* and *alphavirus* genus. These preliminary results shed light on a class of South-East Asian arthropods whose study has been largely neglected, both for their diversity as well as their potential role in pathogen transmission.

Response to health alert

Project coordinator: Marc Grandadam

Background

In March 2014, the Ebola virus emerged in four Western African countries, leading to an unprecedented situation as the virus spread from selvatic foci to urban areas. Difficulties in containing the epidemics led the World Health Organization (WHO) to implement surveillance and alert programs worldwide to prevent a pandemic expansion of the virus. The Institut Pasteur du Laos has been mandated by the Lao Ministry of Health to set up a laboratory plan to investigate locally suspected cases. The transfer of viral hemorrhagic fever virus diagnostic tools has been performed with the help of the French WHO collaborative centre for arbovirus and viral hemorrhagic fevers co-hosted by the Institut Pasteur teams in Paris and Lyon. A response team has been trained to handle samples under high security conditions on a 24/7 basis (Figure n°2). Internal and external global alert exercises have been organized to test lab capacities and coordination between the partners in the surveillance system. This capacity building has been partially supported by the WHO Western Pacific office.

The laboratory response capacity has been assessed by organizing full-size internal and external exercises. The mean time to obtain a first RT-PCR result is 5 hours 30 minutes.

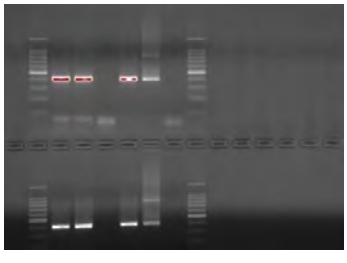


Figure 2:
Agarose gel analysis of VHF amplicons.

Consulting activities

IP Laos teams were asked to carry out a risk assessment of French schools in Vientiane (Lycée Hoffet and the French nursery school) to provide recommendations for vector control methods. On-site visits were organized in the presence of the different school staff including management, the bursar, housekeepers and gardeners. Alternative strategies were proposed to reduce the use of chemical compounds in order to minimize the toxic exposure of staff and children and the emergence of insecticide resistance in mosquitoes.

Support activities:

The A&EVD laboratory benefited from the support of members of the International Pasteur Network (IP Sénégal, IP Madagascar, IP Hong Kong and NIHE, Hanoi) to launch its research activities. As technical platforms are now fully operational, the lab can now respond to requests for reagents and reference materials from other Institutes within the Pasteur Network. Table II summarizes biological material transfers in 2014.

Consignee	Biological material
IP Madagascar	Antigens: Dengue; West Nile; Chikungunya; Japanese encephalitis
IP Cambodia	Zika virus and anti-Zika HIMAF (obtained from IP Sénégal) Ebola positive control (419 bp transcript obtained from IP, Paris) FHV primers
IP Guadeloupe	Antigens: Dengue; West Nile; Chikungunya Japanese encephalitis ; Zika.
National reference centre for Arbovirus, IRBA, Marseille, France	Antigen: Chikungunya

Table II: Biological material transfer activity in 2014

Educational activities

Scientific communications

Marc Grandadam. Dengue and chikungunya. Towards a laboratory network for improved diagnosis in Lao PDR. Centre d'infectiologie Christophe Mérieux au Laos (CICML). 25-27 November 2013. Vientiane, Lao PDR.

Marc Grandadam. The emergence of chikungunya virus in Laos. Emerging infectious diseases in South East Asia. Phnom Penh, Cambodia. 9-11 March 2014.

Marc Grandadam. Dengue laboratory surveillance in Lao PDR. Regional Workshop Renewing National Dengue Strategies in GMS
Innovative approaches to dengue prevention and control – identifying new solutions for an old problem. Luang Prabang, Lao PDR, 26-27 March 2014.

Marc Grandadam. A Land of Arboviral Emergence: A New Frontier for Virus-Vector Research. Symposium of the Institut Pasteur International Network. Paris, France. September 10-13, 2014.

Training of students

Name	Degree	Institution	Time period	Subject
Phouxai POUPHACHAN	-	Epidemiology and Prevention centre of the Lao Army	December 2013 - February 2014	General medical virology and arbovirology
Natnicha INTHAVONG	Volunteer (Master 2)	Melbourne University of Sciences	February 2014	Discrimination of dengue 3 genotypes II and III by PCR-RFLP
Julie BOBICHON	Master 2	Université J Fourier, Grenoble, France	January-July 2014	Development of chikungunya virus IgM ELISA test
Siriphone	Master 2	Institut de la Francophonie pour la Médecine Tropicale	March-September 2014	Cohort follow up for Chikungunya and helminthes infections
Mingyuan Li	PhD	University of Hong Kong – Li Ka Shing Faculty of Medicine	June-July 2014	Interaction of KDLER with flavivirus (dengue serotypes-West Nile) cellular life cycles

Training of Table III: Synthesis of students and guest researchers welcomed in 2014.

Scientific publications

Malayvanh Lao, Valérie Caro, Jean-Michel Thiberge, Phaitong Bounmany, Khamsing Vongpayloth, Philippe Buchy, Veasna Duong, Chansamone Vanhlasy, Jean-Marie Hospied, Manichanh Thongsna, Khamla Choumlivong,Phonesavanh Vongkhamchanh, Bounleua Oudavong, Paul T. Brey and Marc Grandadam. Co-circulation of Dengue virus type 3 genotypes in Vientiane capital, Lao PDR. PlosOne. In press.

Okabayashi T, Sasaki T, Masrinoul P, Chantawat N, Yoksan S, Nitatpattana N, Chusri S, Morales Vargas R, Grandadam M, Brey PT, Soegijanto S, Mulyantno K, Churrotin S, Kotaki T, Faye O, Faye O, Sow Ab, Sall A, Puiprom O, Chaichana P, Kurosu T, Kato S, Kosaka M, Ramasoota P, and Ikuta K. Detection of chikungunya virusantigen by a novel rapid immunochromatographic test.J Clin Microbiol. In press

Demanou M, Pouillot R, Grandadam M, Boisier P, Kamgang B, Hervé JP, Rogier C, Rousset D, Paupy C. Evidence of dengue virus transmission and factors associated with the presence of anti-dengue virus antibodies in humans in three major towns in Cameroon.PLoS Negl Trop Dis. 2014 Jul 10;8(7)

Training sessions

- Information on Chikungunya virus disease and diagnosis: Champasak Provincial Hospital, 103 Military Hospital and district hospitals (3 days, March 2014).
- Dengue virus diagnosis: Training sessions for staff and students (2 days, Mahosot Hospital, Vientiane Capital).
- Arbovirus et arboviroses: Institut de la Francophonie pour la Médecine Tropicale (1 journée, Novembre 2013).

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

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

Head of Laboratory: Dr. Paul BREY, PhD

Scientists:
Sébastien MARCOMBE, PhD

Junior Scientists:
Khamsing VONGPHAILOTH, MD
Phoutmany THAMMAVONG, MD
Julie BOBICHON, MS

Technicians:
Boutsady SOMPHONG
Lae THUTKHIN, MS
Khanthalak KHOUNSOMBAT, BS

PhD. Student:
Julie ANNE TANGENA, MS

Master Student:
Abigial JORDAN

Projects

- Insecticide resistance in malaria vectors in Lao People's Democratic Republic and Thailand and capacity building in medical entomology (MALVEC)
- Risk Of Vector-Borne Diseases In Relation To Rubber Plantations In Lao PDR (ECOMORE)
- Vector mapping, characterization of insecticide resistance of Aedes populations, and entomology capacity development in Lao PDR (ARBOVEC)
- Tick and tick-borne diseases in Lao PDR (Tick-borne)

Insecticide resistance in malaria vectors (MALVEC)

Project coordinator: Dr Sebastien Marcombe and Dr Paul Brey
Member of staff: Julie Bobichon(Junior scientist),
Boutsady Somphong (Technician)

Background

In Lao PDR, a recent national survey on the distribution of malaria showed that 65% of the population was still living in transmission areas (Jorgensen et al., 2010). This study also showed the predominance of *Plasmodium falciparum* particularly in the southern part of the country was associated with a high risk of transmission.

In 2004, an entomological survey showed that *Anopheles dirus* was an important malaria vector despite its low density and that the role of *An. minimus* in the transmission varied over time and space (Trung et al., 2004). However, the successive appearance in tropical forest areas of *An. minimus* during the dry season and *An. dirus s.s.* during the second part of the rainy season allows a sustainable malaria transmission. More worrying, the recent environmental modifications linked to agriculture and forestry culture (e.g. rubber plantations) may change the status of several vectors, secondary and major, by giving them appropriate ecological conditions to thrive (Osbomer *et al.,* 2007). Insecticide bioassays showed that *An. minimus* was resistant to pyrethroids in northern Vietnam and Thailand and *An. epiroticus* was resistant to DDT and pyrethroids in Cambodia and southern Vietnam (Van Bortel *et al.,* 2008). It is possible that the use of agricultural insecticides may be at the origin of the selection of these resistances and so constituting a danger for the implementation of effective vector control strategies. Unfortunately, there is a paucity of data available on the insecticide resistance of the main malaria vectors in Lao PDR.

The “hot-spots” of transmission being located in border zones (Thailand, Cambodia, Vietnam...), there is an important risk of dispersal of the population of vectors and the associated aresistances in the surrounding areas. In Lao PDR no data are available regarding the impact of agriculture pesticides on the resistance selection. The only available means of control of the transmission is the use of pyrethroid treated bed-nets, but in Laos, 30 to 50% of the people at risk sleep under treated bed-nets. We do not know if the malaria vectors from Thailand and Lao PDR are endophagic or exophagic. For example, *An. dirus* is known to be exophagic, biting people at twilight at a time of day when that is not protected by treated bed-nets. Hence, it is necessary to understand the vectors biology in Lao PDR and Thailand to adapt the vector control strategies.

The risk of distribution of the insecticide resistances of vectors in South-East Asia represents a serious threat to the good results recorded these last years in the control of malaria. It is urgent to identify the distribution, the levels and the mechanisms of resistance of the vectors in the lower Mekong countries with the aim of helping the health authorities to develop more effective strategies of prevention and control of the disease.

Objectives and outcomes

This project has 4 fundamental objectives:

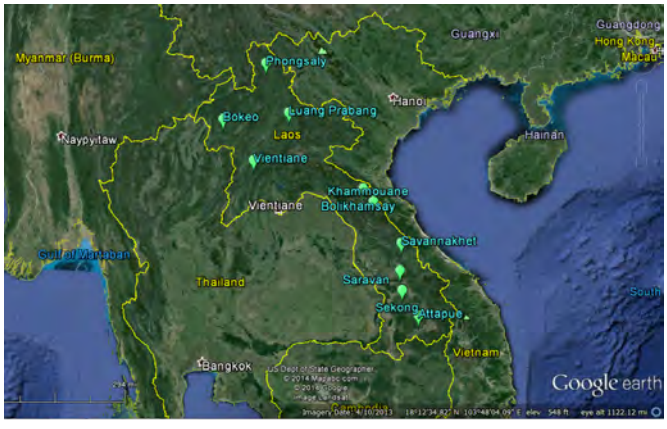
- Evaluation of vectors bionomics and distribution and their role in malaria transmission
- Evaluation of the levels, types and mechanisms of insecticide resistance
- Evaluation of the impact of environmental factors on vector dynamic and resistance selection
- Capacity building in medical entomology in Lao PDR

Expected outcomes:

- Set up a comprehensive map representing the "hot spots" for malaria transmission in Lao PDR and Thailand (border area)
- Generate an Insecticide Resistance database in the main malaria vectors
- Address the dynamics and gene flows between malaria vectors populations
- Guide public health authorities in the design and implementation of Insecticide Resistant Management strategies
- Capacity strengthening of Lao and Thai students in medical entomology and vector control

Methods

Figure 1. Sample locations in Lao PDR



Field sites

After consultation with the different partners (CMPE, IRD, KU) and the Lao Ministry of Health, it was decided to study in 10 provinces distributed throughout Lao PDR and 5 districts in Ubon Ratchathani province in Thailand (Figure 1). This enables us to have a global vision of the situation in the country. CMPE already have an important collaboration network with all the health district departments, which is a fundamental parameter for the success of the project.

Mosquito collection

Every village was divided into four zones from a central axis to select at random 1 house by zone. The selected houses were distant from each other by at least 30 meters. For every house a collector was placed inside and another one outside from 6:00 pm in the evening till 6:00 am in the morning on 4 consecutive nights. Mosquitoes were collected with glass tubes (Figure 2). Mosquitoes were also collected overnight with the cow bait collection method. A long mosquito net was disposed around the animal and adult mosquitoes were collected on it (Figure 3).

Figure 2. Human landing catching in Khammouane province

Figure 3. Cow bait collection in Bokeo province

Figure 4. Field laboratory in Attapue province

Mosquito identification

The mosquitoes collected were morphologically identified to the species or complex using microscopes and following the identification keys (Medical Important Anophelines of Southeast Asia) (Figure 4). The Anopheles collected were then separated by species for the insecticide resistance tests.

Insecticide resistance

Insecticide bioassay (tube tests) were performed following WHO protocols to measure the insecticide susceptibility of the different mosquito species collected (WHO 2013). Adult female were exposed to DDT (4%), deltamethrin (0.05%), and permethrin (0.75%), the main insecticides used in public health in Lao PDR.

Results 2014

Mosquito abundance and diversity

More than forty thousand mosquitoes (*Culex sp., Aedes sp., Anopheles sp*. …) were collected on human and cow bait in Thailand and Lao PDR with nineteen different *Anopheles* species. The mosquito collections in Laos (10 provinces, dry season) resulted in 394 *Anopheles* mosquitoes of 14 different species (Table 1, Figure 5). Among them 2738 were collected on cow bait (CBC) and 394 on humans (HLC). The two most abundant species were *Anopheles minimus*, *An. maculatus* (primary vectors, n=1128 and n=966 respectively) and An. aconitus (secondary vector, n=549). The mosquito collection during the rainy season (8 provinces) resulted in 462 *Anopheles* mosquitoes (HLC, Table 2). *Anopheles minimus* was the most abundant species collected on human (n=128).

The two other primary vectors, *An. maculatus* and *An. dirus,* were also found but in different proportions from the dry season (n=20 and n=23 respectively; Figure 6). Fourteen different *Anopheles* species were collected but were different from the one found during the dry season. The collection in Vientiane and Borlikhamsay provinces are underway.

The mosquito collections in five villages in Ubon Ratchathani (13-31 March 2014), Thailand resulted in 378 *Anopheles* mosquitoes of at least 14 different species. Among them 286 (76%) were either primary or secondary vectors. Of the three primary vectors only *An. maculatus* (n=35) and *An. minimus* (n=4) were collected. The two most abundant species were *An. philippinensis* (n=100) and *An. barbirostris* (n=83), both which are considered secondary malaria vectors (Figure 7).

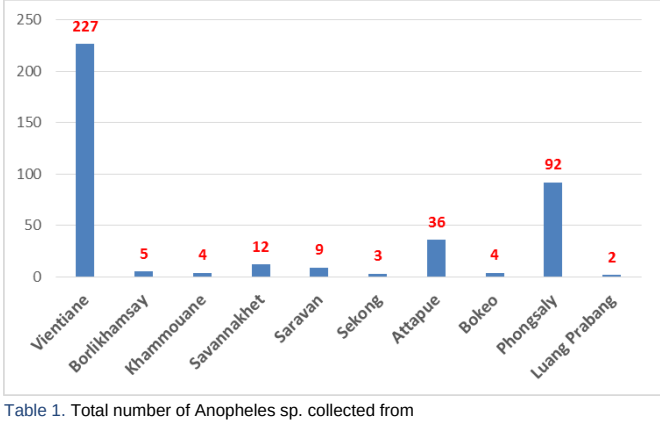


Table 1. Total number of Anopheles sp. collected from Human Landing Catching (HLC) during the dry season 2014, Lao PDR.

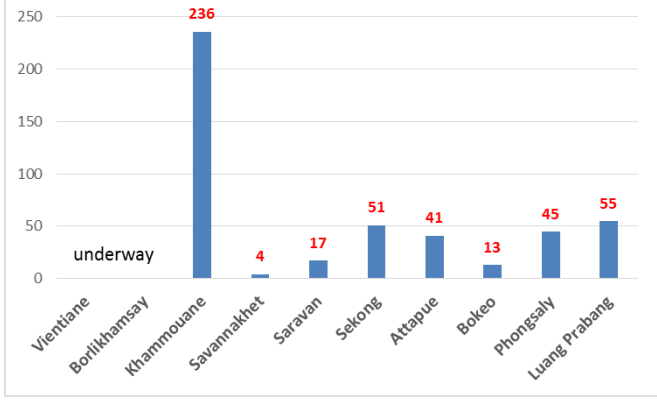


Table 2. Total number of Anopheles sp. collected from Human Landing Catching (HLC) during the rainy season 2014, Lao PDR.

