Institut Pasteur du Cambodge

2018 scientific report and 2019 prospects

Rapport 2018 et programmation scientifique 2019

Dr Didier Fontenille, DRCE, HDR

Directeur, Institut Pasteur du Cambodge

Sommaire / Content

1 RESUME / SUMMARY 5

2 ENJEUX DE L'INSTITUT PASTEUR DU CAMBODGE / CHALLENGES FACING THE INSTITUT PASTEUR DU CAMBODGE 9

2.1 OBJECTIFS A COURT TERME / SHORT-TERM OBJECTIVES 10

2.1.1 ENJEUX INSTITUTIONNELS / INSTITUTIONAL CHALLENGES 10

2.1.2 ENJEUX SCIENTIFIQUES / SCIENTIFIC CHALLENGES 12

2.2 OBJECTIFS A MOYEN TERME / MID-TERM OBJECTIVES 14

2.2.1 ENJEUX INSTITUTIONNELS / INSTITUTIONAL CHALLENGES 14

2.2.2 ENJEUX SCIENTIFIQUES / SCIENTIFIC CHALLENGES 15

3 ACTIVITIES IN 2018 AT INSTITUT PASTEUR DU CAMBODGE 17

3.1 MALARIA MOLECULAR EPIDEMIOLOGY 17

3.1.1 FUNCTIONAL STRUCTURE OF THE UNIT 17

3.1.2 RESEARCH PROGRAMS – MAJOR ACHIEVEMENTS IN 2018 18

3.1.3 RESEARCH PROGRAMS – PROSPECT 2019 21

3.1.4 SUPPORT TO NATIONAL AUTHORITIES 24

3.1.5 TEACHING AND TRAINING 24

3.1.6 PUBLICATION LIST 25

3.2 EPIDEMIOLOGY & PUBLIC HEALTH 26

3.2.1 FUNCTIONAL STRUCTURE OF THE UNIT 26

3.2.2 RESEARCH PROGRAMS IN 2018 27

3.2.3 RESEARCH PROGRAMS IN 2019 33

3.2.4 SUPPORT TO NATIONAL AUTHORITIES 34

3.2.5 TEACHING AND TRAINING 34

3.2.6 PUBLICATION LIST 35

3.3 IMMUNOLOGY UNIT 38

3.3.1 FUNCTIONAL STRUCTURE OF THE UNIT 38

3.3.2 RESEARCH PROGRAMS-MAJOR ACHIVMENT IN 2018 39

3.3.3 RESEARCH PROGRAMS –PROSPECT 2019 42

3.3.4 SUPPORT TO NATIONAL AUTHORITIES 45

3.3.5 TEACHING AND TRAINING....45
3.3.6 PUBLICATION LIST....46
3.4 VIROLOGY....46
3.4.1 FUNCTIONAL STRUCTURE OF THE UNIT....46
3.4.2 RESEARCH PROGRAMMES – MAJOR ACHIEVMENTS IN 2018....47
3.4.3 RESEARCH PROGRAMMES – PROSPECT 2019....56
3.4.4 SUPPORT TO NATIONAL AUTHORITIES....58
3.4.5 TEACHING AND TRAINING....60
3.4.6 PUBLICATION LIST....60
3.5 MEDICAL ENTOMOLOGY....62
3.5.1 FUNCTIONAL STRUCTURE OF THE UNIT....62
3.5.2 RESEARCH PROGRAMS – MAJOR ACHIEVEMENTS IN 2018....62
3.5.3 RESEARCH PROGRAMS – PROSPECT 2019....63
3.5.4 SUPPORT TO NATIONAL AUTHORITIES....64
3.5.5 TEACHING AND TRAINING....64
3.5.6 PUBLICATION LIST (2018)....65
3.6 ENVIRONMENT AND FOOD SAFETY LABORATORY ANALYSIS LABORATORY (LEFS) 65
3.6.1 FUNCTIONAL STRUCTURE OF THE UNIT....65
3.6.2 ROUTINE ACTIVITY 2018....66
3.6.3 RESEARCH PROGRAMS - MAJOR ACHIEVEMENTS IN 2018....68
3.6.4 RESEARCH PROGRAMS - PROSPECT 2019....69
3.6.5 SUPPORT TO NATIONAL AUTHORITIES....70
3.6.6 TEACHING AND TRAINING....70
3.6.7 PUBLICATIONS LIST 2018....71
3.7 MEDICAL LABORATORY....71
3.7.1 FUNCTIONAL STRUCTURE OF THE UNIT....71
3.7.2 ROUTINE ACTIVITY AND EVENTS 2018....72
3.7.3 RESEARCH PROGRAMS – MAJOR ACHIEVEMENTS IN 2018....74
3.7.4 RESEARCH PROGRAMS AND ROUTINE ACTIVITIES – PROSPECT 2019....75
3.7.5 SUPPORT TO NATIONAL AUTHORITIES....76
3.7.6 TEACHING AND TRAINING....76
3.7.7 PUBLICATION LIST (2017 AND 2018)....77
3.8 OTHER SERVICES OF THE INSTITUT PASTEUR DU CAMBODGE....78

3.8.1 RABIES PREVENTION CENTER FOR 2018 78
3.8.2 INTERNATIONAL VACCINATION CENTER FOR 2018 80
3.8.3 VOLUNTARY COUNCELING AND FREE TESTING CENTER (VCTC) FOR 2018 80
4 CONCLUSION 81

1 RESUME / SUMMARY

- English version below

En 2018, l'Institut Pasteur du Cambodge (IPC), avec 235 employés permanent (et jusqu'à 290 personnes sur le campus, avec les scientifiques en accueil et les stagiaires), s'est développé dans ses trois missions : recherche, formation et services. L'institut Pasteur du Cambodge a connu plusieurs évènements marquants en 2017.

- Des projets de recherche ambitieux se sont terminés et ont donné lieu à des séminaires de clôture, des rapports, des recommandations et rédaction d'articles (SEAe, Aspergiloses, Panic, Birdy, ANRS Statis).
- D'autres projets de recherche se poursuivent ou ont été renouvelés et se développent donnant des résultats applicables dans des recommandations de santé publique (résistance aux antipaludéens, Dengue Ecomore 2 (AFD), Paludisme ICEMR (NIH), Hépatite B Ta Prohm (ANRS), coqueluche Perilic (Total), Tuberculose mi RNA et TB Speed (ANRS, Unitaid et Initiative 5%), grippes avaires et grippe saisonnière.
- De nouvelles activités de recherche et de nouveaux projets, nécessaires pour la santé publique du Cambodge, ont été initiés et développés en 2018 en partenariat national et international : Arboviroses émergentes, transmission du paludisme forestier, moustiques de forêts, ..
- Le rythme, qualité et quantité, de publications a été maintenu (>50).
- Le plan Rage de l'IPC, en lien avec les Ministères de la Santé ; de l'Agriculture des pêches et des forêts, et de l'Education, Jeunesse et sport, s'etait développé en 2017 avec l'écriture et la diffusion massive d'un livre éducatif, et des campagnes de communication. En 2018 les activités se sont considérablement accélérées avec l'ouverture du centre de vaccination de Battambang et la formation de personnel.
- L'Audit pour la certification Iso 15189 du laboratoire de biologie médicale a eu lieu avec succes.
- Les collaborations régionales ont été renforcées à travers des projets de recherche: Vietnam, Chine, Thaïlande, Myanmar, Laos, Philippines, en particulier.
- La formation scientifique continue a été soutenue à travers de nombreux stages, y compris à l'étranger.
- L'IPC a augmenté significativement ses liens avec les Universités du Cambodge, en particulier UHS, RUPP, RUA, ITC, et son activité d'encadrement et de formation d'étudiants et de cadres scientifiques cambodgiens et internationaux (près de 100 étudiants accueillis).
- Le projet de Master international en Infectiologie UHS – Université de Paris Saclay, auquel l'IPC contribue, a connu un grand développement et devrait démarrer en octobre 2019 après un retard d'un an.
- La communication scientifique a été renforcée, avec des séminaires hebdomadaires ouverts à toute la communauté scientifique du Cambodge, des conférences grand public, une amélioration des sites web et Facebook.
- La communication de résultats, d'informations scientifiques, de recommandations au Ministère de la Santé, au Ministère de l'Agriculture, à l'OMS et la FAO (selon les agents pathogènes) ont été maintenus à un haut niveau, lors de rencontres, de groupes de travail, ou de notes mémo.
- Les évolutions de carrières pour les cadres scientifiques et administratifs ont été renforcées.
- De nouvelles responsabilités scientifiques et administratives ont été confiées à des cadres cambodgiens (LEFS, Paludisme, LBM, Direction Générale)
- Le laboratoire de microbiologie alimentaire et des eaux, rebaptisé Laboratory Environment and Food Safety (LEFS) a continué à se développer dans nouveaux laboratoires modernes et spacieux (près de 300 m2)..
- Les laboratoires d'immunologie et d'entomologie ont été restructurés et sont des Unités de recherche.
- Deux laboratoires BSL2 ont été rénovés en 2018. L'Institut héberge 7 BSL2 et un BSL3.
- La modernisation de l'accueil du laboratoire de biologie médicale a été terminée et inaugurée.

L'institut Pasteur du Cambodge, membre du Réseau International des Instituts Pasteur (RIIP), est un établissement reconnu d'utilité publique à but non lucratif, qui contribue au diagnostic, à la recherche et à la prévention des maladies infectieuses au Cambodge et dans la région. L'Institut, qui a signé en 1992 une convention avec le Gouvernement Royal du Cambodge, renouvelée par avenant en 2013,

est placé sous le haut patronage du Ministère cambodgien de la Santé pour lequel il réalise des activités de santé publique, de recherche, de formation et d'expertise. L'IPC est une des composantes du réseau d'instituts et d'universités du Ministère de la Santé du Cambodge, avec qui il a des collaborations très étroites, ainsi qu'avec d'autres partenaires nationaux et internationaux, et avec plusieurs Instituts du RIIP, dont en premier lieu l'Institut Pasteur à Paris.

A la pointe de la veille et de la recherche biomédicale en microbiologie (bactériologie, virologie, paludisme, parasitologie, mycologie, immunologie, entomologie, épidémiologie) ses laboratoires sont parmi les plus performants dans le sud-est asiatique, dans le domaine du diagnostic et de l'étude de la transmission des agents pathogènes, dans le développement de marqueurs biologiques, dans l'étude des résistances des microorganismes aux antimicrobiens, dans la prévention et dans la recherche clinique. Des avancées scientifiques majeures ont été obtenues en 2018, qui ont été possibles grâce à la sagacité et à la persévérance des chercheurs dans l'obtention de contrats et la réalisation des recherches, à l'implication forte des personnels techniques et administratifs de l'IPC, au soutien du Ministère de la Santé du Cambodge, et grâce à la confiance et à l'aide indispensable du MESR, du MEAE de France, et de l'IP Paris.

Les résultats des recherches et expertises ont fait l'objet de recommandations, de notes d'information, ou d'exposés lors de réunions de groupe de travail thématiques à l'adresse du Ministère de la Santé, Ministère de l'Agriculture, de l'OMS, et agences partenaires du Ministère de la Santé. Ils ont fait l'objet de communications et plus de 50 publications dans de grandes revues scientifiques ou ouvrages.

Les résultats scientifiques, les formations, les recommandations issues de ces recherches sont présentés dans les chapitres de chaque unité.

L'Institut est très impliqué dans la formation des étudiants de niveau universitaire. En 2018, 78 étudiants (70 Cambodgiens, 8 étrangers) ont bénéficié d'une formation à l'IPC, incluant des étudiants en DES de biologie, de futurs ingénieurs biomédicaux, des étudiants en Licence, Master, et PhD de différentes universités du Cambodge, de France et internationales et ont été accueillis à l'IPC pour des périodes de quelques semaines à 36 mois. La stratégie de l'IPC comporte une promotion des carrières des jeunes scientifiques cambodgiens en leur procurant un environnement scientifique compétitif de niveau international. Des MoU avaient été signés avec l'UHS et l'ITC. Les relations avec RUA, RUPP, l'Université Puthisastra ont été encore renforcées en 2018.

En 2018, l'IPC comptait plus de 235 employés, dont 26 expatriés (ainsi que deux chercheurs du CIRAD), plus les stagiaires de recherche. L'institut est composé d'un département administratif et financier, de services (LBM, LEFS, Vaccinations internationales, centres de vaccination anti-rabique post exposition (Phnom Penh, Battambang, et en 2019 Kampong Cham) et CDAG), d'unités de recherche : immunologie, entomologie médicale, virologie, paludisme, et épidémiologie / santé publique. Son budget de fonctionnement est abondé par des conventions de recherche, des ventes et prestations de services, par la subvention d'état du MESR et l'aide du MEAE, et d'autres ressources (la part respective des 3 principales composantes était : 2012 : conventions, prestations, MESR : 40%, 30%, 20% ; 2013 : conventions, prestations, MESR : 48%, 27%, 17% ; 2014 : conventions, prestations, MESR : 57%, 21%, 14%, 2015 : conventions, prestations, MESR : 58%, 25%, 12%. 2016 : conventions, prestations, MESR : 60%, 29%, 11%, 2017 : conventions, prestations, MESR : 57 %, 32 %, 11 %, 2018 : conventions, prestations, MESR : 57 %, 32 %, 11 %). Le Gouvernement Royal du Cambodge contribue en exonérant l'IPC des taxes.

Les activités de l'année écoulée et les perspectives de l'IPC sont présentées chaque année devant le Conseil de liaison présidé par SE M. le Ministre de la Santé, en présence de SE Mme l'Ambassadrice de France, du Directeur Général de l'IP Paris et de plusieurs autorités nationales (Ministères des Finances, de l'Agriculture, de l'Education, de la Santé) et internationales (OMS, UNICEF, ..). Les activités scientifiques sont évaluées tous les 18 mois par un Conseil scientifique. Le dernier Conseil scientifique s'est tenu les 6 et 7 décembre 2018. La politique scientifique est ajustée en fonction des recommandations formulées par ces conseils, concernant notamment le périmètre scientifique et l'aménagement des laboratoires, le développement, la réduction et la pérennisation d'activités ou de compétences, le transfert de technologies et de compétences vers les institutions cambodgiennes de la santé, les liens avec les partenaires en Asie et dans le monde.

En conclusion, l'IPC, qui réalise des recherches d'intérêt international, se veut une tête de pont de la recherche d'inspiration pasteurienne en santé au Cambodge et en Asie du sud-est. Tout n'est cependant pas parfait, il y reste de très nombreuses voies d'amélioration possibles, avec le soutien des tutelles. Le directeur de l'IPC est résolument engagé sur cette voie.

In 2018, the Institut Pasteur du Cambodge (IPC), with 235 employees (up to 290 with trainees and visiting scientists), expanded in its three missions: research, training and services. The Institut Pasteur du Cambodge experienced a number of significative changes in 2017.

- *Ambitious research projects have been completed and have resulted in closing seminars, reports, recommendations and articles (SEAe, Aspergiloses, Panic, Birdy, ANRS Statis)*
- *Several research projects were greatly expanded, and conducted to public health recommendations (Dengue Ecomore 2 (AFD), Malaria ICEMR (NIH), Hepatitis B Ta Prohm (ANRS), pertussis Perilic (Total), Tuberculosis mi RNA et TB Speed (ANRS, Unitaid and Initiative 5%), avian flu and seasonal flu).*
- *New research activities needed for public health in Cambodia were initiated or developed in 2018 in national and international partnership: emerging Arbovirosis, malaria forested transmission, mosquitoes from the forest, .*
- *Continuous scientific training for staff has been encouraged through numerous workshops and internships, including abroad.*
- *The quality, and "quantity" of IPC's publications were maintained in 2018 (>50).*
- *The IPC's Rabies Plan, in partnership with the Ministries of Health; Agriculture, Fisheries and Forestry, and Education, Youth and Sport, had been developed in 2017 with the writing and mass distribution of an educational book, and communication campaigns. In 2018, activities accelerated considerably with the opening of the vaccination centre in Battambang and the training of staff.*
- *The Iso 15189 accreditation for the Medical Biology Laboratory has been successfully granted.*
- *Laboratories and support services were streamlined and expanded, such as renovation of two BSL2.*
- *Regional collaborations have been developed via collaborative research projects (Vietnam, China, Thailand, Myanmar, Laos, Philippines, etc…).*
- *The IPC has significantly increased its partnerships with Cambodia's universities, in particular UHS, RUPP, RUA, ITC, and its activity of mentoring and training Cambodian and international students and professionals (about 100 students hosted).*
- *The project for an international Master's degree in Infectiology, UHS - University of Paris Saclay, to which the IPC contributes, has seen great development and should start in September 2019.*
- *Scientific communication was reinforced, with weekly seminars open to the entire Cambodian scientific community, with publication of project or disease sheets, via a redesign of the website and facebook.*
- *The communication of results, scientific information, recommendations to the Ministry of Health (and Ministry of Agriculture), WHO, FAO (depending of pathogens) has been maintained at a high level through meetings, working groups or memo notes.*
- *Career developments for scientific and administrative staffs have been strengthened.*
- *New scientific and administrative responsibilities have been entrusted to Cambodian managers (LEFS, Malaria, LBM, General Direction)*
- *The food and water microbiology laboratory, renamed Laboratory Environment and Food Safety (LEFS), has continued to develop and has integrated new modern, spacious laboratories (almost 300 m2).*
- *Immunology and medical entomology laboratories have been restructured into research units.*
- *Two BSL2 laboratories were renovated in 2018. The Institute hosts 7 BSL2 and one BSL3.*
- *Modernization of the reception area for the medical biology laboratory ended and the new LBM was inaugurated.*

The Institut Pasteur du Cambodge, a member of the Instituts Pasteur International Network (IPIN), is a government-approved non-profit institution. The institute signed an agreement with the royal government of Cambodia in 1992, which was renewed by an endorsement in 2013. It is under the senior patronage of the Cambodian Ministry of Health, on behalf of which it carries out public health, expert studies, research and training activities. The IPC is part of the network of institutes and universities

under the Cambodian Ministry of Health. The institute has developed these missions during the past 15 years in both the academic and applied science streams. It is contributing to the diagnosis of, research in and prevention of the priority infectious diseases in Cambodia and throughout the region. It plays a major role in microbiological monitoring and research in cooperation with national and international partners, as well as several IPIN institutes, first and foremostly the Institut Pasteur in Paris.

At the cutting edge of biomedical research in microbiology (bacteriology, virology, malaria, parasitology, mycology, immunology, epidemiology, medical entomology) its laboratories are among the most efficient in Cambodia and Southeast Asia in the field of diagnosis and the study of pathogen transmission, analysis of biological and genetic markers of severity or the antimicrobial resistance of microorganisms, prevention and clinical research. Significant scientific advances that have been published in more than 50 scientific articles (and book chapters) in 2018 have been possible due to the insight and perseverance of researchers in securing contracts and carrying out research, the heavy involvement of the IPC's technical and administrative personnel, support from the Cambodian Ministry of Health and the trust of and vital aid from France's MESR (ministry of higher education and research), MEAE (ministry of foreign affairs), and Institut Pasteur Paris.

The findings of research and expert studies have come out in the form of recommendations or briefing notes submitted to the Ministry of Health, MAFF and WHO. Detailled results and activities are presented hereafter in unit's reports.

The institute is deeply involved in university-level training. Students in biology, biomedical sciences, engineers, master and PhD students from different universities in Cambodia, France and other countries were hosted by the IPC. In 2018, in total 78 graduate students had long term training (from several weeks up to 36 months) at IPC (70 Cambodian students, 8 foreign students).The IPC's strategy focuses on the promotion of careers for young Cambodian scientists by providing them with a competitive, world-class scientific environment. MoU have been already signed with UHS and ITC. Relationships with RUA, RUPP, Puthisastra University have been reinforced in 2018.

The IPC has a staff base of over 235, including 26 expatriates on long-term contracts (plus two researchers from CIRAD) and hosts tens of interns.

The institute is composed of an administrative and financial department, several services (LBM, LEFS, international vaccinations, post-exposure rabies vaccination (Phnom Penh, Battambang, and Kampong Cham in 2019) *and VCTC), of research units: immunology, medical entomology, virology, malaria, and epidemiology/public health*
Its operating budget is supplemented by research contracts, service delivery agreements, MESR and MEAE government grant aid and other resources (the respective share of the three main components was: 2012: research contracts, service deliveries, MESR: 40%, 30%, 20%; 2013: research contracts, service deliveries, MESR: 48%, 27%, 17%; 2014: research contracts, service deliveries, MESR: 57%, 21%, 14%, 2015: research contracts, service deliveries, MESR: 58%, 25%, 12%; 2016: service deliveries, MESR: 60%, 29%, 11%; 2017: service deliveries, MESR: 57%, 32%, 11%), 2018: service deliveries, MESR: 57%, 32%, 11%).. The Royal Government of Cambodia contributes by giving tax exemption to the IPC.

The IPC's activities over the past year and prospects are outlined annually to the Liaison Board chaired by HE the Minister of Health, in the presence of HE the Ambassador of France, the President of IP Paris and representatives of several national authorities (Ministries of finances, agriculture and education) and international agencies (WHO, UNICEF, etc.). The scientific activities are assessed every 18 months by a scientific board (last session on 6-7 December 2018). The scientific policy is adjusted based on the recommendations of these boards with regard to such things as the scientific scope and management of the laboratories, the development, reduction or extension of activities, technology and skills transfer to Cambodia's healthcare institutions, links with partners in Asia and worldwide.

In conclusion, the IPC, with its focus on research of international interest, is a bridgehead of Pasteur-inspired research in healthcare in Cambodia and Southeast Asia. Of course, not everything is perfect, so there is plenty of room for improvement, with the backing of various supporting bodies. The IPC's director is firmly committed to carrying things forward.

2 ENJEUX DE L'INSTITUT PASTEUR DU CAMBODGE / CHALLENGES FACING THE INSTITUT PASTEUR DU CAMBODGE

- English version below

Les enjeux et perspectives relèvent des trois missions de l'IPC : (1) les activités de services et de santé publique aux particuliers et aux institutions, (2) les activités de recherche, (3) les activités d'encadrement et de formation. Les objectifs tiennent compte des recommandations du conseil de liaison et du conseil scientifique de l'année précédente.

A. Commentaires majeurs du précédent conseil de liaison (07 May 2018)

Les commentaires du précédent conseil de liaison insistent sur la qualité des résultats scientifiques obtenus par l'IPC et leur intérêt pour le Ministère de la santé.
Le programme du Master d'infectiology avec l'UHS rentre bien dans l'objectif du Ministère de la Santé et du Ministère de l'Education, de la Jeunesse et des Sports.
Le conseil de liaison encourage l'IPC à travailler sur la résisatnce aux antibiotiques.
Le conseil de liaison recommande de développer le laboratoire de sécurité alimentaire et de l'environnement, en particulier en toxicologie et en chimie, pour répondre aux nombreux évènements de « food safety » au Cambodge.
Le conseil de liaison recommande de développer les centres provinciaux de vaccination contre la rage pour que la rage soit éliminée.

B. Commentaires majeurs du précédent conseil scientifique SAB (6 et 7 décembre 2018)

Le SAB considère que les activités de recherche et de service sont bonnes et productives. Les publications sont satisfaisantes.
Le SAB a apprécié la restructuration des unités d'entomologie médicale et d'immunologie ;
La formation à l'IPC est de qualité. L'implication dans le nouveau Master avec UHS est une très bonne initiative.
De nouveaux projets de recherche sur la résistance aux antibiotiques et les aspergilloses sont très utiles au système cambodgien de santé publique.
Plusieurs projets de recherche portent sur des questions de santé publique très pertinentes (tuberculose, rage, hépatite, dengue, paludisme,...).
Les activités de services sont très pertinentes : LEFS, Rage PEP.
Le SAB félicite IPC pour l'accréditation ISO 15189.
Les nouvelles responsabilités du personnel et des scientifiques cambodgiens constituent une évolution positive.

Cependant le SAB a quelques préoccupations :
IPC doit obtenir de nouvelles subventions pour 2019-2021.
Il manque toujours de bons statisticiens et bio-informaticiens à l'IPC.
L'IPC doit améliorer son attractivité pour les étudiants diplômés.
Le turnover des expatriés peut être un problème
L'IPC devrait organiser des ateliers sur la rédaction et la publication et élaborer un programme de tutorat avec les responsables de la recherche.
Le SAB recommande de mettre sur pied une communauté d'étudiants et d'anciens élèves de l'IPC.
Le SAB recommande de renforcer la communication avec les autorités de santé publique, les universités et les ministères.

The challenges and prospects come under the three IPC missions: (1) service and public healthcare activities targeting individuals and institutions, (2) research activities, (3) teaching and training activities. The objectives include the recommendations made the previous year by the liaison board and scientific advisory board.

A. *Major comments from the last liaison board meeting (*07 May 2018*)*

The comments of the previous Liaison Council emphasise the quality of the scientific results obtained by the IPC and their relevance to the Ministry of Health.
The Master of Infectiology program with the UHS is in line with the objectives of the Ministry of Health and the Ministry of Education, Youth and Sports
The Liaison Council encourages the IPC to work on Antibiotic resistance
The Liaison Council recommends that the Food and Environmental Security Laboratory, particularly in toxicology and chemistry, be developed to respond to the many food safety events in Cambodia,
The Liaison Council recommends that provincial rabies vaccination centers be developed to eliminate rabies.

B. *Major recommendations of the scientific advisory board meeting (6 - 7 décember 2018)*

SAB considers that research and service activities are good and productive. Publications are satisfactory.
SAB appreciated restructuration Medical entomology and Immunology Units;
Training at IPC is good. Involvement in the new Master degree with UHS is worth.
New research projects like AMR and Aspergilosis have strong value for Cambodian public health surveillance system.
Several research projects address highly relevant issues for public Health (TB, Rabies, Hepatitis, Dengue, Malaria, ..).
Services activities are very relevant: LEFS, Rabies PEP.
Sab congratulates IPC for ISO 15189 accreditation.
New responsibilities for Cambodian staff and scientist is a positive evolution.

SAB raised some concerns:
IPC must secure new grants for 2019-2021.
Lack of good statistician and bio-informatician at IPC.
IPC must improve attractiveness for graduate students.
Turnover of expatriates.
IPC should organize writing/publishing workshops and develop a mentorship program with research leaders.
SAB recommends to building a supportive IPC student / alumni community.
SAB recommends strengthening communications with the Public Health Authorities, Universities and Ministries.

2.1 OBJECTIFS A COURT TERME / SHORT-TERM OBJECTIVES

Les objectifs à court et moyen terme de l'IPC suivent les recommandations présentées par SE M. le Ministre de la Santé du Cambodge durant le 39ieme congrès national de la Santé, et concernant les structures sous sa supervision

The short and medium-term objectives of IPC follow the recommendations presented by HE the Minister of Health of Cambodia during the 39th National Health Congress and concerning the structures under his supervision.

2.1.1 ENJEUX INSTITUTIONNELS / INSTITUTIONAL CHALLENGES

MAINTENIR L'IPC DANS SON CONTEXTE SCIENTIFIQUE ET MEDICAL NATIONAL ET REGIONAL / TO MAINTAIN THE IPC IN ITS NATIONAL AND REGIONAL SCIENTIFIC AND MEDICAL CONTEXT

L'IPC, sous tutelle du Ministère de la Santé du Cambodge et de l'Institut Pasteur Paris est au cœur du dispositif de recherche et d'expertise du Cambodge en santé (universités, centres de recherche,

hôpitaux, secteur privé), avec une dimension régionale et internationale. Plus de 90% du personnel, incluant les cadres, est cambodgien. L'IPC a vocation à être mobilisé, et soutenu, par ces différents partenaires, cambodgiens et étrangers, conjointement, afin qu'il continue à remplir ses missions efficacement.

The IPC, reporting to the Cambodian Ministry of Health and Institut Pasteur Paris, is at the heart of Cambodia's healthcare research and expert study apparatus (universities, research centers, hospitals, private sector), with a regional and international dimension. More than 90% of the staff base, including senior researchers and managers, is Cambodian. The determination of the IPC is to be called upon—and supported by—these different partners, Cambodian and foreign alike, so that it can continue to fulfill its missions in a meaningful way.

DEVELOPPER LES CAPACITES / DEVELOPING CAPACITIES

Contribuer encore plus intensivement à la formation et à l'encadrement d'étudiants de masters, d'école d'ingénieurs et de doctorats (PhD, Docteur Pharm, Docteur Med, Vet), inscrits dans les universités au Cambodge et à l'étranger. Rendre plus visible cette offre de formation et d'encadrement. Contribuer acivement à l'ouverture du master en Infectiologie de USS – Paris Saclay en 2019.
Développer les séminaires hebdomadaires ouverts sur la communauté scientifique et universitaire au Cambodge.

Contribute even more intensively to the training and coaching of master's degree, engineering and doctorate-level students (PhD, PharmD, DMedSc,DVM) enrolled in Cambodian and foreign universities. Make these training and supervising activities more visible. Contribute significantly to the opening of the Master's degree in Infectiology from USS - Paris Saclay in 2019.
Develop weekly seminars open to the Cambodian scientific and academic community.

DEVELOPPER LES INFRASTRUCTURES / DEVELOPING INFRASTRUCTURES

Continuer à améliorer les laboratoires et les infrastructures des différentes unités et services, épidémiologie SP et centres vaccination rage à Phnom Penh, Battambang et Kampong Cham en particulier.

Continue to improve laboratories and infrastructure for epidemiology, virology, Malaria, entomology, Immunology, LBM, LEFS unit and vaccination centers (Phnom Penh, Battambang and Kampong Cham Rabies vaccination centers).

RENDRE LES CARRIERES A L'IPC ATTRACTIVES / BOOSTING THE ATTRACTIVENESS OF CAREERS WITH THE IPC

Développer les conditions d'attractivité des emplois, en particulier des cadres, à l'IPC, par la mise en place de plans de carrières, de motivations financières, et de reconnaissance sociale. Communiquer auprès des étudiants diplomés. Donner des responsabilités scientifiques et managériales aux jeunes chercheurs cambodgiens prometteurs. Communiquer sur les possibilités de carrières et les besoins de l'IPC.

Develop conditions to make IPC job opportunities attractive, particularly at the executive level, by putting in place career plans and financial incentives, as well as by securing social recognition for such. Communicate and inform graduate students. Have scientific and managerial responsibilities entrusted to promising young Cambodian researchers.
Communicate on career opportunities and on the needs of IPC.

ASSURER L'ÉQUILIBRE BUDGETAIRE / KEEPING THE BUDGET BALANCED

L'IPC est un institut sans but lucratif. La recherche et l'obtention de financements et de ressources, par des projets de recherche et des activités de services, en particulier LBM, LEFS et vaccinations internationales, sont essentielles et doivent être développées.

The IPC is a non-profit institution. Fundraising and the availability of funding and resources through research projects and service activities (including LBM, LEFS and International Vaccinations) are vital and must be developed further.

ASSURER LA TRANSITION DE LA DIRECTION EN 2019 / ENSURE BRIDGING OF IPC MANAGEMENT

Effectuer une bonne transition de la direction générale et de la direction administrative et financière de l'IPC, en raison des fins de contrats du Directeur (D Fontenille), et du DAF (G. Daufresne) en Aout 2019.

Make a good transition of the management of the IPC, due to the termination of the contracts of the General Director (D Fontenille), and the administrative and financial director (G. Daufresne) in August 2019.

2.1.2 ENJEUX SCIENTIFIQUES / SCIENTIFIC CHALLENGES

INNOVER ET VISER L'EXCELLENCE SCIENTIFIQUE / INNOVATION AND EXCELLENCE IN SCIENCE

Soutenir et favoriser l'émergence de nouveaux projets de recherche ambitieux et innovants issus des unités de recherche et des services de santé publique, en particulier dans le domaine de la santé-environnement, des interactions hôtes-pathogènes, de la résistance aux médicaments, des hépatites, de l'entomologie médicale, des biomarqueurs. Poursuivre la dynamique d'excellence scientifique sur les thèmes majeurs de santé publique en infectiologie : paludisme, encéphalites, dengue, VIH, hépatites, grippes, rage, tuberculose, vecteurs, etc.
Encourager les équipes à publier dans les meilleurs journaux scientifiques, et à diffuser les résultats de la recherche vers les services concernés du Ministère de la Santé, vers l'OMS et vers le grand public.

Support and promote the emergence of new, ambitious and innovative research projects arising from research and public healthcare service units, particularly in the area of health and environment, host-pathogen interactions, drug resistance, hepatitis and medical entomology.

Pursue the dynamics of scientific excellence on the major issues of infectious diseases in public healthcare: malaria, encephalitis, dengue, HIV, hepatitis, influenza, rabies, tuberculosis, vectors, biomarkers, etc.

Encourage the teams to publish their research findings in the best scientific journals and have them disseminated among the concerned departments of the ministry of health, to WHO and to the public at large.

DEVELOPPER LES ACTIVITES POUR LUTTER CONTRE LA RAGE / DEVELOPPING ACTIVITIES FOR RABIES CONTROL

En accord avec le Ministère de la Santé du Cambodge et le MoH CDC, en partenariat avec l'OMS, la FAO et le Ministère de l'Agriculture, renforcer les recherches sur la rage, développer un plan de communication et d'éducation contre la rage, développer les centres de vaccination antirabique de Battambang et de Kampong Cham avec le PHD.

In collaboration with the Ministry of Health of Cambodia, the MoH CDC, the Ministry of Agriculture, the WHO, the FAO, strengthen research on rabies, develop a communication and education plan against rabies, develop the PHD-IPC Battambang and Kampong Cham centers.

ETUDIER LES RESISTANCES / STUDYING RESISTANCE

L'étude, le diagnostic et la gestion des résistances aux médicaments : bactéries, mycobactéries, paludisme, HIV, autres viroses, infections fungiques et aux insecticides pour les vecteurs de dengue et de paludisme, seront développés, soutenus et renforcés. Un « jeune groupe de recherche » sera créé en bactériologie.

The study, diagnosis and management of drug resistance—bacteria, mycobacteria, malaria, HIV, other viral diseases, fungal infections and insecticide resistance for dengue and malaria vectors —will be developed, supported and reinforced. A "young research team "will be created in bacteriology.

ESTIMER LA TRANSMISSION / ESTIMATING TRANSMISSION

La connaissance des mécanismes de transmission, vectorielle, directe, nosocomiale, à partir de sources, réservoirs ou foyers, symptomatiques ou asymptomatiques, anthropiques ou sauvages, est un point critique pour l'estimation du risque et le contrôle de maladies (paludisme, dengue, encéphalite japonaise, autres encéphalites, virus influenza, rage, coqueluche, sepsis, champignons..). L'IPC doit poursuivre son engagement dans cette voie, y compris par des approches de modélisation.

Knowledge of the mechanisms of transmission—vectorial, direct, nosocomial—from reservoirs or transmission foci; symptomatic or asymptomatic, anthropic or wild, is critical for disease risk assessment and disease control (malaria, dengue, Japanese encephalitis, other types of encephalitis, influenza virus, rabies, pertussis, sepsis, fungal infections). The IPC is set to pursue its commitment on this way, including modeling approaches.

DEVELOPPER LES PLATES-FORMES / DEVELOPING PLATFORMS

Permettre le développement de technologies modernes de diagnostics, d'expérimentations, et d'analyses épidémiologiques en poursuivant le développement et l'équipement les laboratoires et en favorisant la mutualisation et le développement des compétences, en particulier en entomologie, immunologie, bio-banking, modélisation, statistique, bio-informatique, génomique et pour l'accès à des équipements lourds partagés déjà disponibles ou a acheter (séquenceurs, cytomètres de flux, trieur de cellules, chromatographie en phase gazeuse - Spectrométrie de masse, Maldi-Tof, etc.).

Enable the development of modern technologies for epidemiological diagnosis, testing and analysis by maintaining the development and equipment of laboratories and by promoting the sharing and development of skills, notably in entomology, immunology, bio-banking, bio-informatics, statistics, genomics and modelling, as well as through access to shared heavy equipment already available or to buy (sequencing machines, flow cytometers, cell sorter, Gas Chromatography Mass Spectrometry, Maldi-Tof, etc.).

COMBLER LES POINTS FAIBLES / ADDRESSING THE WEAKNESSES

Renforcer l'IPC dans le domaine de la modélisation, des biostatistiques, de la génomique, de la chimie, de la bioinformatique, par la formation, le recrutement et le partenariat avec les universités. S'appuyer sur les collaborations internationales, en particulier l'IP à Paris, pour combler ces lacunes.

