

# Institut Pasteur du Cambodge

## 2017 scientific report and 2018 prospects Rapport 2017 et programmation scientifique 2018



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## 1 RESUME / SUMMARY

- English version below

En 2017, l'Institut Pasteur du Cambodge (IPC), avec 230 employés, s'est développé dans ses trois missions : recherche, formation et services. L'institut Pasteur du Cambodge a connu plusieurs événements marquants en 2017.

- Des projets de recherche ambitieux se sont terminés et ont donné lieu à des séminaires de clôture, rapports, recommandations et rédaction d'articles (Ecomore1, Lacanet, ComAcross, Defeat Dengue, DVI).
- D'autres projets de recherche se poursuivent et se développent donnant des résultats applicables dans des recommandations de santé publique (SEAE, DHHS, résistance aux antipaludéens, Birdy, ANRS Statis).
- De nouvelles activités de recherche et de nouveaux projets, nécessaires pour la santé publique du Cambodge, ont été initiés et développés en 2017 en partenariat national et international : hépatites, antibiorésistance, tuberculose, coqueluche, entomologie médicale, infections fongiques, paludisme. (projets : Dengue Ecomore 2 (AFD), Paludisme ICEMR (NIH), Hépatite B Ta Prohm (ANRS), coqueluche Perilic (Total), Tuberculose mi RNA et TB Speed (ANRS, Unitaïd et Initiative 5%).
- Le Ministère de l'Education de la Jeunesse et des Sport avait soutenu en 2016 les activités de recherche et de formation de 2 chercheurs Cambodgiens de l'IPC. Les dossiers de 3 autres chercheurs ont été sélectionnés en 2017 par le MEJS, mais le Ministère des finances n'a pas autorisé le financement.
- Le rythme, qualité et quantité, de publications a été maintenu (>45).
- 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'est considérablement accéléré avec l'écriture et la diffusion massive d'un livre éducatif, et la préparation de l'ouverture du centre de vaccination de Battambang.
- La préparation de l'Audit Iso 15189 pour le laboratoire de biologie médicale prévu en mai 2018 a été finalisée.
- Les collaborations régionales ont été renforcées : 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 (plus 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 septembre 2018.
- 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 (et au Ministère de l'Agriculture) et à l'OMS ont été maintenues 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, Direction Générale)
- Le laboratoire de microbiologie alimentaire et des eaux, rebaptisé Laboratory Environment and Food Safety (LEFS) a continué à se développer et a intégré de nouveaux laboratoires modernes et spacieux (près de 300 m2).
- Le laboratoire de virologie VIH – Hépatite a intégré l'unité de Virologie comme nouvelle équipe, dans de nouveaux laboratoires.
- Les laboratoires d'immunologie ont déménagé dans de nouveaux laboratoires, attenants à l'unité de virologie.

- Les laboratoires de l'unité d'épidémiologie du paludisme ont déménagé dans de nouveaux laboratoires, mieux adaptés, au premier étage de la plateforme régionale de recherche (PPR Asie).
- Deux laboratoires BSL2 ont été rénovés en 2017. L'Institut héberge 5 BSL2 et un BSL3.
- La modernisation de l'accueil du laboratoire de biologie médicale a été initiée.

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 2017, 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 et du MEAE de France.

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 45 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 2017, 108 étudiants (93 Cambodgiens, 15 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. Il y a eu une augmentation très sensible des stagiaires diplômés accueillis pour des stages longs. 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 et RUPP ont été renforcées en 2017.

L'IPC compte plus de 230 employés, dont 25 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, centre de vaccination anti-rabique post exposition et CGAG), d'unités et de plateforme de recherche : immunologie (incluant un groupe à 4 ans), entomologie médicale, virologie, HIV/Hépatites (qui a fusionné fin 2017 avec la 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 %). 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 M. l'Ambassadeur de France, du Directeur 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 8 et 9 juin 2017. La politique scientifique est ajustée en fonction des recommandations de 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, est 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.

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*In 2017, the Institut Pasteur du Cambodge (IPC), with 230 employees, expanded in its three missions: research, training and services. The Institut Pasteur du Cambodge experienced a number of significative changes in 2017.*