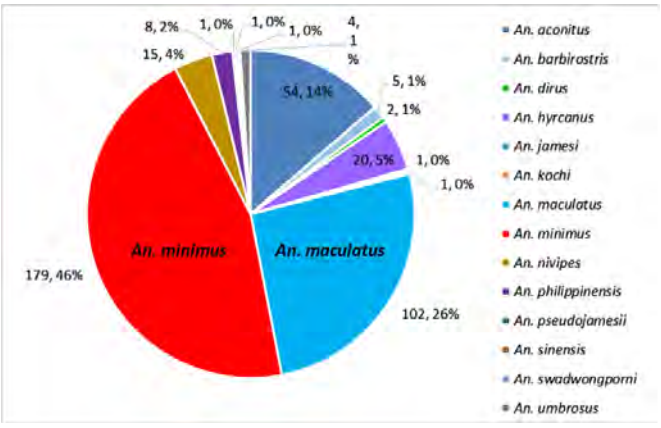


Figure 5. Total number of *Anopheles sp.* collected from HLC during the dry season 2014, Lao PDR.

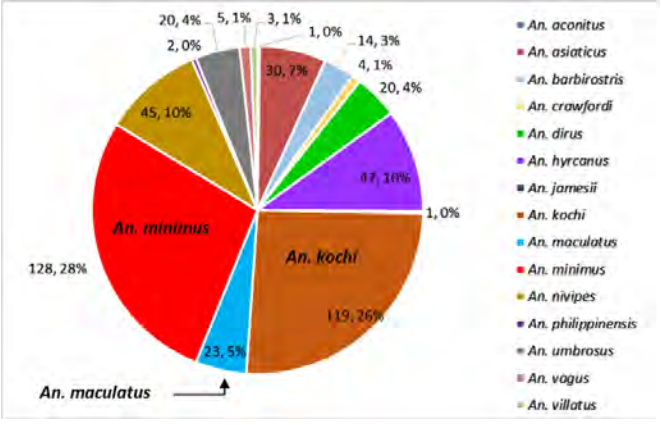


Figure 6. Total number of *Anopheles sp.* collected from HLC during the rainy season 2014, Lao PDR.

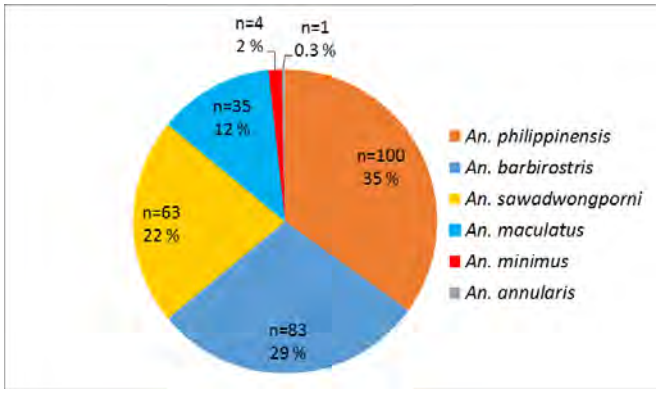


Figure 7. Total number of Anopheles sp. collected from HLC during the rainy season 2014, Ubon Ratchathani province, Thailand.

Insecticide resistance

In Lao PDR, no insecticide resistance was detected in *An. minimus* and *An. maculatus* against deltamethrin, and permethrin. No insecticide resistance was detected in *An. minimus* against DDT, but resistance is suspected in An. maculatus in Attapue province, Hadoudomxay village (n=76, 92% mortality, Figure 8). In the same village, *An. vagus* showed a strong resistance to DDT and resistance to deltamethrin (Figure 9). In Thailand, *An pediteaniatus* a secondary vector, showed resistance to the three insecticides tested while *An. nivipes* and *An. barbirostris* were susceptible.

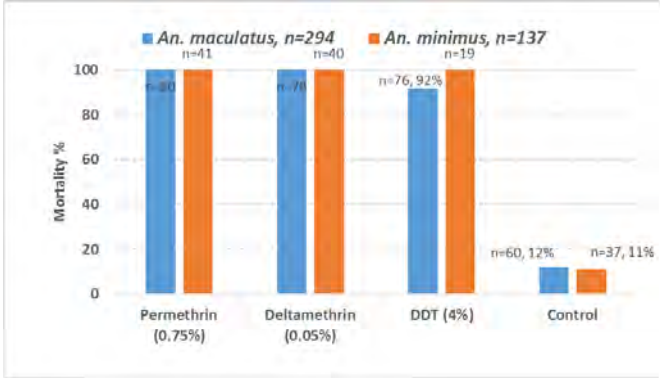


Figure 8. Resistance status of *An. maculatus* and *An. minimus* against permethrin, deltamethrin and DDT, Attapue province, Lao PDR 2014.

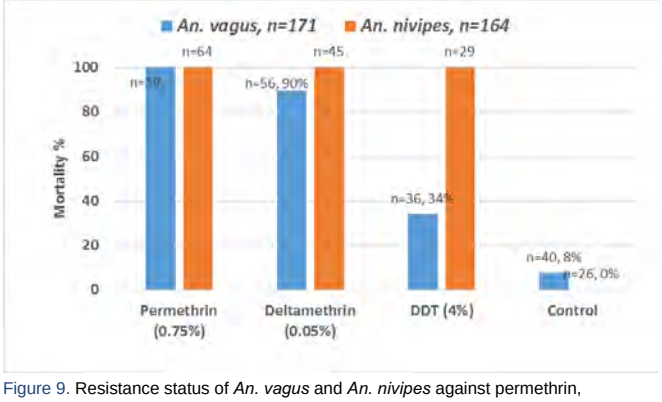


Figure 9. Resistance status of *An. vagus* and *An. nivipes* against permethrin, deltamethrin and DDT, Attapue province, Lao PDR 2014.

Discussion and perspectives

The results from Lao PDR showed that *An. minimus* and *An. maculatus*, primary vectors of malaria, and several secondary vectors, are biting humans constantly during the night both indoors and outdoors. This emphasizes the need for use of bed nets when people are sleeping and personal protection when people are outside. However insecticide resistance tests showed that several Anopheles are resistant to DDT and to pyrethroids (used for bed nets coating) in several provinces of Lao PDR emphasizing the need for a constant monitoring of insecticide resistance in malaria vectors in the area. In 2015, a second round of collections will be implemented in the same villages during the dry and rainy season. Molecular work to identify the sibling species of the different group/complex of *Anopheles* species will start on the mosquitoes collected in 2014. Plasmodium detection in these mosquitoes will also be implemented. Furthermore the possible mechanism involved in insecticide resistance, metabolic and target mutation, will be researched.

Partners

- National Center of Malariology, Parasitology and Entomology (CMPE), Vientiane, Lao PDR
- Institut de Recherche pour le Développement (IRD) IRD-MIVEGEC, IRD UMR-MD3, Bangkok Thailand
- Kasetsart University, Department of Entomology, Bangkok, Thailand
- Institut de Médecine Tropicale d'Anvers (IMTA), Belgium
- University of Life Sciences (ULS), Oslo, Norway
- Bureau of Vector Born Diseases (BVBD), Ministry of Health, Thailand
- World Health Organization (WHO)

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Van Bortel W, Trung HD, Thuan le K, *et al*: The insecticide resistance status of malaria vectors in the Mekong region. *Malar J* 2008, 7:102.

Risk of Vector-Borne Diseases in Relation to Rubber Plantations in Laos

Project coordinator: Dr Paul Brey
Member of staff: Julie-Anne Tangena, Phoutmany Thammavong and Honglakhone Xaiyasing

ECOnomic development, ecosystem mOdifications, and emerging infectious diseases Risk Evaluation

Background

China is the second largest global economic power and bordering countries are benefitting from this increased wealth. In 2009 alone 341 billion US dollars' worth of products were imported from neighbouring countries into China (IMF, 2010). One of the products in high demand in China is rubber. Rubber is used for many products including conveyer belts and many adhesives, but mostly for car tyres. In China alone the number of cars has grown 20 fold in the past decade with 18.5 million cars sold in 2011 (Branigan, 2012). With only 3% of the 1.3 billion Chinese population owning a car at the moment (Madslien, 2012), the car industries are not expected to slow in their growth any time soon. During 2012 consumption of rubber increased by 4% with Asia consuming the biggest proportion of natural rubber (Sumernet, 2009). It has encouraged countries to invest in rubber plantations in Asia where 93% of all natural rubber is produced. In the last few decades the number and area of rubber plantations have increased rapidly. Rubber plantations are now found largely in Thailand, Indonesia and Malaysia, together accounting for 72% of all natural rubber production (ANRPC, 2010).

It takes about five to eight years for the trees to mature, from when the trees can be tapped for rubber for up to 30 years. The whitish latex used for rubber production is present outside the phloem in latex vessels of the bark. These vessels are curved at a 30o angle up the tree in a right-handed spiral. This spiral makes tapping latex very difficult and requires a certain skill. A sequence of thin slices of bark are cut without damaging the growing layer. See figure 1. Every worker is able to tap between 300-450 trees per night equivalent to 1 ha of rubber plantation.

A

B

C

Discussion and perspectives

Lao PDR has seen a high increase in rubber plantations (table 1), where relatively low numbers of plantations are present compared to neighbouring countries (Sumernet, 2009; Hurni, 2008; Li and Fox, 2012). This is a new kind of mass farming not seen in Laos before. The area of rubber plantations will continue growing with currently 342,400ha of land designated for rubber plantations (NAFRI, 2011).

Year	Mature rubber plantations in Lao PDR (hectare)
2010	900
2011	6,900
2013	35,960
2015	147,500

Table 1: Mature rubber plantations in Lao PDR (hectare)

Vector-borne diseases can increase or decrease due to changing land use (de Castro *et al*., 1999; Yasuoka and Levins, 2007). In some areas the massive clearing of the rain forest has resulted in a decrease in the typical habitat for *Anopheles dirus* and malaria has declined (Do Manh *et al*., 2010). However, tree crop plantations can provide the preferred habitat for *An. dirus* again with canopy cover and ample human hosts. Rubber plantations are known to be a significant site for malaria transmission (Singhasivanon et al., 1999; Yasuoka and Levins, 2007). In an area of Thailand it was estimated that 25.5% of all malaria cases were caused by work on commercial plantations, including fruit, rubber and teak plantations (Singhasivanon *et al.*, 1999). Many thousands of people are employed in the commercial forest industry, most of whom spend considerable time in and close to these tree crop plantations when *An. dirus* is biting. Rubber plantation tappers are expected to be most at risk as they work outdoors at night when the malaria vector is most active. In 2012 malaria incidence in the south of Lao PDR was three times as high as 2011 (WHO, 2013). This increase in malaria incidence is not well understood but could be related to the increase in rubber plantations, with malaria risk three times higher for people working in forested areas then people living in residential villages (Erhart *et al*., 2005).

The influence of rubber plantations in Lao PDR on the spread of dengue and chikungunya remains unclear. *Aedes albopictus* and *Ae. aegypti* are known to easily adapt to new environments. In rubber plantations discarded containers used for collecting latex are common and may provide ideal breeding sites for these mosquitoes. Furthermore, depending on the vector species able to invade the rubber plantations and the proximity to forests, emerging infectious diseases could be of interest.

Seasonal workers at the plantations could create a whole new dynamic to the vector-borne diseases in the plantations. Currently 37,500 people work on rubber plantations in Lao PDR of which 95.4% are Lao (NAFRI, 2011). The increase in rubber plantations in the coming decade is expected to create work for another 177,700 people. This will increase the need for seasonal workers from other areas. These temporary workers may not have immunity against the local diseases and are more likely to develop serious adverse effects. Additionally, these workers can spread the disease when they travel to areas where the vectors are already established. Even more worrying is the possibility to introduce a drug resistant strain, like ACT resistance, in an area by either returning home with a resistant strain or transporting the resistant strain to the place of work. It is suggested that the presence of high numbers of different vector mosquitoes combined with the increase in seasonal rubber workers and their high exposure to the vector mosquitoes is creating a ‘perfect storm’ in and around rubber plantations for future disease outbreaks.

Rationale

The area of land cultivated for rubber is expanding rapidly in Lao PDR. We anticipate that the changes in ecology from primary and secondary rainforest, to rubber cultivation and the maturation of these rubber trees is likely to result in an altered risk from vector-borne diseases; predominantly malaria, dengue and chikungunya. It is envisaged that this study will provide an opportunity to understand the vector ecology in rubber plantations and be able to advise organizations on how to decrease vector-borne disease incidence. This study will be of relevance to public health workers, governments and those working in the rubber industries of Lao PDR and other countries in SEA.

All methods were approved by the Lao Ethics committee and Durham University ethics committee. The comparison study was also discussed with the CORC-ethics committee of Institut Pasteur and approved.

Overall goal: To assess the potential risk of vector-borne disease infections arising in rubber plantations

Methods

Mosquito ecology in rubber plantation

To understand the ecology of the mosquitoes in the rubber plantations, we compared the mosquito number and diversity of the plantation with the forest and village for one year. Last year we established three areas of around 4km^2 in Luang Prabang province, in bordering Nan and Xieng Ngeun distict where the following four habitats were present.; immature rubber plantation, mature rubber plantation, village and forest. The villages consist of Lao Loum and a mix of minorities including Khamou, Yao and Hmong.

We collected mosquitoes in the different habitats using the double bednet collection method which does not expose participants to mosquitoes. The double netting method entails the use of a small net covering the participant and a larger net covering the smaller net with a gap of 30cm at the bottom. Mosquitoes are attracted to the presence of the participant and fly towards them, mostly flying low to the ground. These mosquitoes encounter the smaller bednet and try to gain access by flying up. They are now in between the two bednets and will have difficulty escaping. See figure 2. Every hour the participant within the net collects all the mosquitoes in between the two bednets and puts them in a collecting cup. In 2013 we sampled mosquitoes with three participants every hour for two days and two nights in the three study areas every month from July to November. In 2014 this was carried out every two months from January to July. We therefore collected data on the density and diversity of mosquitoes every hour, both day and night, every month in all four habitats. With these data we can analyse the behaviour of mosquito species attracted to humans and their abundance in the different habitats. A total of 3888 hours of data were collected in each habitat during the collection period of 2013-2014.

Figure 2: images of the double netting method (A) participants setting the collection method (B) a participant resting inside the bednet (C) a participant collecting the mosquitoes between the two nets and aspirating them into a collection cup

Resistance study

For possible implementation of vector control methods in the future, it is of vital importance to understand the resistance status of the important vector mosquitoes. As *Anopheles* species were too low for good resistance analysis and malaria cases were not found in our study area, we focussed on the dengue vector *Ae. albopictus*. These mosquitoes were collected from the secondary forest in Village Thin Keo by villagers using the double netting method. Two participants collected mosquitoes in the forest for three consecutive days in 6 different months, both in 2013 and 2014. These mosquitoes were exposed to insecticides in WHO tubes for one hour in our field laboratory. Knock down and mortality was noted. The insecticides used were DDT (4%), permethrin (0.75), Deltamethrin (0.05%) and Malathion (5%).

We also collected larvae of *Ae. albopictus* and *Armigeres kesseli* mosquitoes from the rubber plantation area. The Aedes larvae are currently reared in the insectary for large numbers. After a sufficient number of generations the female mosquitoes will be exposed to the same insecticides as the wild caught mosquitoes.

These mosquitoes will be of a known age and are therefore standardized. The *Armigeres* larvae, which are suggested to be possible vectors for some arboviral diseases, were reared in our field laboratory with 4-6 days old non-blood fed females exposed to the insecticides.

To understand where mosquitoes breed in the rubber plantations, a preliminary survey was conducted in 2013. Using this information an extensive Standard Operation Procedure (SOP) was written. This SOP details all the steps to be taken during the survey. A systematic approach is used to identify all water bodies. Data on water quality of all identified water bodies, their size, turbidity, location, presence of mosquito larvae and more are noted. Mosquito larvae are collected and reared at the field laboratory for identification purpose. Surveys are conducted monthly in villages, mature and immature rubber plantations of all three study areas with the help of two villagers. See figure 3. The survey was started in August 2014 and is currently still taking place.