Strengthen the IPC in the area of modelling, immunology, chemistry, biostatistics, as well as in genomics, bioinformatics, through training, recruiting and partnerships with Universities. Build upon international cooperation arrangements, particularly with the Paris IP, to address these weaknesses

2.2 OBJECTIFS A MOYEN TERME / MID-TERM OBJECTIVES

2.2.1 ENJEUX INSTITUTIONNELS / INSTITUTIONAL CHALLENGES

RENFORCER LE PARTENARIAT / STRENGTHENING THE PARTNERSHIP

L'IPC seul, n'est rien. Seul un partenariat bien compris, à bénéfice mutuel, en premier lieu avec les institutions au Cambodge du Ministère de la Santé et d'autres Ministères (éducation, agriculture, environnement, …) telles que les Universités, les centres de recherche, les hôpitaux, le secteur privé ; avec les Instituts Pasteur du réseau Pasteur international ; et avec les partenaires internationaux de la sous-région ASE, et du reste du monde, permettra à l'IPC de remplir ses missions.

On its own, the IPC doesn't amount to anything. Only a well-understood partnership, with mutual benefits, firstly with Cambodian institutions under the ministry of health and other ministries (education, agriculture, environment, etc.) (universities, research centers, hospitals, private sector), with the Pasteur Institutes of the international Pasteur network and with international partners in the Southeast Asia sub-region and the rest of the world, will enable the IPC to perform its role.

DEVELOPPER LES CAPACITES ET LES COMPETENCES / SKILLS AND CAPACITY BUILDING

Participer à des masters nationaux (au Cambodge), et internationaux, et à des formations spécifiques thématiques (immunologie, entomologie, biostatistiques, essais cliniques..).

Contribuer à la création en 2019 d'un master sur les maladies infectieuses avec l'USS et l'université Paris Saclay.

Intervenir dans les formations des établissements d'enseignement supérieur au Cambodge (voire dans la sous-région) : USS, URA, URPP, ITC, en particulier. Attirer des étudiants cambodgiens et étrangers et les accueillir en formation et stage à l'IPC. Faire connaître les possibilités d'encadrement et de recrutement de l'IPC.

Reformuler l'offre de formation continue de l'IPC pour ses agents, afin de mieux répondre aux besoins conceptuels et techniques immédiats et à long terme.

Contribute to national (in Cambodia) and international master's degree streams and to specific issue-based training initiatives (immunology, entomology, biostatistics, clinical trials, etc.).

Contribute to the creation in 2019 of a master's degree on infectious diseases with the UHS and university Paris Saclay.

Get involved in the training dispensed in Cambodia's institutions of higher education (and even in the sub-region), notably the University of Health Sciences, Royal University of Agriculture, Royal University of Phnom Penh and the Institut de Technologie du Cambodge. Attract Cambodian and international students and host their training at the IPC. Advertise the IPC's training and hiring opportunities.

Streamline the IPC's ongoing training offer for its staff with a view to better responding to both immediate and long-term needs, both conceptual and technic.

AUGMENTER LA VISIBILITE DE I'IPC / MAKING THE IPC MORE VISIBLE

Rendre l'IPC encore plus visible, utile et nécessaire au niveau national, régional et international, à travers une meilleure communication sur les activités scientifiques et les services rendus. Développer l'animation scientifique, dans et hors les murs. Développer les liens avec les médias. Améliorer les sites Web et Facebook.

Make the IPC even more visible, meaningful and necessary at the national, regional and international levels, through better communication on the scientific activities and services delivered. Develop scientific facilitation both internally and externally. Develop links with the media. Upgrade the web and Facebook sites.

FORMALISER LE MANAGEMENT DE L'IPC / FORMALIZING MANAGEMENT OF THE IPC

Faire vivre et valoriser le comité Hygiène, Sécurité et Qualité (c'est une priorité 2019).
Faire vivre et valoriser le bio-banque.
Faire évoluer la qualité de vie et l'esprit d'entreprise dans l'IPC en s'appuyant sur le dialogue avec le personnel et les délégués du personnel.
Maintenir la consultation régulière avec les responsables d'unités et de services et les cadres de l'IPC.
Rappeler régulièrement les missions et les valeurs de l'IPC.

Give life to and enhance the Hygiene, Safety and Quality Committee (it is a priority in 2019).
Give life to and enhance the biobanking Committee.
Improve the quality of life and entrepreneurial spirit within the IPC based on dialogue with the staff and staff representatives.
Maintain regular consultation with the officers in charge of the units and departments and IPC executive staff.
Regularly highlight the IPC's missions and values.

AUGMENTER NOS CAPACITES D'ACCUEIL / INCREASING OUR INTAKE CAPACITY

Continuer à aménager les laboratoires, et les services de soutien et support, ainsi que les services de santé publique (rage, LEFS), pour permettre le développement de nos activités de recherche et de services, à travers l'accueil de nouveaux jeunes chercheurs et étudiants cambodgiens, de la sous-région ASE, français et internationaux.

Continue to eensure development of the laboratories, help and support services, as well as public healthcare services (rabies, LEFS) so as to promote the development of our research and service activities by taking in new young researchers and students from Cambodia, the Southeast Asia sub-region, France and other countries.

ACCREDITER ET LABELISER DES SERVICES DE l'IPC / ACCREDITATION AND LABELING OF IPC SERVICES

L'IPC a obtenue l'accréditation ISO 15189, pour l'unité de biologie médicale. L'objectif est d'étendre progressivement (en 4- 5 ans) cette labélisation à d'autres services, dont la microbiologie alimentaire. D'autres laboratoires sont des références nationales ou internationales (dengue, grippe aviaire, rage).

The IPC was awarded ISO 15189 for the medical biology unit. The objective is to gradually extend (over 4 to 5 years) this labeling to other services, including food microbiology. Other laboratories are national or international referral laboratories (dengue, avian influenza, rabies).

2.2.2 ENJEUX SCIENTIFIQUES / SCIENTIFIC CHALLENGES

POURSUIVRE LE DEVELOPPEMENT DU LABORATOIRE DE MICROBIOLOGIE ALIMENTAIRE / CONTINUE TO DEVELOP THE FOOD MICROBIOLOGY LABORATORY

Le Cambodge, pays en grand développement économique, compte sur son industrie touristique et agro-alimentaire pour augmenter ses ressources. Dans ces deux secteurs, la qualité microbiologique et chimique des produits (produits agro-alimentaires bruts ou transformés, eaux de piscine, eaux de traitement, repas) doit être vérifiée et certifiée. L'IPC grâce à son expérience et à ses capacités propose

ce service au Cambodge, et un plan de développement du laboratoire de microbiologie et de chimie des eaux et des aliments (LEFS) a été initié (formation, nouveaux responsables, suivie de la qualité, nouveaux laboratoire).

Cambodia, a country on a fast-track economic development path, depends on its tourism and agri-food industries to increase its resources. In both of those sectors, the microbiological and chemical quality of the products (raw or processed agri-food products, swimming pool water, drinking water, meals) require testing and certification. The IPC provides these services in Cambodia based on its experience and facilities. To successfully carry this out, a development plan for the microbiology and water and food chemistry laboratory (LEFS) was initiated (training, new managers, quality control, new laboratory).

ANTICIPER LES EMERGENCES DE MALADIES INFECTIEUSES / ANTICIPATING THE EMERGENCE OF INFECTIOUS DISEASES

De nombreux agents pathogènes pour l'homme ou les animaux domestiques vont ré-émerger ou émerger dans les années à venir (EV71, virus grippaux (H7N9 ?), Chikungunya, Zika, arenavirus, coronavirus..). Les résistances aux traitements vont se développer (tuberculose, entérobactéries, coqueluche, paludisme, grippe, VIH, hépatites, etc.). La possible éradication de *Plasmodium falciparum*, va conduire à une contribution plus importante de *Plasmodium vivax* dans le paludisme au Cambodge. Les hépatites B et C sont devenues un problème majeur de santé publique. L'hépatite E est un problème sous-estimé. Zika, Chikungunya, Nipah, Coronaviruses restent une menace. Les infections fungiques sont un problème en devenir. La résistance des moustiques aux insecticides augmente. L'IPC grâce à son expertise, son expérience et ses infrastructures (dont son laboratoire de sécurité BSL3), a un rôle majeur à jouer dans la détection et la mesure du risque, le suivi de l'émergence et les mesures de contrôle, non seulement pour le Cambodge, mais pour l'Asie.

Many human and domestic animal pathogens are bound to re-emerge or emerge in the next few years (EV71, avian flu viruses (H7N9 ?), chikungunya, zika, arenaviruses, coronaviruses, etc.). Resistance to treatment is likewise bound to develop (tuberculosis, enterobacteriaceae, bordetella, malaria, influenza, HIV, hepatitis, etc.). The possible eradication of Plasmodium falciparum will lead to a higher input of Plasmodium vivax in malaria in Cambodia. Hepatitis B and C have become a major public health issue. Hepatitis E is an under-estimated problem. Zika, chikungunya, Nipah, Coronaviruses continue to be a threat. Fungal infections are becoming a problem. Insecticide resistance of mosquito is increasing. With its expertise, experience and infrastructure (including its BSL3 security laboratory), the IPC has a major role to play in risk detection and scoping, monitoring emergence and control measures, not only for Cambodia, but for Asia.

AMELIORER LES DIAGNOSTICS / IMPROVING DIAGNOSIS

Les efforts déjà initiés dans la mise au point de nouveaux tests de diagnostic rapide (virus, bactéries et mycobactéries, paludisme, etc.), et le développement de biomarquers seront poursuivis.

The effort already being deployed to fine-tune rapid diagnostic tests (RDT) (viruses, bacteria and mycobacteria, malaria, vector control, etc.) and biomarkers development will be continued.

TESTER DE NOUVEAUX MEDICAMENTS, DE NOUVEAUX VACCINS ET DE NOUVELLES STRATEGIES / TESTING NEW DRUGS, VACCINES AND STRATEGIES

Plusieurs nouvelles approches thérapeutiques voient le jour (nouvelles stratégies d'utilisation de médicaments, nouveaux médicaments, nouveaux vaccins, nouvelle lutte antivectorielle). L'IPC souhaite apporter sa contribution dans ces recherches depuis les phases 2, jusqu'aux essais cliniques en phase 3 (paludisme, dengue, tuberculose, hépatites, rage, EV71, lutte antivectorielle, etc.)

Many new therapeutic or diseases control approaches are being discovered (new strategies for drug use, new drugs, new vaccines, new vector control tools). The IPC will fully play its role in the relevant

research from phase 2 up to the phase 3 clinical trials (malaria, dengue, tuberculosis, hepatitis, rabies, EV71, vector control, etc.).

DEVELOPPER L'APPROCHE "UNE SEULE SANTÉ" / DEVELOPING THE "ONE HEALTH" APPROACH

L'émergence des maladies relève d'un concept « One Health ». Plus de 80 % des maladies humaines ont une origine animale. Par ailleurs, biodiversité, agriculture et santé, y compris pour les maladies infectieuses, sont liées. L'IPC a réalisé des recherches dans ces domaines (programmes Lacanet, ComAcross, Ecomore, Predict, AMR) et va poursuivre cet engagement en collaboration avec des partenaires des secteurs vétérinaire, agronomique, économique et des sciences humaines.

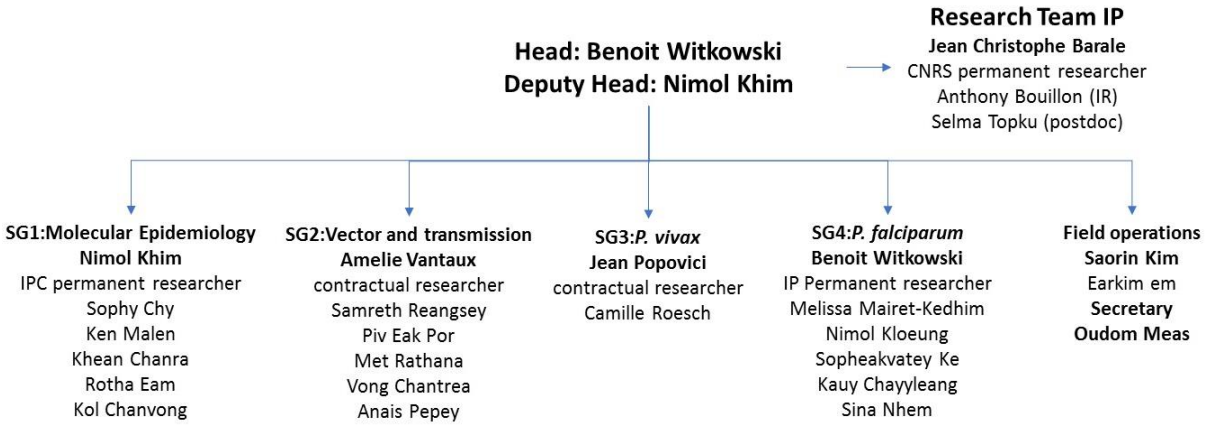
Disease emergence is part of a "one health" concept. Over 80% of human diseases are of animal origin. Moreover, biodiversity, agriculture and health, including infectious diseases, are linked. The IPC condusted research in these fields (Lacanet, ComAcross, Ecomore, Predict, AMR programs) and intends to pursue this commitment in cooperation with partners in the veterinary, agriculture, economic and human science sectors.

3 ACTIVITIES IN 2018 AT INSTITUT PASTEUR DU CAMBODGE

3.1 MALARIA MOLECULAR EPIDEMIOLOGY

3.1.1 FUNCTIONAL STRUCTURE OF THE UNIT

Since September 2017 the unit is directed by Benoit Witkowski. The Unit is organized around four thematic: *Plasmodium falciparum* blood stages, *Plasmodium vivax* blood stages, Molecular Epidemiology and Malaria transmission. The Unit is composed of one head of Unit (B. Witkowski-IP permanent researcher), one deputy head (Nimol Khim-IPC permanent researcher), two contractual researchers (Amelie Vantaux & Jean Popovici), five PhD student (Melissa Mairet-Khedim, Camille Roesch, Anais Pepey, Kutub Ashraf, Mirco Sandfort) and seventeen technical & administrative staff. Since December 2016, the Malaria Unit at IPC is joined with Jean Christophe Barale Unit (Pasteur Institute in Paris) across a single structure: Malaria Translational Research Unit (MTRU).



Malaria Molecular Epidemiology Unit collaborator	
national	international
CNM	Insitut Pasteur
Ministry of Health	Pasteur International Network
Partner for Development (Cambodia)	WHO
ITC	MMV
URC	University of Georgia
UHS	MORU
	UCSF
	Insitut Cochin
	MSF
	University of Gottenburg
	INSERM
	CNRS
	TAD
	SANOFI
	NIMPE
	Lao NMCP
	Myanmar NMCP
	WEHI
	CWRU

3.1.2 RESEARCH PROGRAMS – MAJOR ACHIEVEMENTS IN 2018

i. Drug resistance & antimalaria therapy

Drug resistance epidemiology

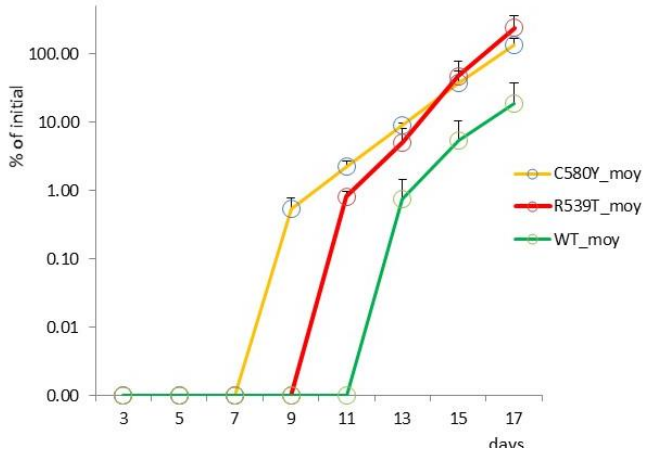
MMEU is involved in molecular epidemiology programs intending to determine parasites resistance prevalence in Cambodia, Vietnam, Myanmar and Laos. This has been carried out in association with MSF and WHO. Results obtained have demonstrated the high prevalence of PPQ-R in Laos despite absence DHA-PPQ in Lao treatment policy. Additionally, it have been demonstrated a notable rate of coartem treatment failure independently to known molecular markers. These first approaches open for research question regarding spread of parasite from Cambodia to Laos and on the molecular signature associated to lumefantrine resistance. Results in Cambodia have demonstrated an evolution in term of resistance profile to MQ in parasites collected in 2016-2017. Additionally, these results have revealed the existence in Cambodia of parasites presenting a triple pattern for ART, PPQ and MQ resistance. These results have been published in the journal Lancet Infectious Disease. Further investigation are ongoing to characterize the resistance profile of these isolates and to determine whether they may present a notable issue for further antimalarial treatments guidelines. In 2017-2018, MMEU coordinate the KARMA3 project which involve a resistance surveillance in GMS, Middle East and some African sites. Among results generated an emergence of strains presenting K13 non synonymous mutation have been noticed in Eastern Africa. This particular result tends to show a possible appearance of ART-R in Africa and will be consider for complementary investigations.

***P. falciparum* treatment efficacy**

In collaboration with WHO and CNM, MMEU is involved in biological investigations of therapeutic efficacy studies. Among results generated we have noticed the existence of amodiquine resistance in Cambodia despite the non-deployment of ASAQ and a putative and limited use of AQ in the 80's, the isolates tested have presented a wide range of susceptibility to AQ with upper values corresponding to what is designed as AQ-R in literature. A clear association between AQ susceptibility and clinical outcome was noted through AQSA which enable to conclude on an insufficient clinical efficacy of ASAQ because of the AQ-R of the parasites in Cambodia. Confirmation of AQ-R in Cambodia is particularly important since it is not exclusive (it could coexist with other resistance patterns) and since ASAQ is widely used in Africa. These data are currently under investigation in order to characterize the AQ-R molecular signature. Additionally, we have performed extensive investigation on pyronaridine resistance in Cambodia. We have demonstrated that no cross resistance exist with other drugs and the absence of susceptibility association with described molecular patterns resistance. These investigation published in AAC (Leang & Mairet Khedim et al.).

As previously mentioned, there is currently an urgent need in the implementation of novel association that can potently malaria infection with multiresistant parasites. Among drugs in the pipeline the association OZ439-FQ (ferroquine) seems particularly promising. The OZ439 is an endoperoxide such artemisinin but with the property to remains several days in the blood. FQ is a chloroquine derivative, however, its efficacy appear to be unaltered by known resistance patterns (*Mairet-Khedim et al. under submission*). Moreover, this association is designed to be administered in one unique dose. A phase II study focused on this association is currently on going in Vietnam. MMEU is involved in this project. The objective will be to determine the resistance patterns of the strains infecting enrolled patients to drugs that are in use to date. This is achieve trough in vitro culture of isolates collected in the study and trough determination of their in vitro chemo-susceptibility profiles. Additionally, we perform a molecular characterization of resistance markers of such strains. This project is conducted in association with Sanofi laboratories and MMV. The results obtained are confidential and cannot be exposed in this report.

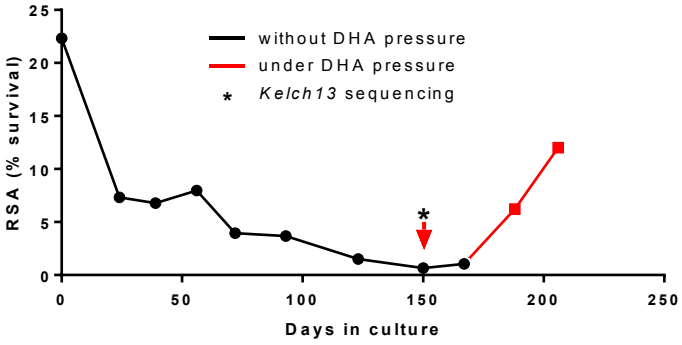
Drug resistance Mechanism



MMEU in collaboration others have demonstrated previously the role of K13 as a marker of artemisinin resistance.

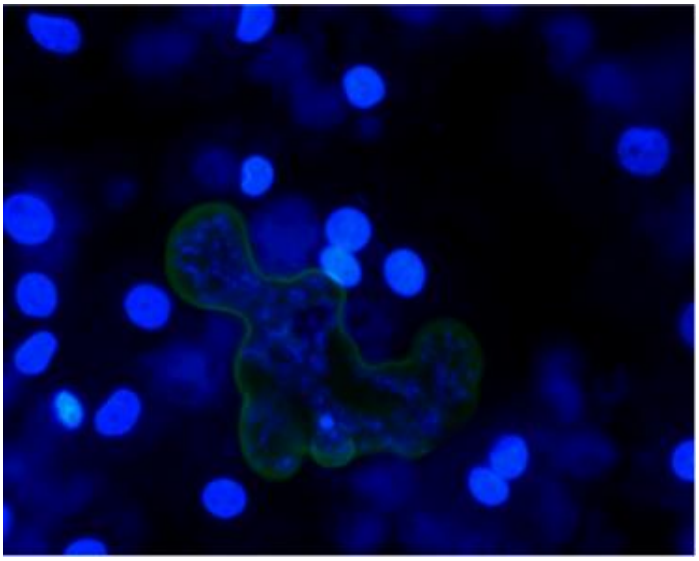
Our objective is now to investigate more deeply the ART mechanism, to explain the selection of only certain K13 haplotype and determine if other genes may exist. Regarding selection of K13 in Cambodia, we have developed an in vitro assay mimicking in host drug exposure to evaluate the dormancy or persistence of in response to ART exposure. Using this assay and genetically edited strains differing only from their K13 haplotypes, we have observed that the K13 mutant C580Y confer a better survival to artemisinin. This experiment is still ongoing but would be likely to explain why C580Y mutation represent now a prevalence close to 100% in Cambodia.

In addition, we have performed in vitro experimental evolution of K13 ART-R mutant parasites. The strain selected initially displayed all features of ART-R parasites: mutation in the K13 gene (C580Y haplotype) and in vitro survival after ART exposure using the RSA. After months of in vitro growth in absence of ART exposure, the RSA value gradually decreased until reaching values seen for susceptible wild-type parasites. K13 sequencing was performed after 150 days of culture and result have shown the conservation of the C580Y haplotype (Fig3). Following re-exposure to ART during several development cycles, the RSA value eventually increased again. While this experimental evolution experiment is still ongoing, the data obtained so far are clearly indicative that K13 mutation is not the only parasite determinant involved in the modulation of response to ART opening research perspectives for the identification of novel ART-R protagonists. All these investigation were done in collaboration with JC Barale in the context of MTRU.

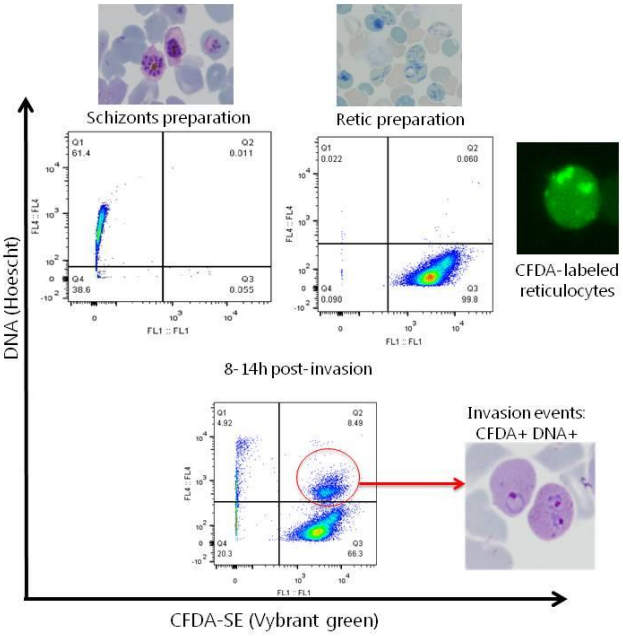


Therapeutic options against P. vivax liver stages

The main characteristic of Plasmodium vivax is to develop in patient's liver particular cellular stages named hypnozoites. These hypnozoites remain silent for a variable duration until they exit their dormancy. Since awaken the hypnozoites develop to hepatic shizonts that finally led to blood stage infection, reactivation of symptoms and transmissibility of the disease. Because of this feature, *P. vivax* will be tremendously difficult to eradicate. Very few treatment exist for targeting dormant stages and all of them belong to amino-8-quinoline family. The most famous compound is called primaquine and is currently in usage worldwide. However, in certain patients presenting G6PD deficiency, primaquine treatment led to severe hemolysis that may be responsible for patient death. Unfortunately, Cambodia is one of the malaria hotspots where G6PD deficiency is the most widespread. For these patients primaquine usage is unsafe or present an unfavorable risk/benefit balance. For these reasons the development of new drug active against these parasites stages without presenting toxic effect, will be essential for vivax elimination in Cambodia. In this context, a collaborative project was started late 2017 between IPC, University of Georgia (D. Kyle) and MMV (Brice Campo). Methodologically, we use the blood from *P. vivax* infected patients to feed anopheles mosquito. Thereafter mosquito develop sporozoites that are used, after vector dissection, for infecting human hepatocytes maintained in vitro. A that time, a variety of drugs can be assessed for their anti-hypnozoite effect. Feasibility of this project has been successfully demonstrated in 2017. In 2018 MMEU have developed a platform for assessing high throughput drugs screening against *P. vivax* hypnozoites. To date a panel >1500 molecules have been already tested. The first results have been accepted for publication in the journal ''Nature Communication''.



ii. Plasmodium vivax invasion biology

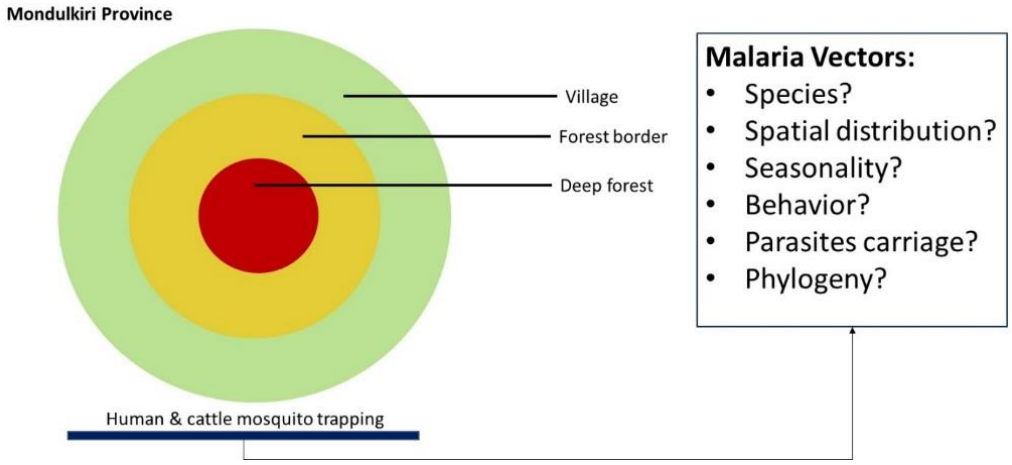


Plasmodium vivax is responsible for chronic infections that drastically limit the disease control operational range, at least with the existing tools. One innovative strategy would be to develop vaccine against *P. vivax*. Among possible targets, development of antibodies directed against blood stage would be extremely relevant. However, blood stages are intracellular and this makes the choice for exposed antigens mandatory. Proteins that comply with this last point are the receptor involved in red blood cell invasion. Technically, MMEU have developed protocols enabling to measure whether an antigen could affect parasite invasion. This methodology is based on short term culture of P. vivax field isolates associated to flow cytometry measurement of reinvasion. MMEU have setup collaborative plan for 2018 with Dr. Wai-Hong Tham (WEHI) and Pr. Chris King (CWRU) for the assessment of human monoclonal antibodies against *P. vivax* reinvasion. In addition to that, the impact of antibodies screened to be active will be measured on different strains of *P. vivax* presenting target polymorphism to understand whether the parasites could evades this strategy. Ultimately, data collected will enable to decipher vivax invasion pathways which

will help to propose and design the most relevant vaccine strategies. Achievement of this topic could be the basis for development of new malaria control options to act against *P. vivax*.

Malaria & malaria vectors epidemiology……..……..…….Funding: NIH

MMEU is part of the Asia-Pacific International Centre of Excellence in Malaria Research (Asia-Pacific ICEMR PI I Mueller) aims addressing the key challenges to malaria elimination in the Asia-Pacific by conducting a coordinated set of in-depth studies into the epidemiology, entomology and biology of residual malaria transmission in 3 sites spanning the entire Asia-Pacific transmission gradient from moderately and high transmission in Papua New Guinea to low, highly focal transmission in Cambodia. In this context a cross sectional study have been setup in Mondulkiri province. Screening for malaria infection will be achieve on >4000 volunteers. This study will enable, beyond epidemiological considerations, to investigates the foci of transmission, the immunology of individuals in Cambodia, to define the parasite population structuration and ultimately to rationalize further operational research initiatives. This project has been conducted in Cambodia in association between MMEU and Epidemiology Unit of IPC. The results have provided a clear snapshot of malaria in Keoseima district. Notably a gradient of prevalence have been noticed depending proximity of villages with nearby forest. Ultimately we have determined that prevalence of malaria in Cambodia is focused but not low since the highest rate showing a prevalence of about 40% of parasite carriage, mainly represented by P. vivax. These results will have important implication especially in the setup of intervention intending to control malaria. Vector is the main denominator of malaria transmission. No malaria elimination initiatives were and will be successful without a strong focus on the vector itself.



Dataset available in Cambodia are unfortunately very fragmented and further malaria elimination attempts highlight the need for a clear understanding of the malaria vector in this country. In this context, MMEU is involved ICEMR-Asia Pacific program (International Center of Excellence in Malaria Research-direction I. Muller). Aims of this program will be to bring a better understanding of vectors in Cambodia. This project that will address several medical entomology questions can be regroup under vector epidemiology. Aspect that are ongoing to be investigated are the spatial distribution of the anopheles, their behavior, their phylogeny, tropism and ultimately their potency to carry human parasites. This project is conducted in Mondulkiri province (high malaria prevalence) and expected outcome will enable design of the further vector control initiatives. The first results indicates that anopheline initially described as main vector (A. dirus) are not sole responsible for malaria transmission. Additionally, vector behavior investigation have revealed that day bitting represent a notable proportion of human-vector contact. Finally we have identified a gradient in parasite carriage with epicenter in deep forest area. These data will be submitted soon for publication (*Vantaux et al*.).

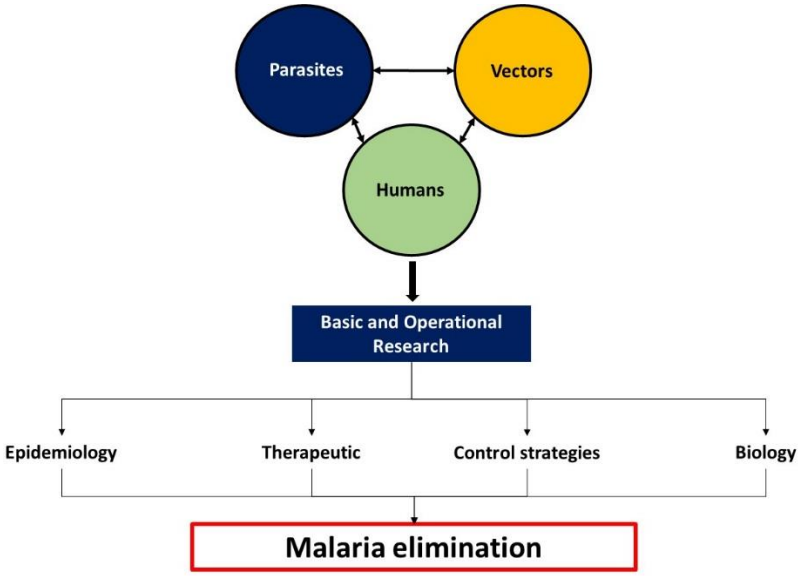
3.1.3 RESEARCH PROGRAMS – PROSPECT 2019

Research plan overview

It have been officially proposed an elimination of malaria (all genus) in Cambodia by 2025. In addition to malaria burden in Cambodia and its public health impact, eradication of malaria in Cambodia takes

on a global importance to avoid further emergence and spread of antimalarial resistances. However, drug resistance is only one side of malaria problematic in Cambodia and several question need to be addressed to reach a rationalized understanding of this disease. Thus, MMEU research plan is oriented toward whole malaria problematic and structured around four main research axis that aims to:

a) **Determine the dynamic of malaria in Cambodia**
b) **Explore the therapeutic options & the drug resistance (multi-centric)**
c) **Strategize the malaria control in Cambodia**
d) **Understand the parasite biology**



i. Dynamic of malaria in Cambodia

Cohort study……..……Funding source NIH

Previous investigation (cross sectional) have demonstrated locally a very high prevalence in malaria. To better address and understand the question of the malaria dynamic we will setup a longitudinal study in Keoseima district (Eastern Cambodia). This study will start in December 2018 for a duration of one year. 1,000 individuals will be followed monthly and screened for their malaria positivity. This study will help us to understand how the asymptomatic/ symptomatic status of the individual evolve. It will indicate us what could be the immunological shift and what could be the weight of asymptomatic in the transmission. Ultimately, this study will show the infection dynamic in the population accurately for P. vivax and falciparum and will help to setup better control strategies.

Vector-Human geospatial study……..……Funding source NIH

In order to quantify human-vector contact patterns resulting in residual malaria transmission in the Asia-Pacific ICEMR field sites in Cambodia we will use state-of-the-art electronic data collection tools such GPS-loggers to capture motion patterns of humans in endemic areas. In addition to molecular detection and genotyping of *Plasmodium spp*. infections we will use novel analysis tools to quantify human exposure to mosquito bites by assaying blood samples against anopheline salivary gland proteins (aSGPs) as markers of exposure. Geospatial information system (GIS) technology and spatial analysis methods will be used to quantify and visualize human-vector contact. The research undertaken as part of this project will enhance our understanding of host and parasite factors that are contributing to residual malaria transmission and complicating control and elimination programs. The two research aims will develop or validate methods that could significantly improve the efficiency of malaria control, especially for medium and low transmission settings. Developing and implementing such methods will be particularly crucial for addressing the challenged inherent in eliminating malaria in the Asia-Pacific.

ii. Therapeutic & Drug resistance

Drug resistance epidemiology……..……..…....……Funding: WHO

Historically, Cambodia is known to be a hotspot for antimalarial drug resistance development. Previous experience have demonstrated that resistance may spread owing to population movement. Thus, efficient tracking of antimalarial drug resistance have to be considered as two staged: active surveillance of drug efficacy and molecular resistance in host post of resistance to determine the evolution and worldwide monitoring in the way to early detect resistance spread. In this context and in collaboration with WHO, MMEU will be in charge of molecular investigation regarding drug resistance in GMS, in Middle East and in certain African country. Objective is to characterize the presence or no of known resistance markers and to determine the rate of treatment failures in listed area. Additionally, this initiative will be a basis for deciphering putative new resistance markers and for providing new insight of drug resistance mechanisms & epidemiology.

New therapeutic options……..……..……Funding: MMV/UGA

One of the main target in drug research will be the liver stage of P. vivax. The collaboration that have been setup in 2017 between MMV/UGA/IPC will be resumed & strengthen for the 2019 period. We will notably implement at IPC high throughput screening capabilities. The objective will be to spot out compounds that could actively target P. vivax hypnozoites. The second main target is the multiresistant strains of P. falciparum. Malaria Unit have developed a field of expertise in malaria drug resistance determination. Thus and over last years, it have been isolated several hundreds of clinical isolates from Cambodia characterized for their chemo-susceptibility and their genotype of resistance. One of the major objective for next years is the development of new antimalarial able to tackle resistant strains. In this context MMEU have developed a collaboration to investigate the in vitro efficacy of promising preclinical lead compound against resistant *P. falciparum* strains from Cambodia.

iii. Strategize malaria control

Intervention……..……Funding: 5% initiative/Global Fund

It is now assumed that malaria foci in Cambodia are located in sylvatic area. However, very few science based evidence support such assumption and, mainly, prevalence of malaria vectors and human carriers are largely unknown. MMEU is involved in a collaborative project lead by IPC Epidemiology Unit (PI: P. Piola) that will investigates the question of malaria in forest. Objectives will be, first, to determine over one year the characteristic of malaria in forest. Particularly we will investigate parasitological and entomological aspect of this feature. Secondly, these data will serves to setup an intervention phase in the studied area that should enable to reach malaria control. Specifically, MMEU will be involved in the entomological and molecular diagnostic measurement of intervention impact. This project will start mi-2018 and should provide in term of outcome new insights enabling to going toward malaria elimination in Cambodia.

In addition, a complementary approach have been approved (Jan 2019). This approach will target malaria in forest on a large scale based on ‘’mass screen and treatment’’ strategy (MSAT) under a cluster randomized trial design. Secondarily, the question of the chemoprevention will be addressed and its feasibility will be monitored (adverse event, observance, efficacy, resistance) in the context of a controlled individual study design. This project will be directed by IPC Epidemiology Unit (PI Piola) in collaboration with MMEU (co-PI Witkowski).

Malaria diagnostic……..……Funding: PMI

Malaria diagnostic is primordial for elimination attempts. Notably, malaria infection are often asymptomatic with low parasites density. These infections are poorly monitored due to conventional methods sensitivity and may be substantial contributors to malaria transmission. A collaborative project have been setup between URC, CDC, CNM and IPC regarding the effectiveness of novel ultra-sensitive RDT in the context of malaria pre-elimination in Battambang and Pailin district in western Cambodia. In this project usefulness of these new test and their performance will be addressed over a pro-active case

detection design. *P. falciparum* detection is mainly based on HRP2 protein detection. It have been proved that strains deleted for HRP2 exist and circulates (especially South America). These strains cannot being detected by usual diagnostic method and this feature must be considered in a context of malaria case management and elimination. Data on HRP2 deletion in Cambodia are extremely limited and we will address this question in the future retrospectively and prospectively. South East Asia is a main foci of human G6PD deletion. This genetic trait implicates severe adverse event under certain drug administration and especially primaquine. To date, no satisfactory test exist for being implemented in Cambodia. We will therefore evaluate novel G6PD field test (quantitative) and determine whether these devices can safely spot out G6PD deficient individuals. Funding for that project is under discussion.

iv. Understand parasite Biology

Parasite response to immunity…….Funding: IPC (MTRU)

In 2018 we have developed a robust methodology to assess transmission blocking efficacy of antibodies direct against P. vivax invasion proteins. In 2019 we will address the question of the parasite polymorphisms/differential expression against host immune response. We will compare antibodies relative efficacy and determine whether parasite genetic modification may impact clinical outcome in patients. Secondarily, in collaboration with Toulouse University hospital we will conduct a study on the capacity of invasion of P. vivax in Duffy negative individuals. This will enable to generate hypothesis regarding the presence of P. vivax in Africa and possibly to define unknown invasion pathways. The data that will be globally generated will help to understand and design novel immunologic approach in the control of P. vivax.

3.I.4 SUPPORT TO NATIONAL AUTHORITIES

MMEU is part, such like all IPC research unit, of Cambodian ministry of health. Specifically, MMEU is a main collaborator and a main technical support of Cambodia national malaria control program hold by CNM. Particularly, MMEU offer its support to drug efficacy studies that are conducted yearly in Cambodia. Additionally, MMEU is involved since 2014 in the malaria infection screening for Cambodian troops deployed in UN mission context in Africa.

3.I.5 TEACHING AND TRAINING

i. Students

PhD students:

Melissa Mairet-Khedim: *P. falciparum* resistance (January 2017)
Camille Roesch: biology of *P. vivax* invasion (January 2017)
Kutub Ashraf: *P. vivax* liver stage (April 2018)
Anais Pepey: Ecology of malaria transmission (November 2018)
Mirco Sandfort: Epidemiology of malaria in Cambodia (November 2018)

Master student:

Neangsothea Sothy (M2, November 2018) Prevalence of HRP2 deletion in Cambodia
Rotha Eam (M1, January 2019) Polymorphism of PfCARL in Cambodia

Interns

Iona Gyuomarch (august 2018) Setup of capillary electrophoresis technique
Chloé Bannier (july-august 2018) P. vivax invasion
Mathieu Gendrot *(November 2018)* Methods in drug resistance screening
Beatrice Tappy *(February 2019)* Synergy of antimalarial drugs

RIIP researcher Mobility

Sandrine Nsango *(March 2019)* DHA-PPQ resistance in Cameroon

ii. Teaching

The Malaria Molecular Epidemiology Unit staff will be involved in the setup and further teaching for initiative of international Epidemiology Master Plan between UHS and Paris Saclay University.

iii. Training

- Parasitology training

MMEU have held a workshop in January 2017 dedicated to methodology to monitor PPQ resistance. This was achieve at international level involving scientific staff from Vietnam & Thailand (OCRU & MORU). MMEU have realized capacity building trainings in Vietnam (4 field sites, July & September 2017) aiming to implement methodologies for parasites sampling in clinical trials context. This was achieve at national level involving NIMPE (NMCP) and Ministry of Health staffs. MMEU have realized a capacity building in Cambodia (December 2017) focused on blood sampling in remote health centers. This was achieve at national level involving CNM & Provincial Health department staffs.

- Entomology Training

Thanks to the support of the Rotary Club from Versailles and the Rotary Club from Phnom Penh, Institute Pasteur in Cambodia has now acquired a mobile insectary to develop entomological researches in Cambodian Provinces. Fully autonomous, the mobile insectary is equipped with a room allowing mosquito rearing, a secured room to carry out mosquito experimental infections with the blood of malaria parasite carriers and a third room to welcome patients and withdraw blood. Since January 2017 this mobile insectary is operating in Mundolkiri Province, supporting public health researches. In addition to this tool, entomological training workshops have been carried out in the field during the year 2017. From a general introductory course on vectors and mosquito biology, to practical on mosquito rearing, mosquito dissections, experimental infections, mosquito trapping methods and mosquito identifications.

3.1.6 PUBLICATION LIST

2017

1. Dwivedi A, Reynes C, Kuehn A, Roche DB, Khim N, Hebrard M, Milanesi S, Rivals E, Frutos R, Menard D et al: Functional analysis of Plasmodium falciparum subpopulations associated with artemisinin resistance in Cambodia. Malar J 2017, 16(1):493.
2. Imwong M, Suwannasin K, Kunasol C, Sutawong K, Mayxay M, Rekol H, Smithuis FM, Hlaing TM, Tun KM, van der Pluijm RW et al: The spread of artemisinin-resistant Plasmodium falciparum in the Greater Mekong subregion: a molecular epidemiology observational study. Lancet Infect Dis 2017, 17(5):491-497.
3. Kheang ST, Sovannaroth S, Ek S, Chy S, Chhun P, Mao S, Nguon S, Lek DS, Menard D, Kak N: Prevalence of K13 mutation and Day-3 positive parasitaemia in artemisinin-resistant malaria endemic area of Cambodia: a cross-sectional study. Malar J 2017, 16(1):372.
4. Kim A, Popovici J, Vantaux A, Samreth R, Bin S, Kim S, Roesch C, Liang L, Davies H, Felgner P et al: Characterization of P. vivax blood stage transcriptomes from field isolates reveals similarities among infections and complex gene isoforms. Sci Rep 2017, 7(1):7761.
5. Lu F, Culleton R, Zhang M, Ramaprasad A, von Seidlein L, Zhou H, Zhu G, Tang J, Liu Y, Wang W et al: Emergence of Indigenous Artemisinin-Resistant Plasmodium falciparum in Africa. N Engl J Med 2017, 376(10):991-993.
6. Menard D, Dondorp A: Antimalarial Drug Resistance: A Threat to Malaria Elimination. Cold Spring Harb Perspect Med 2017, 7(7).
7. Phuc BQ, Rasmussen C, Duong TT, Dong LT, Loi MA, Menard D, Tarning J, Bustos D, Ringwald P, Galappaththy GL et al: Treatment Failure of Dihydroartemisinin/Piperaquine for Plasmodium falciparum Malaria, Vietnam. Emerg Infect Dis 2017, 23(4):715-717.
8. Rossi G, De Smet M, Khim N, Kindermans JM, Menard D: Emergence of Plasmodium falciparum triple mutant in Cambodia. Lancet Infect Dis 2017, 17(12):1233.
9. Rossi G, De Smet M, Khim N, Kindermans JM, Menard D: Performance of Rapid Diagnostic Testing in Patients with Suspected Malaria in Cambodia, a Low-Endemicity Country Aiming for Malaria Elimination. Clin Infect Dis 2017, 65(10):1769-1770.
10. Watson J, Taylor WR, Menard D, Kheng S, White NJ: Modelling primaquine-induced haemolysis in G6PD deficiency. Elife 2017, 6.

11. Witkowski B, Duru V, Khim N, Ross LS, Saintpierre B, Beghain J, Chy S, Kim S, Ke S, Kloeung N et al: A surrogate marker of piperaquine-resistant Plasmodium falciparum malaria: a phenotype-genotype association study. Lancet Infect Dis 2017, 17(2):174-183.

2018

1. Roth A, Maher SP, Conway AJ, Ubalee R, Chaumeau V, Andolina C, Kaba SA, Vantaux A, Bakowski MA, Luque RT, Adapa SR, Singh N, Barnes SJ, Cooper CA, Rouillier M, McNamara CW, Mikolajczak SA, Sather N, Witkowski B, Campo B, Kappe SHI, Lanar DE, Nosten F, Davidson S, Jiang RHY, Kyle DE, Adams JH. A comprehensive model for assessment of liver stage therapies targeting Plasmodium vivax and Plasmodium falciparum. Nat Commun. 2018;9(1):1837. doi: 10.1038/s41467-018-04221-9. PubMed PMID: 29743474; PMCID: PMC5943321.
2. Ross LS, Dhingra SK, Mok S, Yeo T, Wicht KJ, Kumpornsin K, Takala-Harrison S, Witkowski B, Fairhurst RM, Ariey F, Menard D, Fidock DA. Emerging Southeast Asian PfCRT mutations confer Plasmodium falciparum resistance to the first-line antimalarial piperaquine. Nat Commun. 2018;9(1):3314. doi: 10.1038/s41467-018-05652-0. PubMed PMID: 30115924; PMCID: PMC6095916.
3. Popovici J, Vantaux A, Primault L, Samreth R, Piv EP, Bin S, Kim S, Lek D, Serre D, Menard D. Therapeutic and Transmission-Blocking Efficacy of Dihydroartemisinin/Piperaquine and Chloroquine against Plasmodium vivax Malaria, Cambodia. Emerg Infect Dis. 2018;24(8):1516-9. doi: 10.3201/eid2408.170768. PubMed PMID: 29798745; PMCID: PMC6056113.
4. Popovici J, Pierce-Friedrich L, Kim S, Bin S, Run V, Lek D, Hor Daryl HK, Lee Soon UL, Cannon MV, Serre D, Menard D. Recrudescence, reinfection or relapse? A more rigorous framework to assess chloroquine efficacy for vivax malaria. J Infect Dis. 2018. doi: 10.1093/infdis/jiy484. PubMed PMID: 30102351.
5. Popovici J, Friedrich LR, Kim S, Bin S, Run V, Lek D, Cannon MV, Menard D, Serre D. Genomic Analyses Reveal the Common Occurrence and Complexity of Plasmodium vivax Relapses in Cambodia. MBio. 2018;9(1). doi: 10.1128/mBio.01888-17. PubMed PMID: 29362233; PMCID: PMC5784252.
6. Paloque L, Witkowski B, Lelievre J, Ouji M, Ben Haddou T, Ariey F, Robert A, Augereau JM, Menard D, Meunier B, Benoit-Vical F. Endoperoxide-based compounds: cross-resistance with artemisinins and selection of a Plasmodium falciparum lineage with a K13 non-synonymous polymorphism. J Antimicrob Chemother. 2018;73(2):395-403. doi: 10.1093/jac/dkx412. PubMed PMID: 29177421.
7. Gupta S, Singh S, Popovici J, Roesch C, Shakri AR, Guillotte-Blisnick M, Huon C, Menard D, Chitnis CE. Targeting a Reticulocyte Binding Protein and Duffy Binding Protein to Inhibit Reticulocyte Invasion by Plasmodium vivax. Sci Rep. 2018;8(1):10511. doi: 10.1038/s41598-018-28757-4. PubMed PMID: 30002416; PMCID: PMC6043553.
8. Bilgo E, Vantaux A, Sanon A, Ilboudo S, Dabire RK, Jacobs-Lorena M, Diabate A. Field assessment of potential sugar feeding stations for disseminating bacteria in a paratransgenic approach to control malaria. Malar J. 2018;17(1):367. doi: 10.1186/s12936-018-2516-x. PubMed PMID: 30333029.
9. C Roesch, J Popovici, S Bin, V Run, S Kim, S Ramboarina, E Rakotomalal, R Rakotoarison, T Rasoloharimanana, Z Andriamanantena, A Kumar, M Guillotte-Blisnick, C Huon, D Serre, C Chitnis, I Vigan-Womas, D Menard. Genetic diversity in two Plasmodium vivax protein ligands for reticulocyte invasion. Plos One (in press)

3.2 EPIDEMIOLOGY & PUBLIC HEALTH

3.2.1 FUNCTIONAL STRUCTURE OF THE UNIT

The Epidemiology and Public Health (EPH) unit performs operational research studies on major public health challenges in Cambodia. Dr Patrice PIOLA is the head of this unit.
The Rabies Prevention Center and the International Vaccination Center from the Institut Pasteur du Cambodge are also part of the EPH unit and under the direct responsibility of the deputy head of the EPH Unit, Dr. Sowath LY.

The EPH research unit is structured around three main groups:

- the Community Epidemiology Group (CEG), led by Dr. Sowath LY, has an extensive experience in research projects on rabies, dengue, avian flu and outbreak investigations.
- the Clinical Research Group (CRG), led by Dr. Laurence Borand, has a long history of guideline changing trials to improve diagnosis and treatment of patients living with HIV, tuberculosis and hepatitis B. This group also runs hospital-based studies addressing antibiotic resistance and whooping cough.
- the Veterinary Epidemiology Group (VEG), led by Dr. Veronique Chevalier, is part of a collaboration between the CIRAD (French Agricultural Research Centre for International Development) and IPC. Its main focus is on zoonotic diseases with a strong modeling component. Diseases addressed by the VEG include rabies, Japanese encephalitis and Nipah Virus. Most CIRAD projects include a human component implemented by the CEG and/or IPC lab units.

Almost all research activities of the Epidemiology Unit rely on close collaborations with the IPC laboratory units as well as the Ministry of health: NCHADS, CENAT, NaVRI, SHCH, Cambodian CDC,

and CNM to a name a few. The unit's projects would neither be possible without the interest and contribution of several reference hospitals in Phnom Penh and across the country, such as the Calmette Hospital, National Maternal Child Health Center (NMCH), Kompong Cham &Takeo Provincial Hospitals, Sihanouk Hospital Center of Hope, and Kantha Bopha Hospital. Robust partnerships with the University of Health Sciences and the Institute of Technology of Cambodia (ITC) were also strengthened through collaborative projects.

Finally, most research projects result from partnerships with international agencies or research groups including the Agence Nationale de Recherche sur le SIDA (ANRS), Dengue Vaccine Initiative (DVI), the International Vaccine Initiative (IVI), the European Union, Fondation Total, INSERM, AIRD, Division International of Pasteur Institutes, Institut Pasteur in Paris, CIRAD, Pasteur Foundation, MSD Avenir, Gillings Public Health Fellowship, the World Health Organisation, UNITAID, the French Initiative 5% (Expertise France), and the Agence Française de Développement (AFD).

In 2018, the EPH unit team was composed of approximately 50 persons in order to successfully conduct the broad variety of projects and to meet quality standards. The epidemiology unit is at the cross-road of almost all IPC laboratories, providing epidemiological support both methodologically and in study sites.

Three important grant proposals on operational research developed by the epidemiology unit were accepted: two from the French Initiative 5% (one on malaria and one on tuberculosis) and the third on malaria from the Resistance to Artemisinin Initiative (Global Fund).

3.2.2 RESEARCH PROGRAMS IN 2018

3.2.2.1 VIRAL DISEASES

a. RABIES

Rabies PEP immune response study (RESIST 2)

Data is available showing that the immune response at D14 after a one-week protocol of three ID injections at D0, D3 and D7 is comparable to a 5-dose regimen. The number of doses administered in the one-week, three session protocol, remains however high at 12 ID-doses. Clinical, epidemiological and biological data are being gathered to estimate whether the existing IM and ID protocol can be shortened (three-session, one-week regimen; without D28 session) and reduced to doses (6 instead of 8 total doses) in PrEP and in PEP protocols, at no risk to patients. Current status: One article was finalized and was submitted to a scientific journal. Main partners in Cambodia: MoH

EPH Unit Team leaders: S. Ly, L. Borand, A. Tarantola. Financial support: International Division

Rabies One Health

The purpose of this project is to create a One Health network for rabies research and control in dogs and human populations in Cambodia, Lao PDR and Vietnam. This 3-years project is organized into 3 research packages and includes several activities: i) research and working visits between partners, ii) joint seminars and workshops aimed at expert opinions, knowledge exchange and mutual learning, and iii) field surveys (dog demography, vaccination and vaccine coverage follow-up in Kandal province). A workshop on FAVN diagnostic has been organized by IPC in 2018, in collaboration with ANSES Nancy. Preliminary results on dog demography show a high human/dog ratio, a young dog population, a high bite incidence, with children younger than 15 years old being more at risk, a good acceptability of dog vaccination

EPH Unit Team leaders: Véronique Chevalier, Sowath Ly. Financial support: Swedish Research Council

Control of Rabies in Battambang province, Cambodia

The global objective of this project is to reduce rabies induced mortality in Battambang Province, through 5 main activities: (i) information, education and communication; (ii) improvement of cases monitoring and management (iii) estimation of demographic parameters of the dog population (iv) evaluation of the vaccination efficacy in dogs, both at the individual and population level (v) recommendation on vaccination strategies based on demographic parameters, owner acceptability and feasibility. Communication tools, such a s leaflets or web communication, have been settled. A second PEP center has been open by IPC in September 2018. 3000 dog are being monitored and vaccinated, with a sub-batch of 800 dog being sampled every 6 months.

EPH Unit Team leaders: Veronique Chevalier. Financial Support : Région Occitanie

b. DENGUE AND ZIKA

ECOMORE 2/PANIC: Mosquito control and education in schools to reduce dengue burden in the community

A cluster randomized controlled trial (2017-2019) covering two dengue seasons was established to measure the impact of an integrated school-based strategy combining mosquito control and education programs on the transmission of dengue disease in their surrounding communities. One study cluster is a geographic area composed of one school and several neighboring villages from which most children go to that school. Interventions will consist in a school-based strategy combining: (1) larvicide (Bti) usage in big containers, (2) physical destruction of breeding sites, (3) use of dissemination insecticide (Pyriproxyfen in2Care) and (4) COMBI education method. In March 2018, a total of 24 clusters was followed-up, of which 12 were randomly allocated the school-based intervention and 12 were controls. In all 24 study clusters, active community-based surveillance of dengue-like illnesses among children aged 5-15 years old and saliva serological follow-up among school children of same age were performed as effectiveness outcomes.

Financial support: Funded by AFD and PANIC

Surveillance of Zika-like syndromes and microcephaly

The Zika pandemic that started in 2015 in the Americas caused thousands of microcephalies in newborns from infected pregnant women. In mid-2018, an active surveillance of Zika-like syndromes in pregnant women attending the ante-natal clinic of Calmette Hospital has been initiated. All pregnant women with a WHO case definition of Zika-like syndrome are tested by PCR by the IPC virology unit for confirmation. Mothers from newborns with microcephalies in Calmette hospital will be serologically tested from Zika. This light sentinel surveillance aims at detecting an emerging ZIKV circulation in Cambodia, with a focus on the most vulnerable group. Few pregnant mothers presented Zika Like syndromes since the beginning of the surveillance, and none had a Zika infection confirmed by PCR.

EPH Unit Team leaders: P. Piola, S. IV. Funding: French Ministry of Foreign Affairs

c. HIV AND/OR TUBERCULOSIS INFECTION

STATIS ANRS 12290

Despite the initiation of HAART, many patients die of tuberculosis within the first month of treatment. The STATIS (Systematic vs. Test- tuberculosis guided Anti TB Treatment Impact in Severely immuno-suppressed HIV-infected adults initiating antiretroviral therapy with CD4 cell counts <100/mm3) is a multicentric randomized controlled trial aiming to compare two experimental strategies to reduce the mortality and occurrence of severe bacterial infections (incl. tuberculosis) at 6 months in severely immunodeficient adults infected with HIV (CD4 < 100/mm3): 1) a strategy for intensive screening and repeated tuberculosis through workable tests during the day (Xpert MTB / RIF, LAM urinary, chest radiography); and, 2) a strategy of systematic empirical anti-tuberculosis treatment initiated two weeks

before the start of HAART. Current status: Recruitment and follow-up of patients is completed: 199 patients included in Cambodia amongst a total 1050 included. Valorisation is ongoing. Main partners in Cambodia: NCHADS, CENAT, SHCH

Team leader: L. Borand. Financial support: ANRS

miRNA as prediction and/or prognostic markers of IRIS in TB-HIV co-infected patients. ANRS 12358

The role of miRNAs in HIV disease and tuberculosis is yet to be completely defined. The objectives of this study are to 1) Identify miRNA expression profile as potential novel predictive and prognostic biomarkers for IRIS. 2) Identify the miRNA expression profile in TB patients and HIV/TB co-infected patients. Current status: Patients' recruitment and follow-up ongoing. Main partners in Cambodia: Sihanouk Hospital Center of Hope.

EPH Unit Team leaders: L. Borand. Financial support: ANRS

IL-1Ra Study - ANRS 12394: Rapid decrease in Interleukin-1 receptor antagonist plasma concentration following tuberculosis treatment initiation: a proof of concept study in Cambodia and Cote d'Ivoire

In a pilot study done previously with Cambodian patients from the CAMELIA clinical trial, we found that IL-1Ra plasma concentrations dropped dramatically after two months of TB treatment. The objective of this current proof-of-concept study is to demonstrate that IL-1Ra concentrations significantly decrease within two weeks following TB treatment initiation in adults with documented TB. Current status: Study preparation is ongoing. Main partners in Cambodia: Sihanouk Hospital Center of Hope. EPH Unit Team leaders: L. Borand. Financial support: ANRS.

TB-Speed Research Project

The majority of children with TB are not diagnosed/not reported and do not benefit from appropriate treatment. The TB-Speed is a multicentre (seven countries) Research Project, aiming at improving the diagnosis of childhood tuberculosis through decentralization of TB diagnosis and systematic tuberculosis diagnostic in vulnerable children. Current status: This research project is currently under preparation. Main partners in Cambodia: CENAT, National Pediatric Hospital, Kampong Cham & Takeo Hospitals, Batheay and Angrokar districts health facilities

EPH Unit Team leaders: L. Borand. Financial support: UNITAID/ Global Fund 5% Initiative.

LILAC-TB - ANRS 12394 Lowering InterLeukin-1 receptor Antagonist Concentrations after TB treatment onset: a proof of concept study in Cambodia and Ivory Coast

The aim of this proof-of-concept study is to demonstrate that IL-1Ra concentrations significantly decrease within two weeks following TB treatment initiation in adults with tuberculosis. Current status: This study is currently under preparation. Main partners in Cambodia: Sihanouk Hospital Center of Hope

EPH Unit Team leaders: L. Borand - Financial support: ANRS

Optimizing Latent TB Treatment Among People Living With HIV in Cambodia

The aim of the project is to improve Latent Tuberculosis Infection (LTBI) Treatment uptake in people living with HIV in Cambodia by addressing the barriers and assessing the impact of 3HP use in the LTBI treatment uptake and completion as part of a comprehensive intervention. Current status: This study is currently under preparation. Main partners in Cambodia: NCHADS, CENAT, CHAI

EPH Unit Team leaders: L. Borand - Financial support: Global Fund 5% Initiative

d. HEPATITIS

Tenofovir As PRevention Of Hepatitis B transmission for Mothers (TA PROHM - ANRS 12345)

Despite effective primary prophylaxis, HBV remains a substantial health problem both internationally and in Cambodia where neonatal transmission still occurs. WHO recommends immediate administration of Hepatitis B vaccine and immunoglobulin in newborns to HBsAg+ mothers. Reported failure rates range from 1– 14%, despite serovaccination. Factors associated with failure include HBeAg positivity and high HBV DNA viral loads in mothers. Antivirals can be utilized to further decrease the risk of vertical transmission, especially in areas where WHO-recommended serovaccination is inaccessible. This project aims to prevent MTCT by reducing the HBV viral load in mothers by antivirals, typically initiated starting week 24 of pregnancy. Current status: Patients' recruitment and follow-up ongoing. Main partners in Cambodia: Calmette Hospital, NMCH, Jayavarman VII hospital, National Pediatric Hospital, Kampong Cham &Takeo Provincial Hospitals

EPH Unit Team leaders: L. Borand, P.Piola. Financial support: ANRS

e. ENCEPHALITIS

SEAe (Southeast Asia encephalitis)

Encephalitis, an acute inflammation of the central nervous system associated with neurologic dysfunction, is of major Public Health concern in South East Asia as it represents a frequent cause of paediatric hospitalization (8/100,000 patients-year) and leads to a high mortality and long-term neurological sequelae. The Southeast Asia encephalitis project aims to (i) improve encephalitis diagnosis by strengthening hospital clinical teams and labs and to (ii) identify encephalitis aetiologies in 4 countries (Cambodia, Laos, Vietnam, and Myanmar). This prospective study started mid 2015 (mid 2016 in Myanmar) and over 750 patients were included. In the Mekong Region, preliminary results show that unidentified causes of encephalitis remain high (39%) while Japanese encephalitis is the main cause (32%) followed by Herpes simplex virus, tuberculosis, scrub typhus and Dengue which are of similar proportions (3%). Partners: LOMWRU, Institut Pasteur Paris, National Institute of Epidemiology and Hygiene in Vietnam, National Health Laboratory in Myanmar, CIRAD, IRD

3.2.2.2 BACTERIOLOGIAL DISEASES AND ANTIBIOTIC RESISTANCE

BIRDY (Bacterial Infections and antibiotic-Resistant Diseases among Young children in low-income countries) - 2014-2018 + ACIP *klebsiella pneumoniae*

Little is known about bacterial infections and resistances in pediatrics and developing countries. Antimicrobial resistance (AMR) is one of the biggest threats worldwide. BIRDY is an international (Madagascar, Senegal, Cambodia) multicentric and prospective cohort study aiming to estimate bacterial infections and AMR incidence among neonates and young children from rural and urban community settings, to describe and characterize pathogenic and colonizing bacteria, and assess the burden of AMR. In Cambodia, among 815 mothers, group-B streptococcus vaginal carriage was low: <1%, digestive carriage of *Klebsiella pneumoniae* was high: 68% and digestive carriage of ESBL-producing enterobacteria was extremely high: 75%. Neonatal mortality was low compared to national data, 6.2 vs 14.0/1000 live-births respectively. Incidence of neonatal sepsis was ~5.2/1000 live-births with 3/4 (75%) isolates resistant to antibiotic recommended by WHO. Partners: Cambodian CDC, 2 Health centers, 3 Hospitals.

EPH Unit Team leaders: A. de Lauzanne, L. Borand. Financial support: IP Paris, MSD Avenir.

PEEC NIC (Producing Extented-Spectrum Beta Lactamase *Enterobacteriaceae* Carriage in Newborns and Infants in Cambodia) – 2016-2018

In low-resources settings, the spread of ESBL-producing Enterobacteriaceae (ESBL-E) in the community is a public health concern. Data are scarce, especially in newborns where the burden of sepsis is high. The objectives of this study are to 1) determine early prevalence of ESBL-E fecal carriage in newborns, 2) follow acquisition during the first year of life, 3) identify risk factors and investigate ESBL genes in Cambodia. 147 newborns from two urban and rural community settings were enrolled and followed 1 year. Preliminary results: At day 3 of life, the prevalence of ESBL-E fecal carriage among newborns was 53% [95%IC: 45-61], remaining stable up to 12 months of life: 52% [95%IC: 44-60]. Most frequently detected ESBL-genes were *bla*$_{\text{CTXM-15}}$ *bla*$_{\text{CTXM}}$ -55 and *bla*$_{\text{CTXM-27}}$. Urban setting, delivery at hospital/private clinic and a household of <6 people were positively associated to carriage. This particularly high and precocious prevalence of ESBL-E carriage increases the risk of ESBL-E neonatal infection in Cambodian newborns. Partners: French NRC for carbapenem resistance.

EPH Unit Team leaders: A. de Lauzanne. Financial support: BIRDY project, Dr Inghammar-Lund University-Sweden.

Pertussis Immunization programs in Low Income Countries (PERILIC)

Infection by Bordetella pertussis or Bordetella parapertussis occurs in epidemic cycles and can cause severe acute respiratory diseases especially in infants. Incidence of its clinical form has declined by more than 90% in the industrialized world. However, WHO listed pertussis as a major cause of death in infants in 2014, coincident with a global resurgence in pertussis incidence. The aim of this study is to document contamination processes, clinical characteristics and prevalence rates of pertussis cases in children under 6 months old suspected of whooping cough (WP1) and to assess immunization status among household contacts and children from 3-15years old (WP2). Current status: WP1: Ongoing participants' recruitment, WP2: Participants' recruitment completed. Main partners in Cambodia: NIP, NPH, Several provincial hospitals, private clinics and health centers.

EPH Unit Team leaders: L. Borand. Financial support: Fondation Total.

A hospital based case-control study to identify risk factors of leptospitoris and to improve post-disaster management of emerging diseases

While extreme weather events, such as floods, are associated with leptospirosis outbreaks, little is known about the magnitude of leptospirosis incidence in Myanmar where floods are a priori increasingly frequent. Leptospirosis in Myanmar is suspected to be endemic, but it remains underdiagnosed. We contributed to the design of a multicenter hospital-based case-control study, exploring socio-demographic and environmental risk factors of urban leptospirosis in Yangon region. This study will improve leptospirosis surveillance in Myanmar. Partners: National Health Laboratory of Myanmar, Institut Pasteur de Nouvelle Calédonie

EPH Unit Team leaders: P**.** Piola (technical advisor). Funding: AFD

SEPSIS and antibiotic resistance in adults project in Calmette Hospital

Despite a National Action Plan issued in 2014 to face the emergence of antimicrobial resistance (AMR) in Cambodia, epidemiological and bacteriological data from sepsis cases remain scarce. Evidence lies mostly in retrospective microbiological studies, but few explore patients' clinical history and outcomes. Early recognition of sepsis is a challenge in Emergency Room settings, where most life-threatened cases are referred.

A partnership between Institut Pasteur du Cambodge and Calmette Hospital (adult hospital) of Phnom Penh started in mid-2018. Calmette Hospital has the most attended adult Emergency Room (ER) in Cambodia.

A prospective observational study aimed at including all adult sepsis arriving at the ER to estimate risk factors (including bacteremia and AMR) of poor outcomes and death. The Third International Consensus Definitions for Sepsis and Septic Shock (2016) was used to detect cases. Systematic blood cultures on admission provided the prevalence of most common multidrug-resistant pathogens. We also aim at identifying risk factors for infection with multidrug-resistant pathogens. Adequacy of initial treatment, length of stay in ICU and hospital, mortality and other complications are currently analyzed, with outcomes evaluated after 28 days.

EPH Unit Team leaders: P. Piola, S. Bory (*Calmette Hospital*), P-H Wicky. Funding: Institut Pasteur du Cambodge

3.2.2.3 PARASITOLOGICAL DISEASES

Understanding of malaria epidemiology and malaria elimination inside forests

Malaria elimination is a priority in Cambodia, where P.*falciparum* strains are resistant to artemisinin derivatives and to nearly all partner drugs. However, the main reservoir of parasites in Cambodia is inside its forests. While 2017 underwent a doubling of malaria cases, there is still a very limited understanding of malaria epidemiology and transmission inside forests; and hence no malaria elimination strategies specific to this environment. A study aims at an in-depth understanding of malaria transmission inside three forests totalling 200km² (Year 1) followed by an intervention (Year 2) to eliminate in-forest malaria. Selected individuals from the high-risk group, forest goers, will be trained to develop the necessary skills to work and control malaria inside forests, hopefully leading to an elimination of malaria inside forests and their fringe villages.

EPH Unit Team leaders: P. Piola, Sophea IV, Amber Kunkel (in collaboration with Malaria Unit). Funding: French Initiative 5%. Partners: Partners for Development, CNM.

3.2.2.4 ZOONOTIC DISEASES

Modelling and assessment of combining gilt vaccination, vector control and pig herd management to control Japanese Encephalitis virus transmission in Southeast Asia

Despite existence of human vaccines, Japanese Encephalitis (JE) remains a prominent public health problem in Southeast Asia (SEA). JE is caused by a Flavivirus which is transmitted between pigs, the main amplifying hosts, by Culex mosquito bites. Therefore, sow vaccination, pig herd management and vector control –or a combination of these three potential control measures, might constitute additional control measures contributing to reduce JE health impact in humans, and economic losses in pig farms. We built a deterministic metapopulation model, combining a pig and a Culex mosquito vector population, to represent JE virus (JEV) transmission dynamic within a pig herd. The dynamic of the epidemiological systems resulted from an infectious process, operating in continuous time, combined with the pig breeding process that was modeled based on discrete events occurring instantaneously. We used this model to simulate JEV transmission within a continuum of plausible pig breeding systems encountered in SEA, ranging from backyards to semi-commercial systems. We then analyzed the joint effects of the three tested control measures, namely sow vaccination, pig herd management and vector control, on several indicators characterizing (i) the ability of different pig breeding systems to be simultaneously profitable and allow JEV eradication in the herd, (ii) the impact of JE on pig production and the profitability of gilt vaccination, and (iii) the risk for human beings living in the vicinity of pig herds and/or near pig slaughterhouses. According to our model, herd management has no effect on JEV circulation. Vector control alone is a major control tool but shows paradoxical effects that should be considered in any mosquito based control strategy. Combining sow vaccination and vector control could be an alternative or an additional measure to human vaccination to efficiently reduce both JE incidence in humans and the economic impact of JE infection on pig farms. Financial support: ComAcross (EU), CIRAD, IPC

Influenza Surveillance in Cambodia

EPH Unit team: Malen Chan

This study (2017-2019) lead by the Virology Unit, IPC, aims to map poultry supply networks and identify supply areas with a high prevalence of avian influenza viruses through tracking of middlemen and poultry movement (GPS/GSM tracking device) and testing of poultry samples at different steps of supply chain. The project also aims to establish avian influenza viruses (AIVs) and swine influenza viruses (SIVs) surveillance in Cambodian border regions to obtain a greater understanding of the dynamics of cross-border movements of AIVs into Cambodia through follow-up of cohorts of individuals.

Financial support: FAO, USAID

3.2.3 RESEARCH PROGRAMS IN 2019

Effectiveness of Malaria elimination inside forests

The overarching objective of this study is to eliminate malaria infections inside forests (and consequently in surrounding villages) within a year. We think this could be achieved through quarterly in-forest active screenings (MSATs with HS-RDTs) and continuous passive detection to efficiently treat all malaria infections and provide them with a vector control kit. Estimates of the malaria incidence from all the Health Centers (HCs) neighboring intervention forests will be compared to the incidences of approximately 100 HCs neighboring other non-intervention forests in Cambodia (control forests) in similar transmission areas. A significant drop in malaria notifications among HCs surrounding intervention forests compared to control forests would strongly suggest the effectiveness of the abovementioned interventions inside forests, where the parasite reservoir is in Cambodia. This would open new perspectives in malaria elimination strategies in South East Asia.

Forest goers are a mobile community accustomed to the harsh working condition inside forests and are the best placed to become a new type of malaria workers: Forest Malaria Workers (FMWs). A tailored Android application will be developed to support FMWs across all components of this forest project. The data uploaded from this MHealth application will be used in near real-time to monitor, track (GPS) and evaluate all field activities and malaria indicators.

EPH Unit Team leaders (in collaboration with Malaria Unit): P. Piola, Amber Kunkel and a team to be hired. **Funding**: Resistance to Artemisinin Initiative (Global Fund). **Partners**: Partners for Development, Malaria Consortium, Institut de Technologie du Cambodge, CNM.

SEAdogSEA

The aim of Sea-dog-Sea “Socio-Ecological Approach of Dog-borne zoonotic diseases in SE Asia » is to study social and ecological dimensions of dog zoonotic diseases in rural vs semi-urban sites in Cambodia, Indonesia and Thailand. The surveys will comprise: i) prevalence in owned and stray dog populations of rabies, leptospirosis, internal helminths and rickettsia; ii) census, distribution and movements of owned and stray dogs; iii) perceptions and practices of local populations regarding dog keeping and management: iv) modelling of zoonotic risks associated with dogs (v) evidence-based recommendations for improved management of dog populations and better prevention of spill-over risks.

EPH Unit Team leaders: Véronique Chevalier. Partners: CIRAD/ASTRE (France); ITM (Belgium); UGM (Indonesia) and KU (Thailand) Financial support: ANR

AMR Pigs

The main objectives of the project are to provide (i) a comprehensive report on knowledge, attitudes and practices related to antimicrobial use and antimicrobial resistance among stakeholders working with Cambodian pig production including the characterization and mapping of the distribution pathways

base on the identified key stakeholders (ii) recommendations on good management practices to limit the need for antimicrobials and good antimicrobial usage for preventing AMR.

EPH Unit Team leaders: Véronique Chevalier. Financial support: FAO Cambodia

PTR FITERAD

The aim of the FiTeRaD project (Field Tests for Rabies Diagnostic) is to develop and to validate, both in laboratory conditions and in field settings, the first point of care tests (POCTs) for the rapid detection of the etiological agent of rabies (with rabies virus - RABV) in humans.