Larval survey

Figure 3: Larval survey conducted by our team and local villagers

Trapping comparison

For further entomological surveys in rural areas of Laos it is important to understand the benefits and costs of different surveying methods, especially when human participants are used. Many different trapping methods are possible for surveys, including the CDC light trap, the BG sentinel trap and the Suna trap. In 2013 we already showed that the double bednet method collected a larger diversity and higher number of mosquitoes then all the electric traps. This year we compared the double bednet method with the Human Landing Catches (HLC) method which is considered the gold standard method for collecting mosquitoes. We have just concluded this study and are in the middle of our analysis. No concrete results are available yet.

Results JUNE 2013- SEPTEMBER 2014

Mosquito ecology in rubber plantation

The described data below are a summary of 3888 collection hours in each habitat (village, forest, mature rubber plantation and immature rubber plantation) throughout one year. We finished our longitudinal study in July 2014. We identified a total of 123 different mosquito species in the different habitats combined with the highest species diversity in the forest. A total of 1250 males were collected of which 890 were *Ae. albopictus*. A total of 24,920 female mosquitoes were collected of which 6305 were *Ae. albopictus,* followed by *Heizmania mattinglyi* (4398), *Culex vishnui* (3562) and *Armigeres kesseli* (2621). A total of 1341 *Anopheles* mosquitoes were collected.

Possible vectors of dengue, chikungunya, malaria, Japanese encephalitis and Lymphatic Filariasis were found in the rubber plantation. See table 2. Currently we are in the process of preparing the *Ae. albopictus* samples for molecular analysis to identify pathogens. About 75% of all *Ae. albopictus* mosquitoes collected during the longitudinal study have been cut and pooled.

Rubber plantation	Total no. of mosquitoes collected	Species diversity	*Aedes albopictus (% of total)*	Potential vectors found
Mature	3649	74	1332 (36.5)	Malaria, dengue, chikungunya, Japanese encephalitis and filariasis
Immature	5322	88	1248 (23.4)	Malaria, dengue, chikungunya, Japanese encephalitis and filariasis

Table 2: Summary of mosquito data collected in the rubber plantations

Apart from identifying the different species present in the rubber plantations, it is important to understand if and when rubber workers are exposed to these vector mosquitoes. Latex tapping in Lao PDR generally occurs from 21:00 to 5:00 and collecting of latex from the cups occurs between 10:00-16:00. Combining this information with the number of mosquitoes collected each hour we have been able to make preliminary figures showing the host seeking behavior of the important vector species in the mature plantation and when the rubber workers are active in these plantations.

Figure 4: Shows the activity of the Japanese encephalitis vector mosquito, *Culex vishnui*, throughout the day and night in the mature plantation with the blue and green

areas showing when the rubber workers are working in the plantations. The mean number of mosquitoes is lower than one as we collected less than one mosquito an hour on average during the surveying period. However, it does show that when workers are present in the plantation for longer than one hour, the chance of getting bitten by *Cx vishnui* increases. The rubber workers are active in the plantations when the *Cx vishnui* mosquitoes are searching for a blood meal with highest exposure for the worker when latex tapping is started. However the highest mosquito activity is before latex collecting is started between 18:00 and 20:00. As often the rubber workers are living in the plantations, the workers will be exposed to the mosquitoes during the peak of 18:00 to 20:00 when they are cooking food and bathing outside their houses.

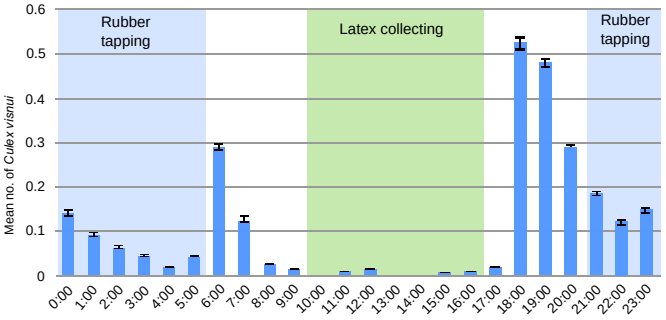

Figure 4:Japanese encephalitis vector exposure by rubber workers: mean number of Cx visnui mosquitoes collected in the mature rubber plantation with the blue areas showing the times when rubber workers are in the mature plantation tapping latex and the green area showing when the workers are in the plantations to collect the latex. The error bars show the 95% confidence interval.

Figure 5: Shows the activity of the dengue and chikungunya vector, Ae. albopictus, throughout the day and night in the mature plantation with the blue and green areas showing when the rubber workers are present in the plantations. The rubber workers are active in the plantations when the mosquitoes are searching for a blood meal with highest exposure for the worker when latex is collected during the day. Although the peak of mosquito activity is after the latex collecting time, the exposure for rubber workers is still high during latex collection with at 16:00 almost one bite per hour.

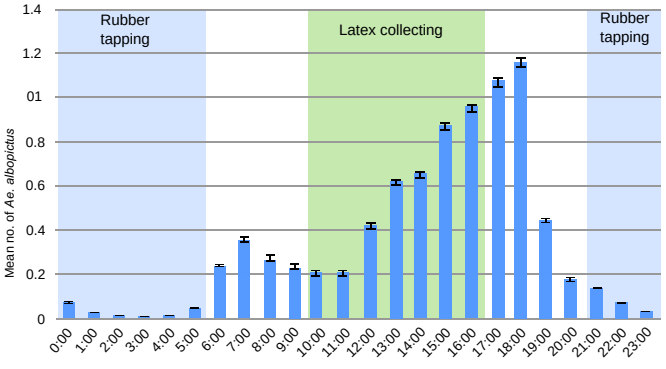

Figure 5: Dengue and chikungunya vector exposure by rubber workers: mean number of Ae. albopictus mosquitoes collected in the mature rubber plantation with the blue areas showing the times when rubber workers are in the mature plantation to tap latex and the green area showing when the workers are in the plantations to collect the latex. The error bars show the 95% confidence interval.

Figure 6: Shows the activity of the malaria vectors throughout the day and night in the mature plantation with the blue and green areas showing when the rubber workers are present in the plantations. The rubber workers are active in the plantations when the mosquitoes are searching for a blood meal with highest exposure at the beginning of the latex tapping period at 21:00. Numbers of *Anopheles* were low throughout the collection period in mature rubber plantations with low exposure risk throughout the day and night. However low exposure risk does not mean that malaria risk is non-existent with many examples in Africa where malaria is endemic with very low vector population.

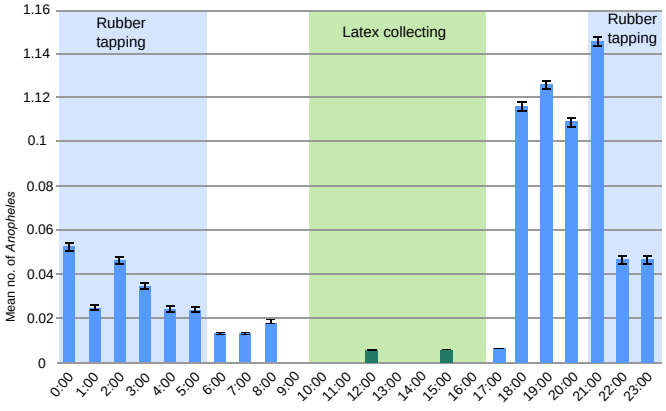

Figure 6: Malaria vector exposure by rubber workers: mean number of *Anopheles* mosquitoes collected in the mature rubber plantation with the blue areas showing the times when rubber workers are in the mature plantation to collect latex and the green area showing when the workers are in the plantations to collect the latex. The error bars show the 95% confidence interval.

In general the rubber workers are exposed to the important vector mosquitoes during their work in the plantations. Even though the exposure is often less than one mosquito per hour, if this exposure risk is taken for the entire period the workers are in the plantations and multiplied by the days they are working in the plantations this exposure does become noteworthy and we can conclude the workers are exposed to the important vector species in the rubber plantations. Interestingly, the highest mosquito activity for all three vector species was between latex collection and latex tapping from 17:00-20:00. During this time rubber workers are often in

and around their houses in the plantations cooking food, bathing and relaxing. Therefore, the presence of their housing in the rubber plantations during these times is potentially increasing their exposure risk. As this is still very preliminary data further analysis will lead to more concrete conclusions.

Resistance study

We tried to expose at least 100 *Ae. albopictus* mosquitoes to each insecticide during multiple occasions to identify their susceptibility to the different insecticides in our area. Currently we are still in the process of exposing wild caught mosquitoes to the different insecticides. Below is a summary of our current data. Susceptibility tests were conducted in August, September and November in 2013 and July, August and September in 2014 using wild caught *Ae. albopictus* from Thin Keo. In total 21 insecticide exposures were conducted in the different months with an average of 20 mosquitoes in the exposure tubes. These were three Bendiocarb, six DDT, eight permethrin, two deltamethrin and two malathion exposures. See table 3. All control exposure tubes consisted of at least 12 female *Ae. albopictus* mosquitoes. No control mosquito died during the 24hrs after exposure for any of the tests.

	total number of exposures	total no. mosquitoes exposed	knock down after 15 min	knock down after 30 min	knock down after 45 min	knock down after 60 min	Dead after 24hrs	Percentage dead after 24hrs
bendiocarb	3	56	12	47	56	56	56	100
DDT	6	114	3	14	61	97	113	99
permethrin	8	165	97	163	165	165	165	100
deltamethrin	2	46	39	46	46	46	46	100
malathion	2	47	0	20	46	46	47	100

Table 3: WHO tube test results for *Ae. albopictus* exposed to bendiocarb, DDT, permethrin, deltamethrin and malathion

Our preliminary results show that there is no resistance to the exposed insecticides. For the DDT exposure one mosquito survived. This mosquito will be analysed for its possible resistance mechanism. We still need to continue our exposure tests for three insecticides shown in table 3 to reach the >100 mosquitoes exposed. Furthermore the knockdown data will be further analysed.

Apart from the *Ae. albopictus* mosquitoes collected from the forest, we also exposed *Armigeres kesseli* mosquitoes collected as larvae in the rubber plantations to DDT, permethrin and malathion. The exposure of *Armigeres* mosquitoes in the WHO tubes has not been standardized as they are not known to be an important vector species. Therefore we used the same exposure method as for *Aedes* species. The mosquitoes were all 4-6 days old non-blood fed mosquitoes. In total 15 insecticide exposures were conducted in three days of August 2014 with an average of 20 mosquitoes in the exposure tubes. See table 4. All control exposure tubes consisted of at least 19 female mosquitoes. No control mosquito died during the 24hrs after exposure for any of the tests.

	total number of exposures	total no. mosquitoes exposed	knock down after 15 min.	knock down after 30 min.	knock down after 45 min.	knock down after 60 min.	Dead after 24hrs	Percentage dead after 24hrs
DDT	6	116	5	10	18	37	110	95
permethrin	5	97	5	72	96	97	97	100
malathion	4	74	3	8	32	68	74	100

Table 4: WHO tube test results for Armigeres kesseli mosquitoes exposed to DDT, permethrin and malathion

The summary of the results show that the *Armigeres kesseli* mosquitoes are susceptible to permethrin and malathion. However for DDT there is only 95% mortality, indicating a possible resistance. The mosquitoes that survived the exposure will be analysed for their possible resistance mechanism. It has to be kept in mind that, as there is no standardization of the *Armigeres* species for WHO tube exposure, it is difficult to link clear consequences to these results.

Larval survey

Mosquito larvae have been collected in the immature rubber plantation, mature rubber plantation and villages using a systematic approach to identify all water bodies. We have started the larval survey in August 2014 and will continue until December 2014. So far more than 300 water bodies have been identified in the three study areas and more than 2000 mosquito larvae collected. Currently no analysis is available for the preference of larvae breeding sites in rubber plantations as data will be collected until December 2014. However, we can confirm we have found mosquito larvae breeding in latex collection cups in the mature rubber plantations. Furthermore breeding sites are numerous in the mature plantations with often the border cropping with banana trees providing many breeding sites. See figure 7.

A

B

C

Figure 7: Images of the larval survey (A) Surveying latex collecting cups in the mature rubber plantations (B) the banana trees bordering the mature rubber plantations (C) a close-up of a latex collecting cup containing latex with a layer of water with mosquito larvae of different stages, including pupae and adult circled in red

Communication

We have had several important meetings with key people from the village, district and province throughout this year. Apart from these regular meetings we wanted to further communicate our project goals towards our stakeholders in Lao PDR and across the border. We therefore organized a national stakeholders meeting in Vientiane to which important representatives of health, agriculture, industry, government of Lao PDR were invited. Furthermore we gave a presentation of our project at the 2014 international conference on rubber in Thailand which was attended by international health, agriculture and industry representatives, and government officials of Thailand. Ms Julie Anne Tangena received an award at the conference for 'outstanding oral presentation'. Apart from communication with our stakeholders we also shared our project and the dangers of mosquitoes with villagers from our study area. We distributed stickers, flyers and posters provided by the Ministry of Health to the villagers showing the dangers of dengue and how to decrease the mosquito breeding sites. Furthermore, we talked to young students at their school to explain the dangers of mosquitoes and the importance of cleaning garbage.

Future activities

We will continue our work in the field, do the molecular analysis on mosquitoes collected in our longitudinal study and analyse data collected in the field this year. Focus in the next few months will be on re-identifying and confirming the species collected during our longitudinal study to ensure we are confident with the high diversity of mosquitoes found. Furthermore, we will pin 10 mosquitoes of each species found in our study site for future reference and place them in the 'IP Laos' collection of medically important arthropods. The molecular analysis will take place in Vientiane for viral detection and in Bangkok for species identification. We will continue surveying the rubber plantations and the villages for larval breeding sites and continue our resistance test. We will additionally measure important environmental factors in the three different forested areas with the help of the National Agriculture and Forestry Research Institute (NAFRI); immature rubber plantation, mature rubber plantation and secondary forest. This information will be used for all our comparison data to distinguish the different habitats from each other. We will also have short group discussions with the rubber workers in our rubber plantations to have both an opportunity to share information on our preliminary findings and discuss their problems with mosquitoes, their methods of prevention and how they would like to be helped.

In the long term, emphasis will be on analyzing all the data collected during the 2013 and 2014 field periods to be able to share clear conclusions with the stakeholders. We will be working closely together with NAFRI and the ministry of health to identify how to communicate and implement our findings and recommendations concerning the vector-borne disease risks for the people working in and around the rubber plantations.

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ECOMORE (see www.ecomore.org)

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participation of villagers in identifying areas with high number of mosquitoes village Silalek

Talking to rubber owners during the larval survey in the rubber plantation

Measuring the rainwater quality in collecting cups in which mosquito larvae were found

Vector mapping, characterization of insecticide resistance of *Aedes* populations in Laos

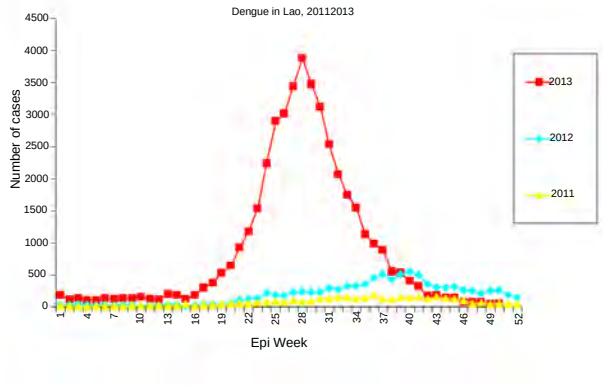
Project coordinator: Dr S. Marcombe, Dr Ian Sutherland and Dr Paul Brey
Member of staff: Khampsing Vongpayloth, Noy Khounsombat and A. Jordan

Background

Because of global changes (environment, climate) and increasing transportation, in the past decades we have seen a dramatic resurgence of dengue and chikungunya throughout regions where *Aedes* mosquito vectors are present and this has led to major public health problems (WHO 2006, Benedict *et al*., 2007). In 2013, Lao PDR faced one its most severe dengue outbreak in years (Figure 1). The mosquito *Aedes aegypti* is the main dengue virus vector in Lao PDR (Dr Vongphayloth pers com.). Another species, *Aedes albopictus*, is a secondary vector of dengue, but is also the vector of the chikungunya virus that is reemerging in Southeast Asia and in Lao PDR. Because there is still no vaccine or specific treatment available against these viruses, vector control remains the only strategy for reducing dengue or chikungunya transmission. Effective vector control measures rely on active community participation, health education programs, and environmental management that include improvement of water supplies and storage, solid waste management, and modification of human-made larval habitats (Erlanger *et al.*, 2008). During inter-epidemic periods or when the elimination of breeding habitats of the mosquito is not easily achievable, insecticide application in larval habitats is routinely conducted by public health services in many countries including Lao PDR (Thavara *et al*., 2004). Space spraying applications are conducted during epidemics or when the entomological indices of mosquitoes are high. For both l arviciding and adulticiding, organophosphates and pyrethroids are the insecticide families of choice worldwide, as well as in Lao PDR. Unfortunately, many dengue vector control programs are threatened by the development of insecticide resistance in *Aedes* populations across the world. Insecticide resistance is associated with mutations in the sequence of the target protein that induce insensitivity to the insecticide (target-site resistance, knock-down resistance mutation, *kdr*), and/or the up-regulation of detoxification enzymes (metabolic-based resistance, P450 monooxygenases (P450s), glutathione S-transferases (GSTs) and carboxy/cholinesterases (CCEs)). Strong levels of resistance to organophosphates and pyrethroids have been detected in *Aedes aegypti* population in Southeast Asia (Ranson, 2010). Resistance to these same families of insecticide has also been detected in *Aedes albopictus* populations worldwide (Kamgang et al 2010, Marcombe *et al.* 2014). The organophosphate temephos (larvicide) and insecticides from the pyrethroid family (permethrin, deltamethrin; adulticides) have been used in Lao PDR for decades to reduce the vector populations during important dengue epidemics but to date, compared to its neighboring countries there is no information available on the resistance status of Aedes populations and the possible impact of the resistance on vector control operations in the country.

The risk of insecticide resistance in dengue vectors in South-East Asia represents a serious threat to the achievements seen in dengue control during recent years. It is urgent to identify the distribution, the levels, the mechanisms, and potential environmental factors of resistance in dengue vectors in the lower Mekong countries to assist health authorities to develop more effective strategies of prevention and control of the disease.

Figure 1. Dengue cases in Lao PDR, 2011 – 2013. Data from Arbovirology Lab IPL. Source: http://www.wpro.who.int/emerging_diseases/documents/dengue.updates.2013/en/



Objectives and expected results

The purpose of this project is to provide entomology capacity building, identify circulating levels of insecticide resistance (IR), and improve data on vector risk profiles in high priority location in Lao PDR.

Objectives:

- Evaluation of the levels of insecticide resistance in the vector populations in Lao PDR.
- Evaluation of the types and mechanisms of insecticide resistance (i.e. metabolic or target site) in Laos.
- Evaluation in semi-field trials of common insecticide formulations used in Lao PDR versus candidate insecticides for larval control
- Capacity building in medical entomology in Lao PDR.

Expected outcomes:

- Set up a comprehensive map of the levels of insecticide resistance in Lao PDR
- Generate an Insecticide Resistance database in the main dengue vectors in Lao PDR
- Guide public health authorities of Laos in the design and implementation of Insecticide Resistant Management strategies.
- Capacity strengthening in medical entomology and vector control in Lao PDR.

Methods

Mosquito collection

It was decided to focus specifically on 3 provinces for the study of insecticide resistance in dengue vectors (Vientiane, Luang Prabang and Attapue). For each province, larval collection were implemented in rural and urban areas. Contact was made with several provinces to obtain larvae from the field. In July 2014, collections were made in Xanyabouly and Luang Prabang provinces (Figure 3) and larvae collected by collaborators from Savanakhet (CMPE and district staff) were sent to the Institut Pasteur du Laos. In Xanyabouly province, Borten district, larvae were collected in breeding habitats in several villages and brought back to the laboratory for rearing. Larvae were collected in the forest surrounding Tinkheo village and in rubber plantations (rural areas) in Luang Prabang province. Larvae were also collected in Luang Prabang city in households and in Temples (urban area).

Figure 3. Larval collection of dengue vectors in Xanyabouly and Luang Prabang provinces, July 2014

Morphological mosquito identification

For all the mosquito populations collected, larvae were reared until adults (F1 generation). After adults identification, mosquitoes obtained were separated by species and locations. Only *Aedes aegypti* and *Ae. albopictus* were kept for breeding. Female mosquitoes were then blood fed and eggs obtained were kept for the future larval and adult bioassays. Approximately 5,000 of *Aedes aegypti* larvae and 3,000 of Aedes albopictus larvae were collected from all study sites.

Insecticide resistance

We started to test the susceptibility of *Ae. aegypti* mosquitoes to a range of insecticides representative of those historically and currently used for mosquito control in Lao PDR (i.e. DDT, temephos, deltamethrin and permethrin). Larval and adult bioassays were performed following WHO guidelines (WHO 2005, 2006) on mosquito populations from Vientiane capital (Figure 4).

Figure 4. Adult bioassay

Semi-field trial

The trial started in October 2014 in Vientiane at the Pasteur Institute of Laos. According to WHO procedures (WHO 2005), the effects of temephos, Bti, diflubenzuron and spinosad formulations will be evaluated and compared. Plastic containers with a high capacity (200L) were used because they are widely found for water storage in Lao PDR. These containers were filled with domestic water and covered with a mosquito net to prevent oviposition by wild female mosquitoes in the area and to prevent the deposition of debris. Containers were placed under a shelter to prevent direct exposure to rain and sunlight (Figure 5). The formulations are tested at the WHO-recommended dosage for the control of mosquito arvae (WHO 2006). A total of 15 containers (3 replicates of each insecticide and 3 controls) are used. Groups of 100 third-instar larvae of the F1 generation of the Pasteur strain (IPL strain) were added to each container with one l gram of food (dry cat food) at time 0, and then every 10 days until the efficacy, i.e., the emergence inhibition rates of the mosquitoes (EIs) decrease to < 80%.

Figure 5. Semi field trial at the Pasteur Institute of Laos. Efficacy of conventional versus alternative insecticide formulations used for vector control are tested.

Partners

U.S. Naval Medical Research Center - Asia
National Center of Malariology, Parasitology and Entomology (CMPE), Vientiane, Lao PDR

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Ticks and tick-borne diseases in Laos

Pricipal Investigators : Dr. Ian Satherland (NMRC-A) and Dr. Paul Brey
Project coordinator: Dr. Kahmsing Vongphayloth
Member of staff: Lae Thutkhin

Introduction

Ticks are important hematophagous ectoparasites, which play an important role as pests and vectors of a wide range of viral, bacterial and protozoan diseases of humans, livestock, pets, and wild animals. Ticks not only play a role as disease vectors, but also cause direct impact on their hosts, such as blood loss and tick-induced paralysis (Pfaffle et al. 2009, Patil et al. 2012) and cause significant economic losses as a constraint to animal production (Jongejan and Uilenberg 2004).

In Southeast Asia, taxonomically accurate knowledge of tick species and the risk distribution of tick-borne diseases are very poorly characterized. About 104 species of ticks from 12 genera have been known in Southeast Asia, and the most species-rich genus is *Haemaphysalis*, with about 52 species or 30% of the world fauna (Petney et al. 2007). The relationship between ticks and tick-borne pathogens in this region is largely unknown, even though the presence of these pathogens has been recognized for many years and the number of new pathogens discovered in ticks has increased markedly (Yu et al. 2011).

In Lao PDR, vector borne-diseases are often misdiagnosed and underestimated because of inadequate surveillance networks and laboratory capacity. Up until the present, tick identification, distribution, and their vector status remained unknown. The Nam Theun 2 watershed includes the Nakai Nam Theun National Protected Area (NNT NPA), known as Watershed Management and Protection Authority area (WMPA area) located in Nakai district, Khammoune province, covering a total area of 423 000 hectares. This area is considered as a biodiversity hotspot in SE Asia About 6,900 people live in NNT clustered in 31 villages; a density of about 1.95 persons/km^2. Villagers in the area mainly rely on a number of forestry-agriculture practices including hunting and gathering of forest and stream products. Water buffalo, cattle, pigs, chickens and dogs are important domestic animals for their livelihood and these valuable animals are often maintained close to human dwelling (http://www.nt2wmpa.gov.la/en/people-and-nature/). Such practices increase the risk of tick-borne disease transmission and other vector borne diseases. Furthermore, several forest workers and scientists working in this zone have been diagnosed and treated for rickettsial disease. Mites and ticks are believed to be the vectors of these diseases and potentially other new emerging infectious diseases such as arboviral diseases. In order to assess tick-borne diseases in Lao PDR, an in-depth assessment of tick species and their pathogens was carried out.

Objectives

- To describe species composition and distribution of the ticks in the research area.
- To describe the ticks and their vector status (putative vectors for viruses, rickettsia and bacteria).
- To develop vector map for ticks and tick-borne diseases in order to provide information on the diseases surveillance and control.
- To develop local capacity building and competencies on ticks systematics/bionomics and tick-borne disease identification.

Methodology

Building of local capacity and competencies

Training/local capacity building

As previously reported, to improve local capacity and competencies, the first training was held in Vientiane, Lao PDR at IPL from 24th to 28th February 2014, under the title "Intensive Training Course/Workshop on the Taxonomy and Identification of Ticks, Mosquitoes and Sand flies from Lao PDR".

The course was instructed by Dr. Pollie L.M. Rueda, Ph.D., Research Entomologist, WRBU, Smithsonian Institute (ruedapol@si.edu), Dr. Khamsing Vongphayloth, M.D., Research Entomologist, IPL (k.vongphayloth@pasteur.la), Dr. Ian Sutherland, Ph.D., MSPH, Chief of Entomological Sciences, NMRC-A (ian.sutherland@fe.navy.mil) and Dr. Paul Brey, Ph.D., Research Entomologist, the director of the Institut Pasteur du Laos (p.brey@pasteur.la).

Sixteen participants from Department of Disease Control (Ministry of Health); CMPE; 103 Military Hospital; Military Institution for Disease Prevention and Control; and 9 provincial divisions of CMPE attended the course (Fig. 1). To our knowledge, this was the first-ever, intensive course dedicated to the taxonomic identification and vector biology of medically important arthropods to be conducted in Lao PDR.

Civilian and military public health professionals from across Lao PDR participated in the joint NMRC-A, IPL, and WRBU Medical Entomology Workshop.

Figure 1: Pictures from February 2014 course.

Institut Pasteur du Laos: Medically important arthropod collection room

In order to preserve specimens for future research and training purposes, a room of medically important arthropod collection has been established with 2 Cornell Cabinets; with 20 drawers each for dry insect specimens such as mosquito adults and one cabinet for specimens preserved in ethanol and slide mounted specimens such as ticks, sand-flies, and mosquito larvae (Fig. 2).

Medically important arthropod collection room in the Institut Pasteur du Laos, with 2 Cornell Cabinets (brown) each with 20 drawers for dry insect specimens, and a cabinet (white) for specimens preserved in ethanol and mounted slides

Figure 2: Medically important arthropod collection room of the Institut Pasteur du Laos.

Tick collection sites

In order to broaden our scope for tick collection and define hot spot areas in Nakai district, Khammoune province, we sampled as many sites as possible, according to the allotted time and logistic difficulties. We investigated the upstream and downstream forest areas of the Nam Theun, Nam Noy rivers, Markfueng and Korbong villages inside WMPA area, and Natan Village in Phou Hin Poun National Protected Area (Fig. 3).

The NNT NPA is under management by the WMPA authority. It is home to wide range of wildlife species including reptiles and amphibians (20 amphibian species, and 31 reptile species), more than 400 species of birds, 92 species of mammals (elephants, muntjac, deer, pangolins, etc.) (http://www.nt2wmpa.gov.la/ en/ecology/). Natan village is located in the Phou Hin Poun National Protected Area (Phou Hin Poun NPA) in Khammouane Province and enclosed by karstic limestone formations and forested mountains. Phou Hin Poun NPA is one of only two NPAs in Lao PDR covering representative samples of the Central Indochina Limestone.

Tick collection was conducted during the dry season; from December to April. The first sampling was started in December 2012 and the last was in April 2014, see Table 1 for the detail of our tick collection sites. During the course of collection in March 2014, temperature for the Nam Noy field site (forest) ranged from 18.5-39°C with a relative humidity (RH) range of 49.5-89.5%. In April 2014, Makfeug village temperature ranged from 19.5-41.5°C with a RH range of 37-80%, while the Korbong village field-site temperature ranged 21-33.5°C with a RH range of 51.5-88%. This is within the normal season averages for this region in the same month [25.5°C; range (20.5-30.5°C), 67%; range (51-83%)] (Lao PDR climate data, 2011).

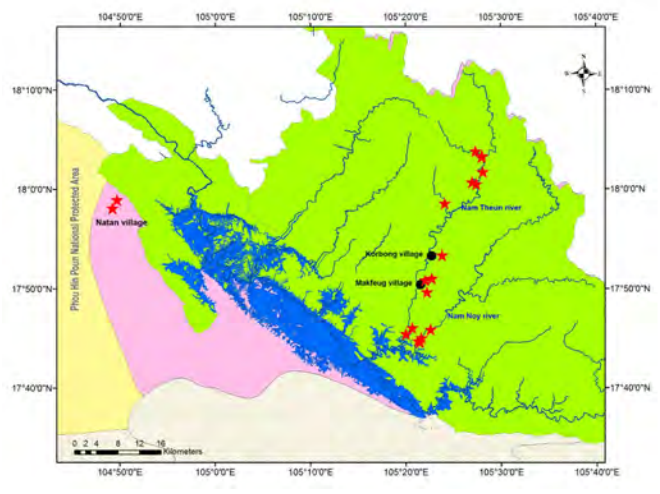

Figure 3: Map of field collection sites in Khammoune Province, Lao PDR.

Field site	Collection date	Name	Latitude	Longitude	Altitude (meter)
Nam Noy river	11 December 2012 5-7 February 2013 19-23 March 2013 9-16 February 2014 2-3 March 2014	Downstream Nam Noy	17.75084	105.3604	542
	12 December 2012	Downstream Nam Noy	17.74395	105.3579	579
	12 December 2012	Downstream Nam Noy	17.74556	105.3547	564
Nam Theun river	13 December 2012	Downstream Nam Theun	17.76768	105.3445	625
	13 December 2012	Downstream Nam Theun	17.75742	105.3337	564
	18 December 2012 16 February 2013	Vang Chang village	17.97563	105.4017	626
	16 February 2013	Upstream Nam Theun	18.00798	105.4570	579
	18 December 2012	Upstream Nam Theun	18.01137	105.4497	621
	20 December 2012	Upstream Nam Theun	18.02897	105.4685	598
	12 February 2013	Upstream Nam Theun	18.05362	105.4664	651
	13 February 2013	Upstream Nam Theun	18.06337	105.4557	690
	14 February 2013	Upstream Nam Theun	18.05567	105.4674	656
Ban Natan	5-7 March 2014	Ban Natan	17.967977	104.819818	178
	5-7 March 2014	Secondary forest near TamLuang cave	17.981883	104.829076	203
Makfeung village	30 March 2014	Secondary forest	17.847732	105.367612	597
	1 April 2014	Secondary forest	17.850140	105.378981	576
	31 March 2014	Primary forest	17.827494	105.370127	676
Korbong village	3-5 April 2014	Primary forest	17.889201	105.396963	687

Table 1: Field site collection coordinates and collection dates.

Tick sampling procedure

Dragging method was used for tick sampling from forest floor and vegetation. White heavy cotton sheets were cut into many sizes: 50 cm, 70 cm, and 100 cm widths X 100 cm long. These makeshift dragnets were swept along the forest ground at around 1-2 m long intervals around natural animal trailways before being examined for ticks. Ticks were removed from the sheets using forceps, transferred to 1.5 ml labeled cryo–tubes, and then transported to the Nakai field Laboratory (Ticks were also collected from some domestic animals by direct hand removal with forceps during our surveys).

In the Lab, ticks were killed by putting in the freezer -20° C for 10 minutes. Then adults, nymphs, and larvae of tick were separated, counted, and subsequently stored at -80°C for further analysis (species identification, pathogen detection, and discovery). All study sites were data-logged for full GPS parameters (see Table 1).

Tick identification

Ticks were identified and grouped under microscope in cooling conditions (on ice pack) by using reference determinations from Dr. Richard G. Robbins of the US Armed Forces Pest Management Board (AFPMB) and together with related keys from Japan, Korea and the Ryukyu Islands (Yamaguti et al. 1972), L. E. Robinson keys for genus *Amblyomma* (Nuttall et al.) and Thailand (Tanskull and Inlao 1989) for adults of *Haemaphysalis* ticks. As there are no morphological identification keys available for pre-imago forms of *Haemaphysalis* spp., larval and nymph stages in this genus were grouped by using their main morphological characteristics, especially the capitulum.

Laboratory procedure for pathogen screening

Sample preparation and RNA/DNA extraction

Tick samples were pooled in to groups of one to ten by species or genus, sex, stage of development, collection period, and site. Specimens were placed in a 1.5 ml vial 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 5 min at 3000 rpm. Total nucleic acid (both DNA and RNA) for bacterial and viral detection by polymerase chain reaction (PCR), 100 µl of each pool were extracted and purified by using NucleoSpin® 8 Virus extraction kit following manufacturer's protocol. The remaining 400 µl of each pool were stored at –80°C for pathogen isolation.