EPH Unit Team leaders: Véronique Chevalier. Financial support IPP

ARCAHD

The objective of this project is double: to identify the sources of emergence and spread of resistant bacteria in Cambodia using a One Health approach; to evaluate if the MinION technology could be used as diagnostic tool.. The expected results will represent the baseline for the setting up of a surveillance system, will allow stakeholders to implement efficient control strategies and will help determine the capacity of MinION technology to be used as diagnostic tool.

EPH Unit Team leaders: Véronique Chevalier. Financial support: ANR

3.2.4 SUPPORT TO NATIONAL AUTHORITIES

- Opening of IPC-PHD Rabies Prevention Center at Battambang province (Epidemiology and Public Health Unit, IPC)
- Contribution to the development of « Rabies surveillance and response guideline » for Cambodia. This work was coordinated by WHO, FAO, Ministry of Agriculture and Ministry of Health (Dr LY Sowath)
- Participation to the writing of National Guidelines for Rabies Control in Cambodia (OMS, FAO, C-CDC, IPC, GDAPH)(Dr Véronique Chevalier)
- Contribution to the national surveillance program on whooping cough: Data on whooping cough cases identified in the PERILIC study were shared with the National Immunization Program (Dr Laurence BORAND/ Dr LENG Chanthy)
- Participation to monthly meeting for AMR working group - MoH Cambodian CDC (Agathe de Lauzanne).
- Participating to the Multisectoral (MoH-MAAF-MoE) National Action Plan on AMR (MSNAP) – tripartite support of WHO-FAO-OIE (Dr Agathe de Lauzanne)
- Participating to the Zoonosis Working Group (Drs Ly Sowath, Véronique Chevalier, Patrice Piola)
- Participating to the Scientific Committee of 1st Research Meeting on AMR – To be held during the MSNAP Launching in Phnom Penh in June 2018 (Dr Agathe de Lauzanne)
- Participating to the Technical Working Group on the development of the National Latent TB Infection Standard Operating Procedure (Dr Laurence BORAND/ Dr DIM Bunnet)
- Participating to the Technical Working Group on the development of the National Pediatric TB curriculum (Dr DIM Bunnet)

3.2.5 TEACHING AND TRAINING

- A grant is funded by the Bourse du Gouvernement Français (BGF) for the PhD in Epidemiology of Ms. Sophea IV (supervised by P. Piola, on the French Initiative 5% project on malaria elimination in forests).
- Master degree of Clinical Trials (LSHTM) funded by the ANRS for Dr Dim Bunnet

- Several training given on clinical research and Good clinical Practices (Clinical Research Group)
- Master II (InterRisk) supervision, student Sorn Sopheak (Dr Sowath Ly)
- Lecture sessions at Applied Epidemiology Training (AET) program (Dr Sowath Ly)
- Supervision of two students in Cambodian Veterinarian Applied Epidemiology Training (CAVET) (Dr Sowath Ly)
- Microbiology & Neonatal Sepsis Workshop. 16 partcipants collaborators and monitors of BIRDY program. Angkor Children Hospital - Siem Reap, November 6th – 7th 2018.
- Public Health Bachelor Degree students from UHS internship (Dr Laurence Borand)
- PhD supervisor: « Modelling of Japanese Encephalitis in Cambodia and optimisation of control strategies » Direction V. Chevalier, co-direction D. Fontenille, IPC director. ED GAIA Montpellier II. Cofunding CIRAD/ IP Paris.
- Starting of « Modelling of JE in Cambodia; simulation of the virus introduction in La Reunion Island and consequences « Co-direction wth Dr Benoit Durand (ANSES Maisons Alfort).
- L. Pitou. Master II InterRisk (Kasetsart/Toulouse): Rabies control in Cambodia: Analysis of the dog population demography and preliminary model of rabies transmission in Kandal province (Cambodia)

3.2.6 PUBLICATION LIST

2018

1. Laurence Borand, Agathe de Lauzanne, Nguyen Ngoc Lan, Sokleaph Cheng, Hang Pham Thu, Sara Eyangoh, Abdoul-Salam Ouedraogo, Vibol Ung, Philippe Msellati, Mathurin Tejiokem, Boubacar Nacro, Malin Inghammar, Bunnet Dim, Christophe Delacourt, Sylvain Godreuil, Stéphane Blanche, Olivier Marcy, for the ANRS 12229 PAANTHER 01 Study Group “Isolation of Non-tuberculous Mycobacteria in South-East Asian and African HIV-infected Children with Suspected Tuberculosis”. Clin Infect Dis.
2. Laureline Berteloot, Olivier Marcy, Bang Nguyen, Vibol Ung, Mathurin Tejiokem, Boubacar Nacro, Sophie Goyet, Bunnet Dim, Stéphane Blanche, Laurence Borand, Philippe Msellati, Christophe Delacourt, for the ANRS 12229 PAANTHER 01 Study Group. Value of Chest X Ray in the diagnosis of tuberculosis in HIV-infected children living in resource limited countries. Results from the ANRS 12229 - PAANTHER 01 study. Int J Tuberc Lung Dis. 2018 Aug 1;22(8):844-850. doi: 10.5588/ijtld.18.0122.
3. Malin Inghammar, Laurence Borand, Sophie Goyet, Blandine Rammaert, Vantha Te, Patrich Lorn Try, Bertrand Guillard, Philippe Buchy, Sirenda Vong, Eap Tek Chheng, Philippe Cavailler, Charles Mayaud, Arnaud Tarantola. Community-acquired pneumonia and Gram-negative bacilli in Cambodia– incidence, risk factors and clinical characteristics. The Royal Society of Tropical Medicine and Hygiene 2018 Feb 1;112(2):57-63. doi: 10.1093/trstmh/try022.
4. “Acquisition of extended spectrum beta-lactamase-producing enterobacteriaceae in neonates: A community based cohort in Madagascar”. Herindrainy P, Rabenandrasana MAN, Andrianirina ZZ, Rakotoarimanana FMJ, Padget M, de Lauzanne A, Ndir A, Kermorvant-Duchemin E, Garin B, Piola P, Collard JM, Guillemot D, Huynh BT, Delarocque-Astagneau E; BIRDY study group. PLoS One. 2018 Mar 1;13(3):e0193325.
5. “Combating Global Antibiotic Resistance: Emerging One Health Concerns in Lower- and Middle-Income Countries”. Nadimpalli M, Delarocque-Astagneau E, Love DC, Price LB, Huynh BT, Collard JM, Lay KS, Borand L, Ndir A, Walsh TR, Guillemot D; Bacterial Infections and antibiotic-Resistant Diseases among Young children in low-income countries (BIRDY) Study Group . Clin Infect Dis. 2018 Mar 5;66(6):963-969.
6. Malin Inghammar, Youlet By, Christina Farris, Thong Phe, Laurence Borand, Alexandra Kerleguer, Sophie Goyet, Vonthanak Saphonn, Chanleakhena Phoeung, Sirenda Vong, Blandine Rammaert, Charles Mayaud, Bertrand Guillard, Chadwick Yasuda, Gavin Ford, Steven Newell, Ung Sam, Buth Sokhal, Sok Touch, Paul Turner, Jan Jacobs, Mélina Messaoudi, Florence Komurian-Pradel, Arnaud Tarantola. Serotype distribution of clinical Streptococcus pneumoniae isolates before the introduction of the 13-valent pneumococcal conjugate vaccine in Cambodia. American Journal of Tropical Medicine & Hygiene 2018 Jan 8. doi: 10.4269/ajtmh.17-0692.
7. Alpha Oumar II Diallo, Véronique Chevalier, Julien Cappelle, Didier Fontenille, Veasna Duong, Raphaël Duboz. Does direct transmission between pigs contribute to Japanese Encephalitis virus circulation? A modelling approach in Cambodia .2018. PLosOne. https://doi.org/10.1371/journal.pone.0201209
8. Juliette Di Francesco, Rithy Choeung; Borin Peng; Long Pring; Senglong Pang; Raphaël Duboz; Sivuth Ong; San Sorn; Arnaud Tarantola; Didier Fontenille; Vaesna Duong; Philippe Dussart Dussart; Véronique Chevalier; Julien Cappelle. Comparison of the dynamics of Japanese encephalitis virus circulation in sentinel pigs between rural and peri-urban settings in Cambodia PLoS Negl Trop Dis. 2018 Aug 23;12(8):e0006644. doi: 10.1371/journal.pntd.0006644. eCollection 2018 Aug.
9. A-S Ruget, C. Beck, A. Gabassi, K. Trevennec, Ho Thu Huong, S. Lecollinet, V Chevalier and J Cappelle. Japanese Encephalitis circulation pattern in swine of northern Vietnam, and consequences for swine’s vaccination recommendations. Transbound Emerg Dis. 2018 May 8. doi: 10.1111/tbed.12885.
10. Horwood PF, Horm SV, Suttie A, Thet S, Y P, Rith S, Sorn S, Holl D, Tum S, Ly S, Karlsson EA, Tarantola A, Dussart P. Co-circulation of Influenza A H5, H7, and H9 Viruses and Co-infected Poultry in Live Bird Markets, Cambodia. Emerg Infect Dis. 2018 Feb;24(2):352-355. doi: 0.3201/eid2402.171360.
11. Auerswald H, Boussioux C, In S, Mao S, Ong S, Huy R, Leang R, Chan M, Duong V, Ly S, Tarantola A, Dussart P. Broad and long-lasting immune protection against various Chikungunya genotypes demonstrated by participants in a

cross-sectional study in a Cambodian rural community. Emerg Microbes Infect. 2018 Feb 7;7(1):13. doi: 10.1038/s41426-017-0010-0.
12. Hampson K, Ventura F, Steenson R, Mancy R, Trotter C, Cooper L, Abela-Ridder B, Knopf L, Ringenier M, Tenzin T, Ly S, Tarantola A, Moyengar R, Oussiguéré A, Bonfoh B, Narayana DA, Sudarshan MK, Muturi M, Mwatondo A, Wambura G, Andriamandimby SF, Baril L, Edosoa GT, Traoré A, Jayme S, Kotzé J, Gunesekera A, Chitnis N, Hattendorf J, Laager M, Lechenne M, Zinsstag J, Changalucha J, Mtema Z, Lugelo A, Lushasi K, Yurachai O, Metcalf CJE, Rajeev M, Blanton J, Costa GB, Sreenivasan N, Wallace R, Briggs D, Taylor L, Thumbi SM, Huong NTT. WHO Rabies Modelling Consortium. The potential effect of improved provision of rabies post-exposure prophylaxis in Gavi-eligible countries: a modelling study. Lancet Infect Dis. 2018 Nov 21. pii: S1473-3099(18)30512-7. doi: 10.1016/S1473-3099(18)30512-7.
13. Tarantola A, Ly S, Chan M, In S, Peng Y, Hing C, Taing CN, Phoen C, Ly S, Cauchemez S, Buchy P, Dussart P, Bourhy H, Mary JY. Intradermal rabies post-exposure prophylaxis can be abridged with no measurable impact on clinical outcome in Cambodia, 2003-2014. Vaccine. 2018 Nov 16. pii: S0264-410X(18)31420-8. doi: 10.1016/j.vaccine.2018.10.054
14. Tarantola A, Blanchi S, Cappelle J, Ly S, Chan M, In S, Peng Y, Hing C, Taing CN, Ly S, Bourhy H, Buchy P, Dussart P, Mary JY. Rabies Postexposure Prophylaxis Noncompletion After Dog Bites: Estimating the Unseen to Meet the Needs of the Underserved. Am J Epidemiol. 2018 Feb 1;187(2):306-315. doi: 10.1093/aje/kwx234.
15. Nikolayeva I, Bost P, Casademont I, Duong V, Koeth F, Prot M, Czerwinska U, Ly S, Bleakley K, Cantaert T, Dussart P, Buchy P, Simon-Lorière E, Sakuntabhai A, Schwikowski B. A Blood RNA Signature Detecting Severe Disease in Young Dengue Patients at Hospital Arrival. J Infect Dis. 2018 Feb 27. doi: 10.1093/infdis/jiy086
16. Champagne C, Paul R, Ly S, Duong V, Leang R, Cazelles B. Dengue modeling in rural Cambodia: Statistical performance versus epidemiological relevance. Epidemics. 2018 Aug 29. pii: S1755-4365(17)30170-6. doi: 10.1016/j.epidem.2018.08.004.
17. Alpha Oumar II Diallo*, Véronique Chevalier*, Julien Cappelle, R. Duboz, D. Fontenille, B. Durand. Assessment of combining gilt vaccination, vector control and pig herd management to control Japanese Encephalitis virus transmission in Cambodia? (Preprint) Plos Neglected Tropical Diseases
18. Olivier Ségéral; Dieynaba S N'Diaye; Sophearot Prak; Janin Nouhin; Samsorphea Chhun; Wootichai Khamduang; Kearena Chhim; Anne Marie Roque Afonso; Patrice Piola; Laurence Borand; Nicole Ngo-Giang-Huong; Francois Rouet. Usefulness of a serial algorithm of HBsAg and HBeAg rapid diagnosis tests to detect pregnant women at risk of HBV mother-to-child transmission in Cambodia, the ANRS 12328 pilot study. Journal of Clinical Virology 2018
19. Changguang Xiao, Chenxi Li1, Di Di, Julien Cappelle, Lihong Liu, Xin Wang, Linlin Pang1, Jinpeng Xu, Ke Liu, Beibei Li, Donghua Shao, Yafeng Qiu, Lihong Liu, Frederik Widén, Véronique Chevalier, Jianchao Wei and Zhiyong Ma. Differential replication efficiencies between Japanese encephalitis virus genotype I and III in avian cultured cells and young domestic ducklings. PLoS Negl Trop Dis. 2018 Dec 18;12(12):e0007046. doi: 10.1371/journal.pntd.0007046. eCollection 2018 Dec.
20. DKW Chu, K Hui, M Perera, E Miguel, A Traore, O Fassi-Firhi, G Ayelet, J Oladipu, MG Hemida, MC Chan, JM Nicholls, RJ Webby, F Roger, LLM Poon, V Chevalier*, M Peiris* (2018)., Genetic and phenotypic characterization of MERS coronaviruses from North, West and East Africa. Proc Natl Acad Sci U S A. 2018 Mar 20;115(12):3144-3149. doi: 10.1073/pnas.1718769115. Epub 2018 Mar 5.
21. Sebastian Napp; Veronique Chevalier; Núria Busquets; Paolo Calistri; Jordi Casal; Mohamed Attia; Rehab Elbassal; Heba Hosni; Hatem Farrag; Noura Hassan; Rasah Tawfik; Shahin Bayomy (2018) Understanding legal trade of cattle and camels and the risk of Rift Valley Fever introduction into and transmission within Egypt. Plos Neg Trop Dis https://doi.org/10.1371/journal.pntd.0006143

2019

1. “Meat and Fish as Sources of Extended-Spectrum β-Lactamase-Producing Escherichia coli, Cambodia.” Nadimpalli M, Vuthy Y, de Lauzanne A, Fabre L, Criscuolo A, Gouali M, Huynh BT, Naas T, Phe T, Borand L, Jacobs J, Kerléguer A, Piola P, Guillemot D, Le Hello S, Delarocque-Astagneau E; BIRDY study group. Emerg Infect Dis. 2019 Jan;25(1).
2. “CTX-M-55-type ESBL-producing Salmonella enterica are emerging among retail meats in Phnom Penh, Cambodia.” Nadimpalli M, Fabre L, Yith V, Sem N, Gouali M, Delarocque-Astagneau E, Sreng N, Le Hello S; BIRDY study group. J Antimicrob Chemother. 2019 Feb 1;74(2):342-348.

Abstracts

- Prevalence and risk factors for early ESBL-producing enterobacteriaceae fecal colonization among newborns from Cambodian community settings. de Lauzanne A., Bernabeu S., Gouali. M., Borand L., Huynh BT., Delarocque-Astagneau E., Naas T. Submitted for ESPID 2018, Malmö. (Dr A. de Lauzanne)
- Antibiotic Resistance: from research to action. 59th Colloquium of the Institute of Tropical Medicine. 5-7th Dec. 2018
- Antibiotic Resistance: from research to action. 59th Colloquium of the Institute of Tropical Medicine. 5-7th Dec. 2018- Antibiotic Resistance: from research to action. 59th Colloquium of the Institute of Tropical Medicine. 5-7 December 2018. Evidence for meat and fish as sources of community exposure to ESBL-producing Escherichia coli in Cambodia. M. Nadimpalli, Y. Vuthy, A. de Lauzanne, L. Fabre, A. Criscuolo, M. Gouali, BT. Huynh, T. Naas, T. Phe, L. Borand, J. Jacobs, A. Kerléguer, P. Piola, D. Guillemot, S. Le Hello, E. Delarocque-Astagneau.
- 22nd International AIDS Conference (AIDS 2018), 23-27 July 2018, Amsterdam, the Netherlands: “Systematic empirical versus test-guided anti-tuberculosis treatment impact in severely immunosuppressed HIV-infected adults initiating antiretroviral therapy with CD4 cell counts <100/mm : final results from the STATIS (ANRS 12290) randomized trial.” F.X. Blanc , A.D. Badje , M. Bonnet , D. Gabillard , E. Messou , C. Muzoora , S. Samreth , D.B. Nguyen , L. Borand , A. Domergue , N. Natukunda , S. Juchet , S. Eholie , S. Domoua , X. Anglaret , D. Laureillard
- 1st National AMR conference in Cambodia. Phnom Penh, 23 May 2018. Extremely high prevalence of extended-spectrum βeta-lactamase-producing enterobacteriacae fecal carriage among women at time of delivery in cambodia. de Lauzanne Agathe, Foucaud Elsa, Sok Touch, Heng Soda, Borand Laurence, Kerléguer Alexandra, Huynh Bich-Tram, Delarocque-Astagneau Elisabeth.

- Bacterial Infections and antibiotic Resistant Diseases among Young children in low income countries (BIRDY): a focus in Cambodia. de Lauzanne. A, Huynh BT. MSD Avenir Scientific Day 28 Nov. 2018. (Dr A. de Lauzanne)

Presentations

- Combating resistance: microbes and vectors. 2018 Institut Pasteur International Network Symposium, Paris, France, 15-16 Nov 2018. High prevalence of ESBL producing enterobacteriacae fecal carriage within the community in Cambodia: results of the BIRDY and PEEC NIC studies. A. De Lauzanne, N. Sreng, E. Foucaud, S. Bernabeu, T. Sok, T. Chon, C. Yem, V. Hak, P. Long, S. Born, M. Nadimpalli, S. Le Hello, MAN. Rabenandrasana, JM. Collard, M. Gouali, S. Heng, S. Meng, A. Kerléguer, P. Piola, M. Inghammar, E. Delarocque-Astagneau, BT. Huynh, T. Naas, L. Borand.
- Combating resistance: microbes and vectors. 2018 Institut Pasteur International Network Symposium, Paris, France, 15-16 Nov 2018. Klebsiella pneumoniae carriage in low-income countries: antimicrobial resistance, risk factors and genomic diversity. Bich-Tram Huynh, Virginie Passet, Aina Rakotondrasoa, Leonardo Panunzi, Alexandra Kerleguer, Agathe de Lauzanne, Patrice Piola, Perlinot Herindrainy, Awa Ndir, Thierno Diallo, Abdoulaye Seck, Elisabeth Delarocque-Astagneau, Didier Guillemot, Laurence Borand, Raymond Bercion, Muriel Vray, Jean-Marc Collard, Sylvain Brisse.
- American Society of Tropical Medicine and Hygiene. New Orleans, USA, Oct 28 – Nov 1, 2018.Neonatal infections in low income countries: incidence, etiology, risk factors and outcomes, Evidence from a multicentric community-based cohort study in Madagascar, Senegal and Cambodia. Huynh BT., de Lauzanne A., Herindrainy P., Borand L., Kermorvant-Duchemin E., Vray M., Seck A., Ndir A., Padget M., Tarantola A., Touch S., Piola P., Kerléguer A., Randrianirina F., Collard JM., Guillemot D. , Delarocque-Astagneau E.
- Oxford-Pasteur Antimicrobial Workshop. Oxford, England, 25 June 2018. High prevalence of ESBL producing enterobacteriacae fecal carriage within the community in Cambodia: results of the BIRDY and PEEC NIC studies. A. De Lauzanne, N. Sreng, E. Foucaud, S. Bernabeu, T. Sok, T. Chon, C. Yem, V. Hak, P. Long, S. Born, M. Nadimpalli, S. Le Hello, MAN. Rabenandrasana, JM. Collard, M. Gouali, S. Heng, S. Meng, A. Kerléguer, P. Piola, M. Inghammar, E. Delarocque-Astagneau, BT. Huynh, T. Naas, L. Borand.
- 1st National AMR conference in Cambodia. Phnom Penh, 23 May 2018. Prevalence and Risk Factors for Early ESBL-Producing Enterobacteriaceae Fecal Colonization Among Newborns from Cambodian Community Settings. Preliminary Results of the PEEC NIC Study. de Lauzanne A., Bernabeu S., Chon T., Pring L., Chan M., Gouali M., Borand L., Huynh BT, Delarocque-astagneau E., Naas T.
- 1st National AMR conference in Cambodia. Phnom Penh, 23rd, May 2018. Meat and fish as sources of exposure to ESBL-producing Escherichia coli in Cambodia. M Nadimpalli, Y Vuthy, A de Lauzanne, L Fabre1, A Criscuolo, M Gouali, BT Huynh1, T Naas, T Phe, L Borand, J Jacobs, A Kerleguer, P Piola, D Guillemot, S Le Hello, E Delarocque-Astagneau, On behalf of the BIRDY study group.
- Pig-to-pig direct transmission of Japanese encephalitis: its importance in two pig cohorts in Cambodia. Alpha Oumar II Diallo, Véronique Chevalier, Julien Cappelle, Didier Fontenille, Raphaël Duboz. ModAH. Modeling in Animal Health, Nantes, France
- CROI Conference 2018, March 4-7, Boston, USA “Systematic Vs Test-guided tuberculosis treatment: Data of the STATIS randomized trial” François-Xavier Blanc, Anani D. Badje, Maryline Bonnet, Delphine Gabillard, Eugène Messou, Conrad Muzoora, Sovannarith Samreth, Duc Bang Nguyen, Laurence Borand, Anaïs Domergue, Naome Natukunda, Serge Eholie, Serge Domoua, Xavier Anglaret, Didier Laureillard.
- 22nd International AIDS Conference (AIDS 2018), 23-27 July 2018, Amsterdam, the Netherlands: “Systematic empirical versus test-guided anti-tuberculosis treatment impact in severely immunosuppressed HIV-infected adults initiating antiretroviral therapy with CD4 cell counts <100/mm : final results from the STATIS (ANRS 12290) randomized trial.” F.X. Blanc, A.D. Badje, M. Bonnet, D. Gabillard, E. Messou, C. Muzoora, S. Samreth, D.B. Nguyen, L. Borand, A. Domergue, N. Natukunda, S. Juchet, S. Eholie, S. Domoua, X. Anglaret, D. Laureillard
- AO Diallo*, V. Chevalier* (*first co-authorship), J. Cappelle, R. Duboz, D. fontenille, B. Durand« Modelling the spread of Japanese encephalitis virus on swine farms in Cambodia: Exploring for control strategies and cost-effectiveness. Conférence internationale « 3rd Mathematical Biology Modelling days of Besançon » 19-22 Juin 2018
- V. Chevalier and S. Boyer. “Trophic behavior of Japanese Encephalitis vectors in Cambodia.” Nov 2018, conference ISVEE Chiang Mai.
- Application of an Exponential Random Graph Model to determine of Nomadic herders' movements in Senegal. Jaber Belkhiria, Modou Moustapha Lo, Fafa Sow, Beatriz Martínez-López, Veronique Chevalier. 2018. ISVEE conference, Chiang Mai, Thalinad, Nov 2018
- Rabies surveillance and control programs in Cambodia. Philippe Dussart, Sowath Ly, Véronique Chevalier, Didier Fontenille. 2018 OIE Twinning Project for Rabies was Launched in Taiwan to Establish Asian Disease Prevention Network through Laboratory Proficiency Testing Center for Rabies Diagnosis within Asian Network. Dec 2018, Taiwan
- AO Diallo*, V. Chevalier* (*first co-authorship), J. Cappelle, R. Duboz, D. Fontenille, B. Durand « Modelling the spread of Japanese encephalitis virus on swine farms in Cambodia: Exploring for control strategies and cost-effectiveness”. Nov 2018, conference ISVEE Chiang Mai.
- LY Sowath “Rabies in Cambodia : an under estimated public health problem.” Colloque du Réseau Francophone sur les Maladies Tropicales Négligées, Agropolis, Montpellier, 22-23 octobre 2018
- Combating resistance: microbes and vectors. 2018 Institut Pasteur International Network Symposium, Paris, France, 15-16 Nov 2018. High prevalence of ESBL producing enterobacteriacae fecal carriage within the community in Cambodia: results of the BIRDY and PEEC NIC studies. A. De Lauzanne, N. Sreng, E. Foucaud, S. Bernabeu, T. Sok, T. Chon, C. Yem, V. Hak, P. Long, S. Born, M. Nadimpalli, S. Le Hello, MAN. Rabenandrasana, JM. Collard, M. Gouali, S. Heng, S. Meng, A. Kerléguer, P. Piola, M. Inghammar, E. Delarocque-Astagneau, BT. Huynh, T. Naas, L. Borand.
- Combating resistance: microbes and vectors. 2018 Institut Pasteur International Network Symposium, Paris, France, 15-16 Nov 2018. Klebsiella pneumoniae carriage in low-income countries: antimicrobial resistance, risk factors and genomic diversity. Bich-Tram Huynh, Virginie Passet, Aina Rakotondrasoa, Leonardo Panunzi, Alexandra Kerleguer, Agathe de Lauzanne, Patrice Piola, Perlinot Herindrainy, Awa Ndir, Thierno Diallo, Abdoulaye Seck, Elisabeth Delarocque-Astagneau, Didier Guillemot, Laurence Borand, Raymond Bercion, Muriel Vray, Jean-Marc Collard, Sylvain Brisse.
- American Society of Tropical Medicine and Hygiene. New Orleans, USA, Oct 28 – Nov 1, 2018.Neonatal infections in low income countries: incidence, etiology, risk factors and outcomes, Evidence from a multicentric community-based cohort study in Madagascar, Senegal and Cambodia. Huynh BT., de Lauzanne A., Herindrainy P., Borand L., Kermorvant-

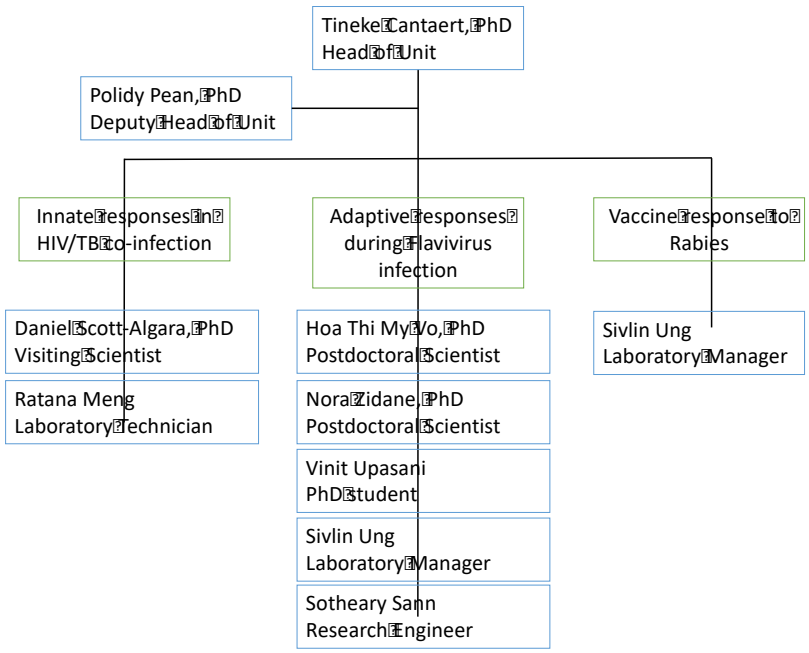
Duchemin E., Vray M., Seck A., Ndir A., Padget M., Tarantola A., Touch S., Piola P., Kerléguer A., Randrianirina F., Collard JM., Guillemot D., Delarocque-Astagneau E.

- Oxford-Pasteur Antimicrobial Workshop. Oxford, England, 25th June 2018. High prevalence of ESBL producing enterobacteriacae fecal carriage within the community in Cambodia: results of the BIRDY and PEEC NIC studies. A. De Lauzanne, N. Sreng, E. Foucaud, S. Bernabeu, T. Sok, T. Chon, C. Yem, V. Hak, P. Long, S. Born, M. Nadimpalli, S. Le Hello, MAN. Rabenandrasana, JM. Collard, M. Gouali, S. Heng, S. Meng, A. Kerléguer, P. Piola, M. Inghammar, E. Delarocque-Astagneau, BT. Huynh, T. Naas, L. Borand.
- 1st National AMR conference in Cambodia. Phnom Penh, 23rd May 2018. Prevalence and Risk Factors for Early ESBL-Producing Enterobacteriaceae Fecal Colonization Among Newborns from Cambodian Community Settings. Preliminary Results of the PEEC NIC Study. de Lauzanne A., Bernabeu S., Chon T., Pring L., Chan M., Gouali M., Borand L., Huynh BT, Delarocque-astagneau E., Naas T.
- 1st National AMR conference in Cambodia. Phnom Penh, 23rd, May 2018. Meat and fish as sources of exposure to ESBL-producing Escherichia coli in Cambodia. M Nadimpalli, Y Vuthy, A de Lauzanne, L Fabre1, A Criscuolo, M Gouali, BT Huynh1, T Naas, T Phe, L Borand, J Jacobs, A Kreleguer, P Piola, D Guillemot, S Le Hello, E Delarocque-Astagneau, On behalf of the BIRDY study group.
- CROI Conference 2018, March 4-7, Boston, USA “Systematic Vs Test-guided tuberculosis treatment: Data of the STATIS randomized trial” François-Xavier Blanc, Anani D. Badje, Maryline Bonnet, Delphine Gabillard, Eugène Messou, Conrad Muzoora, Sovannarith Samreth, Duc Bang Nguyen, Laurence Borand, Anaïs Domergue, Naome Natukunda, Serge Eholie, Serge Domoua, Xavier Anglaret, Didier Laureillard.
- Antibiotic Resistance: from research to action. 59th Colloquium of the Institute of Tropical Medicine. 5-7th Dec. 2018. Evidence for meat and fish as sources of community exposure to ESBL-producing Escherichia coli in Cambodia. M. Nadimpalli, Y. Vuthy, A. de Lauzanne, L. Fabre, A. Criscuolo, M. Gouali, BT. Huynh, T. Naas, T. Phe, L. Borand, J. Jacobs, A. Kerléguer, P. Piola, D. Guillemot, S. Le Hello, E. Delarocque-Astagneau.

3.3 IMMUNOLOGY UNIT

3.3.I FUNCTIONAL STRUCTURE OF THE UNIT

Tineke Cantaert, PhD, Head of Unit
Polidy Pean, PhD, Deputy Head of Unit
Thi My Hoa Vo, PhD, Postdoctoral Researcher
Nora Zidane, PhD, Postdoctoral Researcher
Vinit Upasani, MSc, PhD student
Sivlin Ung, MSc, Laboratory Manager (end of contract: October 31, 2018)
Sotheary Sann, MSc, Research Engineer
Ratana Meng, Laboratory Technician



3.3.2 RESEARCH PROGRAMS-MAJOR ACHIVMENT IN 2018

Axis 1: Adaptive Immune responses During Flavivirus Infection

Introduction:

Dengue viruses (DENV) infect up to 390 million individuals each year, of which 500 000 cases require hospitalization. Since 2012, dengue is the most important vector-borne viral disease of humans and likely more important than malaria globally in terms of morbidity and economic impact. The mosquito vectors, *Aedes aegypti* and *Aedes albopictus* both thrive well in populated urbanized areas, contributing to the spread of DENV. DENV is a member of the family *Flaviviridae*, and consists out of 4 related serotypes (DENV-1 to DENV-4). Dengue virus infection results in a range of clinical outcomes, from asymptomatic infection, to classic dengue fever (DF), to dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). Most primary infections are mild and probably provide lifelong protection against the infecting serotype. In contrast, secondary infection with a heterologous DENV serotype can result in more severe dengue, suggesting that primary DENV infection triggers a host memory immune response that can result in either protection or enhancement of subsequent infection. Recently, enhanced risk of hospitalization has been attributed to the presence of low titers of anti-dengue IgG in longitudinal studies. Due to the incomplete understanding of the relevant adaptive immune responses leading to protection or enhancement of disease in secondary infection and the absence of conclusive biomarkers for protection, vaccine development has been severely hampered.

AIM1: Investigation of immune responses in asymptomatic DENV-infected individuals

Most DENV infections cause few or no symptoms. Asymptomatic DENV-infected patients provide a unique opportunity to decipher the host immune responses leading to virus elimination without negative impact on the individual's health. We used an integrated approach of transcriptional profiling and immunological analysis comparing a Cambodian population of strictly asymptomatic viremic individuals with clinical dengue patients.

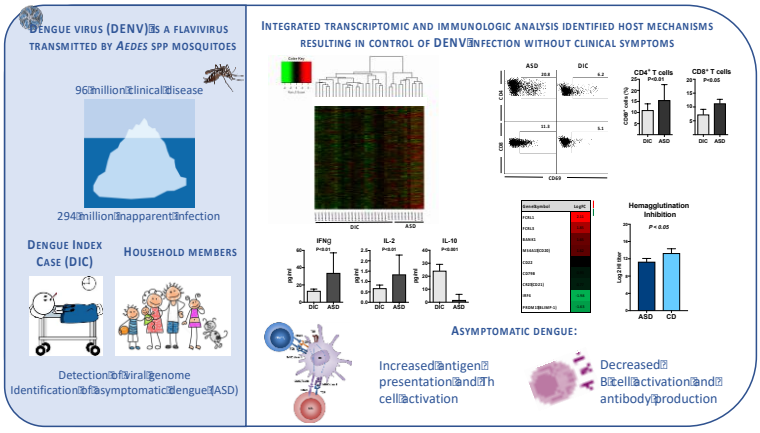


Figure 1: Graphical abstract of Simon-Loriere, Duong *et al*, Scie Transl Med, 2017 (*20*).

Whereas inflammatory pathways and innate immune responses were similar between asymptomatic individuals and clinical dengue patients, expression of proteins related to antigen presentation and subsequent T and B cell activation pathways were differentially regulated, independent of viral load or previous DENV infection (Figure 1). Taken together, our data illustrate that symptom-free DENV infection in children is determined by increased activation of the adaptive immune compartment and proper control mechanisms leading to elimination of viral infection without excessive immune activation, having implications for novel vaccine development strategies.

In 2018, we have repeated the field study in order to collect a new cohort of asymptomatic, acute infected individuals and a cohort of hospitalized patients in collaboration with the Virology Unit and Epidemiology and Public Health Unit, IP Cambodia. We screened a total of 1213 individuals in a household based cluster investigation, of which 58 individuals were DENV+ by RT-qPCR. In addition, we screened 84 patients with dengue-like symptoms at hospital admittance, of whom 39 were DENV+ by RT-qPCR.

	Individuals included/ DENV+	classification	Serotype (DENV1:2:3:4:UN)	Immune status (prim:sec:UN)
Asymptomatic cohort	1213/58 (0.05%)	14 asymptomatic 44 mild disease	3:3:0:5:2	8:5:1
Hospitalized cohort	84/39 (46%)	32 Dengue Fever 7 Dengue Hemorrhagic Fever	5:21:1:11:1	9:27:4

Table 1: Biobanked samples in 2018

Project Ongoing

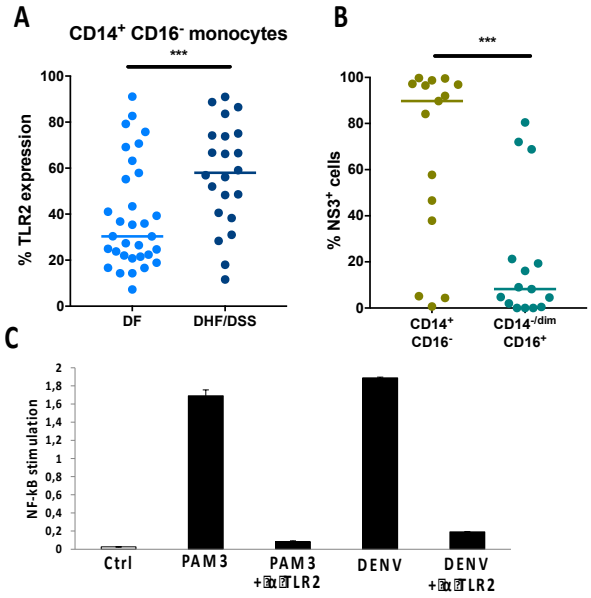
Collaboration with: Virology Unit, IP Cambodia (Philippe Dussart, Veasna Duong), Epidemiology and Public Health Unit, IP Cambodia (Sowath Ly)

Funding: HHMI-Wellcome Trust International Research Scholars Program

AIM2: Define novel biomarkers of severe disease early in the disease course.