Reverse transcriptase polymerase chain reaction for viral screening

Multiple sets of primers have been selected and tested for screening arthropods specimens for the presence of flavivirus and alphavirus sequences by means of conventional nested RT-PCR as previously described (Sanchez-Seco et al. 2001 and 2005). In order to improve the sensitivity of the flavivirus screening, a real time RT-PCR system is under evaluation (Moureau 2007). As planned in the project, positive samples for any of these two arbovirus genera detected through an initial screening were re-analyzed by specific real time RT-PCR to attempt to identify the viral species. Such methods are already available for detection of the main arboviruses of human health concern in SE Asia: dengue (all serotypes), Japanese encephalitis, and chikungunya virus.

Virus isolation

Positive samples for any viral markers were inoculated onto both mosquito (*Aedes albopictus* derived C6/36) and mammalian (African green monkey VERO E6) cell lines. Briefly, sub-confluent cells monolayers prepared in 25 cm2 culture flasks were washed once with 3 ml of 1X DPBS. After removing the washing solution, cells were inoculated with 100 µl of each positive pool supernatant diluted with 1 ml of cold culture medium filtered through a 0.22 µm Millipore filter unit. After two hours of contact, the inocula were removed and replaced by 5 ml of culture media completed by fetal calf serum. Cultures were maintained for 5 days and checked daily under a microscope for presence of cytopathic effect (CPE). Up to four blind passages were performed to follow up of culture negative for CPE. Cells and supernantant were preserved frozen at -80°C for a retrospective control of the culture by pan-flavi or pan-alpha RT-PCR.

Bacterial screening

To investigate the occurrence of Spotted Fever Group (SFG) Rickettsia in ticks in Laos a molecular screening approach targeting the 17kDa gene was taken (Jiang et al. 2004). In order to identify novel as well as known human pathogenic species a previously established and validated quantitative real-time PCR (qPCR) assay was adapted for the use with DNA from tick pools (Jiang et al. 2004, Mayxay et al. 2013). After severe inhibition of the qPCR reaction was identified in preliminary experiments the final protocol was adapted for the use with diluted (1:10) tick-DNA and the inclusion of bovine serum albumin (BSA) to minimizing the effects of inhibitors.

Further, a subset of pools was investigated for the presence of *Anaplasma phagocytophilum* (136/768, 17/7%; Joshua et al. 2004), *Ehrlichia chaffeensis* (85/768, 11%; Amanda et al. 2003) and *Coxiella burnetii* (68/768; 8.8%; Fournier et. al. 2010), all significant human pathogens in many parts of the world (Pritt et al. 2011, Dumler et al. 2005).

The validity of results was ensured by the inclusion of positive and negative results into each run. The possibility for cross-contamination was limited due tostrict physical separation of DNA preparation, pre-and post PCR areas as well as the use of uracil-DNA glycosylase.

Results

Tick species composition and distribution

During our course of study, 14,735 ticks were collected by sweeping/dragging method. Total specimens by life-stage included adults 174 (1%), nymphs 5,637 (38%), and larvae 8,924 (61%). Only 12 ticks were collected from domestic animal dogs and cows in Korbong village, WMPA area. Overall, 6 tick genera were present: *Amblyomma*, *Demacentor*, *Haemaphysalis*, *Ixodes*, *Rhipicephalus*, and *Boophilus*. *Rhipicephalus haemaphysaloides* and *Boophilus* spp. were only collected from domestic animals. From sweeping/dragging collections, Haemaphysalis spp. represents the most abundant genus with high diversity, representing 73.62% (10,848/14,735), followed by *Amblyomma testudinarium* 23.78% (3,505/14,735), *Demacentor auratus* 2.54% (375/14,735), and Ixodes spp. 0.04% (7/14,735) (cf. Table 2).

* 1 female of *Demacentor steini* (Schulze 1933), and 1 female of *Haemaphysalis colasbelcouri* (Santos Dias 1958) were also collected but the total number reported here did not include these specimens and also other reference specimens that were deposited to the IP-Laos Arthropod Collection Room after confirmation by Dr. Richard G. Robbins.

Genus/species*	Development stage					Trap method	Host
	Female	Male	Larval	Nymph	Total		
Makfeung village, WMPA area						Dragging	Ground/Vegetation
Amblyomma testudinarium	0	1	0	41	42		
Haemaphysalis aborensis	0	2	0	0	2		
Haemaphysalis spp. Group 2	0	0	0	10	10		
Haemaphysalis hystricis	3	4	0	0	7		
Haemaphysalis spp.	2	0	0	0	2		
Haemaphysalis spp. Nymph group 1.1	0	0	0	2	2		
Haemaphysalis spp. Nymph group 1.2	0	0	0	8	8		
Total	**5**	**7**	**0**	**61**	**73**		
Nam Thuen river, WMPA area							
Amblyomma testudinarium	1	1	1,507	863	2,372		
Demacentor auratus	0	1	1	15	17		
Haemaphysalis aborensis	5	1	0	0	6		
Haemaphysalis spp. Group 1	0	0	2,264	160	2,424		
Haemaphysalis spp. Group 2	0	0	2,525	457	2,982		
Haemaphysalis hystricis	6	8	0	0	14		
Haemaphysalis spp.	28	26	810	0	864		
Haemaphysalis spp. Nymph group 1.1	0	0	0	59	59		
Haemaphysalis spp. Nymph group 1.2	0	0	0	754	754		
Ixodes spp.	0	0	0	7	7		
Total	**40**	**37**	**7,107**	**2,315**	**9,499**		
Nam Noy river, WMPA area							
Amblyomma testudinarium	0	0	0	200	200		
Demacentor auratus	4	4	262	84	354		
Haemaphysalis aborensis	11	14	0	0	25		
Haemaphysalis spp. Group 1	0	0	1,505	1,116	2,621		
Haemaphysalis spp. Group 2	0	0	21	17	38		
Haemaphysalis hystricis	18	11	0	0	29		
Haemaphysalis spp.	10	13	0	0	23		
Haemaphysalis spp. Nymph group 1.1	0	0	0	89	89		
Haemaphysalis spp. Nymph group 1.2	0	0	0	545	545		
Total	**43**	**42**	**1,788**	**2,051**	**3,924**		
Korbong village, WMPA area							
Amblyomma testudinarium	0	0	1	0	1	Remove from host	Dogs
Haemaphysalis hystricis	1	0	0	0	1		
Rhipicephalus haemaphysaloides	1	0	0	0	1		
Amblyomma testudinarium	1	3	1	0	5		Catle
Boophilus spp.	1	0	0	0	1		
Haemaphysalis hystricis	2	0	0	0	2		
Rhipicephalus haemaphysaloides	2	2	0	0	4		
Total	**6**	**5**	**1**	**0**	**12**		
Grand Total	**94**	**91**	**8,925**	**5,637**	**14,747**		

Table 2: Tick species composition, distribution, trap method and host.

Laboratory screening for pathogens

A total of 13,571 ticks from sweeping/dragging collection were pooled into 1,492 pools. So far, 768 (6,692 ticks) pools have been processed for viral screening by RT-PCR targeting alphaviruses and flaviviruses. Of the unfed 6,692 ticks in 768 pools, 23 (3%), 280 (36%) and 465 (61%) were adult, larval and nymph pools respectively. The most abundant genus was *Haemaphysalis* spp. (59.89%, 460/768), followed by *Amblyoma testudinarium* (36.32%, 279/768); and *Demacentor auratus* (3.77%, 29/768). None of *Ixodes* spp., *Rhipicephalus haemaphysaloides*, and *Boophilus* spp. pools has been processed for DNA/RNA extraction for pathogen screening.

Virus detection

Of the 768 pools analyzed, 17/768 pools were positive by pan-alphaviruses in 8 pools of *Amblyoma testudinarium* (2 larval and 6 nymphal pools), 8 pools of *Haemaphysalis* spp. (3 larval and 5 nymphal pools) and 1 pool of nymphal *Demacentor auratus*. 3 pools of *Haemaphysalis* spp. were found positive by Pan-Flaviviruses [2 larval pools (one from Nam Theun and one from Nam Noy area) and 1 nymphal pool from Makfeung village]. See Table 3.

Stage of development/species	Total No. pools	Total No. ticks	Pan Alpha Positive		Pan Flavi Positive	
			No. pools	No. ticks	No. pools	No. ticks
Female	**11**	**26**	0	0	0	0
Demacentor auratus	1	1	0	0	0	0
Haemaphysalis aborensis	3	6	0	0	0	0
Haemaphysalis hystricis	3	15	0	0	0	0
Haemaphysalis spp.	4	4	0	0	0	0
Male	**12**	**26**	0	0	0	0
Amblyomma testudinarium	1	1	0	0	0	0
Demacentor auratus	2	2	0	0	0	0
Haemaphysalis aborensis	4	13	0	0	0	0
Haemaphysalis hystricis	3	5	0	0	0	0
Haemaphysalis spp.	2	5	0	0	0	0
Larval	**280**	**2,687**	5	50	2	20
Amblyomma testudinarium	61	600	2	20	0	0
Haemaphysalis spp. Group 1	194	1,871	3	30	2	20
Haemaphysalis spp. Group 2	25	216	0	0	0	0
Nymph	**465**	**3,953**	12	108	1	1
Amblyomma testudinarium	217	1,959	6	60	0	0
Demacentor auratus	26	91	1	3	0	0
Haemaphysalis spp. Group 1	184	1,591	4	38	0	0
Haemaphysalis spp. Group 2	22	173	1	7	0	0
Haemaphysalis spp. Nymph group 1.1	1	2	0	0	0	0
Haemaphysalis spp. Nymph group 1.2	15	137	0	0	1	1
Total	**768**	**6,692**	**17**	**158**	**3**	**21**

Table 3: Arboviral screening by RT-PCR targeting Alphaviruses and Flaviviruses from ticks collected.

The second screening by specific flavivirus (dengue; Japanese encephalitis; West Nile) and alphavirus (chikungunya) real time RT-PCRs did not identify any of these targeted viruses within the positive pools. Pan-genus PCR products have been purified on agarose gels for subsequent sequencing. Future sequences will be subjected to blast analysis against genome data bank to

establish whether the sequence(s) detected correspond to known virus not detected yet in Laos or to new viral species.

Up to now, two blind passages were performed for all positive pools but none of the cultures displayed any CPE as of yet. Follow-up passages will be performed up to passage 4 after which all cultures will be tested by pan-genus RT-PCR.

Bacteriology

Of the 768 pools analyzed, 94/768 (12.2%) pools were positive for 17kDa Pan Rickettsia, in 31 pools of *Amblyoma testudinarium* (15 larval and 16 nymphal pools), 58 pools of *Haemaphysalis* spp. (31 larval, 26 nymphal pools and 1 female pool) and 5 pools of nymphal *Demacentor auratus*. Overall 4.3% (1/23) of adult pools, 16.4% (46/280) of larval pools, and 10.1% (47/465) of nymphal pools were positive for Rickettsia spp. by PCR. Overall *Demacentor auratus* tick pools were found most frequently positive for Rickettsia spp. pathogens (19.2%), followed by *Amoblyomma* spp. (17.6%) and *Haemaphysalis* spp. (11.1%; Table 4).

Stage of development/species	Total No. pools	Total No. ticks	Pan Rickettsia (17kDa)	
			No. pools	% of pools
Female	**11**	**26**	**1**	**0.9**
Demacentor auratus	1	1	0	0
Haemaphysalis aborensis	3	6	0	0
Haemaphysalis hystricis	3	15	1	33
Haemaphysalis spp.	4	4	0	0
Male	**12**	**26**	**0**	**0**
Amblyomma testudinarium	1	1	0	0
Demacentor auratus	2	2	0	0
Haemaphysalis aborensis	4	13	0	0
Haemaphysalis hystricis	3	5	0	0
Haemaphysalis spp.	2	5	0	0
Larval	**280**	**2,687**	**46**	**16.4**
Amblyomma testudinarium	61	600	15	24.6
Haemaphysalis spp. Group 1	194	1,871	30	15.5
Haemaphysalis spp. Group 2	25	216	1	4
Nymph	**465**	**3,953**	**47**	**10.1**
Amblyomma testudinarium	217	1,959	16	7.4
Demacentor auratus	26	91	5	19.2
Haemaphysalis spp. Group 1	184	1,591	25	13.6
Haemaphysalis spp. Group 2	22	173	0	0
Haemaphysalis spp. Nymph group 1.1	1	2	0	0
Haemaphysalis spp. Nymph group 1.2	15	137	1	6.7
Total	**768**	**6,692**	**94**	**12.2**

Table 4: *Rickettsia* spp. screening by qPCR from ticks collected.

A subset of tick pools was additionally investigated for *C. burnetii*, *A. phagocytophilum* and *E. chaffeensis* all pathogens frequently described in ticks. None of the tick pools (0%; 0/68) were positive for *C. burneti*i, 2.4% (2/85) for *E. chaffeensis* (both *Amblyomma testudinarium* nymph stage), and 0.7% (1/136) for *A. phagocytophilum* (*Amblyomma testudinarium* nymph stage).

In the 68 pools (617 ticks) that have been tested for all investigated viral and bacterial pathogens in the subset of nymphs *Rickettsia* spp. pathogens were detected most frequently followed by E. chaffeensis (Table 5).

		Pan Alpha virus	Pan Flavi virus	*C. burnetii*	*A. phagocytophilum*	*E. chaffeensis*	*Rickettsia* spp.
	No. pools	Positive pools (%)	Positive pools (%)	Positive pools (%)	Positive pools (%)	Positive pools (%)	Positive pools (%)
Nymph	**68**	**0 (0)**	**0 (0)**	**0 (0)**	**0 (0)**	**2 (2.9)**	**4 (5.9)**
Amblyomma testudinarium	35	0 (0)	0 (0)	0 (0)	0 (0)	2 (5.7)	4 (11.4)
Demacentor auratus	3	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Haemaphysalis spp. Group 1	30	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Table 5: Overview of positivity rates for investigated viral and bacterial pathogens.

Discussion

As information on the ticks present in Lao PDR is scarce and there are no morphological identification keys available for pre-imago forms of *Haemaphysalis* ticks, morphological identification to species has been and will continue to be difficult if not impossible. In SE Asia, the only keys available for identifying adults of *Haemaphysalis* ticks are the keys of Thailand (Tanskull and Inlao 1989). The application of these keys to identify our tick specimens may not be appropriate because all ticks were collected from the area close to the Laos-Vietnam border. Nevertheless, identification of this tick group will be continued as far as possible through collaborative assistance with WRBU, AFPMB, and NMRC-A. The combination of morphological identification and molecular technique may be an alternative method to describe the presence of *Haemaphysalis* ticks in Lao PDR.

Our results of arboviral detection by RT-PCR showed that 2.21% (17/768) pools were positive by pan-alphavirus from *Amblyoma testudinarium*, *Haemaphysalis* spp., and *Demacentor auratus*.; and 0.39% (3/768) were found positive by pan-flavivirus from *Haemaphysalis* spp. This is the first time that arboviruses were observed in ticks from Laos. An in-depth study on these viral species diversity warrants further investigation. All our tick samples that were processed in this investigation for arboviral detection were all from unfed specimens collected from ground and vegetation by the dragnet method. These finding may provide information on their vector competence and it will be interesting to continue to analyze the remaining and fed tick samples collected from dogs and cows in order to better understand the

ecology of these pathogens. Our findings also indicated that these arboviruses were detected from both larval and nymphal pools, so it is possible that these pathogens may be vertically transmitted or transmitted by cofeeding. However, additional experimental data are needed to confirm this hypothesis. The fact that none of the adult pools were positive, may be due to our very limited sample size, only 23 pools (52 adult ticks) were analyzed. As *Haemaphysalis* longicornis was incriminated as the vector of severe fever with thrombocytopenia syndrome virus (SFTSV) in Southern China and South Korea, a member of the family *Bunyaviridae*, genus *Phlebovirus* (Yu et al. 2011, Park et al. 2014, Yun et al. 2014), it would be very interesting to continue to screen our tick samples for this viral genus.