Early detection of severe cases will help to identify patients that benefit from intensive therapy. Until today, no prognostic marker has been identified and early diagnosis relies on multi-parameter interpretation by the health care provider. We aim to identify novel biomarkers predictive for the development of severe dengue within 72 hours after onset of symptoms in a clinically relevant setting.

We have identified an 18-gene RNA signature that can detect severe cases among young Cambodian secondary-infected dengue patients at hospital presence. This signature can yield new pointers into the underlying pathogenesis of severe disease. In this cohort, we have presented evidence that the detection is robust for both PBMCs and whole blood (*22*).



In a separate project, we could show that enhanced TLR2 expression on circulating monocytes is associated with severe disease at the early stages of disease development. In addition, increased expression of TLR2, an innate sentinel, generally associated with bacterial infections, leads to an increase in DENV-infected cell mass (Figure 2). In addition, our functional analyses uncover the ability of TLR2 to sense DENV infection and to fuel infection-mediated inflammatory responses leading to activation of the human vascular endothelium.

Figure 2: TLR2 expression on monocytes is associated with more severe disease and facilitates DENV infection. A: Increased percentages of TLR2 on classically activated monocytes of DENV-infected patients as determined by flow cytometry. B: Detection of DENV non-structural protein 3 in classically activated monocytes from DENV-infected patients by flow cytometry. C: NF-κB activation in HEK-BlueTM hTLR2 reporter cells pretreated for 2h with αTLR2 before exposure to PAM3 (positive control) or DENV2 for 24h. Manuscript submitted in revised form.

Project Ongoing

Collaboration with: Virology Unit, IP Cambodia (Philippe Dussart); Department of Molecular Medicine, Instituto Pasteur Rome (Carolina Scagnolari); Department of Virology, Groningen University (Izabella Rodenhuys-Ziebert)

Funding: HHMI-Wellcome Trust International Research Scholars Program

Axis 2: Innate responses to HIV/TB co-infection

Aim 1: miRNA as predictor and/or prognosis marker of IRIS and morbidity of HIV infection with tuberculosis

MicroRNAs (miRNAs) are small, typically 22 nucleotides, non-coding (nc), endogenous, single-stranded RNAs. miRNAs have been reported as powerful regulators of post-translational gene expression. It could be used as biomarkers in many diseases including infectious diseases. The role of miRNAs in HIV disease is yet to be completely defined. Host miRNAs target certain HIV genes, thus can affect HIV replication and participate in viral control. A set of miRNA expression can characterize HIV disease phenotype, as has been shown in HIV elite controllers. Several study have been characterized miRNA from *Mycobacterium tuberculosis* infected individuals but, so far, common biomarkers have not been identified. However, the studies of miRNA in acute HIV infection and co-infections like tuberculosis are lacking. In addition, there is a great need of using plasma miRNA as biomarkers in clinical application. One of the limitations of detection of miRNA is the technique, which is time consuming and required the specialized laboratory equipped with molecular technique (qPCR, high throughput sequencing technique). Flow cytometry has been developed in routine clinical laboratory and is well-standardized technique. For the routine detection of miRNA, flow cytometry could be the best way to perform high throughput screening with affordable price.

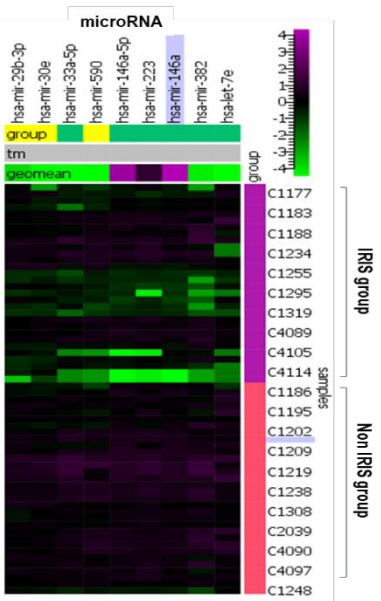


Figure 3: Heat map represents plasma microRNA expression profile in IRIS versus non IRIS patient

In this study, our aims are to evaluate by flow cytometry whether circulating miRNA pattern might be applicable as potential biomarkers in HIV disease. To assess the miRNA expression profile in a cohort of HIV and tuberculosis co-infected patients and correlate it with their clinical evolution and the occurrence of immune reconstitution inflammatory syndrome (an inflammatory reaction to tuberculosis following antiretroviral treatment in severely immunosuppress HIV patient). From March 2018, we enroll, as control group, 20/20 healthy donor, 20/20 HIV mono-infected patients CD4 count less than 200mm3 and 17/20 tuberculosis without HIV infection at VCCT of Pasteur Institute in Cambodia and Sihanouk Hospital Center of Hope in Phnom Penh. Frozen plasma of 74 HIV-TB co-infected (35/74 IRIS) enrolled during CAPRINK/CAMELIA study were assess for 27 microRNA expression (in plasma and exosome) profile using kit Fireplex miRSelect (Abcam) by flow cytometry. Preliminary, we observed 9/27 microRNA tested were significantly lower in HIV-TB co-infected patient who had experienced of IRIS when compared with those who had no IRIS (Cf. figure). Thus, a dysregulation of microRNA expression may play a role in occurrence of IRIS syndrome in HIV/TB co-infected patients following antiretroviral treatment.

Project Ongoing

Collaboration with: Lymphocyte Cell Biology Unit, IP Paris (Daniel Scott-Algara), Epidemiology and Public Health Unit, IPC (Laurence Borand)

Funding: ANRS

Axis 3: Humoral responses to Rabies vaccination

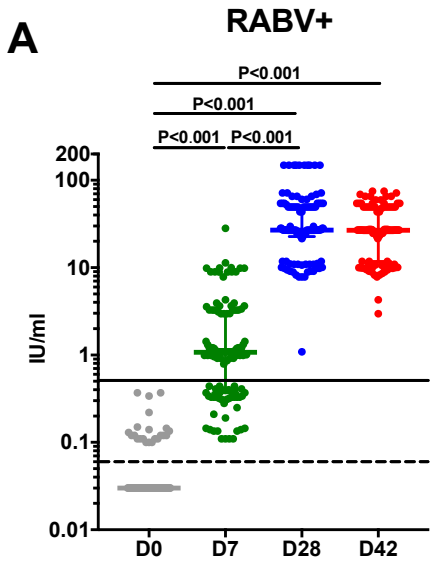


Figure 4: Rabies virus neutralizing antibody titers reach protective levels in all vaccinated individuals at D_{28} and are not different from levels at D_{42}. Neutralizing antibody titers as determined by RFFIT of patients bitten by DFAT-confirmed rabies-infected dogs (n=116). Manuscript submitted.

The international health authorities are backing an effort to eliminate canine-mediated rabies in humans by 2030. This will require improving access to adequate and timely post-exposure rabies prophylaxis (PEP) as the compliance is low with previously WHO recommended regimens (4 to 5 visits, one month). Access could be dramatically improved by an abridged regimen to reduce doses, direct and indirect costs and improve vaccine equity by better sharing available vaccine. In this project, we assessed rabies virus neutralizing antibodies using rapid fluorescent focus inhibition test in 116 persons bitten by dogs with laboratory-confirmed rabies and 20 control individuals. Percentages of circulating plasmablasts were determined by flow cytometry. All individuals had referred to the rabies prevention clinic at Institut Pasteur in Cambodia (IPC) and received two-dose intradermal PEP at days 0, 3, 7 and 28 ("Thai Red Cross regimen") with or without equine rabies immunoglobulin (eRIG), as per 2010 WHO recommendations. We could show that all individuals demonstrated rabies virus neutralizing antibody titers considered protective (≥ 0.5 IU/ml) and plasmablast activation at day 28, immediately before the last injection. Protective titers were reached notwithstanding eRIG use, age, sex, nutrition status or dog infective status. Titers or plasmablast percentages did not increase between the day of the last injection and two weeks later. Therefore, we can conclude that the fourth vaccine session at day 28 provides no additional benefit. Rabies PEP can be abridged to a two-dose, three-sessions, one week (D0, D3, D7), dose-sparing intradermal "IPC" regimen to improve PEP coverage and equity at no risk to patients. Based on these results the WHO endorsed changes in its April 2018 guidelines. This "IPC protocol" is the first one-week PEP regimen to be recommended and the shortest and most vaccine-sparing rabies PEP protocol endorsed. Above all, it will reduce direct cost of vaccination, transportation and other indirect costs to vaccines and therefore now constitutes the most cost-effective regimen for PEP.

Project Finalized

Collaboration with: Virology Unit, IP Cambodia (Philippe Dussart), Epidemiology and Public Health Unit, IP Cambodia (Arnaud Tarantola, Sowath Ly); Lyssavirus epidemiology and neuropathology Unit, Institut Pasteur Paris (Herve Bouhry)

Funding: Institut Pasteur International Network

3.3.3 RESEARCH PROGRAMS –PROSPECT 2019

Axis 1: Adaptive Immune responses During Flavivirus Infection

Characterization of adaptive immune responses in asymptomatic DENV infected individuals

In order to confirm and extend our previous observations (see AIM1), we have collected additional samples of asymptomatic dengue-infected individuals during the 2018 dengue transmission season. The availability of PBMC from asymptomatic individuals will allow us to further characterize in depth by novel cutting edge techniques the humoral B cell responses, antibody-independent B cell functions and T cell responses in these individuals.

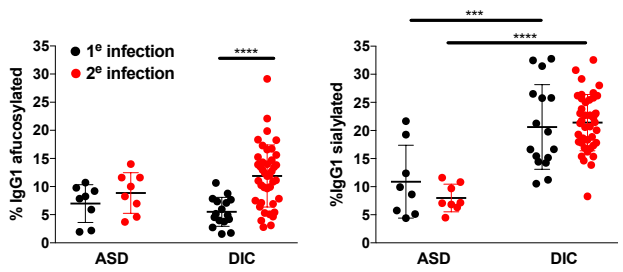


Figure 5: Glycosylation profling in asymptomatic and hospitalized cases. A. Increase of afucosylated total IgG1 in secondary infection is only observed in hospitalized cases. **B.** Total IgG from hospitalized dengue index cases show decreased abundance of sialylated IgG. ASD : asymptomatic dengue, DIC : hospitalized dengue index cases. Mean and standard deviation are shown. Data generated in collaboration with Prof. Jeffrey Ravetch, Rockefeller University, USA. Mean and SEM are shown. *** P < 0.001, **** P< 0.0001

Antigen specificity, crossreactivity and neutralization will be evaluated by micro neutralization assays, ELISA and droplet microfluidics using a whole array of DENV-derived antigens (obtained in collaboration with Institut Pasteur Paris). Antibody effector functions will be evaluated by *in vitro* cell based assays (including antibody-dependent enhancement (ADE)) and glycosylation pattern of antigen-specific antibodies will be interrogated by nanoLC-MS/MS in asymptomatic individuals and hospitalized cases (Figure 5,6).

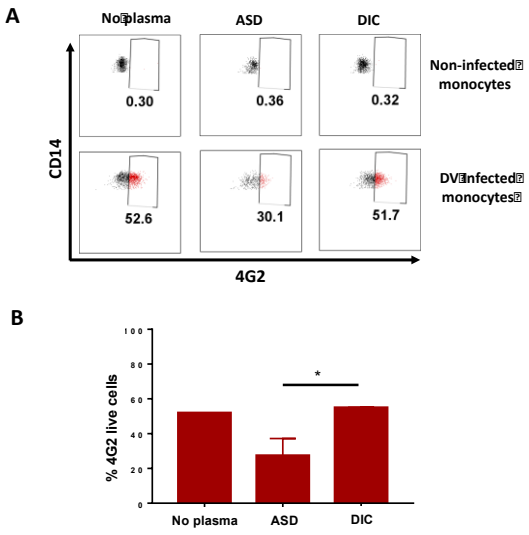


Figure 6: Increased ADCC activity in asymptomatic dengue-infected individuals. Co-cultures of DENV1-infected primary monocytes were incubated with primary NK cells and plasma from acute infected asymptomatic individuals (N=4) or hospitalized DIC (N=3) undergoing secondary infection. No plasma control was added to correct for spontaneous killing. **A.** Representative dotplots where percentage of infected $CD14^+$ monocytes was determined by flow cytometry. **B.** Bar graph summary of the data showing mean and SD. *P<0.05

T cell responses in asymptomatic individuals shall be investigated by single-cell RNAseq, functional assays such as Treg suppression assays and cytotoxic T cell assays. DENV-specific T cells will be enriched after peptide stimulation and TCRVβ repertoire will be analyzed by next generation sequencing.

Collaboration with: Structural Virology Unit, IP Paris (Giovanna Barba-Spaeth); Antibodies and Therapy Unit, Institut Pasteur (Pierre Bruhns); Laboratory of Molecular Genetics and Immunology, Rockefeller University, NY, USA (Jeffrey Ravech); Laboratory of translational Immunomodulation, Hasselt University, Belgium (Markus Kleinewietfeld)

Funding: HHMI-Wellcome Trust International Research Scholars Program.

Identification of novel mechanisms contributing to DENV immunopathogenesis.

In the blood, DENV is tropic for monocytes and dendritic cells (*25-27*) and a few reports have suggested that B cells support viral replication (*28-30*). The consequence of B cell infection by DENV on B cell functionalities remains unknown. B cells are not only precursors of plasma cells, but perform other functions such as antigen presentation, cytokine production and immunomodulation, as shown by us and others (*31-33*). These functions could be altered due to DENV infection. We are able to detect DENV-infected B cells in a patient cohort of Cambodian children (Figure 3). In addition, we have setup an *in vitro* model investigating DENV infection in primary human B cells, aiming to identify possible mechanisms of viral entry and consequences of DENV infection on B cell functions.

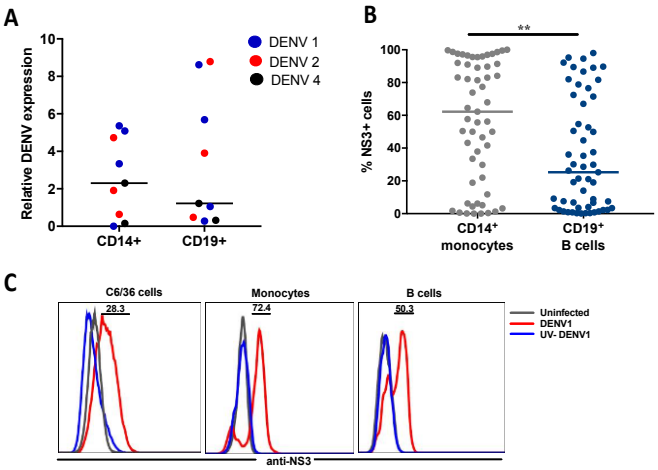


Figure 4: B cells are susceptible to DENV infection in vivo. A: Detection of viral RNA in sorted monocytes and B cells from DENV-infected patients. B: detection of DENV non-structural protein 3 in monocytes and B cells from DENV-infected patients by flow cytometry. C: Presence of NS3 protein indicates successful infection as in vitro infection with inactivated UV-DENV in insect cells, B cells and monocytes treated with UV-DENV does not show any anti-NS3 reactivity.

Project Ongoing

Collaboration with: Virology Unit, IPC and University of Groningen, The Netherlands

Funding: Institut Pasteur International Network (G4 and Calmette-Yersin)

Axis 2: Innate responses to HIV/TB co-infection

Lowering interleukin-1 receptor antagonist concentrations after TB treatment onset: A proof of concept study in Cambodia and Ivory Coast

Tuberculosis (TB) is one of the most common opportunistic infections among HIV-infected patients especially in resource-limited countries. In Africa and Asia, TB is also the most common cause of mortality in HIV-infected population. Diagnosis of pulmonary TB currently relies on evaluation of clinical symptoms, X-ray and detection of *Mycobacterium tuberculosis* (Mtb) in respiratory samples such as sputum. However, sputum smears are poorly sensitive and a high proportion of TB cases are smear negative. Although nucleic acid amplification tests are more sensitive for diagnosing TB, numerous TB cases remain undiagnosed. Thus, additional tools are urgently needed not only to help diagnose TB, but also to assess the response to TB treatment. Monocytes and macrophages are innate immune cells that play a pivotal role in the pathogenesis of TB. Activated monocytes release soluble forms of receptors including CD14 and CD163, and IL-1 receptor antagonist, IL-1Ra, a competitive inhibitor of the pro-inflammatory cytokine IL-1. In a pilot study with Cambodian patients from the CAMELIA clinical trial, we found that IL-1Ra plasma concentrations dropped dramatically after two months of TB treatment. **The objective of this proof-of-concept study is to demonstrate that IL-1Ra concentrations significantly decrease within two weeks following TB treatment initiation in adults with documented TB**. We hypothesize that, in response to the rapid mycobacterial load decrease following effective TB treatment, monocytes will be less activated and consequently plasma concentrations of monocyte activation biomarkers will rapidly decrease after treatment initiation. 100 adults with documented active TB will be enrolled in 2 ANRS sites, Cambodia (n=50) and Côte d'Ivoire (n=50).

Project Ongoing

Collaboration with: INSERM UMR 1149 (Laurence Weiss), Ivory-Coast PACCI/MEREVA (Raoul Moh)

Funding: ANRS

3.3.4 SUPPORT TO NATIONAL AUTHORITIES

Tineke Cantaert, PhD and Polidy Pean, MD, PhD: Part of steering committee international Master infectiology (University of Health Sciences, Phnom Penh, Cambodia and Universite Paris Saclay, Paris, France). Coordinators of Immunology Module in Master year 1.

Vaccine responses to rabies Post-Exposure Prophylaxis: Based on the results discussed here the WHO endorsed changes in its April 2018 guidelines. This “IPC protocol” is the first one-week PEP regimen to be recommended and the shortest and most vaccine-sparing rabies PEP protocol endorsed (https://www.who.int/rabies/resources/who_wer9316/en/). Above all, it will reduce direct cost of vaccination, transportation and other indirect costs to vaccines and therefore now constitutes the most cost-effective regimen for PEP. This PEP-vaccination scheme has been implemented in the rabies vaccination centers of Institute Pasteur du Cambodge.

3.3.5 TEACHING AND TRAINING

Tineke Cantaert, PhD and Polidy Pean, MD, PhD:

1/ 10 hours/year each of teaching in the Immunology Module, Master Medical Biology, University of Health Sciences

2/ Member of the Steering Committee International Master Infectious Diseases and coordinator of the Immunology Module.

3/ Member of Organizing committee and teaching at “Innate Immunity to Infectious Diseases” course (October 22-November 02 2018), Institut Pasteur International Network, Phnom Penh Cambodia

PhD student:

Vinit UPASANI 2017-2019: The student is supported by a Calmette-Yersin grant from the Institut Pasteur International Network. The student is enrolled at the University of Groningen, The Netherlands.

Internship/thesis students:

Thimoro CHENG, Northern Illinois University, USA: Bachelor Biochemistry: June-August 2017 and June-August 2018
Sokhoun ENG, Royal University of Phnom Penh, Cambodia: Bachelor thesis Biochemistry: January-June 2018
Axelle VANDERLINDEN: University of Antwerp, Belgium: Master thesis Biomedical Sciences: November 2018-June 2019
David GUERRERO GOMEZ: University of Antwerp, Belgium: Master thesis Biomedical Sciences: November 2018-June 2019
Sokchea LAY: University of Health Sciences, Phnom Penh, Cambodia: Master thesis Biomedical Sciences: October 2018-May 2019

3.3.6 PUBLICATION LIST

Awards and Grants Approved in 2018

- Southeast Asia-Europe Joint funding scheme for Research and Innovation (03/2018-02/2020) DEZI: A single component pentavalent dengue-zika vaccine preventing antibody-dependent enhancement PI: Anavaj Sakuntabhai Co-investigator: Tineke Cantaert
- ANRS12358 (2019-2020): miRNA as predictor and/or prognosis marker of IRIS and morbidity of HIV infection with tuberculosis PI: Daniel Scott-Algara, Co-investigator: Polidy Pean

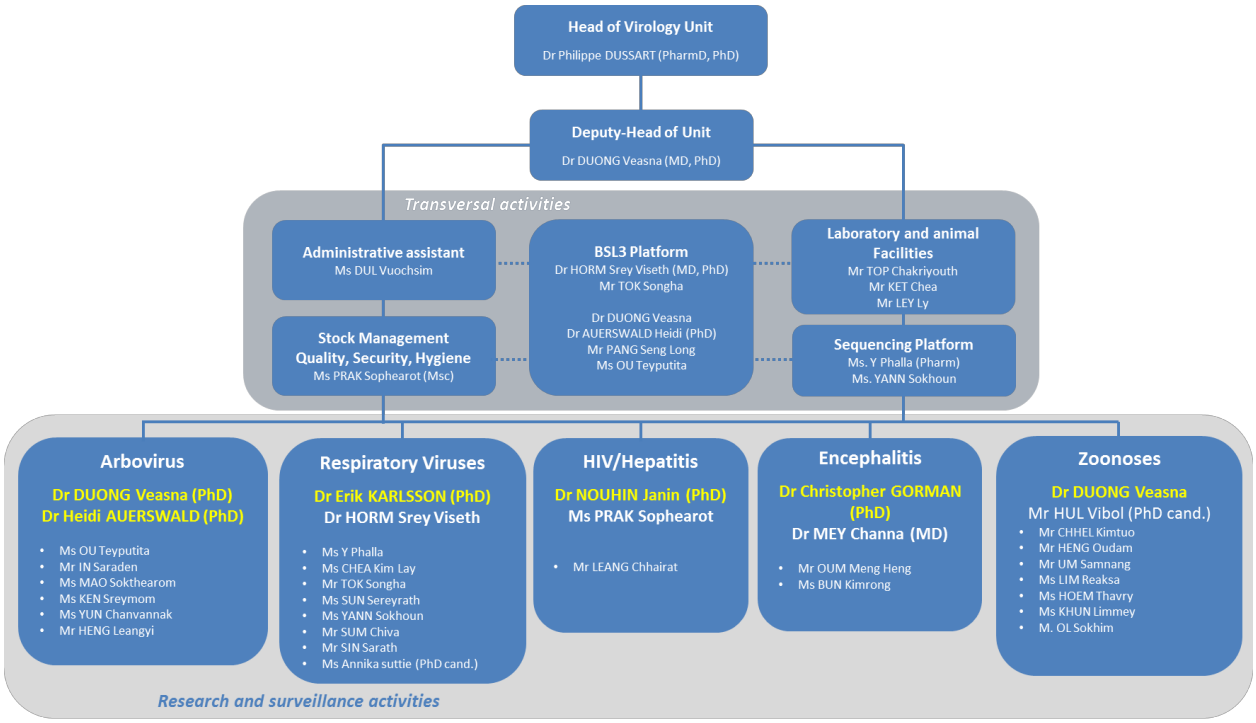
Publication list 2018

1. Nikolayeva I, Bost P, Casademont I, Duong V, Koeth F, Prot M, Czerwinska U, Ly S, Bleakley K, Cantaert T, Dussart P, Buchy P, Simon-Loriere E, Sakuntabhai A, Schwikowski B. A Blood RNA signature detecting severe disease in young dengue patients at hospital arrival. J Infect Dis 2018, J Infect Dis. 2018;29(384). doi: 10.1093/infdis/jiy086.
2. Manet C, Roth C, Tawfik A, Cantaert T, Sakuntabhai A, Montagutelli X. Host genetic control of mosquito-borne Flavivirus infections. Mamm Genome. 2018 Aug;29(7-8):384-407. doi: 10.1007/s00335-018-9775-2. Epub 2018 Aug 25. Review.

3.4 VIROLOGY

3.4.1 FUNCTIONAL STRUCTURE OF THE UNIT

The activities of IPC's Virology Unit are directed towards biomedical research and the surveillance/monitoring of infectious diseases. They can be divided into five main components: (1) arboviruses (ex: dengue, Zika, chikungunya and Japanese encephalitis), (2) respiratory syndromes (mainly seasonal and avian influenza), (3) HIV and viral hepatitis (since January 2018), (4) viral encephalitis (Japanese encephalitis, EV-A71), and (5) zoonotic and emerging pathogens (ex: coronaviruses and Nipah virus). Six senior researchers are involved in these surveillance and research activities with approximately a total of 40 persons working in Virology Unit. Within each of these topics, the Virology Unit has developed numerous research programmes. Most of these programmes are conducted in collaboration with the IPC's Epidemiology and Public health Unit (EPH). All these programmes focus on infectious diseases of interest to the Cambodian population.



3.4.2 RESEARCH PROGRAMMES – MAJOR ACHIEVMENTS IN 2018

3.4.2.1 ARBOVIRAL DISEASES

DENFREE study

Virology Unit Team leader: V. Duong

DENFREE is a study funded by the European Union (FP7) that ended in 2016 with the objective of examining dengue infections from different angles, including immunology, genomic evolution, human genetic predisposition, entomology, diagnostic development, etc. with a special focus on asymptomatic infections. The work led by the Virology Unit at IPC (in collaboration with Etienne Simon-Lorière, IP Paris) is ongoing with the following objectives: (1) to study genetic characteristics of DENV in relation to DENV evolution, (2) to study the evolution variations in vector and in human host, and. DENV strains obtained from human and mosquitoes during an outbreak in Kampong Cham in 2012-2013 are being sequenced using High Throughput Sequencing HTS technology at IP Paris. Preliminary results obtained in 2017 are highlighted by the co-detection of dengue virus and Phasi Charoen-like virus (PCLV), a mosquito-specific virus. More sequencing are on-going to confirm this preliminary observation. Primers and probe were designed to detect and confirm the presence of PCLV in *A. aegypti* and *A. albopictus* collected in Cambodia. All of *A. aegypti* mosquitoes were infected with MSV while the virus was present only in a small number of *A. albopictus*.

Partners: S. Ly and coll. (EPH Unit), A. Sakuntabhai, R. Paul, E. Simon-Lorière (IP).

Financial support: IPC – Institut Pasteur, Paris.

ACIP DEN-Gen: Dengue virus genotype replacements: investigating viral fitness differences driving the evolution of dengue epidemics

Virology Unit Team leader: V. Duong

This project aims to better understand the evolutionary mechanisms driving DENV genotype replacements typically observed during the course of dengue epidemics. Understanding the causes and consequences of genotype replacements has implications for vaccine design because DENV lineages may differ in their antigenic properties. The specific objectives of this study are: (1) Characterize recent DENV genotype diversity and evolutionary dynamics in Cambodia and New Caledonia in relation with the epidemiological profile; (2) evaluate the potential role of vector-driven selection in DENV genotype replacements by investigating vector-virus interactions *in vivo*; (3) measure the relative ability of DENV genotypes to replicate in mammalian cells *in vitro* and to produce subgenomic flavivirus RNAs (sfRNAs).

For the consistency between Cambodian and New Caledonia, we chose to work on DENV-1 and looked the replacement of genotypes and/or lineages in the past 10-20 years. We observed that genotype 1 of DENV-1 was the only genotype circulating in Cambodia since year 2000. However, we often saw lineage replacement and at least 5 lineages were found co or circulating in the country. For this study, 27 DENV-1 isolates were selected among the 3 lineages of interest (lineage 3, 2005-2007, lineage 4, 2005-2016 and lineage 5, 2007-2016) and sent to IP Paris for full genome sequencing. Additionally, the isolates will be used for the experiment in objective 3. For objective 2, 2 isolates from each of the 3 lineages will be used for competition vector competence study using 50/50 virus ratio in blood feeding. Real-time RT-PCR primers and probes were designed to detect and differentiate the virus from the 3 lineages in mosquitoes.

Partners: M. Dupont-Rouzeyrol (IPNC), L. Lambrechts (IP, Paris).

Financial support: Institut Pasteur, Paris.

Dengue vaccine initiative (DVI): study on burden of dengue infection in Cambodia (2014-2016).

Virology Unit Team leader: V. Duong, P. Dussart

This project, led by the Epidemiology Unit, involved a hospital- and community-based study in two provinces (Kampong Cham and Kampot) with a total of 3000 subjects. This study took a multidisciplinary approach from the epidemiological, economic, and health-seeking behavioral perspectives to build a package of evidence for the disease burden of dengue in rural Cambodia.

Of the 2087 participants involved, 624 (30%) tested IgG positive at baseline survey. The proportions of IgG positive by village ranged from 5% to 57%. The positivity of IgG testing increased by age group from 3% among age 1-4 years to over 15% among age 25-29 years. Among the 1463 subjects with negative IgG at baseline, 260 seroconverted within approximately 6 months after baseline sample and 107 of the remaining 988 negative subjects at month 6 seroconverted by month 15. The samples from this study will be used to feed another European project, ZIKAlliance, for the investigation of seroprevalence against Zika virus (ZIKV) in Cambodian rural communities.

Partners: S. Ly and coll. (EPH Unit).

Financial support: DVI.

Detection, molecular evolution and vector competence of Zika viruses (ZIKV) from Africa, Asia and the Pacific islands

Virology Unit Team leader: V. Duong, P Dussart

ZIKV is a relatively recent topic of research in the Virology Unit, participating in the ACIP ZIKA (2014-2017) in collaboration with others Institute partner of the Institut Pasteur International Network (IPIN). The objectives of this study were to optimize and standardize ZIKV diagnostic tools, to study ZIKV diversity in Africa, South East-Asia and in the Pacific by analyzing the spatio-temporal evolution dynamic of the virus, and to evaluate the competence of local vectors to different ZIKV strains from the three contents. ZIKV was first detected in Cambodia in 2010 by NAMRU-2. Using the diagnostic tools developed in this study, we have conducted a retrospective study to look at the ZIKV circulation in Cambodia in the last 10 years (2007-2016) among patients with dengue-like symptoms. We have shown that ZIKV is endemic in the country with low impact on public health and the virus falls into the Asian genotype (Duong V et al. Emerg Infect Dis, 2017). Vector competence study of Cambodian Aedes mosquitoes (*A. aegypti* and *A. albopictus*) against African, South East-Asian and Pacific Zika strains is underway. Our preliminary data shown that ZIKV from Africa infected better Cambodia *A. aegypti* mosquitoes than ZIKV strain from Cambodia and New Caledonia.

Partners: M. Dupont-Rouzeyrol (IPNC), O. Faye (IPD), M. Grandadam (IPL), VM. Cao-Lormeau (Institut Louis Malardé) and AB Failloux (IPP).

Financial support: Institut Pasteur, Paris (ACIP).

Zikalliance

Virology Unit Team leader: V. Duong

Zikalliance is a large consortium composed of 49 organizations and institutes and is funded by the EU (H2020). IPC participates in WP6 and the Virology Unit works on Task 6.2 while Task 6.1 is under the IPC Entomology Platform. WP6 associates entomologists, virologists, bacteriologists and immunologists to understand the complexity of arbovirus transmission cycles that can lead to emergence. Our strategy is be based on: (1) identification of vectors involved in ZIKV transmission, (2) assessment of vector competence of different mosquito (wild and domestic) populations, (3) understanding interactions between ZIKV and other flaviviruses with the potential consequence of selecting new epidemic variants of ZIKV, (4) interactions of ZIKV with vectors/vector cells, and (5)

appraisal of insecticide resistance and design of alternative strategies for the control of disease transmission.
These objectives will be achieved through the development of two specific tasks in Cambodia: (1) to define the mosquito species that are involved in urban transmission of ZIKV in Cambodia, and to examine wild mosquitoes in rural environments with a special emphasis at the interface with forested areas, in ZIKV-endemic countries (e.g. Cambodia), and (2) to define the vector competence of different mosquito species to ZIKV (mainly *Aedes*). After establishing standardized protocols for vector competence studies with other partners of the consortium in 2017-2018, mosquitoes collected from different Cambodian areas (urban versus rural areas) will be tested in 2019. Mosquito colonies, *Aedes aegypti* from urban vs. urban (Phnom Penh and Siem Reap) and *Aedes albopictus* from rural vs. urban (Mondulkiri and Phnom Penh) areas were established to study their vector competence to transmit ZIKV from Africa (Senegal), Asia (Malaysia) and South America (Martinique).

Partners: Zikalliance Consortium, S. Boyer (Entomology Unit, IPC).

Financial support: European Union.

3.4.2.2 AVIAN INFLUENZA VIRUSES

Virology Unit Team leader: E. Karlsson

Avian influenza in Cambodia: molecular characterisation of HPAI A(H5N1) virus, virus evolution, drug resistance, Animal-Human-Environment interface survey for HPAI in Live Birds Markets (LBMs)

As the Cambodian National Influenza Centre (NIC) and WHO H5 Reference Laboratory (H5RL), IPC supports the Cambodian Ministry of Health (MoH) and Ministry of Agriculture, Forestry and Fisheries (MAFF) in the confirmation of influenza infections in humans and animals. In 2018, Cambodia experienced seven (7) outbreaks of avian influenza – all subtype A(H5N1) in poultry in Cambodia. Sample screening and detection of A(H5N1) was initially done by the National Animal Health and Production Research Institute (NAHPRI). All influenza positive samples were sent to IPC for confirmation and viral characterisation. Our laboratory testing confirmed the detection of A(H5N1) and sequence analysis of the outbreak strains showed that the viruses were closely related to strains circulating in live bird markets in 2015, 2017 and 2018. Analyses are ongoing and a manuscript detailing connections between seasonality, environmental factors, sociological factors, outbreaks and market samples is in preparation.

- ***Full genome analysis of Cambodian A/H5N1 strains***

To date, full genome A(H5N1) sequences have been generated from influenza strains collected in 2015 and 2016 from the LBMs and isolated in embryonated eggs. Full genome sequences have also been completed for a number of other A(H5N1) strains from 2017 and 2018 with several single HA, NA and M gene sequences also generated. All A(H5N1) sequences generated have clustered with Clade 2.3.2.1c, which was first detected in Cambodia in March 2014. We hypothesise that the introduction of this strain resulted in the replacement of the reassortant Clade 1.1.2 virus, which was associated with a dramatic increase in human cases. Analysis of the deep sequencing data is ongoing between IPC, Hong Kong University and Dr Thomas Friedrich's laboratory at the University of Wisconsin. Through these analyses we are attempting to determine the mutation rate of A(H5N1) when the virus infects humans, compared to a natural host such as a duck. This will provide important information about the emergence risk of mammalian-adapted A(H5N1) strains following human infection. These analyses have been completed and a publication is in preparation for submission in second quarter of 2019.

- ***Sequencing of market environmental samples to investigate the diversity of influenza viruses circulating in Cambodian poultry***

Since 2011, we have conducted LBM surveillance in Orussey market in Phnom Penh to determine the circulation characteristics of avian influenza in Cambodia. Isolates from 2015 to 2018 have been transferred to the WHOCC in Melbourne for full genome sequencing using NGS and have been completed. These analyses will reveal important information about the rate of reassortant events

occurring in LBMs and the risk of emergence of novel AIV strains. Final samples from late 2018 are in final stages of being isolated and surveillance will continue into 2019.

- ***Risk of aerosol exposure to avian influenza viruses in Cambodian live bird markets***

In February 2016, we started a preliminary study to investigate the risk of A(H5N1) aerosol exposure to workers and customers on Cambodian LBMs, where we had conducted previous A(H5N1) research. We used a pump and filtration system that actively filters the air for live virus particles. The system was used to determine the size of the particles to which the viruses were attached. Such system could provide important information on how widely the particles may disperse and determine the threat level of infection deep in the respiratory tract of people working on LBMs. The study has been completed and data is currently being analysed for publication early mid-2019.

- ***Poultry market supply chain study in Phnom Penh, and upstream evaluation of avian influenza viruses' circulation***

In 2017, we began a study to establish the market supply chain of poultry for the main markets in Phnom Penh and Takeo provinces. This study is also designed to determine if there are hotspots of A(H5N1) circulation that can be targeted for intervention. In this study, we propose to map in finer details of the supply networks than has previously been attempted and to establish the supply areas where there is a higher prevalence of avian influenza viruses. During 2017 and 2018, we were able to track the movements of 7 middlemen using GPS/GSM tracking devices and to collect poultry samples at different stages of their transportation, from provincial or district markets and/or stock houses to the central live bird markets located in Phnom Penh and Takeo. Data collection has been completed in 2018 and final analyses are ongoing. So far, our results show a high variability in trade practices and informal activity throughout the study period. We observed both local trade as well as transboundary movement and were able to define novel locations and practices that could impact infection and dissemination of AIV. Heavy viral loads, coinfections, and a diverse range of AIVs were detected in potential hotspots located both upstream and downstream of the LBMs under surveillance. Further sequence analysis is underway to enable back tracing of AIVs. A manuscript of the findings from this study is in preparation and tracking will continue in the first half of 2019.