Rickettsia spp. pathogens were identified in 12.2% of all tick pools, which is a conservative estimate as the real carriage is likely to be underestimated due to the observed inhibition of the qPCR reaction. Consistent with the finding for vial pathogens, only one of the adult pools was positive (0.4%) indicating that the sample sizes within the pools and overall might have been too small to detect pathogen carriage. This is further supported by findings from other regions were *Rickettsia* spp. was more frequently identified in adult ticks (Hildebrand et al. 2010, Kumsa et al. 2014). The highest carriage rate in our study was observed in *Demacentor auratus* (19.2%), consistent with findings at the Thai-Myanmar border where the only Rickettsia spp. positive pools were *Demacentor* ticks (Parola et al. 2003). Rickettsial pathogens, like *Rickettsia typhi* and *Orientia tsutsugamushi*, have recently been identified as significant contributors to the overall fever (Phongmany et al. 2009, Mayxay et al. 2013) and central nervous system disease burden (Dittrich et al. submitted) in Lao PDR. Further, *Rickettsia felis*, a member of the SFG has been described in Lao PDR (Dittrich et al. 2014) and has been suggested as a emerging pathogen in Africa (Parola et al. 2011). Despite our growing understanding of the importance of certain members or the family Rickettsiaceae in the region, this is the first large-scale carriage study investigating this important pathogen group in local ticks, which are likely to serve as vectors. Interestingly, in the small subset investigated *A. phagocytophilum* and *E. chaffeensis* were detected in 1 (0.7%) and 2 (2.4%) of tick pools respectively, while *C. burnetii*, the causative agent of Q-fever, could not be detected (0/68). These are the first ever data available on *A. phagocytophilum, E. chaffeensis* and *C. burnetii* in Lao PDR and further investigations into their role as causes of human disease are needed. Further screenings of relevant bacterial pathogens and speciation of *Rickettsia* spp. organisms will no doubt identify organisms with human disease potential that can be included in targeted diagnostic tests and studies in the future.

More in-depth studies into the carriage rate within different live stages and tick species will allow a greater detailed analysis of pathogen distribution and dissemination in Lao ticks.

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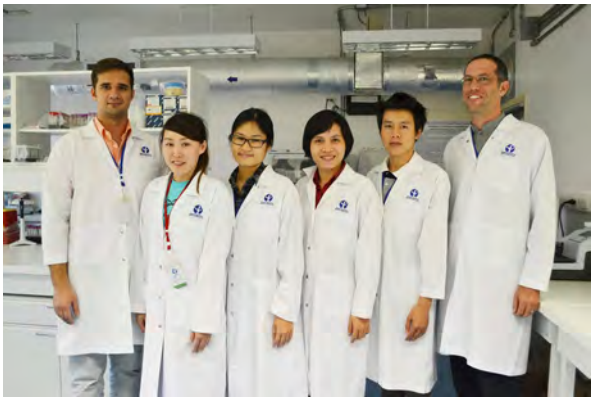
Vaccine Preventable Diseases Laboratory

Lao-Lux joint Lab

The LaoLux Laboratory is operated by the Institute of Immunology in Luxembourg and aims to build capacity for investigations of important human and animal infectious diseases and to initiate and support collaborative research projects in Lao PDR. The laboratory carries out country-specific research in Lao PDR focusing on vaccine-preventable infectious diseases, zoonotic diseases, identification of new viruses and variants and other investigations. These studies are important to estimate the burden of specific infections, to promote virus outbreak control, to improve animal health welfare and productivity, to support public health policies and vaccination programmes and to optimize health strategies.



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Projects

- Mumps seroprevalence and molecular epidemiology.
- Hepatitis B virus in the Lao People's Democratic Republic: a cross sectional serosurvey in different cohorts.
- Diphtheria seroprevalence in Huaphan Province.
- Serosurveillance of vaccine preventable diseases and hepatitis C in healthcare workers from Lao PDR.
- Vaccination effectiveness within the Lao National Expanded Program of Immunisation in provinces of Bolikhamxay, Vientiane and Khammouan.
- Hepatitis B and C virus in Lao blood donors.
- Evaluation of influenza virus circulation and transmission in pigs and free ranging poultry from mixed farms in Lao PDR.
- Childhood respiratory infections in Lao PDR.

The research projects include important components:

- Investigation of public and animal health challenges caused by infectious diseases.
- Training of laboratory and academic staff and students both at IPL and CRP-Santé.
- Implementation of new technologies by technology transfer and by providing equipment.
- Providing international visibility to scientists from Lao PDR and access to the international scientific community.
- Dissemination of research results through scientific publications, presentations and international meetings as well as national and international press releases.
- Technical and scientific support for other laboratories in Lao PDR.
- Teaching/training of laboratory staff from collaborating laboratories.

Areas of research and surveillance include:

- Immunology and genetic/antigenic diversity of viruses including molecular epidemiology.
- Public health issues related to infectious diseases in humans (measles virus, rubella virus, mumps virus, hepatitis virus, respiratory viruses etc).
- Public health and animal welfare issues related to veterinary viruses.

Visiting scientists from Institute of Immunology, Luxembourg:

Dr. Konstantin Evdokimov

Students trained in 2014:

Dr. Kong Sayasinh, Institut de la Francophonie pour la Medecine Tropicale.

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crp SANTÉ
CENTRE DE RECHERCHE PUBLIC
LABORATOIRE IMMUNOLOGIE

Mumps seroprevalence and molecular epidemiology

Project coordinator:Judith Huebschen, Antony Black, Claude Muller
Member of staff: Keooudomphone Vilivong, Chanthasone Souvannaso

Background

Mumps is a vaccine preventable disease caused by mumps virus (family: Paramyxoviridae, genus: Rubulavirus) with a subclinical course in up to one third of all cases. Common manifestations include parotitis and respiratory symptoms; and orchitis in postpubertal males. Laboratory diagnosis relies on detection of specific IgM antibodies or mumps virus RNA. Based on 316 nucleotides covering the small hydrophobic (SH) gene region, 12 different genotypes of mumps virus have been proposed with genotypes C, F, G and H predominating in Asia. In 2012, 120 out of 194 WHO member states (62%) were using mumps vaccine in their national immunization schedule. In Lao PDR, mumps is not a notifiable disease and mumps vaccine is currently not included in the routine childhood immunization program.

Activities and Prospective

In order to assess the burden of disease, we investigated the seroprevalence of mumps-specific IgG antibodies across four provinces. In addition, we continue to genetically characterize mumps viruses from outbreaks and single cases brought to our attention by the National Center for Laboratory and Epidemiology (NCLE) and medical doctors. Blood and/or throat swabs from suspected cases are investigated for specific IgM antibodies or viral RNA.

Mumps cases to date have occurred between March and November in 2011-2013 and 5-15 year olds were most affected. Four sequences from an outbreak in the North of Lao PDR in 2011 were identical and belonged to genotype G. Eight sequences from two outbreaks and wo individual cases from 2012 and 2013 belonged to genotype J. In addition, sera collected from 2379 healthy infants and school pupils between 9 months and 19 years and from pregnant women between 16 and 46 years were investigated for mumps-specific IgG. Overall, 58.2% were positive, 39.5% were negative and the remaining 2.3% were equivocal. The seropositivity increased with age, with the lowest percentage found in the <1 year old infants (9.1%) and the highest in the cohort of pregnant women (69.2%). More females than males were seropositive (60.4 versus 54.9%). There were some differences between the locations.

Swab and serum samples from suspected cases continue to be collected and analyzed in ongoing active mumps surveillance in collaboration with NCLE.

These data will be presented as a poster at the 12th International Conference on Molecular Epidemiology and Evolutionary Genetics of Infectious Diseases, Bangkok, 11-13 December, 2014

Partners

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- Luangprabang Provincial Hospital, Luangprabang, Lao PDR.
- Department of Pathology, Faculty of Medicine, Chiang Mai University, Thailand.
- WHO Lao Country Office, Vientiane, Lao PDR.
- National Center for Laboratory and Epidemiology, Vientiane, Lao PDR.
- Public Health Laboratory Services Branch, Centre for Health Protection, Department of Health, Hong Kong SAR, China.

Publication

Hubschen JM, Vilivong K, Souvannaso C, Black AP, Lutteke N, Samountry B, Phongsavath V, Khamphaphongphane B, Denny J, Sayyavong C, et al: **High prevalence of mumps in Lao People's Democratic Republic.** *Clinical microbiology and infection, February 2014.*

Figure 1:
Map of Lao PDR showing the different sample collection sites for mumps seroprevalence and case molecular characterisation. PV = province; PF = prefecture.

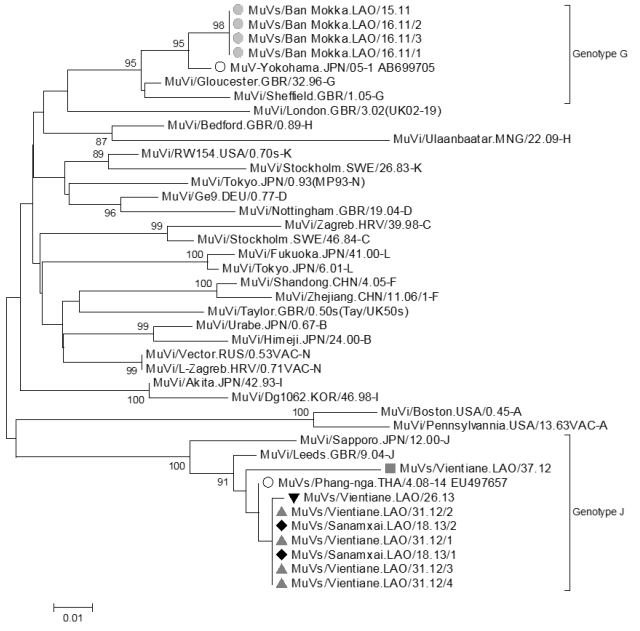


Figure 2:
Phylogenetic tree based on 316 nucleotides of the mumps virus SH gene region and the Kimura 2-parameter model and the Neighbor-Joining algorithm. Light grey dots mark the sequences obtained from the mumps outbreak in 2011, grey triangles highlight sequences from the 2012 outbreak and black diamonds show sequences from the 2013 outbreak. The grey square and the black triangle mark the individual cases from 2012 and 2013, respectively. White dots indicate the closest relatives of the sequences from Lao PDR identified by BLAST.

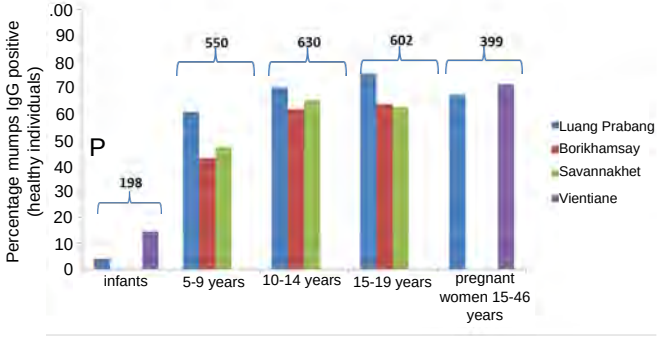


Figure 3:
Seroprevalence of mumps IgG in 4 different locations in Lao PDR.

Hepatitis B virus in Laos a cross sectional serosurvey in different cohorts.

Project coordinator: Antony Black, Claude Muller
Member of staff: Keooudomphone Vilivong, Phonethipsavanh Nouanthong, Did Panyathong

Background

HBV is endemic in the Lao PDR, between 8-10% of blood donors being chronic carriers of HBV, and is the cause of high morbidity and mortality. It is believed that most infections occur during early childhood, e.g. during birth or early family life. If infected at birth, children have a 90% risk of developing chronic infection. This rate decreases to 30% if infected between the ages of one and five and to 5-10% if infected after the age of 5. As screening for hepatitis B surface antigen (HBsAg) in pregnant women and immunoglobulin prophylaxis in newborns are not widely practiced in Lao PDR, routine infant vaccination is the mainstay of the prevention of early childhood infection.

From 2001, there has been a phased introduction of HBV vaccine into the national immunization schedule. Currently, infants are scheduled to receive the HBV birth dose within 24 hours after birth, followed by HBV containing vaccine at 6, 10 and 14 weeks of age in combination with diphtheria, tetanus, pertussis and Haemophilus influenzae b vaccine (DTP- HepB-Hib). The birth dose of HBV vaccine, followed by the timely 3 dose schedule, is assumed 70 to 95% effective in preventing mother-to-child transmission of HBV. However, the HBV birth dose coverage in Lao PDR was only approximately 28% in 2011–2012, largely because the majority of births are unattended home-births (62% in 2010). Furthermore, due to the difficult access to healthcare for a large proportion of the Lao population, only 78% of children less than 1 year old received all three DTP-HepB-Hib vaccinations in 2011.

Activities and Prospective

We performed a cross-sectional survey in four different sub-populations from Lao PDR with different ages and vastly different access to vaccination and general health care; infants, pre-school children, school pupils and pregnant women. Among 192 infants aged 9 to16 months tested in the urban settings of Vientiane and Luang Prabang, only one carried HBsAg while 23 (12%; all were aged 9–10 months) were anti-HBc positive. Anti-HBc positivity, usually indicative of HBV exposure, may also be attributed to passive transfer of maternal antibodies still detectable at this age. Importantly, the low HBsAg prevalence in the infants in our study may be seen as a positive impact of the vaccination policy implemented in the districts. This is also supported by the 59.9% seroprevalence rate of anti-HBs alone, reflecting an active, albeit low-level, immunization campaign in these areas. Our results show that vaccination coverage among infants even in the main cities of Luang Prabang and Vientiane remains inadequate. This is in line with the low vaccination coverage with the HBV birth dose (44-48%) and the DTP-HepB-Hib3 (80-85%) reported from these cities in 2011. It can be expected that in a rural setting the coverage is even lower.

Indeed only 13.6% of our pre-school children aged 1 to 4 years from rural settings in Huaphan province had anti-HBs antibodies alone, suggesting that very few children had been vaccinated. Distance from the nearest health centre was significantly associated with vaccination status, emphasizing the relationship between access and vaccination coverage ($p = 0.01$). In contrast 21.3% were anti-HBc positive, reflecting a prior exposure to HBV in this age group rather than passive transfer of maternal antibodies. Furthermore, 4.5% were HBsAg carriers as a result of early life exposure.

The school pupils in our study were recruited from semi-urban settings in Luang Prabang, Boulhikhamxai and Savannakhet provinces. Those aged 9 and under were born after the introduction of the HBV vaccines. The HBsAg prevalence increased from 4.3% (similar to the 4.5% in the under-fives in Huaphan province) in the 5 to 9 years old to 7.8% between 10 and 14 years. Assuming that most chronic infections are acquired during the first years of life, the lower HBsAg prevalence can be considered as the result of improved vaccination coverage. The increase of anti-HBc prevalence from 19.4% in the 10 to 14 years old to 27.0% in the 15 to 19 years bracket shows that HBV exposure continues and may even intensify after the age of 14 years.

The pregnant women in our study were recruited from urban areas of Luang Prabang and Vientiane. The high HBsAg prevalence of 8.2% in this cohort represents a high risk of vertical transmission of HBV and stresses the need to promote HBV vaccination of all children at birth, a strategy that has proved effective in many other countries within the region.

Lao PDR is heterogeneous in terms of vaccination coverage and access to healthcare. Because of geographical and ethnic heterogenicity and non-randomised sampling it is difficult and even not indicated to extrapolate our seroprevalence data to the entire Lao population. Nevertheless, we clearly show that there is a positive impact of HBV vaccine as evidenced by low prevalence of HBsAg in infants and school-children under 10 years i.e. those born after its introduction.

A significant improvement in immunization coverage of children would require strengthening routine outreach services, by mobile teams, especially in remote areas, paired with a strict management of the cold chain and reliable certification of vaccinations.

These data were presented at the Third National Workshop on Hepatitis, Vientiane, 3-5th November, 2014

Partners

- Lao Red Cross, National Blood Transfusion Centre, Vientiane, Lao PDR.
- Department of Pathology, University of Health Sciences, Vientiane, Lao PDR.
- Department of Pathology, Faculty of Medicine, Chiang Mai University, Thailand.

Publication

Black AP, Nouanthong P, Nanthavong N, Souvannaso C, Vilivong K, Jutavijittum P, Samountry B, Lutteke N, Hubschen JM, Goosens S, et al: Hepatitis B virus in the **Lao People's Democratic Republic: a cross sectional serosurvey in different cohorts.** BMC infectious diseases 2014, In Press.

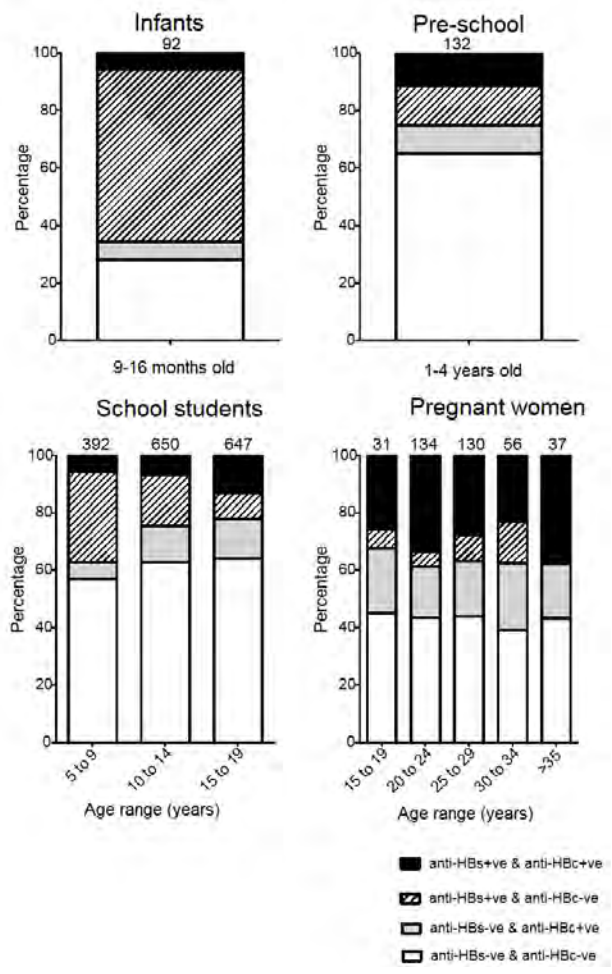


Figure 4:
Hepatitis B seroprofile with age. Excluding 3 participants with no age data and 50 with equivocal anti-HBs data. Numbers above columns represent number per age group.

Diphtheria seroprevalence in Huaphan Province.

Project coordinator: Antony Black, Claude Muller, Yves Buisson
Member of staff: Keooudomphone Vilivong, Naphavanh Nanthavong

Background

Diphtheria is a vaccine-preventable infectious disease caused by the bacterium *Corynebacterium diphtheriae*. The disease is spread primarily by droplets from the nose, throat and eyes and affects all ages but is more prevalent in unvaccinated individuals below the age of 15. In late 2012, there were diphtheria outbreaks in the provinces of Huaphan, Bokeo, Xayabouly, Xieng Khouang and Vientiane and in Vientiane capital.

Activities

In collaboration with the Institut de la Francophonie pour la Medecine Tropicale in Vientiane, we aimed to assess the diphtheria immunization status of children in two districts of the province of Huaphan and to determine the reasons for non-vaccination and lack of seroconversion among vaccinated children.

Following parental informed consent, 132 blood samples were taken from children between the ages of 12 to 59 months in Kuan and Xamtai districts of Huaphan province. Antibodies to Diphtheria toxin were detected in 84 children (63.6%). We also found 49% were infected with intestinal parasites. These data indicate very low vaccine coverage and high intestinal parasite infections in Huaphan province, particularly in rural areas far from health centres.

Prospective

These data were presented in poster format at the National Health Research Forum, Vientiane, October 2013 and have been reported to the Lao MOH. A manuscript of these data is in preparation.

Partners

- Institut de la Francophonie pour la Médecine Tropicale.
- Laos-Japan Joint Laboratory for Parasitology, Institut Pasteur du Laos, Vientiane, Lao PDR.

Serosurveillance of vaccine preventable diseases and hepatitis C in healthcare workers from Laos

Project coordinator: Antony Black, Claude Muller
Member of staff: Phonethipsavanh Nouanthong, Keooudomphone Vilivong, Did Panyathong, Siriphone Virachith

Background

Healthcare workers (HCW) have increased risk of exposure to infectious diseases and infected HCW represent a potential source for onward transmission of pathogens to susceptible patients. It is important that these risks are minimized, both by reducing exposure and by vaccination in the case of vaccine-preventable infections.

Activities and Prospective

In this study, we aim to estimate the susceptibility of healthcare workers from central, provincial and district hospitals to 6 vaccine-preventable infections and against HCV. We have recruited over 1000 participants to the study and begun to analyse sera for the presence of antibodies against hepatitis B, hepatitis C, measles, rubella, tetanus and diphtheria. Preliminary data suggest that a high proportion of Lao HCW remain susceptible to infection with hepatitis B, diphtheria, tetanus and rubella. Data analysis is ongoing.

Vaccination effectiveness within the Lao National Expanded Program of Immunisation three provinces

Activities and prospective

In order to analyse the effectiveness of the routine childhood vaccines we have initiated a study in collaboration with Lux Development to determine the antibody level among vaccinated Lao infants. In total, approximately 1000 child-mother pairs have been recruited from the provinces of Boulhikhamxay, Khammouan and Vientiane. Levels of antibodies specific for several vaccine-preventable diseases will be assessed including hepatitis B, diphtheria, tetanus, measles and rubella. Serological data from the children will be analysed in relation to vaccination status, maternal immune status, nutritional status and parasite load.

Hepatitis B and C virus in Lao blood donors.

Activities and prospective

The LaoLux laboratory, in close collaboration with the Lao Red Cross, has begun to investigate the characteristics of hepatitis infections in Lao blood donors. So far, over 3000 serum samples have been collected from 6 provinces. These samples will be analysed for prevalence of past and chronic infection, occult infections, and further characteristics of the virus and host response to infection or vaccination.

Evaluation of influenza virus circulation and transmission in pigs and free ranging poultry from mixed farms

Activities and prospective

We aim to evaluate the presence and characteristics of influenza viruses circulating in several hosts in Lao PDR. Furthermore we wish to investigate the transmission of influenza strains from one host to another and evaluate the potential health risks associated with the circulating viruses.

In collaboration with the Faculty of Agriculture, University of Laos, further sample collection is ongoing from mixed farms in Vientiane Province. Swabs from pigs, chickens and ducks are being collected and will be assessed in the Lao Lux Laboratory for influenza and other viruses. A researcher from the Faculty of Agriculture will be trained at the Institut Pasteur du Laos in sample processing and PCR.

Childhood respiratory infections in Laos

Activities and prospective

The study aims to determine the pattern of respiratory infection in children in Lao PDR. We also aim to provide a detailed analysis of etiological and risk factors determining the morbidity due to childhood respiratory tract infections such as micro RNA and cytokine profiles. 57 swabs and nasopharyngeal aspirates have been collected to date, with 26% respiratory syncytial virus and 7% influenza positive by rapid test. Sample collection and data analysis are ongoing.

Partners for ongoing studies 2015

- National Center for Laboratory and Epidemiology, Vientiane, Lao PDR.
- Vientiane central hospitals; Mitthapab, Settathirath and Childrens.
- Vientiane district hospitals; Sykhod, Chantabouly, Sysatanak, Xaysetha.
- Provincial Lao hospitals; Huaphan, Khammouan, Luangprabang and Boulhikhamxay.
- Luxembourg Agency for Development Cooperation (Lux Development), Lao PDR.
- National Immunization Programme, Ministry of Health, Lao PDR.
- National Institute of Public Health, Vientiane, Lao PDR.
- Lao Red Cross, National Blood Transfusion Centre, Vientiane, Lao PDR.
- Department of Pathology, University of Health Sciences, Vientiane, Lao PDR.
- National Animal Health Laboratory, Vientiane, Lao PDR.
- Faculty of Agriculture, University of Laos, Lao PDR.
- Department of Pathology, Faculty of Medicine, Chiang Mai University, Thailand.

Parasitology Laboratory

Lao-Japan joint Lab

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: **Shigeyuki KANO, MD, Ph D**

Scientist:

Moritoshi IWAGAMI, Ph D, Laboratory Manager

Junior Scientist:

Phonepadith KHATTIGNAVONG, MD

Technicians:

Pheovaly SOUNDALA
Lavy LORPHACHAN

Visiting Scientists:

Masami NAKATSU, Ph D
Tamotsu NAKASA, MD, MPH
Emilie MATSUMOTO-TAKAHASHI, Ph D
Masamine JIMBA, MD, Ph D
Jun KOBAYASHI, MD, Ph D
Toshihiro MITA, MD, Ph D
Takashi KUMAGAI, Ph D
Satoshi NAKAMURA, Ph D

Projects

- Development of Innovative Research Technique in Genetic Epidemiology of Malaria and Other Parasitic Diseases in Lao PDR for Containment of Their Expanding Endemicity (SATREPS)

Development of Innovative Research Technique in Genetic Epidemiology of Malaria and Other Parasitic Diseases in Laos for Containment of Their Expanding Endemicity

Project coordinator: Dr. Shigeyuki Kano
Member of staff: Moritoshi IWAGAMI,Phonepadith Khattignavong, Pheovaly Soundala, Lavy Lorphachan and other visiting scientists.

Background

Malaria, Schistosomiasis *(Schistosoma mekongi)* and Opisthorchiasis *(Opisthorchis viverrini)* have tremendous health burden on the people in Lao PDR. Although significant reductions in malaria transmission have been reported due to the large-scale insecticide-treated bed nets (ITNs) distribution through the Global Fund to Fight AIDS, Tuberculosis and Malaria, strategies based on the scientific evidence have not been developed to deal with the genetic variation in parasites and vectors population, and drug resistant malaria. In fact, artemisinin resistant malaria was reported in Attapeu province in 2014 (Ashley et al., 2014), and it has to be further surveyed in other provinces especially in the Southern part of this country. Estimation of the geographical origin and spread of its resistance is particularly essential for developing control strategies.

Since prevalence of Schistosomiasis *(S. mekongi)* and Opisthorchiasis *(O. viverrini)* are localized to Lao PDR and surrounding countries, they are often called as neglected tropical diseases. However, the prevalence of Opisthorchiasis is estimated as high as 15-54% in Lao PDR, causing the unacceptable burden on the people. Indeed, little information on the molecular/genetic epidemiology of the Opisthorchiasis is available to develop effective measures for its prevention.

The Government of Lao PDR requested Japan International Cooperation Agency (JICA) to establish the Lao-Japan Joint laboratory within Institut Pasteur du Laos (IPL) for conducting highly technological research on Plasmodium falciparum and P. vivax, S. mekongi and O. viverrini. The joint research will concentrate on genetic epidemiological studies to detect and control the emergence and dissemination of these parasitic diseases. The Project will also contribute to the capacity development of researchers and technicians in Lao PDR through training of field and Lab work, seminar and career development.

Objective

Objectives of this project are (1) to develop more convenient and accurate methods (PCR methods, LAMP methods, etc.) for diagnosis of the diseases, (2) to monitor temporal and spatial epidemiological situations of pathogens and vectors of the diseases, (3) to analyze mechanisms of emergence and expansion of the drug resistant malaria, especially, artemisinin resistance, and (4) to analyze glucose-6-phosphate dehydrogenase (G6PD) activity of Lao population for evaluation of possible usage of primaquine (Howes et al., 2013), utilizing molecular biological techniques. Based on the scientific evidence obtained by this project, health education for the people will be strengthened and endemicity of the diseases will be monitored together with the local Lao Ministry of Health. Research results will also be utilized in government services for sustainable development of Lao PDR.

Study period of the project

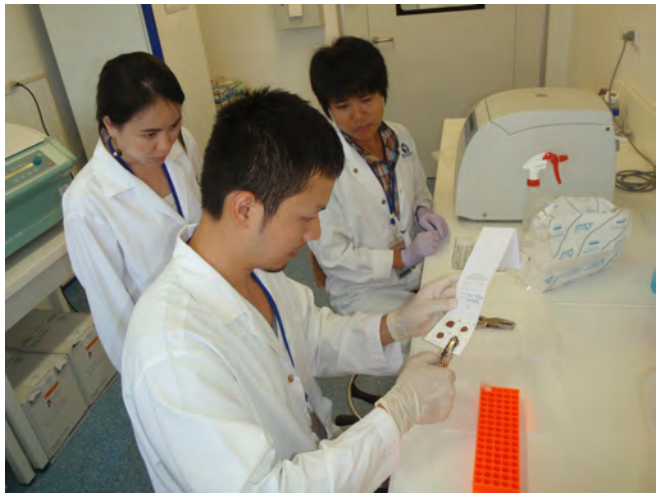
Five years (May 2014 to April 2019)

Study sites of the project

Malaria (*P. falciparum, P. vivax*):
Savannakhet Province, Attapeu Province, Champasak Province, Saravan Province, Sekong Province, Xieng Khouang Province, Phong Sali Province, Louangphrabang Province

Schistosomiasis (*S. mekongi*):
Khong District, Champasak Province

Opisthorchiasis (*O. viverrini*):
Khammuane Province, Champasak Province, Saravan Province, Attapeu Province



Study Design, Procedure of Sample Collection and Analyses

Malaria (*P. falciparum, P. vivax*):

- Microscopic observation of blood smear, malaria rapid diagnostic tests (RDTs), and G6PD activity assay will be performed using blood samples collected by fingertip prick from the people in malaria endemic areas on sites and/or in the IPL. For G6PD activity assay, G6PD Assay Kit (Dojindo Molecular Technologies, Inc, Japan) will be employed.
- Blood sample collection on filter papers by fingertip prick from the people in the malaria endemic areas will be performed for DNA analyses, such as PCR methods, LAMP methods, detection of drug (artemisinin, atovaquone, chloroquine, etc.) resistant mutations, and other genetic epidemiological analyses by DNA sequencing (Iwagami et al., 2012 & 2013).
- Mosquito sample collection in the malaria endemic areas will be performed for identifying species and malaria infection rate by morphological observation and DNA analyses.
- If possible, in vivo drug (Coartem: artemether-lumefantrine) susceptibility test will be performed at hospitals (or villages) in the endemic provinces.
- If possible, 2ml of blood will be taken from malaria patients for in vitro drug (Coartem) susceptibility test through Plasmodium falciparum culture and special DNA analysis, i.e. whole genome sequencing by Next Generation Sequencer to identify new responsible drug resistant gene(s) at NCGM laboratory in Japan.

Schistosomiasis (*S. mekongi*):

- Microscopic observation of stool sample from the people in Schistosomiasis endemic areas will be performed on sites and/or the IPL. Formalin-detergent method for precipitation of the eggs or Mini Parasep® (APACOR, UK) will be employed.
- Blood sample collection on filter papers by fingertip prick from the people in the Schistosomiasis endemic areas will be performed for DNA analyses, such as PCR methods, LAMP methods, and other genetic epidemiological analyses by DNA sequencing.
- Fresh water snail (*Neotricula*) sample collection in the Schistosomiasis endemic areas will be performed for identifying species of the snail and infection rate of *S. mekongi* in the snail by morphological observation and DNA analyses.
- When we find cercaria of *S. mekongi* in the snail, genetic epidemiological analyses will be performed by DNA sequencing.
- Microscopic observation of stool sample from Animals (potential reservoirs, such as, Pig, Cow, Water Buffalo, Dog, Cat, etc.) in Schistosomiasis endemic areas will also be performed on sites and/or in the IPL. Formalin-detergent method for precipitation of the eggs or Mini Parasep® (APACOR, UK) will be employed.

Opisthorchiasis (*O. viverrini*):

- Microscopic observation of stool sample from the people in Opisthorchiasis endemic areas will be performed on sites and/or in the IPL. Formalin-detergent method for precipitation of the eggs or Mini Parasep® (APACOR, UK) will be employed.

- Blood sample collection on filter papers by fingertip prick from the people in the Opisthorchiasis endemic areas will be performed for DNA analyses, such as PCR methods, LAMP methods, and other genetic epidemiological analyses by DNA sequencing.
- Fresh water snail (*Bithynia*) and Fresh water fish samples collection in the Opisthorchiasis endemic areas will be performed for identifying species of vectors and infection rate of *O. viverrini* in the vectors by morphological observation and DNA analyses.
- When we find cercaria or metacercaria of *O. viverrini* in the vectors, genetic epidemiological analyses will be performed by DNA sequencing.

Ethical clearance

The SATREPS project was approved by the National Ethics Committee for Health Research in the National Institute of Public Health (NIOPH), Ministry of Health, Lao PDR in 2014.

Activities

Malaria field survey was carried out in Sepon district, Savannakhet province, in the central part of Lao PDR in August 2014. This field survey was carried out with collaboration with staff from the CMPE, the NIOPH, Malaria Station Savannakhet Provincial Health Department, and a team headed by Professor Kazuhiko Moji in Nagasaki University, Japan. We collected blood samples from people in 3 villages (Kaluk Kao, Kaluk Mai, and Kaleng Kang), Sepon district, for identifying the prevalence and molecular characteristics of malaria. In order to estimate a rate of asymptomatic malaria patient (or malaria carrier), we collected blood samples from both malaria patients and healthy people in the 3 villages (Table 1 & 2). The analysis of the blood samples is ongoing.