- ***Avian, swine, and human influenza surveillance in Cambodia border regions***

In 2017, in collaboration with the FAO and NAHPRI, we sought to establish avian and swine influenza virus surveillance in Cambodian border regions to obtain a greater understanding of the dynamics of cross-border movements of avian, swine and human influenza viruses into Cambodia, to gain an understanding of human exposure human seroprevalence studies, and to obtain molecular profiles of the circulating influenza viruses in Cambodia. During 2017 the 2018 study period, we collected 2,142 poultry samples and 1,087 swine samples with 23.6% and 6.1% influenza positivity respectively. We also collected 751 human serum samples from 237 subjects.

Analyses is complete and several manuscripts are in preparation. One manuscript is currently under review. This collaboration continues for the 2018-2019 season and sampling was initiated in late September 2018.

Investigation of avian influenza A(H5N1) outbreak among poultry in Cameroon and evidence of sub-clinical human infection

From May 2016 to March 2017, 22 poultry outbreaks of avian influenza A(H5N1) were reported in Cameroon, mainly in poultry farms and LBMs. No human cases were reported. In 2017, in collaboration with IP Cameroon, we sought to describe the 2016 A(H5N1) outbreak strain and to investigate the risk of infection in exposed individuals. We found that highly pathogenic influenza subtype A(H5N1), clade 2.3.2.1c from Cameroon is closely related phylogenetically and antigenically to strains isolated in central and western Africa at the time. No molecular markers of increased human transmissibility were noted; however, evidence of infection was detected in some poultry workers. This study was accepted for publication in late 2018.

Partners: S. Ly, M. Chan (EPH Unit), MoH, S. Tum (NAHPRI), WHO, K. Osbjer (FAO), US-CDC, NIPH, Kantha Bopha Hospital, NAMRU-2, R. Njouom (Centre Pasteur du Cameroun).

Financial support: US DHHS – USAID – FAO – WHO – IPC.

3.4.2.3 HIV AND VIRAL HEPATITIS

Virology Unit Team leader: J. Nouhin (until November 2018)

TA PROHM STUDY – ANRS 12345: Test and treat strategy using rapid test and tenofovir treatment to prevent hepatitis B virus (HBV) transmission for HBV-infected pregnant women with positive HBeAg in Cambodia

This study aims to evaluate the effectiveness of a strategy based on the use of RDT for the diagnosis of HBV infection in pregnant women in Cambodia and the use of a treatment by tenofovir ("test and treat" strategy). The study will be conducted with around 300 women HBeAg positive receiving tenofovir and 600 women HBeAg negative without tenofovir treatment. The first woman was enrolled in the study in October 2017. By October, 2018, 298 women (52 with HBeAg+ and 246 HBeAg-) were included in the study. Our team is involved in quantification of HBV DNA viral load.

Partners: L. Borand (Epidemiology and Public Health Unit, IPC), O. Segeral (ANRS), C. Chhun (Calmette Hospital).

Financial support: ANRS.

Characterization of gibbon HBV strains in Cambodia

The main objective of our study is to compare genetically HBV strains from wild-caught pileated gibbons housed at the Phnom Tamao Wildlife Rescue Center (PTWRC) with HBV strains from wild-living pileated gibbons. The study was conducted on plasma samples obtained from 17 captive gibbons living in 11 cages at the Phnom Tamao Wildlife Rescue Center (PTWRC) and on stool samples obtained from 22 wild-living gibbons living in Western (n=16) and Eastern (n=6) provinces of Cambodia. HBV infection status was assessed using HBV DNA and serological markers for captive gibbons and HBV DNA in stool for wild-living gibbons. To characterize and compare HBV strain in captive and wild-living gibbons, all samples with detectable HBV DNA viral load were subjected to HBV DNA amplification and sequencing in the RT-S gene (738 bp). Gibbon species were also demined based on mitochondrial cytochrome b (cytb) gene (1200 bp). Nine out of 17 (52.9%) of captive gibbons living at the PTWRC were chronically infected with actively replicative HBV (HBs Ag+ and HBe Ag+) with plasma HBV DNA VL in median: 8.25 Log10IU/mL). In contrast, only 2 out of 22 (9.1%) wild gibbons had detectable HBV DNA viral load in stools. Phylogenetic analysis revealed that HBV strains isolated from wild and captive gibbons are clustered together. In addition, our HBV gibbon strains are strongly related to other gibbon strains living in Cambodia, however, showed different profiled as compared to human strains.

Partners: N. Marx (Wildlife Alliance).

Financial support: Wildlife Alliance.

In-depth phylogenetic analysis and molecular epidemiology of hepatitis C genotypes circulating in Cambodia

Around 62 million people are living with HCV the Western Pacific region, which represent approximately one-third of all infections worldwide. The prevalence of HCV is particularly high in South-East Asia where HCV genotype 6 is the most predominant with great genetic diversity. Very few data are available in Cambodia compared to other countries in the region. We aimed to analyse phylogenetically all available NS5B sequences obtained at inclusion from HCV antibody (Ab) and HCV RNA-positive patients who were enrolled in the MSF cohort and who received Sofosbuvir and Daclatasvir (or Ledipasvir) through a 12-week regimen, as recommended by the current AASLD/IDSA HCV guidelines.

The secondary objective was to identify risk factors associated with the emergence of a predominant genotype. Between August 2016 and October 2017, 3134 patients (> 18 years old) whose HCV NS5B sequences and clinical data are available were included in the study. The average age was 55 and the F/M ratio was 1.4. In total, 46% of patients were infected with HCV genotype 1 (with the majority of subtype 1b: 94%), genotype 6 (46%), genotype 2 (4%), and non-genotype determined (4%). Sociodemographic information of patients, such as age, risk factors for infection and treatment history, was comparable between genotypes 1 and 6.

Partners: A. Kerleguer, S Heng (Medical Biology Unit, IPC), M. Iwamoto, JP. Dousset, M. Le Paih (Médecins Sans Frontières - MSF), D. Maman (MSF/Epicentre).

Financial support: MSF.

Performance characteristics of a new commercial assay for hepatitis C RNA viral load quantification in plasma in Cambodia

Since 2016, direct-acting antivirals (DAA)-based treatments for hepatitis C are available in Cambodia. Therefore, there is an urgent need for low-cost hepatitis C virus (HCV) RNA viral load (VL) assays for identifying subjects with active HCV infection and for monitoring of treatment efficacy. Here we report the performance in Cambodia of the OMUNIS PUMA HCV kit (Omunis, Clapiers, France), a new commercial low-cost (around 25-30 USD per test) HCV RNA VL test using open real-time PCR machine (lower limit of detection: 70 IU/mL), on plasma harboring mainly HCV genotype 6.

The evaluation was conducted on 185 plasma specimens including 82 HCV infected and untreated patients and 103 HCV uninfected patients, collected and stored at -80°C between 2015 and 2016 at IPC. We used as gold standard a fully automated closed Roche platform (Roche Cobas AmpliPrep/Cobas TaqMan HCV Test, v2.0). HCV genotyping was performed in the NS5B region for samples with detectable HCV RNA VL. All 82 (100%) plasma samples having detectable HCV RNA VL with the Roche TaqMan HCV kit showed detectable VL results with the Omunis PUMA HCV assay. Our results were highly correlated (correlation coefficient R = 0.87) between the two techniques, with a mean difference of 0.36±log IU/mL. HCV genotype distribution was as follows: genotype 6 (55%, mostly subtype 6e), genotype 1b (31%), genotype 2a (12%), and genotype 1a (2%). Finally, the OMUNIS assay presented an excellent (100%) specificity among 103 plasma samples having undetectable VL with the Roche test.

Partners: A. Kerleguer, S. Heng (Medical Biology Unit, IPC).

Financial support: IPC.

Delta hepatitis virus (HDV): serological screening and PCR confirmation in Cambodia

HDV which is responsible for the most severe form in patients infected with HBV, is completely neglected from screening, treatment, and care programs. The global prevalence of HDV is estimated at 15-20 million. In Vietnam, two recent studies have reported a prevalence of 10% of HDV infection in HBsAg carriers. High prevalence of total anti-Delta antibodies have been reported in Thailand and China. Data is lacking in Cambodia. This study aimed to establish the seroprevalence of HDV and to detect HDV RNA, in case of positive anti-Delta antibody. A total of 267 samples positive for HBsAg were tested for total anti-Delta antibodies using ELISA. One out of 267 samples was positive for total anti-Delta antibodies leading to a prevalence of 0.4%. HDV RNA viral load quantification and viral genotype characterization is in progress.

Partners: A. Kerleguer, S. Heng (Medical Biology Unit, IPC); E. Tuaillon (UMR Inserm 1058 Montpellier); N.E. Gordien (Avicenne Hospital, Bobigny).

Financial support: UMR Inserm 1058 Montpellier.

Seroprevalence of hepatitis E virus in Phnom Penh, Cambodia, 1996 – 2017

Hepatitis E virus (HEV) infection is endemic in Cambodia. However, little relevant data were available and there is no clue if HEV is an emerging or decreasing pathogen. Objective: To describe temporal trends of the anti-HEV IgG and IgM seroprevalence during the last two decades (1996 – 2017) in the context of population growth and urbanization in Cambodia. A total of 2004 human plasma samples collected between 1996 and 2017 were tested for anti-HEV IgG and IgM using the commercial Wantai anti-HEV assays. Relevant demographic data was recorded and assessed. Overall, the prevalence rates of anti-HEV IgG and IgM were 41.1% and 2.7%, respectively. The higher risk of HEV infection was independently associated with older age, male-gender, and having urban residency. After age and gender standardizing, the prevalence rates of anti-HEV IgG decreased from 61.3% during the 1996 – 2000 period to 32.3% during the 2016 – 2017 period, suggesting that HEV is not an emerging pathogen, but rather seems to circulate less in Cambodia. Finally, the rate of anti-HEV IgM fluctuated around the overall rate.

Partners: A. Kerleguer (Medical Biology Unit, IPC), Y. Froehlich (Epidemiology and Public Health Unit, IPC), Y. Madec (Unité d'Epidémiologie des Maladies Emergentes, Institut Pasteur), N. Pavio (UMR 1161 Virologie, Anses Laboratoire de Santé Animale).

Financial support: Institut Pasteur International Network (grant no. ACIP-3-2015).

3.4.2.4 NEUROTROPIC INFECTION

South East Asia Encephalitis Project – SEAe

Virology Unit Team leader: P. Dussart, C. Gorman

Infectious encephalitis is a world-wide public health issue, particularly in resource limited settings such as Southeast Asia due to the high level of endemic and emerging infectious diseases. Defining the etiology for encephalitis cases has proven difficult due to the wide spectrum of encephalitic pathogens circulating in the environment, and the difficulty to establish standard laboratory diagnosis. The SEAe Project has expanded on previous encephalitis studies at IPC by including a large array of tests and a more comprehensive range of patient samples. 786 patients were recruited from Cambodia, Lao PDR, Myanmar and Vietnam between 2014 and 2017. Each patient recruited into the project was tested for ~80 pathogens. Pathogens known to be endemic or where treatment is available were prioritised, and results were delivered to the physician within 24 hours. For other pathogens, samples were tested retrospectively. All laboratory tests were standardised between countries and results coupled with clinical, neurological and epidemiological data to ensure a comparable testing strategy across the region. The diagnosis of encephalitis in hospitals is supported by the national centres, by implementing a quality control programme and providing an expansive range of further testing that is applicable to both laboratory diagnosis and discovery of new or unusual pathogens. Regionally, we found Japanese encephalitits virus (JEV) is the most common etiology, followed by herpes simplex virus 1 (HSV1), Dengue virus and eneterovirus A71 (EV-A71). Of the known etiologies, 69% of pathogens were preventable and 22% treatable. Pathogen discovery (NGS) of CSF samples from patients with an unknown etiology and severe outcome revealed interesting and novel findings. The outcome of the recruited patients were: 13% death, 53% sequelae and 34% recovery. In April 2018, a 2-day final meeting was held in Phnom Penh and attended by the consortium and stakeholders. This meeting focused on the major results, their analysis and future strategies. Considering the countries selected for this project are resource-limited, SEAe capacity building in hospitals has strengthened their abilities to test for a larger range of pathogens and provided a system in which samples can be stored for additional studies. The results of this study provide scientific evidence to guide regional and national health policies.

Partners: Kantha Bopha Hospitals (D. Laurent) – SEAe consortium: IPC's Epidemiology Unit (P. Piola, JD Pommier), Institut Pasteur (M. Lecuit, O. Lortholary, M. Eloit), NIHE Hanoi, IRD (X. de Lamballerie), Oxford University Clinical Research Unit Laos (P. Newton), CIRAD (V. Chevalier).

Financial support: Pathoquest – Total Foundation – Institut Pasteur, Paris – AVIESAN Sud – INSERM – IRD.

Prevalence of HFMD and viral diversity study of enteroviruses in Cambodia and study of antigenic and immunogenic features of EV-A71

Virology Unit Team leader: P. Dussart

EV-A71 is a leading public health problem because it causes a range of illnesses from hand-foot-and-mouth disease (HFMD) to severe neurological manifestations. In 2018, the IPC virology unit, in collaboration with the Kantha Bopha hospitals, continued surveillance of hospitalized patients with non-severe or severe HFMD Syndrome with objective to better understang circulation dynamic of EV-A71 strains a regional level. In total, very few patients (n=19) were sampled with the following clinical presentation: HFMD syndrome associated with encephalitis (n=6), HFMD syndrome with encephalitis and cardiopulmonary involvement (n=13). Among these patients, 32% (n=6) had positive detection of enteroviruses (EV-A71, n=1; other HEV, n=5). Considering a previous study showing that EV71 outbreaks occurred in a cyclical pattern in Cambodia and that the virus infected large proportions of immunologically naive children every 2–3 years, we must anticipate a possible severe case upsurge in 2019, the last period of intensive EV-71 circulation associated with severe forms dating back to early 2017.

Partners: Kantha Bopha Hospitals (D. Laurent), Institut Pasteur (F. Delpeyroux, R. Volle, L. Chakrabarti), Institut Pasteur in Shanghai (F. Arenzana).

Financial support: Total Foundation.

3.4.2.5 ZOONOSES

PREDICT 2

Virology Unit Team leader: V. Duong

The PREDICT 2 project has been carried out since 2014 and is now entering its fifth year of surveillance in wild animals (bat and rodents), domestic animals and humans in 2018. Each year, 4 field work sessions (2 in dry and 2 in wet seasons) were conducted in Kampong Cham with sampling of bats from bat farms and in Kandal for animal value chains with sampling of rodents brought to the trade hub in the southern part of Cambodia near the Vietnamese border. Government partners from Forestry Administration (FA), General Directorate for Animal Health and Production (GDAHP) and Department of Communicable Disease Control (CDC) in Cambodia joined all four field missions. Besides wild animals sampling, we also conducted human surveys and domestic animal sampling supported by FAO in surrounding areas where wild animals were collected. A selection of samples have been tested at IPC for Alphavirus, Coronavirus, Filoviridae, Flavivirus, Hantavirus, Influenza A virus, Rhabdoviridae, Paramyxoviridae and Bunyaviridae. Between 2014 and 2018, samples were collected from 1183 bats, 809 rodents, 2714 domestic animals and 683 healthy people living close to animal sampling sites. The first batch of results from samples collected in year 1 (2014) was approved by the government counterpart and released to the public via the PREDICT website (http://www.vetmed.ucdavis.edu/ohi/predict/predict_what-weve-found.cfm) and can be visualized using an interactive map (http://data.predict.global/). In brief, 8.5% of bats and 36.9% of rodents were found positive for at least one of the following virus Coronavirus, Paramyxovirus, Rhabdovirus and Hantavirus. Among 16 viruses identified, 6 were known while 10 were previously unknown. A second batch of result including wildlife and domestic animal samples was recently approved. A total of 2913 wildlife samples were tested from 1839 animals including 1223 bats, and 616 rodents and shrews. Nine viruses were detected in 97 animals, of which 3 are known viruses, and 6 were detected as part of PREDICT 1. Coronavirus was detected in 68 rodents and 3 bats and Paramyxovirus in 25 bats.
A total of 738 samples were tested from 390 domestic animals including 230 poultry/other fowl, 58 cows, 49 dogs, 33 swine, 18 goats, and 2 cats. Samples were tested for up to 6 viral families/genera and included Corona-, Flavi-, Filo-, Influenzas, Orthobunya-, and Paramyxo-, virus families/genera. Tembusu virus, a flavivirus, was found in 2 chickens.

In addition, syndromic surveillance in humans to identify novel agents associated with Influenza-Like Illness (ILI), Severe Acute Respiratory Infection (SARI), Fever of Unknown Origin (FUO), hemorrhagic fever and encephalitis has been set up in three district hospitals (one in Kandal and two in Kampong

Cham) close to the animal sampling sites. Samples from children presenting SARI were also collected from Kuntha Bopha hospital. A total of 895 patients were enrolled. Samples including oral and rectal swabs, urine and blood were collected and testing is on-going.

Partners: Wildlife Conservation Society (WCS), University of California Davis (UC Davis), Forestry Administration, National Animal Health and Production Research Institute (NAHPRI, former NaVRI), Cambodian CDC-MoH.

Financial support: USAID.

The LACANET One Health surveillance and laboratory network project

Virology Unit Team leader: P. Dussart

The LACANET partners initiated a program to strengthen the One-Health capacities in the Kingdom of Cambodia and Lao People's Democratic Republic. To achieve these goals, four integrated project activities were designed to: 1) improve field capacity to conduct disease surveillance; 2) improve laboratory capacity to detect priority pathogens; 3) improve communication and collaboration between One-Health sectors and the two countries; and 4) conduct innovative research projects to investigate drivers of zoonotic disease emergence. The LACANET One-Health Project made significant progress in improving disease surveillance and reporting in Cambodia and Lao PDR. Improved collaborative linkages between the two target countries, and between the human, veterinary and wildlife health sectors, has strengthened the capacities of each country to conduct disease surveillance and outbreak investigation activities. The project has also contributed to the inclusion of the environmental/wildlife sector into the national One-Health strategies, which has allowed significant progress in the establishment of wildlife disease surveillance networks in Cambodia and Laos. Innovative research projects to investigate the role of land-use change and wildlife trade in zoonotic disease emergence have revealed important information about priority pathogens, vector distributions and the risks to humans, livestock and wildlife. The LACANET Project has led to a sustainable improvement in One-Health capacities in Cambodia and Laos that will lead to further cross-sectoral, bilateral activities.

Partners: Wildlife Conservation Society (WCS), National Animal Health and Production Research Institute (NAHPRI, former NaVRI), the Lao P.D.R. National Animal Health Laboratory (NAHL), the Lao-Oxford-Mahosot-Hospital-Wellcome Trust Research Unit (LOMWRU).

Financial support: European Union.

Development of tools to study infection of novel rodent-borne mammarenaviruses found in Cambodia

Virology Unit Team leader: P. Dussart, H. Auerswald, V. Duong

Several rodent species are known for hosting zoonotic viruses. Investigations led throughout the CERoPath project revealed the discovery of two new mammarenaviruses in diverse rat species (Duong V et al., 2016). One of the discovered viruses is a variant of the Wēnzhōu virus (WENV), formerly isolated in Eastern China. This project initially focused on the cultivation of this Cambodian variant of WENV. Several trials under diverse cultivation conditions were conducted using simian, canine and rodent cell lines. The Cambodian WENV isolate was successfully cultivated in macrophage cell lines from *Rattus norvegicus*, the same rat species that was formerly used for experimental infections. However, these cells are not suitable for use in cell-based assays as they barely exhibit a cytopathic effect after infection even with high doses of virus. Further investigations will be carried out in 2019 in collaboration with the University of Aix-Marseille as part of the PhD work of a staff of the virology unit, in order to develop specific diagnostic tools.

Partners: Aix-Marseille Université (Xavier de Lamballerie).

Financial support: Institut Pasteur, Paris – IPC.

3.4.3 RESEARCH PROGRAMMES – PROSPECT 2019

3.4.3.1 ARBOVIRAL DISEASES

The various studies in progress on dengue and Zika viruses will be continued in 2019.

Dengue virus and mosquito specific virus interaction

The preliminary results obtained from NGS on dengue-infected mosquitoes, highlighted co-detection of dengue virus and mosquito-specific virus in the same vector. More sequencing is needed to confirm this preliminary observation and further investigation is needed on the interaction of these pathogens. We are now in preparation of a proposal to seek for funding to study the interaction between mosquito specific viruses and dengue virus or other arboviruses as the presence of these mosquito viruses in nature might or might not affect arbovirus vector competence. This novel work will help the scientific community to better understand factors influencing the outcome of arbovirus vector competence studies.

Zika virus in Cambodia: detection, seroprevalence and vector competence

After standardizing protocols with our partners from the ACIP Zika and Zikalliance consortium in 2017, vector competence experiments on three ZIKV strains from Africa (Senegal), New Caledonia and Asia (Cambodia) were partially conducted for the ACIP project in 2018 and will be achieved in 2019. In parallel, vector competence experiments on three ZIKV strains from Africa (Senegal), Asia (Malaysia) and South America (Martinique) for ZikAlliance using *Aedes* mosquitoes collected in Cambodia. Additionally, serum samples from the DVI study will be utilized to study the seroprevalence of ZIKV in the Cambodian community under the umbrella of the ZIKAlliance project.

3.4.3.2 SEASONAL AND AVIAN INFLUENZA VIRUSES

Investigation of etiology and risks for morbidity and mortality from influenza-associated SARI in Cambodian children

In collaboration with Kantha Bopha Hospital (KBH), IPC has been conducting surveillance for respiratory infections in Cambodian children for over 10 years as a first line strategy for human A(H5N1) detection. While Cambodia has not experienced a human infection with A(H5N1) since 2014, this surveillance picks up a number of severe seasonal influenza infections, especially in children. However, 50-100% of the samples submitted to IPC from the KBH surveillance system are influenza negative and specific etiology has not been determined. Additionally, in 2016 and 2017, we received an increased number of SARI cases positive for seasonal human influenza with severe morbidity and mortality. This increase in severity does not appear to be due to any genetic changes in the virus and could be due to coinfection with other pathogens. Therefore, the goal of this project will be to determine the etiology of unknown samples and the extent of coinfection in influenza positive samples from past and ongoing SARI surveillance at IPC. A cohort of healthy children was recruited (n=367) to monitor background levels of respiratory carriage. Samples from SARI patients between 2014 and 2017 and the cohort of healthy children have been analyzed and the database of information is being finalized. Final etiological, virological and phylogenetic analyses are ongoing and are projected to be completed by mid-2019.

Investigation and risk assessment of influenza viruses isolated from zoonotic transmission events in captive mammals

Through various passive surveillance systems, IPC has detected a number of zoonotic transmission events in mammals, especially in captive populations. One such event occurred in 2015 and another in 2017. In 2017, we were able to isolate these viruses and, in 2018, have commenced full risk assessment on these isolates including phylologenetics/molecular analysis, antigenic testing and mammalian studies in conjunction with Dr. Stacey Schultz-Cherry at St Jude Children's Hospital in Memphis, TN as part of the Centers for Excellence in Influenza Research and Surveillance (CEIRS) network.

Sequencing, molecular, and in vitro work has been completed. In vivo work is commencing in the first quarter of 2019 and the full risk assessment is projected to be completed by mid-2019.

Surveillance of avian influenza and identification of hotspots of spillover between poultry and wild birds

The recent emergence of H7N9 in China, emergence and global spread of H5Nx clade 2.3.4.4. and continual H5N1 outbreaks in domestic poultry highlight the need to understand the prevalence as well as genetic and phenotypic diversity of avian influenza virus circulating in wild bird reservoirs in Southeast Asia (SEA). While countries in SEA, especially Cambodia, have a high prevalence of AIV in poultry, very little is known about prevalence in wild birds. Therefore, in 2018, in collaboration with the Wildlife Conservation Society (WCS), NAHPRI and other international partners, we seek to start a project looking at influenza prevalence in wild birds in the Mekong Delta region to gain an understanding of basic prevalence and to identify potential hotspots of spillover from wild populations to domestic poultry. Discussions between DTRA, NAHPRI and WCS have commenced and sites have been selected for this study. Final funding approval is pending from DTRA; however, preliminary sampling commenced in early 2019.

3.4.3.3 HIV AND VIRAL HEPATITIS

HIV and HCV infections in 2 communes from the Battambang province, Cambodia: prevalence rates, viral strains, and unsafe injection practices (Roka – ANRS 12352):

The main objective of this study is to compare the HIV/HCV prevalence rates and corresponding viral strains in subjects living in Roka commune versus those who are living in another commune from the Battambang Province. The protocol of the study was approved by the National Ethics Committee for Health Research on May 2nd, 2017. The study is planned to start by December 2018.

Partners: V. Saphonn, CV. Mean, K. Khim, P. Pheng P (UHS), PS. Ly, S. Mam, C. Mom (NCHADS).

Financial support: ANRS.

Clinical utility of resitance-associated substitutions characterization in patients with Chronic HCV infection failing direct-acting antivirals treatment in Cambodia

Even DAA treatment is proving a high efficiency, around 5% of patients under treatment are experiencing treatment failure associated with mutations in HCV genome. Currently, very few data are available for HCV genotype 6. In this regard, our team will commence a project aiming to study DAA resistance among patients infected with HCV genotype 6 and failing DAA treatment. The specific objective of the study will be the identification of genetic signature of HCV genome associated with DAA resistance, using genotypic and phenotypic approaches.

Partners: MSF (JP Dousset, M Le Paih); IP Paris (E Simon-Loriere).

Financial support: IPC and MSF.

3.4.3.4 NEUROTROPIC INFECTION

South East Asia Encephalitis Project – SEAe

In 2019, a major publication of the study including clinical, neurological, microbiological and epidemiological findings will be prepared and submitted to a high impact journal. Additional studies accessing the SEAe biobank specimens and their associated data are proposed, with funding secured for at least one investigation.

Prevalence of HFMD and viral diversity study of enteroviruses in Cambodia and study of antigenic and immunogenic features of EV-A71

We will continue to monitor the circulation of EV-A71 but also CV-A16 and CV-A6 viruses and amend our community point prevalence studies accordingly. We will also investigate the genetic variation in enterovirus strains to determine the role of viral pathogenesis in disease severity, and the involvement of host immunological factors as a contributor to disease severity. In parallel we will increase our collaboration in surveillance of EV-A71 with colleagues from Institut Pasteur in Shanghai by comparing the characteristics enteroviruses strains circulating in the region.

3.4.3.5 ZOONOSES

PREDICT 2

By September 2018, all field works were ended including syndromic surveillance in hospitals. Viral family testing is now finished. Confirmation sequencing of positive PCR product and data entry into EIDITH database are ongoing until March 2019.

Development of tools to study infection of novel rodent-borne mammarenaviruses found in Cambodia

Cultivation trials will continue with the Loei River virus (LORV) sample as well as with another novel mammarenavirus detected within the LACANET study. Additionally, efforts towards development of a cell-based assay for the detection of the arenaviruses as well as neutralization by virus-specific antibodies will continue. Finally, we have a Cambodian Ph.D. student working in the Virology Unit on this topic with financial support from the French Embassy in collaboration with Aix-Marseille Université.

3.4.4 SUPPORT TO NATIONAL AUTHORITIES

National dengue surveillance in Cambodia

As part of a collaboration with the WHO and NDCP and within the framework of a national programme on outbreak missions, the Virology Unit's laboratory received, over 2018, samples from six provincial hospitals and the National Pediatric Hospital in Phnom Penh. These surveillance sites are located within high risk areas of dengue haemorrhagic fever (high population density, presence of the vector, history of dengue in the region). Results from the monitoring of haemorrhagic syndromes are reported weekly to the various monitoring programme participants (Director of the NDCP, hospital physicians, etc.).

DENV-1 was the main dengue serotype detected from 2011 to 2015, and 2016 was marked by an increased detection of DENV-2 in Cambodia. In 2017, we continued to detect mainly DENV-2, while DENV-1 was still present in the country with a low circulation of dengue viruses in the country that year. As expected, the recrudescence of dengue in 2018 was much higher compared to previous years, with 24,684 hospitalized cases and 23 deaths (2016: 12,843 cases, 18 deaths; 2017: 6,372 cases, 3 deaths). Due to low herd immunity in the Cambodian population DENV-2 remains the main serotype detected (53%). However detection of DENV-1 (41%) is still high in the country, while DENV-4 (5%) has been sporadically detected.

Cambodian National Influenza Centre and H5 Reference Laboratory

- ***Seasonal human influenza viruses surveillance***

IPC's Virology Unit have been Cambodia's National Influenza Centre (NIC) since 2006. The Virology Unit at IPC was designated as a WHO H5 Reference Laboratory of the WHO Global Influenza Surveillance and Response System (GISRS) in October 2014.

The Influenza-like illness (ILI) surveillance established in 2006, in collaboration with the MoH and WHO, allows for the collection of influenza strains and data on seasonality. Currently, 7 hospitals contribute to ILI surveillance: Kampot, Battambang, Kampong Cham, Mondulkiri, Svay Rieng, Angkor Children's Hospital (Siem Reap) and the National Pediatric Hospital (Phnom Penh). Each hospital randomly collects clinical samples from a maximum of 5 ILI patients per week. Samples are first analysed by the National Institute of Public Health (NIPH) and are then sent to IPC for confirmation. Samples are also received from other institutions in Cambodia which have public health and research activities on influenza, such as the National Institute of Public Health (NIPH), the Naval Army Medical Research Unit (NAMRU-2), and the Armed Forces Research Institute of the Medical Sciences (AFRIMS).

In 2018, 273 samples were received through the national surveillance system that had previously tested positive by the referral laboratories NIPH and AFRIMS. These samples were tested for influenza viruses and subtyped using the PCR methods described above. These results also closely agreed with the results from the NIPH laboratory except for two samples: 1 was reported positive for influenza B/Yam by the referral lab, but was negative by our testing, 1 was reported positive for B/Vic, but was detected positive B/Yam by IPC. These results were confirmed by repeat extraction and PCR testing. We also received 95 negative samples from NIPH for quality control purposes. All of the samples were confirmed as negative in exact concordance with the referral laboratories.

During this period influenza A/H1N1pdm09 viruses was the most commonly detected (n=148, 54.2%), followed by Influenza B/Yamagata (n=103, 37.8%), 17 B/Victoria (n=17, 6.2%) and A/H3N2 (n=2, 0.7%). Influenza A/H1N1 seasonal viruses was not detected. Subtyping of influenza A viruses was achieved through targeted testing using HA1pdm09, HA3, NA1pdm09 and NA2 real-time RT-PCR assays. Analysis of the influenza B viruses revealed that influenza B/Yamagata lineage was predominant circulated in this period. Cell-culture isolation of influenza viruses on MDCK-SIAT cells was done for 256 positive specimens. Of 256 samples attempted for virus isolation, influenza viruses was successful from 87% (n=222) of samples. Isolation was not successful from 20 strains of /H1N1pdm09, 9 strains B/Yamagata, 3 strains of A/H3N2 and 1 strain of B/Victoria.

Additionally, 25 influenza isolates (12 A/H1N1pdm09, 11 B/Yamagata and 2 B/Victoria) were selected for antigenic testing and Neuraminidase inhibition susceptibility analysis at IPC. Neuraminidase inhibition analysis (NIA) to determine the sensibility to the drug Oseltamivir and Zanamivir to these selected 25 isolated in this period showed that all these isolates were sensitive to the drug Oseltamivir and Zanamivir. Moreover, 66 of the viruses collected and analyzed during this period have been sent to the WHOCC in Melbourne, Australia for further analysis: 23 in July 2018 and 43 in mid-January 2019 respectively. All isolates were found to be sensitive to all antivirals tested at the WHOCC. Antigenic characterization by Hemagglutination inhibition (HAI) assay showed that the 12 A/H1N1pdm09 isolates were A/Michigan/45/2015-like, 11 B/Yamagata isolates belong to B/Phuket/3073/201-like and 2 B/Victoria were B/Brisbane/60/2018-like. 43 of the viruses collected and analyzed during this period have been sent in July 2018 to the WHOCC in Melbourne, Australia for further analysis. All isolates were of similar antigenic lineage to those tested at IPC.

- ***Severe acute respiratory illness (SARI) surveillance in humans***

Nasopharyngeal and throat swab samples are collected from patients presenting to Cambodian hospitals from the Kantha Bopha Foundation hospital system (KBH) with severe acute respiratory infections (SARI). In 2018, 88 samples were received from KBH and were screened for influenza A, influenza A/H5N1, influenza A/H7N9 and MERS-CoV. Samples were received from Jayavarman Hospital in Siem Reap (n=36) and Kantha Bopha Hospital in Phnom Penh (n=52). The samples were all negative for A/H5N1, A/H7N9 and MERS-CoV but 23 (26%) SARI were positive for A/H1N1pdm09 influenza viruses, 1 (1%) were positive for influenza B viruses.
Similar to the influenza season in 2017 (rainy season in Cambodia), a large spike in A/H1N1pdm09 positive SARI patients were detected starting in June 2018. In addition to the seasonal ILI samples, we have also sent samples from 19 SARI cases to the WHO Collaborating Centre in Melbourne for further analysis to determine if changes to the virus were responsible for the increase in severe disease associated with these viruses.

Diagnostics for rabies infections

Rabies remains a major public health concern in Cambodia. IPC's Virology Unit have been involved in the diagnosis of rabies infections using a fluorescein-conjugated antibody specific for rabies virus nucleoprotein (Fluorescent Antibody Test – FAT) for almost two decades. This test is routinely performed on samples obtained from suspected rabies infected animals, specifically fresh Ammon's horn or brain samples. When this first-line diagnostic test is negative, a nested RT-PCR can also be utilized for brain samples in advanced state of putrefaction. The Rabies Tissue Culture Infection test (RTCIT) is also available at the Virology Unit.

During 2017, the Virology Unit received 192 brain samples from dogs, an increase of 22% compared to 2016. The positivity rate also increased to 60.9% (n=117) of dog brain tested in 2017 compared to 2016 (50.3%, n=79). In 2018, the number of dog brains tested (n=173) decreased slightly as we received 10% fewer samples than in 2017. However, the number of samples tested positive for rabies remains high at 67.6% (n=117). The average percentage of positive dog heads observed from 2002 to 2018 is currently 51.6%.

3.4.5 TEACHING AND TRAINING

One of IPC's main missions is to contribute to teaching and training activities. The Virology Unit has been proactive in the training of laboratory technicians from partner institutions in the fields of surveillance and research, conducted at the national and regional levels.

In 2018, more than 40 IPC staff (Virology Unit staff representing the majority, but also staff from Entomology platform, Immunology group and Clinical laboratory) followed different types of training, courses and/or workshops organized by IPC scientists (mainly from Virology Unit) or organized at the regional and international levels. Moreover, 4 staff from Myanmar were trained to improve their laboratory capacities in molecular diagnosis in virology. Finally, the Virology Unit also received four foreign students (two Master and two Ph.D. students), as well as eleven Cambodian students (two Master's, six veterinary and three lab technicians' students). In late 2018, we registered one virology staff in Ph.D. program in the field of virology at Aix Marseille Université (Pr. Xavier de Lamballerie).

3.4.6 PUBLICATION LIST

2018

1. Weger-Lucarelli J, Auerswald H, Vignuzzi M, Dussart P, Karlsson EA. Taking a bite out of nutrition and arbovirus infection. PLoS Negl Trop Dis. 2018. 12(3): e0006247.
2. Nikolayeva I, Bost P, Casademont I, Duong V, Koeth F, Prot, M, Czerwinska U, Ly S, Bleakley K, Cantaert T, Dussart P, Buchy P, Simon-Lorière E, Sakuntabhai A, Schwikowski B. A Blood RNA Signature Detecting Severe Disease in Young Dengue Patients at Hospital Arrival. J Infect Dis. 2018. 217(11):1690-1698.
3. Auerswald H, Boussioux C, In S, Mao S, Ong S, Huy R, Leang R, Chan M, Duong V, Ly S, Tarantola A, Dussart P. Broad and long-lasting immune protection against various Chikungunya genotypes demonstrated by participants in a cross-sectional study in a Cambodian rural community. Emerg Microbes Infect. 2018. 7: 13.
4. Lopez-Jimena B, Bekaert M, Bakheit M, Frischmann S, Patel P, Simon-Loriere E, Lambrechts L, Duong V, Dussart P, Harold G, Fall C, Faye O, Sall A, Weidmann M. Development and validation of four one-step real-time RT-LAMP assays for specific detection of each dengue virus serotype. PLoS Negl Trop Dis. 2018. 12(5): e0006381.
5. Di Francesco J, Choeung R, Peng B, Pring L, Pang S, Duboz R, Ong S, Sorn S, Tarantola A, Fontenille D, Duong V, Dussart P, Chevalier V, Cappelle J. Comparison of the dynamics of Japanese encephalitis virus circulation in sentinel pigs between a rural and a peri-urban setting in Cambodia. PLoS Negl Trop Dis. 2018. 12(8):e0006644.
6. Horwood PF, Horm SV, Suttie A, Thet S, Y P, Rith S, Sorn S, Holl D, Tum S, Ly S, Karlsson EA, Tarantola A, Dussart P. Co-circulation of Influenza A/H5N1, H7, and H9 Viruses and Evidence of Co-infected Poultry in Live Bird Markets, Cambodia. Emerg Infect Dis. 2018. 24: 352-355.
7. Suttie A, Yann S, Y P, Tum S, Deng YM, Hul V, Horm SV, Barr I, Greenhill A, Horwood PF, Osbjer K, Karlsson EA, Dussart P. Detection of low-pathogenic influenza virus A(H7N3) during a duck mortality event in Cambodia in 2017. Emerg Infect Dis. 2018; 24(6): 1103-1107.
8. Influenza A(H5N1) viruses with A(H9N2) single gene (matrix or PB1) reassortment isolated from Cambodian live bird markets. Suttie A, Karlsson EA, Deng YM, Horm SV, Yann S, Tok S, Sorn S, Holl D, Tum S, Hurt AC, Greenhill AR, Barr IG, Horwood PF, Dussart P. Virology. 2018. 523: 22-26.
9. Tarantola A, Blanchi S, Cappelle J, Ly S, Chan M, In S, Peng Y, Hing C, Taing CN, Muy S, Ly S, Bourhy H, Buchy P, Dussart P, Mary JY. Rabies postexposure prophylaxis (PEP) noncompletion after dog bites: estimating the unseen to meet the needs of the underserved. Am J Epidemiol. 2018. 187(2): 306-315.