Table 1: Number of participants and malaria positive cases by RDT in the 3 villages in Sepon district, Savannakhet province in 2014

Village Name	Population	No. of Participant (%)	RDT (+)
Kaluk Kao	204	132 (64.7)	0
Kaluk Mai	104	63 (60.6)	0
Kaleng Kang	221	104 (47.1)	5

RDT: Rapid Diagnostic Test (Malaria *Ag Pf/Pv*, Standard Diagnostics Inc. Korea)

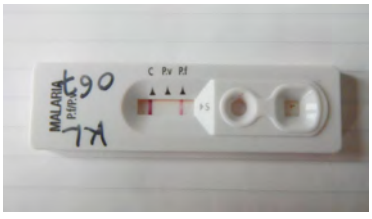
Table 2: Summary of malaria positive cases by RDT in Sepon district, Savannakhet province in 2014

Village Name	ID	Sex	Age	RDT (+)	Body temperature (°C)
Kaleng Kang	KL-22	M	34	Pf (+)	36.6
Kaleng Kang	KL-53	F	10	Pf (+)	36.9
Kaleng Kang	KL-63	F	9	Pf (+)	36.1
Kaleng Kang	KL-67	F	9	Pf (+)	37.0
Kaleng Kang	KL-74	M	7	Pf (+)	37.1

RDT: Rapid Diagnostic Test (Malaria *Ag Pf / Pv*, Standard Diagnostics Inc. Korea)
Pf: Plasmodium falciparum, Pv: Plasmodium vivax

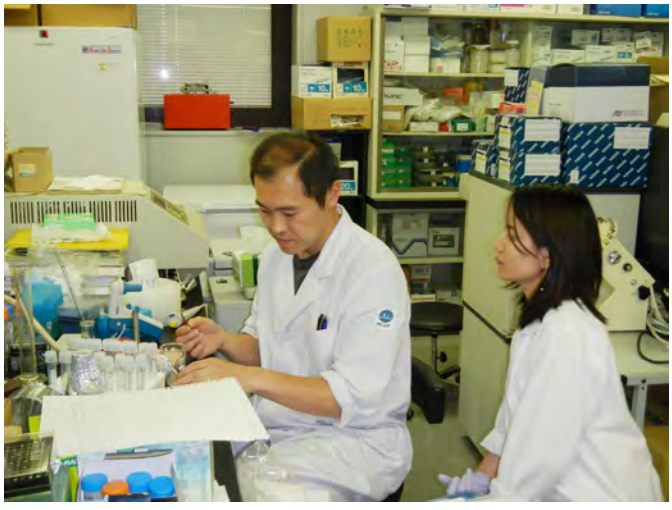
Blood sample collection

A total of 299 blood samples was collected from people (both malaria patients and healthy people) in 3 malaria endemic villages (Kaluk Kao, Kaluk Mai and Kaleng Kang) on filter papers (FTA™ Classic Card, GE Healthcare Life Sciences) by fingertip prick.



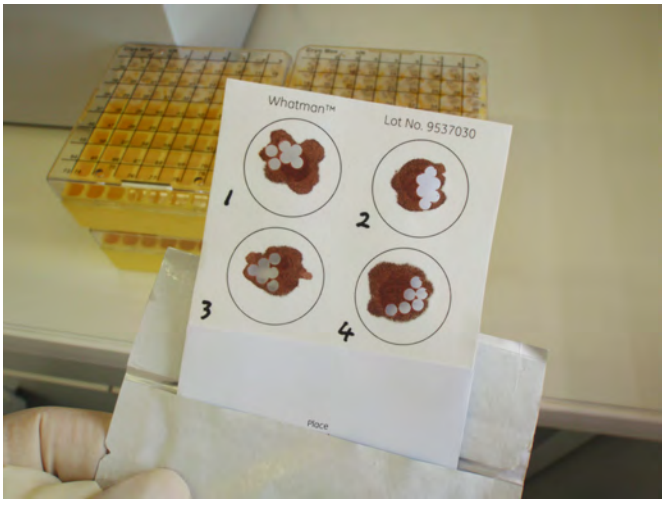
DNA extraction from the blood samples (On going):

Total genomic DNA was extracted from all the filter papers using a QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA) in accordance with the manufacturer's instruction. Six punched-out circles (2 mm diameter) from the each filter paper were used for DNA extraction. The extracted DNA was eluted with 150 µL of elution buffer in the Kit. The DNA will be used for further analyses such as molecular diagnosis, examination of drug resistant mutation, and population genetic analysis.



Prospective

The Lao-Japan joint Laboratory continues to work in collaboration with the NCGM, the CMPE and the NIOPH, (1) to develop more convenient and accurate methods for diagnosis of malaria, schistosomiasis, and opisthorchiasis, (2) to investigate temporal and spatial epidemiological situations of pathogens and vectors of the diseases, (3) to analyze mechanisms of emergence and dissemination of the drug resistant malaria, and (4) to analyze G6PD activity of Lao population. Further sample collection and DNA analyses of the diseases are ongoing. The SATREPS project will provide the scientific evidence for understanding of the diseases and for the diseases control in Lao PDR and other countries in Greater Mekong Subregion.



Partners

- Center for Malariology, Parasitology and Entomology (CMPE), Ministry of Health, Vientiane City, Lao PDR
- NIOPH, Vientiane City, Lao PDR
- National Center for Global Health and Medicine (NCGM), Tokyo, Japan
- The University of Tokyo, Tokyo, Japan
- University of the Ryukyus, Okinawa, Japan
- Juntendo University School of Medicine, Tokyo, Japan
- Tokyo Medical and Dental University, Tokyo, Japan

Scientific communications

Oral presentation:

Shigeyuki Kano. Spread of malaria drug resistance in SE Asia. Research Week for Development, Institut francais, Vientiane City, Lao PDR, October 13th-15th, 2014.

Shigeyuki Kano, Moritoshi Iwagami, Bouasy Hongvanthong, Paul Brey. The project for development of innovative research technique in genetic epidemiology of malaria in Lao PDR for containment of its expanding endemicity. The 8th National Health Research Forum, Vientiane City, Lao PDR, October 16th-17th, 2014.

Moritoshi Iwagami, Masami Nakatsum, Daisuke Nonaka, Tiengkham Pongvongsa, Hisami Watanabe, Jun Kobayashi, Futoshi Nishimoto, Kazuhiko Moji, Panom Phongmany, Bouasy Hongvanthong, Paul Brey, Shigeyuki Kano. Genetic epidemiology of drug resistant malaria in Lao PDR. The 8th National Health Research Forum, Vientiane City, Lao PDR, October 16th-17th, 2014.

Poster presentation:

Shigeyuki Kano, Moritoshi Iwagami, Masami Nakatsu, Jun Kobayashi, Daisuke Nonaka, Hisami Watanabe, Tiengkham Pongvongsa, Panom Phongmany, Bouasy Hongvanthong, Paul Brey. Molecular and genetic epidemiology of chloroquine resistant *Plasmodium falciparum* in Lao PDR under SATREPS project. 13th International Congress of Parasitology, Mexico City, Mexco, August 10th-15ht, 2014.

We wish to thank Dr. Bouasy Hongvanthong, Director of Center for Malariolgy, Parasitology and Entomology (CMPE), Lao PDR, and Project director of SATREPS for his kind support of this project. We thank Dr. Tiengkham Pomgvongsa, Director of Malaria Station, Savannakhet Provincial Health Department, Lao PDR and Professor Kazuhiko Moji, Graduate School of International Health Development, Nagasaki University, Japan for supporting the malaria field survey in Savannakhet. We also thank the staff of the CMPE, the NIOPH, and Sepon Inter-District Hospital, Lao PDR for their contribution to the malaria field survey.

Financial support

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SATREPS

Science and Technology Research Partnership for Sustainable Development Program

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Iwagami M, Hwang SY, Kim SH, Park SJ, Lee GY, Matsumoto-Takahashi EL, Kho WG, Kano S. Microsatellite DNA analysis revealed a drastic genetic change of *Plasmodium vivax* population in the Republic of Korea during 2002 and 2003. *PLoS Neglected Tropical Diseases*, 7: e2522, 2013.

They visited Institut Pasteur du Laos!

5 February 2014 Celebrating the signing of the Memorandum of Understanding (MOU) between the President, Dr. Masato Kasuga, National Center for Global Medicine (NCGM) Tokyo Japan and Dr. Antoine des Graviers, CFO representing IP Laos. Also in attendance were: Dr. Shigeyuki Kano, Dr. Jun Koboyashi, Dr. Darouny Phonekeo, Dr. Phonepadith Khattignavong, This MOU paved the way for the signing of the JICA-JST SATREPS Project.

23 May 2014 The Institut Pasteur du Laos (IPL) and the Disease Prevention Institute of Military Health Department (DPIMHD), Ministry of National Defense signed a Memorandum of Understanding for research and training in the areas of infectious/parasitic disease diagnostics, vector control and biomedical research. IPL and the DPIMHD have previously engaged in training in the area of dengue virus diagnostics and serotyping, as well as vector control. The MOU will allow DPIMHD staff to carry out on-the-job training at IPL and also allow IPL staff to provide training at the Lao Army hospital laboratories and training facilities. The MOU will also allow the parties to write joint grant proposals.

The signatories were Lt. Col. Dr. Xaysaveng Phommatha, Chief of the Disease Prevention Institute, on behalf of the Lao Department of Military Health and Dr. Paul Brey, Director of IP Laos, Lao Ministry of Health. Witnesses were Col. Dr. Bounteun Bandavong Chief of the Department of Military Health and His Excellency, Dr. Ponmek Dalaloy, former Minister of Health of Lao PDR and Honorary Chairman of IP Laos. Representatives of the Cabinet and Department of Communicable Disease Control of the Ministry of Health and Members of the Lao Department of Military Health also attended the ceremony.

19 March 2014 Signing of Memorandum of Understanding between Institut Pasteur du Laos (Dr. Paul Brey, Director) and Luxembourg Development Agency (Peter Heimann, Chief Technical Advisor) for cooperative projects in the area of Vaccine Preventable Diseases. The Government of the Grand Duchy of Luxembourg is a major donor of the Institut Pasteur of Laos.

10 June 2014

A) Visit of Mr. Jean-Christophe Philbe, Director of the South and South East Asia Division of the Electricité de France (EDF) and Mr. François Ailleret, Former Director General of Electricité de France (EDF) and former Chairman of the Board of Directors of Institut Pasteur along with H.E. Dr. Ponmek Dalaloy former Minister of Health of Lao PDR and Honorary Chairman of Institut Pasteur of Laos and Dr. Paul Brey, Director of Institut Pasteur du Laos.

B) Visit of Mr. Jean-Marc Châtaigner, Deputy Director general for Global Affairs, Development and Partnerships, French Foreign Affairs Ministry with Dr. Paul Brey, Director of IP Laos explain research activities.

2 July 2014 Visit of the delegation from Fondation Mérieux. Dr. Marc Grandadam (Head of the Arbovirology and Emerging Viruses lab at IP Laos) explaining the research activities of his group to Mme Mireille Guigaz (Vice Chair of the Global Fund), Prof. David Heymann (Vice President of the Board of Directors of the Fondation Mérieux) and Dr.Paul Brey, (Director of IP Laos).

10 July 2014 Visit of Dr. Shoichiro Tonomura, Executive Director of Japan Science and Technology Agency and Dr. Go Totsukawa, Department of International Affairs Japan Science and Technology Agency with Dr. Paul Brey Director of IP du Laos.

25 September 2014 Visit of H.E. Eksavang Vongvichit, Minister of Health of Lao PDR and Chairman of the Directors of IP Laos with Dr. Darouny Phonekeo, Deputy Director of IP Laos and other members of the Cabinet of the Ministry of Health to inspect the laboratory capacity of IP Laos as the first-line laboratory to diagnose suspected cases of Ebola in Lao PDR.

17 October 2014 Visit of Prof. Dr. Phonethep Pholsena President of the Social and Cultural Affairs Committee of the National Assembly of Lao PDR with his delegation discussing with Dr. Marc Grandadam (Head of the Arbovirology and Emerging Viruses lab at IP Laos) to gather information on the preparedness of IP Laos in the case of a suspected case of Ebola in Lao PDR.

Teaching/Training

12 March 2014 Intensive Training Course/Workshop on the Taxonomy and Identification of Mosquitoes, Ticks and Sandflies from Lao PDR

21 October 2014 First-Aid courses were given to all of the staff who work in the field and remote areas

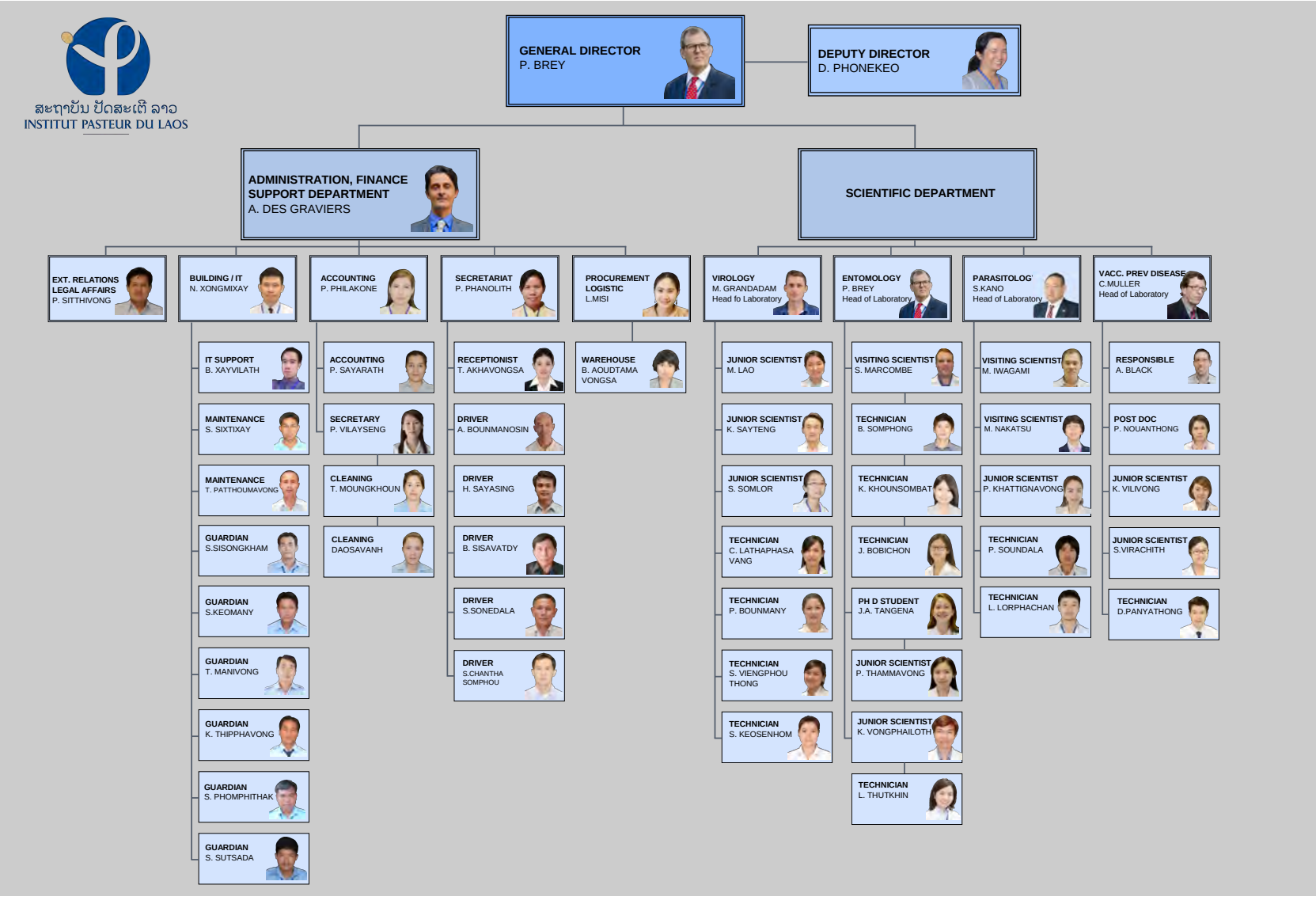
20 June 2014 Identification of parasite egg: Training of IFMTstudent

3-7 November 2014 Since Biosafety and Biosecurity are essential to Lab work, IPL has organized training of trainers for 6 targeted Laboratories from central, provincal and district hospitals

18 Sebtember 2014 Insecticide resitance bioassay training

In-house English lessons are given 6 times a week for 3 groups (beginners, intermediate, advanced)

Main organigram



Institut Pasteur du Laos staff

ສະຖາບັນ ປັດສະເຕີ ລາວ
INSTITUT PASTEUR DU LAOS

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