10. Bharucha T, Sengvilaipaseuth O, Seephonelee M, Vongsouvath M, Vongsouvath M, Rattanavong S, Piorkowski G, Lecuit M, Gorman C, Pommier JD, Newton PN, de Lamballerie X, Dubot-Peres A. Detection of Japanese encephalitis virus RNA in human throat samples in laos - A pilot study. Scientific Reports. 2018. 8(1): 4. 10.1038/s41598-018-26333-4.
11. Champagne C, Paul R, Ly S, Duong V, Leang R, Cazelles B. Dengue modeling in rural Cambodia: Statistical performance versus epidemiological relevance. Epidemics. 2018. pii: S1755-4365(17)30170-6.
12. Diallo AOI, Chevalier V, Cappelle J, Duong V, Fontenille D, Duboz R. How much does direct transmission between pigs contribute to Japanese Encephalitis virus circulation? A modelling approach in Cambodia. PLoS One. 2018 Aug 16;13(8):e0201209.
13. Ten Bosch QA, Clapham HE, Lambrechts L, Duong V, Buchy P, Althouse BM, Lloyd AL, Waller LA, Morrison AC, Kitron U, Vazquez-Prokopec GM, Scott TW, Perkins TA. Contributions from the silent majority dominate dengue virus transmission. PLoS Pathog. 2018 May 3;14(5):e1006965.
14. Dickson LB, Ghozlane A, Volant S, Bouchier C, Ma L, Vega-Rúa A, Dusfour I, Jiolle D, Paupy C, Mayanja MN, Kohl A, Lutwama JJ, Duong V, Lambrechts L. Diverse laboratory colonies of Aedes aegypti harbor the same adult midgut bacterial microbiome. Parasit Vectors. 2018 Mar 27;11(1):207.
15. Nouhin J, Madec Y, Prak S, Ork M, Kerleguer A, Froehlich Y, Pavio N, and Rouet F. 2018. Declining hepatitis E virus antibody prevalence in Phnom Penh, Cambodia during 1996 – 2017. Epidemiology and Infection. 2018. 147 e26, 1–6. Doi:10.1017/S0950268818002790
16. Nouhin J, Bollore K, Castera-Guy J, Prak S, Heng S, Kerleguer A, Rubbo PA, Rouet F, Tuaillon E. 2018. Analytical and field evaluation of the Biocentric Generic HCV Assay on open polyvalent PCR platforms in France and Cambodia. J Clin Virol. 2018 Sep 17;108:53-58. doi: 10.1016/j.jcv.2018.09.011.
17. Rouet F, Nouhin J, Zheng DP, Roche B, Black A, Prak S, Leoz M, Gaudy-Graffin C, Ferradini L, Mom C, Mam S, Gautier C, Lesage G, Ken S, Phon K, Kerleguer A, Yang C, Killam W, Fujita M, Mean C, Fontenille D, Barin F, Plantier JC, Bedford T, Ramos A, Saphonn V. 2017. Massive Iatrogenic Outbreak of Human Immunodeficiency Virus Type 1 in Rural Cambodia, 2014-2015. Clin Infect Dis. 2018 May 17;66(11):1733-1741.
18. Ségéral O, N'Diaye DS, Prak S, Nouhin J, Chhun S, Khamduang W, Chim K, Roque-Afonso AM, Piola P, Borand L, Ngo-Giang-Huong N, Rouet F, ANRS 12328 12345 Study Group. Usefulness of a serial algorithm of HBsAg and HBeAg rapid diagnosis tests to detect pregnant women at risk of HBV mother-to-child transmission in Cambodia, the ANRS 12328 pilot study. J Clin Virol. 2018 Dec;109:29-34. doi: 10.1016/j.jcv.2018.10.007.

2017

1. Somlor S, Vongpayloth K, Diancourt L, Buchy P, Duong V, Phonekeo D, Ketmayoon P, Vongphrachanh P, Brey PT, Caro V, Buisson Y, Grandadam M. Chikungunya virus emergence in the Lao PDR, 2012-2013. PLoS One. 2017 Dec 28;12(12):e0189879.
2. Duong V, Ong S, Leang R, Huy R, Ly S, Mounier U, Ou T, In S, Peng B, Ken S, Buchy P, Tarantola A, Horwood PF, Dussart P. Low Circulation of Zika Virus, Cambodia, 2007-2016. Emerg Infect Dis. 2017; 15,23(2).
3. Duong V, Choeung R, Gorman C, Laurent D, Crabol Y, Mey C, Peng B, Di Francesco J, Hul V, Sothy H, Santy K, Richner B, Pommier JD, Sorn S, Chevalier V, Buchy P, de Lamballerie X, Cappelle J, Horwood PF, Dussart P. Isolation and full-genome sequences of Japanese encephalitis virus genotype I strains from Cambodian human patients, mosquitoes and pigs. J Gen Virol. 2017; 98(9):2287–96
4. Simon-Lorière E*, Duong V*, Tawfik A, Ung S, Ly S, Casadémont I, Prot M, Courtejoie N, Bleakley K, Buchy P, Tarantola A, Dussart P, Cantaert T, Sakuntabhai A.. Increased adaptive immune responses and proper feedback regulation protect against clinical dengue. Sci Transl Med. 2017 ; 30,9(405):eaal5088.
5. Andries A-C, Duong V, Cappelle J, Ong S, Kerleguer A, Ly S, et al Andries AC, Duong V, Cappelle J, Ong S, Kerleguer A, Ly S, Tarantola A, Horwood PF, Sakuntabhai A, Dussart P, Buchy P. Proteinuria during dengue fever in children. Int J Infect Dis. 2017; 55:38-44.
6. Ly S, Horwood P, Chan M, Rith S, Sorn S, Oeung K, Nguon K, Chan S, Y P, Parry A, Tsuyuoka R, Ly S, Richner B, Laurent D, Vong S, Dussart P, Buchy P, Tarantola A. Seroprevalence and Transmission of Human Influenza A(H5N1) Virus before and after Virus Reassortment, Cambodia, 2006-2014. Emerg Infect Dis. 2017; 23(2):300–3.
7. Horwood P, Duong V, Laurent D, Mey C, Sothy H, Santy K, Richner B, Heng S, Hem S, Cheval J, Gorman C, Dussart P, de Jong MD, Kerleguer A, Guillard B, Murgue B, Lecuit M, de Lamballerie X, Farrar JJ, Tarantola A, Eloit M, Buchy P. Aetiology of acute meningoencephalitis in Cambodian children, 2010–2013. Emerg Microbes Infect. 2017; 24,6(5):e35.
8. Crabol Y, Pean P, Mey C, Duong V, Richner B, Laurent D, Santy K, Sothy H, Dussart P, Tarantola A, Buchy P, Horwood PF. A prospective, comparative study of severe neurological and uncomplicated hand, foot and mouth forms of paediatric enterovirus 71 infections. Int J Infect Dis. 2017; 59:69–76.
9. Artois J, Blasdell K, Duong V, Buchy P, Hul V, Morand S, Claude J. Effects of mammarenavirus infection (Wēnzhōu virus) on the morphology of Rattus exulans. Infect Genet Evol. 2017; S1567-1348(17)30184-3.
10. Fillieux C, Chea S, Ramassamy JL, Hul V, Olson S, Fine A, Dussart P, Horwood PF, Pruvot M. Assessment of rodent communities in two provinces of Cambodia. Cambodian J Nat Hist. 2017; 2:147–52.
11. Saphonn V, Fujita M, Samreth S, Chan S,Rouet F, Khol V, Mam S, Mom C, Tuot S, Le LV, Ly PS, Ferradini L, Mean CV. 2017. Cluster of HIV Infections Associated With Unsafe Injection Practices in a Rural Village in Cambodia. J Acquir Immune Defic Syndr. 2017; 75(3):e82-e86.
12. Nouhin J, Pean P, Madec Y, Chevalier MF, Didier C, Borand L, Blanc FX, Scott-Algara D, Laureillard D, Weiss L. 2017. Interleukin-1 receptor antagonist, a biomarker of response to anti-TB treatment in HIV/TB co-infected patients. J Infect. 2017; 74(5):456-465.

3.5 MEDICAL ENTOMOLOGY

3.5.1 FUNCTIONAL STRUCTURE OF THE UNIT

The Medical Entomology Platform was created the 15 September 2016 (N/Réf: N°295/IPC/DIR/2016). The Medical Entomology Platform is managed by Sebastien BOYER (Medical Entomology Platform) under supervision of Didier FONTENILLE. The platform welcomes the Malaria Molecular Epidemiology Unit and Virology Unit.
In 2016, this platform had 2 members.
In 2017, the platform has 5 members.

In 2018, the Unit has 7 members: **Sebastien BOYER** (PhD), head of Unit, **YEAN Sony**, **CHHUM Moeun**, **SUOR Kimhuor**, **CHHUOY Kalyan**, **NHEK Sreynik**, 5 technicians in Medical Entomology, **VUTHY Sreykiuch**, secretary and project manager of the Unit. Iva SONG is a Master 2 student from RUPP: he will be present in 2019.

The Medical Entomology Unit was officially created the 1st October 2018 (N/Réf: N°413/IPC/DIR/2018) at the same time as the recruitment of Sebastien Boyer by Institut pasteur in Paris for a permanent position.

3.5.2 RESEARCH PROGRAMS – MAJOR ACHIEVEMENTS IN 2018

Lacanet project; Comacross project.

These 2 projects aim (in part) to document the burden and the transmission of Japanese Encephalitis virus (SEAe projects, PI: Philippe DUSSART, Virology Unit, and ComAcross project, PI: Véronique CHEVALIER, Epidemiology & Public Health Unit).

Two articles for valorizing these project are under writing:

1. Japanese encephalitis virus mosquito vectors in peri-urban and rural environments in Kandal province Cambodia.
   Sebastien Boyer, Borin Peng, Senglong Pang1, Véronique Chevalier, Veasna Duong, Christopher Gorman, Philippe Dussart, Julien Cappelle, Didier Fontenille

2. Trophic preference of Japanese Encephalitis vectors in rural Cambodia
   Sebastien Boyer, Sony Yean, Cécile Brengues, Didier Fontenille, Julien Cappelle, Véronique Chevalier

Ecomore 2 project; Panic project.

Ecomore 2 project is financed by AFD (Agence française de développement) and Panic project is financed by European Union. The objective of the project, in a development context, is to determine if integrated vector management in localized areas can decrease the incidence of Dengue in the population rural and peri-urban areas. In schools, destruction of breeding sites with the help of students and professors, scientific animation in the school and use of a bacterio insecticide (Bacillus thuringiensis israelensis) and an auto-dissemination insecticide (Pyriproxyfen) will be used. The principal outcome will be the number of like Dengue fever cases in the villages around schools. We will be able to observe if the focalized vector control methods are efficient and also able to decode the main anthropogenic and/or ecological mechanisms responsible for the emergence of infectious diseases. The finality of the project is to propose to the Ministry of Health an applicable intervention strategy.
The project is still ongoing.

Resistance to insecticides.

The Medical Entomology Platform in collaboration with Malaria Consortium studied the insecticide resistance status of the main Dengue virus vector, *Aedes aegypti*. The tested insecticides were temephos (larvicide), permethrin and deltamethrin (adulticide). We demonstrated that Aedes aegypti species (4 populations) are resistant to the three currently used insecticide in Cambodia. The results were communicated to the Ministry of Health (CNM: National Center for Parasitology, Entomology and Malaria Control). These results were also presented in 6 national and 4 international conferences. Actually, no action was taken to a change of insecticide.

The activities continued will the test of new larvicides and adulticides to propose solutions for the Ministry of Health. Now we are testing 15 adulticides and 3 larvicides : The experiments for adulticide tests are over, and need to be analyzed. The larvicide experiment are in progress.

Zikalliance project.

ZIKAlliance is a 3-year project funded by the Horizon 2020 program for research and innovation of the European Union according to the financing agreement n ° 734548. This international consortium aiming a global alliance for Zika virus control and prevention, regroups 53 partners. The project has 3 main objectives: (1) To determine the impact of Zika virus infection during pregnancy and short & medium term effects on newborns, (2) to retrace the natural history of Zika virus infection in humans and their environment in the context of other circulating arboviruses, and (3) to built an overall capacity for preparedness research for future epidemic threats. In this context our objective is both to train scientist here for such a problem and (2) to study the diversity and the possibility of vectors in wild areas (i.e. natural forest) to be able to carry ZIKV. The idea is to see whether the ZIKV cycle exists in natural and preserved areas, and how could the exchange exist between human and natural areas.

In 2018, we obtained the authorization to go in Mondulkiri forest, and we went there to trap mosquitoes. Two field missions were operated in the Mondulkiri with the description of several Stegomyia species, meaning a potential diversity of species able to transmit several families of virus. The presence of Dengue and alphavirus was negative in this specific areas. Further researchs are ongoing on the specimen. Mosquito were then identified and a lot of species, especially Stegomyia species were analyzed. Now, the samples were given to the Virology Unit to be able to detect the presence of virus in the mosquitoes.

3.5.3 RESEARCH PROGRAMS – PROSPECT 2019

DARPA-PREEMPT project

The PREEMEPT project aims to determine the mosquito species and virus families that are present in conserved biodiversity areas. The objectives will be to sample in different area, such as wildlife park, high conservation areas, natural park in order to determine the presence of potential virus. This work will be coordinated with the Ministry of Environment and World Conservation Society in Cambodia.

FSPI project

The objective of this project is to understand how the changing relationships between villages, forests and deforestation activities are impacting the biodiversity of mosquitoes and viruses, and to develop a new method of diagnosis and an associated adapted surveillance. We want to understand these issues through the vector "mosquito", because of the in-depth knowledge it has, and the current major emergences due to mosquitoes.

In addition, the current training situation in Cambodia is alarming, and there is no learning university related to entomology, nor practical work applied to virology. For the public health needs of the country, the Institut Pasteur du Cambodge proposes via this project to train entomologists in the field through a training in Medical Entomology, and also virologists for the national reference center for surveillance of arboviroses in Cambodia.

This work will be coordinated with the Ministry of Environment.

Description of mosquito species and dynamics in Phnom Penh

The diversity, distribution and seasonality of mosquito species in Phnom Penh is totally unknown. To this day, and to my knowledge, there is no data on these various parameters. My team proposes to carry out a weekly follow-up of one year to remedy this lack. We plan to publish an article on the dynamics of Dengue vectors in Phnom Penh. The relative abundance of the different species will be analyzed according to different meteorological parameters, and the different types of urban environment surrounding the pagodas.

After the arrival of Vincent Herbreteau (GIS) at IPC, we will begin before the End of this year to sample and then to evaluate the dynamics, and the risk associated with the potential presence of mosquito vector species. In parallel, in order to assess the method, we also do an estimation of the dynamics of mosquitoes in IPC campus.

This work will be coordinated with the Ministry of Cult and Religion.

3.5.4 SUPPORT TO NATIONAL AUTHORITIES

The article describing the resistance to insecticides of *Aedes aegypti* populations was presented to the Ministry of Health for a change of insecticide use.

3.5.5 TEACHING AND TRAINING

Teaching.

One Master 2 student from RUPP arrived in 2019.

Organization of an international conference.

VECTOLAND is an ASEAN-EU Cooperation in Science, Technology and Innovation. The project was founded by the SEA-EU-NET, an international science cooperation network to deepen science and technology cooperation between Europe and Southeast Asia. Its second meeting was organized by the Medical Entomology Platform at Institut Pasteur du Cambodge, Phnom Penh, Cambodia. The main concerns of the presentations and the workshop was the rapid spreading of some invasive species (i.e. *Ae. albopictus* and Zika Virus), the difficulty of the struggle against malaria in South East India (i.e. insecticide and drug resistance; management of resistance), the communication with authorities and local population.

This meeting was organized in November 2016 with 24 international scientific presentations, regrouping 16 different partners from 7 countries.

Training.

In 2017, the Medical Entomology Platform organized two trainings on mosquito systematics (see previous report for details).

This year, the 4 entomological technicians participated to a training on PCR methods, 2 technicians were trained for P3 used (and used it for vector competence experiment).

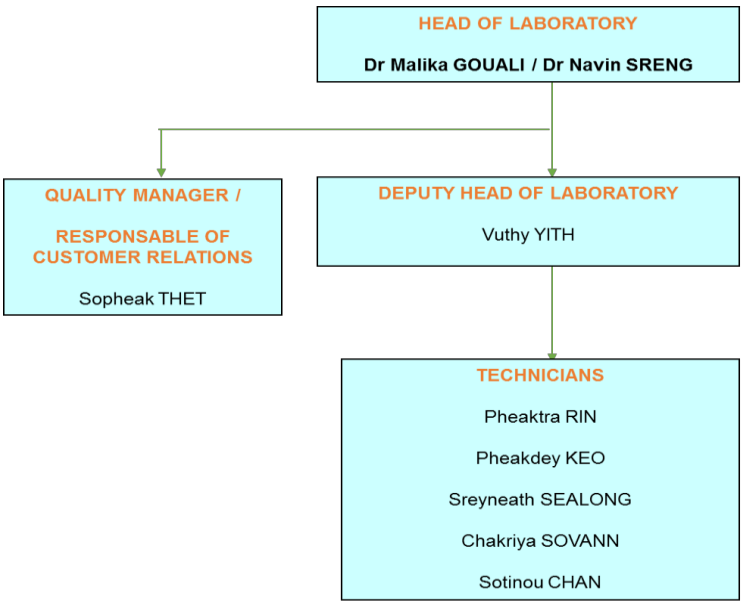
We plan 2 more trainings next year: one training on mosquito systematics, and one training in practical molecular experiment.

3.5.6 PUBLICATION LIST (2018)

1. Boyer S, Calvez E, Chouin-Carneiro T, Diallo D, Failloux AB. An overview of mosquito vectors of Zika virus. Microbes and Infection,2018,1:1-15.
2. Boyer S, Lopes S, Prasetyo D, Hustedt J, Sarady AS, Doum D, Yean S, Peng B, Bunleng S, Rithea L, Fontenille D, Hii J. Resistance of Aedes aegypti (Diptera: Culicidae) populations to deltamethrin, permethrin and temephos in Cambodia. Asia Pacific Journal of Public Health, 2018, 30:158-166.
3. Jean Jose Nepomichene TN, Raharimalala FN , Andriamandimby SF, Failloux AB, Heraud JM, Boyer S. Vector competence of Culex antennatus, Anopheles coustani species with the Rift Valley Fever Virus in Madagascar. Vector-Borne and Zoonotic Diseases, 2018, 32:259-262.
4. Tantely ML, Randrianambinintsoa FJ, Ramasindrazana B, Woog F, Boyer S. Description of one new species of the genus Uranotaenia (Diptera: Culicidae) highlighted by morphological study and wing morphometry. Zootaxa, In Press
5. Harimalala M*, Miarinjara A*, Duchemin JB, Ramihangihajason T, Boyer S. Species diversity and phylogenetic classification of fleas in the Central Highlands of Madagascar. Zoologica Scripta. 2018, 4399:181-196
6. Caron Y, Chheang R, Puthea N, Soda M, Boyer S, Tarantola A, Kerléguer A. Beta-lactam resistance among Enterobacteriaceae in Cambodia: The four-year itch. International journal of Infectious Diseases, 2018, 66:74-79.

3.6 ENVIRONMENT AND FOOD SAFETY LABORATORY ANALYSIS LABORATORY (LEFS)

3.6.1 FUNCTIONAL STRUCTURE OF THE UNIT



Functional and nominative organization chart of LEFS

Updated on 1er November 2018

In 2018, different changes have occurred in the team:

- From July 2017 to the end of April 2018, Dr. Malika GOUALI was appointed interim head of the laboratory pending identification and training of the future Cambodia head of Laboratory.
- On 2018 May 1st, Dr. SRENG Navin is appointed as the head of the laboratory.
- Two staffs were resigned and replaced by two new staffs in November 2018.

3.6.2 ROUTINE ACTIVITY 2018

In 2018, the laboratory has tested 8830 samples comprising 3817 samples of food, 2985 water samples for microbiology testing, 1930 water samples for chemical testing and 98 samples for Pesticide sample preparation. 5 new contracts have be signed with restaurants and hotels during this year. We could provide also 2 trainings on HACCP process and 4 trainings on Personal Hygiene and Food Safety to our customers.

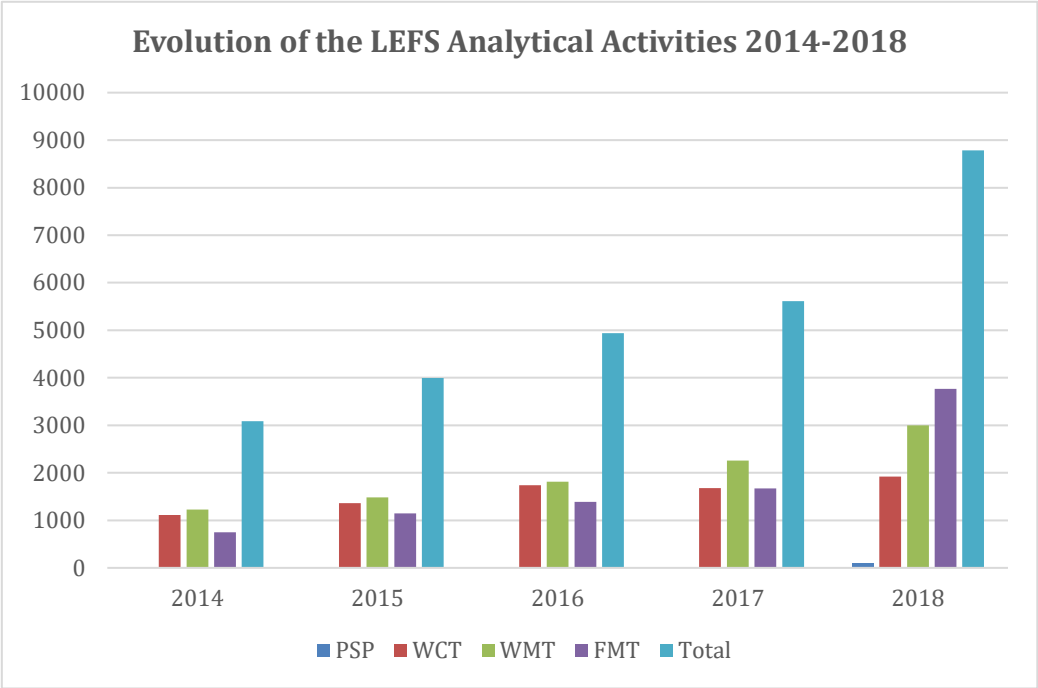
Comparatively to 2017, for microbiology testing, the number of samples increased respectively for food and water by 128% and 32%. For chemical testing, we note an increase of 15%. This increase is due to the increase of customer and contract number, samples from an MOH project, Street food study, and new activities of parasites and air contamination control.

This good and positive progression of analytical activities these 5 last years is showed in table 1 and figure 1 below:

Table 1:

Analytical activity	Years				
	2014	**2015**	**2016**	**2017**	**2018**
Food microbiology testing	750	1147	1390	1674	3817
Water microbiology testing	1224	1485	1810	2256	2985
Water chemical testing	1113	1362	1738	1681	1930
Pesticide sample preparation	-	-	-	-	98
Total	**3087**	**3994**	**4938**	**5611**	**8830**

Figure 1



PSP: Pesticide Sample Preparation, WCT: Water Chemical Testing, WMT: Water Microbiological testing, FMT: Food Microbiological Testing.

If we look more closely to the collected data for each kind of products in terms of quality, we noted that:

- 2.9% of food samples (112/3817) were not satisfied, 23 of which were contaminated by Salmonella which is a pathogen responsible of food poisoning. More than half of food positive for Salmonella were meat products. The other products include seafood, salads, cooked food, and ice cream.
- 15.6% of water samples (467/2985) were not satisfied and were unfit for human consumption because of a fecal contamination.
- 28.6% of ice cubes samples (115/402) served in the restaurants and bars are contaminated by fecal bacteria as coliforms, E. coli and intestinal enterococci.
- 8.1 % of water samples (156/1930) tested for chemical are not satisfied.

In terms of development, several important changes occurred in 2018 in the laboratory as:

- Implementation of international standard methods in microbiology to be in accordance with NF EN ISO 17025 requirements to get an accreditation in the future.
- Improvement of laboratory visibility: regular update of the website, presentation flyer of LEFS activities.
- Organization of an Opening day event to receives our customers for visiting lab and promoting our activities
- Start-up of audit and training activities to respond to some specific customers' requirements
- Renewal of the laboratory equipment
- Setting up a biannual external control of microbiological testing (RAEMA)
- Setting up new activities such as Pesticide sample preparation, Parasite and Air contamination control
- Setting up new parameters analysis such as Cronobacter spp.
- Renewal of customer contract in accordance with NF EN ISO 17025 requirement.
- Setting up the sampling and sample collection service for customers

3.6.3 RESEARCH PROGRAMS - MAJOR ACHIEVEMENTS IN 2018

Ancillary Birdy project 2016-2017

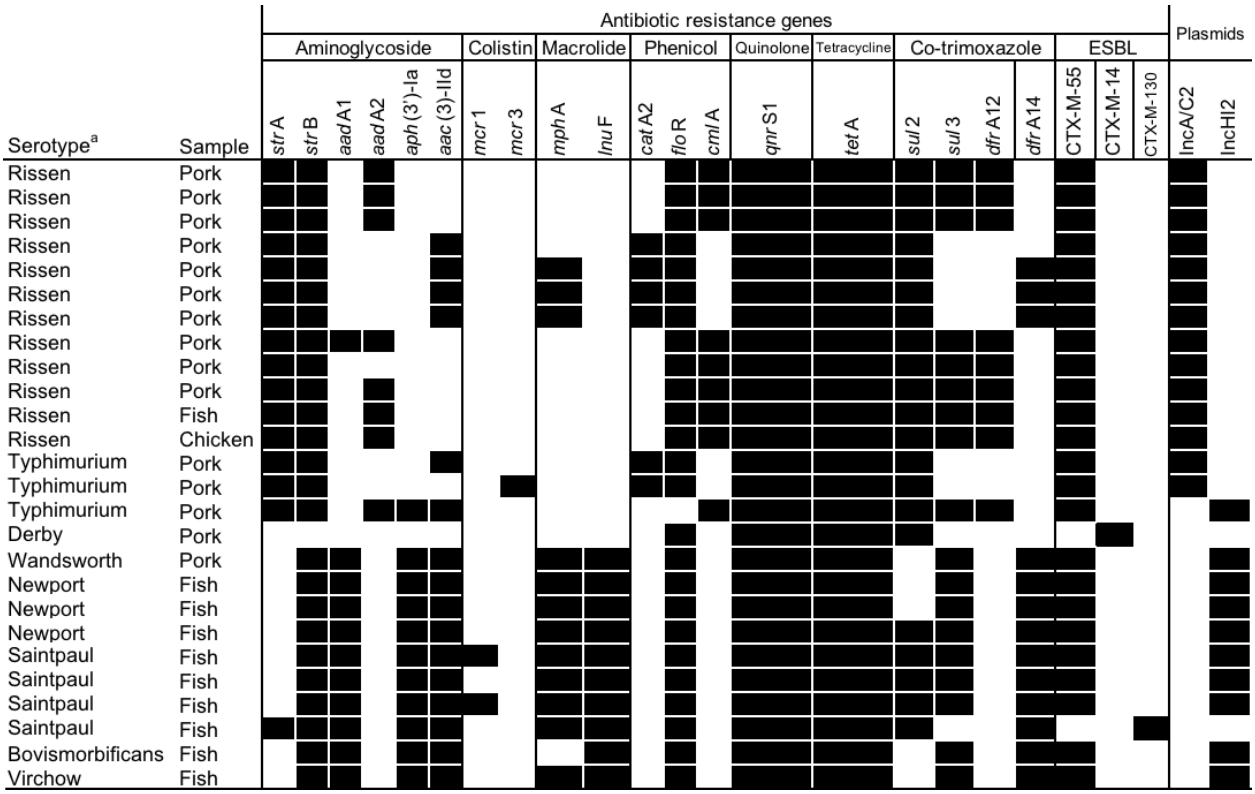
Extended-spectrum β-lactamase-producing *Salmonella enterica*

Salmonella enterica is a leading cause of human gastroenteritis worldwide. Over the past two decades, *S. enterica* that produce extended-spectrum β-lactamases (ESBLs) have emerged among animals and animal products. ESBL-producing *S. enterica* (ESBL-Salm) remain rare in Europe and North America, but less is known about their prevalence among animal-derived foods in countries with weaker food safety protections and unregulated use of antibiotics in animals.

We assessed ESBL-Salm contamination among fish, pork, and chicken sold in two markets in Phnom Penh, Cambodia, from September-December 2016. ESBL-Salm were sequenced and their genomes were characterized.

Twenty-six of 150 fish and meat samples (17%) were positive for ESBL-Salm, including 10/60 fish (17%), 15/60 pork (25%), and 1/30 chicken (3%). Pork-origin ESBL-Salm were primarily serotypes Rissen (10/15) or monophasic Typhimurium 1.4,5,12:i:- (3/15), while Saintpaul (3/10) and Newport (4/10) were more common among fish. Most ESBL enzymes were encoded by blaCTX-M-55 genes.

Figure 2: Acquired antibiotic resistance genes and corresponding plasmid replicon types.



3.6.4 RESEARCH PROGRAMS - PROSPECT 2019

In 2019, the LEFS will focus its activities to reinforce the laboratory competences and develop new research projects in microbiology and chemistry.

Serotyping and antimicrobial susceptibility testing of Salmonella strains isolated from food products in 2016-2017

Between 2016 and 2017, we isolated around 150 strains of Salmonella in different kind of food (meat products, sea food, ready to eat food, vegetables…).

Following the recent results showing the high prevalence of ESBL bacteria present in meat, chicken and fish sampled from 2 different markets in Phnom Penh, we are interested to study the AMR of Salmonella strains isolated in routine in LEFS and evaluate the risk for the consumers.

Indeed, SALMONELLA is one of the major causative agents of foodborne infections. Salmonellosis becomes more dangerous when strains resistant to several antibiotics are found in food, especially in chicken.The study will aim to determine the antibiotic resistance profile and genotypic characteristic of multi-drug resistant (MDR) isolates and study the serotype distribution of Salmonella among different kind of food products.

Prevalence of pesticide residuals in vegetable in Cambodia

The World Health Organization estimates 3 million cases of pesticide poisoning occur every year, resulting in an excess of 250 000 deaths. (WHO, 2004). The main exposure to pesticides for humans is oral ingestion, especially by vegetables and fruits. For instance, a study on fruits and vegetables imported from Southeast Asia into four European countries found pesticide residues above maximum residue limits in 33% of samples from Vietnam, 11% from Malaysia and 9% from Thailand. (Skretteberg et al., 2015).

In many developing countries, agricultural pesticide use is also rapidly increasing, particularly in Southeast Asia (Schreinemachers and Tipraqsa, 2012). Annual growth in Pesticide imports is estimated to be 55% for Lao, 10% for Vietnam and 7% for Thailand. (Schreinemachers et *al.*, 2015).

Cambodia has no pesticide manufacturing capacity of its own, and most available pesticides are imported officially and illegally from neighboring countries such as Thailand and Vietnam. In 2002, Cambodia legally imported approximately 200 tons from Thailand, Vietnam, China, Malaysia, France, Singapore and Taiwan (MOE, 2004). But this figure increased dramatically to 12 000 tons in 2012.

Few studies have been conducted in Cambodia on occupational pesticide exposure and associated health risks. A survey conducted by the Environmental Justice Foundation found that inappropriate pesticide use, including its timing, frequency, concentration, and type of pesticides used, are widespread. Safety measures are often ignored or misunderstood and 88% of 210 pesticide sprayers had experienced symptoms of pesticide poisoning. A report from 2004 by CEDAC found that 33% of pesticides available in the Cambodian market were banned by Cambodian law and that labels were most commonly written in Vietnamese and Thai languages which are incomprehensible to the Cambodian farmers. A small study in Cambodia using qualitative methodologies revealed that untrained sources such as neighbours or pesticide sellers trained farmers in the use of pesticides, there was a lack of appropriate personal protective equipment and that 84% used pesticides which are moderate to extremely hazardous to human health (WHO class Ia, Ib, II). However, there is a need to provide more information on pesticide management practices and to determine the health impacts of pesticide use among Cambodian farmers to improve future health interventions.

In the framework of a doctorate thesis conducted by Mr Yith Vuthy, we plan to study the contamination by pesticides residues in 9 commonly consumed vegetables (Chinese kale, Chinese cabbage, Choy sum, pakchoi, water morning glory, long bean, tomato, cucumber and lettuce) purchased from farms and local markets in Cambodia.

3.6.5 SUPPORT TO NATIONAL AUTHORITIES

For several years, IPC has supported different laboratories in Cambodia, including national public health laboratory, food and drug laboratory of Ministry of Health, Ministry of Industry and Handicraft, Cam Control laboratory, Ministry of Commerce, National veterinary research Institute (NAvRI), Ministry of Agriculture, Forestry and Fisheries and private sectors.

In 2018, the Ministry of Health has contracted to Environment and Food Safety Laboratory to carry out testing of street food sold in Cambodia. Thus, the laboratory received 401 food products and water for microbiology and chemical testing.

Furthermore, as part of a national monitoring program, the Ministry of Health send us, through sampling campaigns many industrial foods imported from south East Asian countries.

3.6.6 TEACHING AND TRAINING

In terms of training, the laboratory, as every year, has supervised 19 trainees coming from different universities in Cambodia and abroad for an internship of 1-3 months.

The details of these internships in terms of university origin, number of students, period and dates are in the table below:

University	Number of students	Scholar Year	Period (month)	Date
University of Health and Sciences (UHS)	7	Year 3	2	29/11/2017 - 18/01/2018 29/11/2017 - 18/01/2018 23/01/2018 - 08/03/2018 24/04/2018 - 14/06/2018 24/04/2018 - 14/06/2018 19/06/2018 - 02/08/2018 19/06/2018 - 02/08/2018
Ecole Nationale Superieure de chimie de Rennes	1	Year 1	2	11/06/2018 - 03/08/2018
TSMC (Technical School for Medical Care)	4	Year 3	1	22/08/2018 - 29/09/2018 22/08/2018 - 29/09/2018 01/10/2018 - 10/11/2018 01//10/2018 - 10/11/2018
ITC (Institute of Technology of Cambodia)	4	Year 5	3	05/03/2018 - 05/06/2018 05/03/2018 - 05/06/2018
		Year 1	1	13/08/2018 - 13/09/2018 03/09/2018 - 28/09/2018
RUPP (Royal University of Phnom Penh)	3	Year 4 & 3	3	03/05/2018 - 03/08/2018 19/06/2018 - 20/09/2018
		Year 3	2	16/07/2018 - 31/08/2018

3.6.7 PUBLICATIONS LIST 2018

2017

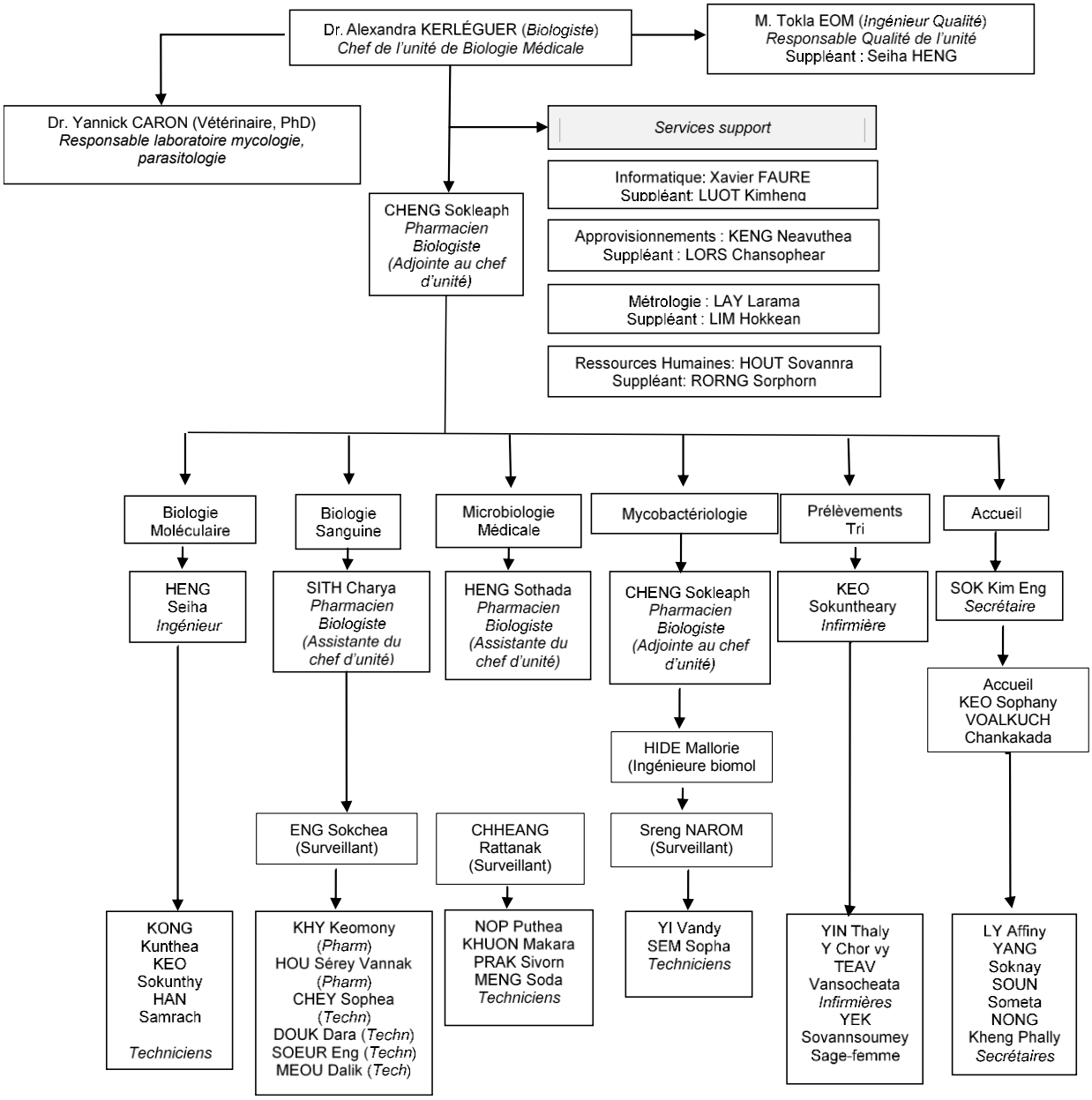
Yith Vuthy, Kruy Sun Lay, Heng Seiha, Alexandra Kerleguer, Awa Aidara-Kane. 2017. Antibiotic susceptibility and molecular characterization of resistance genes among Escherichia coli and among Salmonella subsp. in chicken food chains. Asian Pac J Trop Biomed. 7(7): 670–674

2018

Maya Nadimpalli, Laetitia Fabre, Vuthy Yith, Nita Sem, Malika Gouali, Elisabeth Delarocque-Astagneau, Navin Sreng, Simon Le Hello. CTX-M-55-type ESBL-producing Salmonella enterica are emerging among retail meats in Phnom Penh, Cambodia. J Antimicrob Chemother 2019; 74: 342

3.7 MEDICAL LABORATORY

3.7.1 FUNCTIONAL STRUCTURE OF THE UNIT



3.7.2 ROUTINE ACTIVITY AND EVENTS 2018

1°) The main part of the activity consists of:

- Routine analysis (individuals patients, public and private hospitals, NGOs and Voluntary Counselling and HIV Testing Centre)
- Patients and physicians counseling, as well as in the monitoring of anti-retroviral and anti-tuberculosis treatment

2°) Main Focal Point

Medical laboratory is the first laboratory in Cambodia awarded ISO15189 standard. The search for the satisfaction of our patients and prescribers is the main of our Quality Policy.

3°) Activities Comparison between 2017and 2018

	2017	2018	Balance
Biology	97790	109800	+12,5%
Molecular biology	1015	1620	+58%
Microbiology	8913	7241	-18%
Mycobacteriology	7516	6552	-12%
Total	115234	125213	+40,5%

Due to:

- ISO 15189 accreditation obtaining builds patient confidence
- Renovation of the premises

4°) HIV Screening

	2017	2018
HIV Screening (number)	4167	4246
HIV weakly positive	11	18
HIV highly positive	149 (3.60%)	282 (6.60%)

Distribution	2017	2018
Cambodian people	73,10%	77,98%
Men	68,90%	70,25%
Couple	31,60%	26%
Single	68,40%	74%
High-Risk Sexual Behaviour	73%	76%
Hétérosexual	83,00%	70%
men having sex with men	5,30%	20%

Increasing of the HIV seropositivity and increasing of the MSM proportion

5°) Melioidosis

Year	**Total isolates**	**Total cases**	Blood	Sputum	Bal / ba	Pus	Pleural	Joint	Throat	Other
2011	1	1	1							
2012	7	7			4	3				
2013	5	5	2		1	1		1		
2014	6	6	1		1	3			1	
2015	16	15			2	10	2	1		1
2016	12	8	4	2		6				
2017	19	17	4	2	4	9				
2018	29	25	20	1	3	5				

The cases increase is due to a better knowledge of the disease and also to an improvement in the diagnosis of tuberculosis, allowing differential diagnosis

6°) AMR

- Infectious samples

	2016	2017	2018
Total enterobacteriaceae	505	657	724
ESBLE (n)	166	209	201
ESBLE (%)	33	32	28

It seems that the percentage of ESBLE begins to decrease

- Intestinal carriage: 40.60%

3.7.3 RESEARCH PROGRAMS – MAJOR ACHIEVEMENTS IN 2018

Beta-lactam resistance among *Enterobacteriaceae* in Cambodia: the four-year itch

Although antibiotics are often used inappropriately, few data have been published on antibiotic resistance in Cambodia. Epidemic dissemination and transfer of resistance genes to other bacterial species put the population at risk. The aim of this study was to evaluate the frequency and characteristics of extended-spectrum beta-lactamase enterobacteriaceae (ESBLs-E) isolated in consecutive samples tested at Institut Pasteur du Cambodge from 2012 to 2015. The proportion of ESBLs-E increased from 22.6% to 35% during the study period. Among the ESBLs-E, the proportion of ESBLs-*E. coli* increased significantly from 28.9% in 2012 to 42.7% in 2015 while the rise for *K. pneumoniae* remained below significance (33.7%).

These results were published in International Journal of Infectious Diseases.

Stunting, beyond acute diarrhea: *Giardia duodenalis*, in Cambodia

The adverse outcomes of malnutrition on the development of a child are well acknowledged as are the broad variety of contextual factors that may impact child nutritional status. Adequate nutrient intake and the adoption of appropriate water, sanitation and hygiene measures are largely documented for their positive influence on health. Improved sanitation and protection from human feces can significantly lower the incidence of diarrhea and environmental enteropathy. However, the impact of excessive exposure to animal feces on child health is less well documented. This study tests the hypothesis that there is a positive association between exposure to animal feces, morbidity and anthropometric outcomes in children under 5 years of age, in Cambodia. Data for this study was drawn from the third follow-up round of the MyHealth project cohort study that is conducted in six districts of three Cambodian provinces (Phnom Penh, Kratie and Ratanak Kiri). The analysis included a sample of 639 children under 5 years of age. The presence of livestock and more particularly, pigs near the main household dwelling was found a risk factor associated with *Giardia duodenalis* infection (23%). *Giardia duodenalis* infection was found to be a protective factor for acute diarrhea, yet, associated with stunting in the univariate model.

Collaboration: Unicef Cambodia, these results were published in Nutrients

PTR on Aspergillosis and aspergillus in Cambodia (Yannick CARON)

This PTR get ethical approval from the Cambodian national ethical committee (NECHR) on December, the 26th 2017. It is worth noting that due to ethical, financial, clinical and practical constraint, some adaptation were necessary and a focus on Aspergilloma was decided (new acronyme: “AspA” for aspergilloma in Cambodia). A sponsorship transfer from Institut Pasteur Paris to Institut Pasteur Cambodia was accepted. The main objective of the project was the set up of the first mycology lab in the Pasteur Institute International Network. This objective is fully accomplished. Furthermore a clinical research assistant (SIM Mala) and a technician (YUN Sreyroth) were recruited to help the patient inclusion process, the monitoring of the study and lab experiments. The patient inclusion was finalized in the beginning of October 2018 with six hundreds fourty five included patients: 53.2% of men (age mean of 57.65 years +/- 16.18) and 46.8% of woman (age mean of 59.15 years +/- 15.59). Patelia ELISA® (Golden standard) was positive for 14.54% of the patient (81/557). Eight recombinant proteins (Gel1, Crf1, Cat1, 18Kd, Sod1, A97, H41 and H70) were tested for diagnosis purpose and only 2 permited the differenciation between positive and negative serum control. The AUC was estimated to 0.84% for 18kd and 0.89% for A97. The direct examination of the sputum was positive for 37.7% (210/557) of the patient. Aspergillus specied involved in aspergilloma was evaluated to 42% for *Aspergillus flavus*, 25% for *A. fumigatus* and 22% for *A. niger*. About 350 strains were isolated from patient and 50 from the hospitals environment. A preliminary study seems to show presence of itraconalzole resistance for *A. fumigatus* strain isolated in a patient (MIC > 2 µg/ml instead of 0.12-1 µg/ml for *Aspergillus fumigatus* wild type KU80). The remaining analysis like for example, statistics, molecular identification of the strain and genetic mutation conferring resistance are ongoing. The PTR was extended till the end of April 2019 without financial supplement.

Collaboration: Calmette Hospital (Phnom Penh), Khmer-Soviet Friendship Hospital (Phnom Penh) and Kossamak Hopital (Phnom Penh)

Outbreak of trichinellosis in September 2017 in Kampong Thom Province

On September the 26th 2017, an important outbreak (33 cases including 8 dead) of trichinellosis (*Trichinella* spp.) in Central Kampong Thom Province Hospital was reported and was then diagnosed in Phnom Penh in two hospitals: Calmette and Preah Ket Mealea Hospitals. A proposition to investigate the parasite with the help of the Trichinella Reference Laboratory (Cochin Hospital, Paris, France) to the Communicable Disease Control Department of the Ministry of Health of Cambodia was accepted. We accessed to the complete medical records (clinical symptoms, diagnosis, medical history, treatment, outcome …) of each patients hospitalized in the tird involved Hospital in the frame of this outbreak. Very few scientific data exists in Cambodia on this zoonotic disease and the molecular characterization of the strain involved could be interesting for the management of this important public health issue.

Collaboration: Calmette Hospital (Phnom Penh), Kampong Thom Province Hospital (Kampong Thom), Preah Ket Mealea Hospital (Phnom Penh)

Genetic determinants and evolution of drug resistance in *Mycobacterium tuberculosis* in Cambodia, high tuberculosis burden country

The overall objective of this project is to understand the emergence, spread and evolution of antibiotic resistance in *Mycobacterium tuberculosis* (MTB) in Cambodia by using genetic characterization of MTB isolates. This is a three years study started since September 2016. Until 2018, a total of 473 MTB isolates with known Drug susceptibility testing (DST) for FLDs (Isoniazid, rifampicin, streptomycin and ethambutol), collected between 2012 and 2015 were included in the study. Among them, 143 were sensitive to FLDs and 326 were resistant to at least one FLD. These isolates were genetically characterized using sequencing of genes involving in the resistance of MTB to first and second line anti-TB drugs and genotyping method (spoligotyping and MIRU-VNTR). The laboratory experiment still ongoing and data is being analyzed for publication.

Training: This project data will be used one PhD thesis and 2 DES thesis

Collaboration: National Center for Tuberculosis and Leprosy Control (CENAT), Cambodia

Sponsor:

- Laboratoire Mixte International: Drug Resistance in South East Asia (LMI-DRISA), IRD
- Ambassade de France in Cambodia
- Ministry of Education Youth and Sport
- NIHE : PHC Lotus Project

Laboratoire Mixte International DRISA «Drug Resistance in Southeast Asia»

Through the collaboration with the Institut de Recherche pour le Development, since August 2018, we are hosting a research engineer, Dr. Mallorie HIDE, specialized in molecular biology.
Dr. Hide will base in LBM for at least 2 years in the framework of the LMI DRISA. She will work on drug resistance in *Mycobacterium tuberculosis* and other bacteria in Cambodia and on the application of antimicrobial peptides as alternatives to antibiotics.

3.7.4 RESEARCH PROGRAMS AND ROUTINE ACTIVITIES – PROSPECT 2019

PTR on Tuberculosis: Discovering and understanding the virulence of *Mycobacterium tuberculosis* in Cambodia

Collaboration: Institut Pasteur in Paris

Despite being classified among 30 HBC, little work has been carried out on the genetic diversity of *M. tuberculosis* (Mtb) infecting humans in Cambodia (WHO 2016). Hence further study needs to be conducted to provide more accurate features of the molecular epidemiology and to better understand the pathogenic mechanism of the Mtb isolates circulating in Cambodia. The main goal of this PTR project, is to combine complementary skills, ranging from clinical to fundamental research, with cutting-edge technologies in different scientific fields in order to characterize and to identify the mycobacterial strains causing TB in Cambodia and to get deeper insight into virulent factors that govern the intracellular fate of those clinical isolates strains and the response of the host and drug resistance mechanisms of the strains.

Study of Antibiotic Resistance in Cambodia using a One Health Approach and Evaluation of MinION technology as Diagnostic Tool

Collaboration: Institut de Recherche pour le développement

In the recent years, the situation of antibiotic resistance became critical since multiple and extended resistant bacteria have become more prevalent. Even if we know that the current antibiotic resistance crisis is due to the massive use, overuse and misuse of these drugs, in many countries including Cambodia with a cruel lack of antibiotic usage regulation, still very little data are available and no surveillance system exists. In this context, we submitted a project which aim to identify the sources of emergence and spread of resistant bacteria in Cambodia using a One Health approach and to evaluate if the MinION technology could be used as diagnostic tool. This project will have a huge impact since for the first time, it will explore the spread of antibiotic resistance in humans, animals and environment in Cambodia. The expected results will represent the baseline for the setting up of a surveillance system, will allow stakeholders to implement efficient control strategies and will help determine the capacity of MinION technology to be used as diagnostic tool.

Antimicrobial Resistance in Cambodia: Collaborative program with Institut Pasteur (Cambodia) and Doherty Institute (Australia)

Collaboration: Doherty Institute

The Doherty and Pasteur Institutes are working together to help address this critical Antimicrobial Resistance (AMR) capability gap in the region, by creating a bacteriology collaborative program. This program will focus on addressing important questions around AMR in bacteria of major human health significance, including tuberculosis.

3.7.5 SUPPORT TO NATIONAL AUTHORITIES

- With the CENAT, to develop laboratory technical procedures guideline for the National TB Reference Laboratory including microscopy, Xpert MTB/RIF, Tuberculosis Culture & DST.
- Member of CENAT's TWG on multi-drug resistant tuberculosis.
- With MOH and WHO: modernization of laboratories, modification of the national laboratory policy and surveillance of Multi-Drug Resistant Bacteria
- With MOH Follow-up of melioidosis: in 2017 we isolated 21 Burkholderia pseudomallei: 8 from pulmonary samples, 8 from pus and 5 from blood culture.
  The annual declaration is requested by the Ministry of Health.
- With NCHADS : follow-up of HIV seropositivities

3.7.6 TEACHING AND TRAINING

A. Continuous training for staff
- DU Qualité, accréditation et Audit, Université de Lille
- Molecular Biology course organized by IPC

- MOOC - France Université Numérique on Tuberculoses
- MOOC - France Université Numérique on Resistance to antibacterial agents
- External Quality control
- E-learning : ISO 15189 et accréditation des Laboratoires de Biologie Médicale : découvrez la démarche qualité selon l'ISO 15189
- Safety training for BSL3 and PPE evaluation, IPC
- English language course, IPC

B. Internship

Master of Medical Biology (UHS): 2
Pharmacists (UHS): 20
Laboratory Technicians (UHS & UP):
Bachelor of Bioengineering (RUPP): 2

C. Thesis supervision

One PhD in Health Biology, University of Montpellier: Genetic determinants and evolution of drug resistance in Mycobacterium tuberculosis in Cambodia, high tuberculosis burden country

Four Thesis (1 finished and 3 in progress) for Doctor of Pharmacy degree specializing in Medical Biology, University of Health and Science.

- Étude des entérobactéries productrices de bêta-lactamase à spectre étendu à l'Institut Pasteur du Cambodge.
- Bactéries productrices de carbapénémases, suivi évolutif à l'Institut Pasteur du Cambodge
- Étude des mutations des gènes liés à la résistance de *Mycobacterium tuberculosis* isolées des patients au Cambodge
- Genetic diversity of drug resistant *Mycobacterium tuberculosis* isolates circulating in Cambodia between 2013 and 2015, thesis defensed on December 21, 2018.

D. Participation to the set up of an online upgrading course for the Master of Infectiology

- The Master of infectiology by University of Health Science (UHS, Cambodia), the University of Paris-Saclay (UPS, France) and the Institut Pasteur in Phnom Penh (IPC, Cambodia) is an advanced level program and is intended as a continuation of the French Bachelor degree in Life Science. To enroll in this Master program, all applicants should hold the equivalent of this degree. For students with non-french credentials, they are then asked to successfully complete an upgrading course (Moodle) to ensure that their background is equivalent in terms of course content/standard in the following fields : *Molecular Biology, Cell Biology, Biochemistry, Genetics* and *Statistics*. The course was mainly composed on links to Khan Academy ressources. The course was available for a limited period of time (about 2 months) during which each of the enrolled student (29) must passed a quiz (20 questions, three attemps, > 15/20) for the 5 fields. Nine students validated this upgrading course.

  URL: https://masterinfectiologyupdate.moodlecloud.com/

E. Other

- Agreement with the "French Lycée, René Descartes" to receive the students of 3rd class, to achieve their practical work on bacterial identification.

3.7.7 PUBLICATION LIST (2017 AND 2018)

2018

1. Nouhin J, Bollore K, Castera-Guy J, Prak S, Heng S, Kerléguer A, Rubbo PA, Rouet F, Tuaillon E. 2018. Analytical and field evaluation of the BiocentricGeneric HCV Assay on open and polyvalent PCR platforms in France and Cambodia. J Clin Virol, 108 53-58.
2. Nadimpalli M, Delarocque-Astagneau E, Love DC, Price LB, Huynh BT, Collard JM, Lay KS, Borand L, Ndir A, Walsh TR, Guillemot D; Bacterial Infections and antibiotic-Resistant Diseases among Young children in low-income countries (BIRDY)

Study Group. 2018. Combating Global Antibiotic Resistance: Emerging One Health Concerns in Lower- and Middle-Income Countries. Clin Infect Dis, 66(6):963-969. doi: 10.1093/cid/cix879.
3. Inghammar M, By Y, Farris C, Phe T, Borand L, Kerleguer A, Goyet S, Saphonn V, Phoeung C, Vong S, Rammaert B, Mayaud C, Guillard B, Yasuda C, Kasper MR, Ford G, Newell SW, Sam U, Sokhal B, Touch S, Turner P, Jacobs J, Messaoudi M, Komurian-Pradel F, Tarantola A. 2018. Serotype distribution of clinical Streptococcus pneumoniae isolates before the introduction of the 13-valent pneumococcal conjugate vaccine in Cambodia. American Journal of Tropical Medicine & Hygiene, 98(3):791-796.
4. Rouet F, Nouhin J, Zheng DP, Roche B, Black A, Prak S, Leoz M, Gaudy-Graffin C, Ferradini L, Mom C, Mam S, Gautier C, Lesage G, Ken S, Phon K, Kerleguer A, Yang C, Killam W, Fujita M, Mean C, Fontenille D, Barin F, Plantier JC, Bedford T, Ramos A, Saphonn V. 2018. Massive iatrogenic outbreak of human innunodeficiency virus type 1 in rural Cambodia, 2014-2015. Clinical Infectious Diseases; 66(11):1733-1741.
5. Berteloot L, Marcy O, Nguyen B, Ung V, Tejiokem M, Nacro B, Goyet S, Dim B, Blanche S, Borand L, Msellati P, Delacourt C, for the ANRS 12229 PAANTHER 01 Study Group. 2018. Value of chest X-ray in TB diagnosis in HIV-infected children living in resource-limited countries: the ANRS 12229-PAANTHER 01 study. Int J Tuberc Lung Dis, 22(8):844-850.
6. Marcy O, Tejiokem M, Msellati P, Truong Huu K, Do Chau V, Tran Ngoc D, Nacro B, Ateba-Ndongo F, Tetang-Ndiang S, Ung V, Dim B, Neou L, Berteloot L, Borand L, Delacourt C, Blanche S; ANRS 12229 PAANTHER 01 Study Group. 2018. Mortality and its determinants in antiretroviral treatment-naive HIV-infected children with suspected tuberculosis: an observational cohort study. The Lancet HIV, 5(2):e87-e95.
7. Caron Y, Rattanak C, Puthea N, Soda M, Boyer S, Tarantola A, Kerléguer A. 2018. Beta-Lactam resistance among Enterobacteriaceae in Cambodia: The four-year itch. Int J Infect Dis, 66:74-79.
8. Caron Y, Hong R, Gauthier L, Laillou A, Wieringa FT, Berger J, Poirot E. 2018. Stunting, beyond acute diarrhoea: Giardia duodenalis, in Cambodia. Nutrients.

2017

1. Andries AC, Duong V, Cappelle J, Ong S, Kerleguer A, Ly S, Tarantola A, Horwood PF, Sakuntabhai A, Dussart P, Buchy, P. 2017. Proteinuria during dengue fever in children. Int J Infect Dis, 55, 38-44.
2. Padget M, Tamarelle J, Herindrainy P, Ndir A, Diene Sarr F, Richard V, Piola P, Guillemot D, Delarocque-Astagneau E, BIRDY Study Group. 2017. A community survey of antibiotic consumption among children in Madagascar and Senegal: the importance of healthcare access and care quality. J Antimicrob Chemother, 72, 564-573.
3. Horwood PF, Duong V, Laurent D, Mey C, Sothy H, Santy K, Richner B, Heng S, Hem S, Cheval J, Gorman C, Dussart, P, De Jong MD, Kerleguer A, Guillard B, Murgue B, Lecuit M, De Lamballerie X, Farrar JJ, Tarantola A, Eloit M, Buchy P. 2017. Aetiology of acute meningoencephalitis in Cambodian children, 2010–2013. Emerging Microbes & Infections, 6, e35.
4. Vuthy Y, Lay KS, Seiha H, Kerleguer A, Aidara-Kane, A. 2017. Antibiotic susceptibility and molecular characterization of resistance genes among Escherichia coli and among Salmonella subsp. in chicken food chains. Asian Pacific Journal of Tropical Biomedicine, 7, 670-674 (2017)

Oral/poster presentation

1. Y. Caron. “Parasites: Useful or useless?” Conference at Institut Français du Cambodge, on June 2018. Phnom Penh, Cambodia.
2. Y. Caron. “Les aspergillomes au Cambodge“ Oral presentation in International congress of Cambodian Pulmonary Association (CPA) and Aides aux Insuffisants Respiratoires du Cambodge (A.I.R. Khmer) on October the 30-31th 2018.
3. Janin Nouhin, Sophearot Prak, Seiha Heng, Alexandra Kerléguer, François Rouet Performance characteristics of a new commercial assay for HCV RNA viral load quantification in plasma: Preliminary results in Cambodia. 9th IAS Conference on HIV Science, Paris, 23-26 July 2017
4. Borand L, De Lauzanne A, Inghammar M, Ung V, Cheng S, Pham T.H, Msellati P, Tejiokem M, Ouedraogo A.S, Godreuil S, Delacourt C, Blanche S, Marcy O. High prevalence of respiratory Nontuberculous Mycobacteria Respiratory Infections in Children Living with HIV in South-East Asia., ANRS 12229 PAANTHER 01 Study Group. MOAB0205, The 9th IAS Conference on HIV Science, 23-26 July 2017 Paris, France
5. A. Delauzanne, S. Lach, A. Kerléguer, V. Ngo, S. Touch, A. Tarantola, L. Borand, Birdy study group. High level of Extended Spectrum Beta Lactamase Producing Enterobacteriaceae fecal carriage in mothers and Infants; Preliminary results of the Birdy Program in Cambodia.. Institut Pasteur International Network, Scientific symposium, Paris, November 29th-December 2nd, 2016
6. Cheng S, Heng S, Him V, Sreng N and Kerléguer Comparative performance of “LoopampTM MTBC Detection” kit for rapid detection of complex Mycobacterium tuberculosis: a retrospective study. A. The 6th Conference of IUATLD Asia Pacific Region, 22-25 March 2017

3.8 OTHER SERVICES OF THE INSTITUT PASTEUR DU CAMBODGE

The medical activities performed by Institut Pasteur du Cambodge, commissioned by the government in the agreement with IPC (described as service activities or public health care activities at Institut Pasteur) also provide direct access to patients.

3.8.1 RABIES PREVENTION CENTER FOR 2018

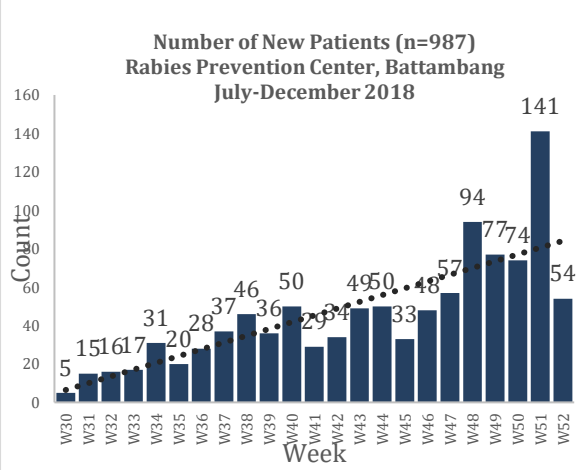
Set up under the terms of Article 7 of the 27 August 1992 convention between the State of Cambodia and Institut Pasteur, the Rabies Prevention Center at the Institut Pasteur du Cambodge is the largest

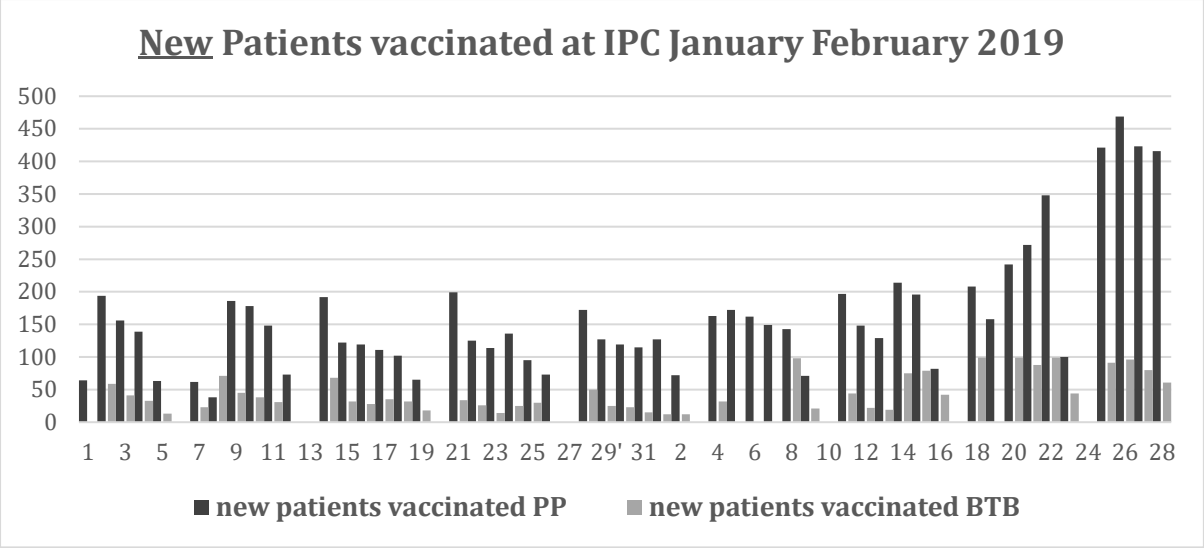
rabies prevention center in Cambodia. It has a medical team of 4.5 full-time equivalents placed under the responsibility of a medical doctor (Dr LY Sowath). This team offers prevention protocol up to WHO norms at a price affordable to the people because the treatment is subsidized by the Institut Pasteur du Cambodge (in 2018 US$15 for a protocol of 2 x3 injections, following new WHO recommanded protocol). The vaccine we use is Verorab produced by Sanofi. In 2015, the Rabies Prevention Center at Institut Pasteur du Cambodge (RPC IPC) had an intake of 21,301 patients for post-exposure management. A total of 185 head of biting animals were examined at the virology laboratory, of which 94 (50.8%) came out positive for rabies under immunofluorescence. In 2016, RPC IPC had an intake of 21,664 patients for post-exposure management. A total of 157 head of biting animals were examined at the virology laboratory, of which 79 (50.3%) came out positive for rabies under immunofluorescence.

In 2017, RPC IPC had an intake of 22,421 patients for post-exposure management. A total of 187 heads of biting animals (185 from dogs and 2 from cats) were examined at the virology laboratory, of which 117 (62.6%), all were from dogs, came out positive for rabies under immunofluorescence.

In 2018 RPC IPC vaccinated for PEP 25,081 people in Phom Penh, most of the time bitten by dogs (82,3%), and 1,201 people in Battambang center. In total of 176 **animal** heads were tested by immunofluorescence for rabies virus at the Virology Unit, IPC, of these 121 (68.8%) were positive for rabies (120 dogs, 1 cat) (tested).

This information is regularly communicated to MoH and MoH CDC, FAO and WHO.
Selection and implementation of the satellite PHD – IPC Rabies Prevention Center in Battambang, close to Provincial Hospital and MoH PHD, had been effective in July 2018. A memorandum of understanding has been signed on 25th of December, 2017, between Battambang Provincial Health department and Institut Pasteur du Cambodge. The center has been inaugurated by HE OR Vandine, General Director for Health and HE the deputy Governor of Battanbang Province, on 28 of September 2018. Activity is increasing week after week in this center.
Selection and implementation of a second satellite PHD – IPC Rabies Prevention Center in Kampong Cham, close to Provincial Hospital and MoH PHD, is in progress (temporary opening on 7 March 2019).





3.8.2 INTERNATIONAL VACCINATION CENTER FOR 2018

Set up under the terms of Article 7 of the 27 August 1992 convention between the State of Cambodia and Institut Pasteur, the International Vaccination Center at the Institut Pasteur du Cambodge has a medical team available of 3.5 full-time equivalents placed under the responsibility of a medical doctor (Dr LY Sowath). It offers the public vaccines from the extended vaccination program and other vaccines or immunoglobulins six days a week. The vaccines are all of international quality and are handled in a cold chain and subject to quality control also up to international standards.

In 2018, the medical team delivered 30915 injections (including the immunoglobulins) in the framework of 18947 vaccine protocols. In 2017, the medical team delivered 28,020 injections (including the immunoglobulins) in the framework of 19,121 vaccine protocols. In 2016, the medical team delivered 27043 injections (including the immunoglobulins) in the framework of 16472 vaccine protocols. In 2015, the medical team delivered 25,255 injections (including the immunoglobulins) in the framework of 14,148 vaccine protocols. In 2014, 26,282 injections (including the immunoglobulins) had been delivered in the framework of 15,849 vaccine protocols, compared with 21,521 injections (+22.1%) for 14,165 vaccine protocols (+14.8%) in 2013.

3.8.3 VOLUNTARY COUNCELING AND FREE TESTING CENTER (VCTC) FOR 2018

The first case of HIV infection was diagnosed in Cambodia in 1991. After a prevalence peak of 1.4% to 1.7% in the population between 2001 and 2003, the estimated prevalence continued to fall to stand at approximately 0.6% at the present time. In 1995, the Institut Pasteur du Cambodge (IPC) was the first center to have set up an anonymous free testing and counseling center (VCTC). This service is financed entirely by the IPC. Over the years, the number of centers countrywide has increased significantly. By late 2010, there were already 246 VCTCs in the country (source NCHADS). However, the IPC's VCTC is a referral center for test confirmations as shown by the high percentage of tests that have come back positive. It has a FTE doctor working under the supervision of another medical doctor.

In 2018 there were 680 pre-test consultations of which 673 were followed up by post-test counselling. In 2017 it was 636 pre-test consultations of which 633 were followed up by post-test counseling in 2017. In 2016 it was 622 pre-test consultations of which 618 were followed up by post-test counselling in 2015).

A total of 74.3 % of the consultants live in Phnom Penh (74.4 % in 2017, 70.9 % in 2016). The grounds for appeal was a risk ratio or practice in 75.4 % of the cases (72.8 % in 2017, 69.1% in 2016). With 129 cases confirmed in 2018 (34 in 2017, 33 in 2016), the prevalence established was 19.0 % (5.3 % in

2017, 5.1% in 2016) among the overall patient cohort, 22.0 % among persons who had never taken a test (6.3 % in 2017, 5.5% in 2016) and 5.4 % among the consultants who came back for their results and who were directed to the public health care system (5.1% in 2017, 5.8% in 2016). No indeterminate result has been rendered since implementation of the Western blot test.

4 CONCLUSION

L'Institut Pasteur du Cambodge, avec plus de 235 personnes, dont plus de 50 cadres scientifiques cambodgiens, français, et d'autres nationalités, et près de 100 étudiants en stage, est parmi les principaux instituts de recherche en santé en Asie du Sud-Est.

Fort de ses près de 50 contrats sur convention, de ses 50 articles scientifiques ou chapitres de livre publiés en 2018, de son expertise en santé publique, des services rendus à travers ses laboratoires d'analyse et ses centres de vaccination et de référence, l'Institut Pasteur du Cambodge est fier de ses activités au service du Cambodge et de la communauté internationale.

Cependant il reste encore beaucoup à faire. De nouveaux champs scientifiques et de santé publique restent à explorer. Les capacités doivent être renforcées dans certains domaines. Des recherches innovantes préliminaires, mais à risque, doivent être initiées sur certains sujets, sur fonds propres de l'IPC. La contribution à la formation et à l'enseignement doit être développée en lien avec les Universités du Cambodge.

Enfin, l'intérêt de l'Institut Pasteur du Cambodge pour l'ensemble de ses partenaires (Ministères, universités, Institut Pasteur, centres de recherche nationaux et internationaux, secteur social et secteur économique) doit être réaffirmé et renforcé.

Tout cela ne sera possible qu'avec un soutien fort et constant des tutelles et des partenaires : Ministère de la Santé du Cambodge, Ministère de l'éducation de la jeunesse et des sports du Cambodge, Institut Pasteur et Ministères de la Recherche et des Affaires Etrangères de France, et avec la confiance des partenaires nationaux et internationaux.

The Institut Pasteur du Cambodge, with a staff of 235 persons, including over 50 scientific supervisors from Cambodia, France and other countries and with about 100 intern students, ranks among the leading health research institutes in Southeast Asia.

With 50 research contracts, 50 scientific articles or book chapters published in 2018, its expertise in public health, the services it delivers through its analysis laboratories and vaccination and referral centers, the Institut Pasteur du Cambodge is proud of what it is doing for Cambodia and the international community.

However, there is much that remains to be done. New scientific and public health fields require exploration. Capacity building is needed in some key areas. Innovative preliminary—but risky—research has to be initiated in certain fields, with the IPC's own-source funding. Contribution to training and education must be developed in partnership with Cambodia's universities.

Finally, the interest and added value that the Institut Pasteur du Cambodge has for all of its partners (ministries, universities, Institut Pasteur affiliates, national and international research centers, the social and economic sectors) must be reaffirmed and strengthened.

All of that will only be possible with the robust and ongoing support of the key agencies: Ministry of Health, Ministry of Education, Youth and Sport of Cambodia, Institut Pasteur and Ministries of Research and Foreign Affairs of France, and with the trust of the national and international partners.

ANNEXE
ORGANIGRAMME 2018 DE L'IPC

Date: 24 October 2018