- *Many ambitious research projects have been finalized (Ecomore1, Lacanet, ComAcross, Defeat Dengue, DVI)*
- *Several research projects were greatly expanded, and conducted to public health recommendations (SEAe, DHHS, Anti malarial drug resistance, Birdy, ANRS Stasis).*
- *New research activities needed for public health in Cambodia were initiated or developed in national and international partnership: hepatitis, antibiotic resistance, tuberculosis, pertusis, medical entomology, fungal infections, malaria. (projets : Dengue Ecomore 2 (AFD), malaria ICEMR (NIH), Hepatitis B Ta Prohm (ANRS), pertusis Perilic (Total), Tuberculosis mi RNA et TB Speed (ANRS, Unitaïd, Initiative 5%).*
- *Continuous scientific training for staff has been encouraged through numerous workshops and internships, including abroad.*
- *Ministry of Education, Youth and Sport supported in 2016 research and supervising activities conducted by 2 Cambodian researchers from IPC. MOEYS selected 3 more applications from IPC in 2017, but Ministry of Finances did not allow the funding.*
- *The quality, and "quantity" of IPC's publications were maintained in 2017.*
- *The IPC project to control rabies, in partnership with the Ministries of Health; Agriculture, Fisheries and Forestry; and Education, Youth and Sport, has accelerated considerably with the writing and mass distribution of an educational book (40 000 samples), and the preparation for the opening of the rabies vaccination centre in Battambang in April 2018.*
- *The Iso 15189 Audit for the Medical Biology Laboratory scheduled for May 2018 has been finalized.*
- *The food microbiology and water lab was extended.*
- *Laboratories and support services were streamlined and expanded, such as renovation of two BSL2.*
- *Regional collaborations have been developed (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 (more than 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 2018.*
- *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) and WHO 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, Directorate General)
- 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 m<sup>2</sup>).
- The HIV-Hepatitis Virology Laboratory has integrated the Virology Unit as a new team in new laboratories.
- Immunology laboratories have moved into new laboratories adjacent to the virology unit.
- The laboratories of the Malaria Epidemiology Unit moved to new, better adapted laboratories on the first floor of the regional research platform (PPR Asia).
- Two BSL2 laboratories were renovated in 2017. The Institute hosts 5 BSL2 and one BSL3.
- Modernization of the reception area for the medical biology laboratory has been initiated.

*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 45 scientific articles (and book chapters) in 2017 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 (ministère de l'Enseignement supérieur et de la Recherche – ministry of higher education and research) and MEAE (ministère de l'Europe et des Affaires étrangères– ministry of foreign affairs).*

*The findings of research and expert studies have come out in the form of recommendations or briefing notes submitted to the Ministry of Health and WHO. Detailed 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 2017, in total 108 graduate students had training (from 6 to 36 months) at IPC (93 Cambodian students, 15 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 and RUPP have been reinforced in 2017.*

*The IPC has a staff base of over 230, including 25 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 and VCTC), of 6 units or research platforms: immunology (including a 4-year group), medical entomology, virology, HIV/Hepatitis (which merged with virology at the end of 2017), 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: v, service deliveries, MESR: 60%, 29%, 11%; 2017: v, 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 the Ambassador of France, the director of IP Paris and representatives of several national authorities (Ministries of finance, agriculture and education) and international agencies (WHO, UNICEF, etc.). The scientific activities are assessed every 18 months by a scientific board (last session on 8-9 June 2017). 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 (26 avril 2017)

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, sur les grandes pathologies mais aussi sur des infections négligées comme les infections fongiques et la rage.

Les membres du conseil de liaison ont insisté aussi sur les développements de la recherche, et des programmes doctoraux, dans les universités du Cambodge et la contribution que l'IPC peut apporter en partenariat.

### B. Commentaires majeurs du précédent conseil scientifique (8 – 9 juin 2017)

**Recherche** : Comme pour les recommandations de 2015, le CS recommande d'élaborer une stratégie de recherche sur la l'antibiorésistance, financée à l'échelle internationale. Le CS recommande la fusion du groupe hépatite-HIV avec l'unité Virologie. La productivité académique de l'IPC est bonne, avec 55 articles scientifiques, mais 40% des articles étaient liés au paludisme en 2016.

**Stratégie**. Le CS a recommandé la formulation d'un plan stratégique quinquennal avec des indicateurs de réussite.

**Management**. Le CS a recommandé de recruter des chercheurs cambodgiens talentueux et de développer les ressources en biostatistique et en bioinformatique.

**Transfert des connaissances** : Le CS a apprécié les améliorations importantes apportées par l'IPC en matière de transfert et d'application des connaissances aux parties prenantes. Le CS a recommandé que l'on porte une attention constante à la recherche de mécanismes permettant de communiquer les résultats des recherches de l'IPC aux décideurs et bénéficiaires locaux pour éclairer les politiques et les pratiques nationales et internationales.

**Bien public.** Le CS a apprécié la contribution à la santé publique au Cambodge de l'IPC, en particulier par les laboratoires de services et les laboratoires de santé publique, y compris le centre de vaccination contre la rage. Le CCS a recommandé de viser l'accréditation ISO du laboratoire de microbiologie et de chimie des aliments et de l'eau.

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*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 (26 April 2017)**

The comments of the previous Liaison Council highlights the quality of the scientific results obtained by the IPC and their interest for the Ministry of Health, not only on major pathologies (TB, HIV, malaria, ..) but also on neglected infections such as fungal infections and rabies.

The members of the Liaison Council also stressed the development of the research, and doctoral programmes, in Cambodian universities and the contribution that IPC can offer in partnership.

#### **B. Major recommendations of the scientific advisory board meeting (8 and 9 June 2017)**

**Research.** Like previous 2015 recommendations, in 2017 SAB recommends to develop a research strategy on AMR, funded internationally. SAB recommended the fusion of the Hepatitis/HIV group with the Virology group. The academic productivity is good, with 55 scientific articles in 2016, but 40% of articles were related to malaria in 2016.

**Strategy.** SAB recommended formulating a 5yr strategic plan and benchmarks of success.

**Management.** SAB recommended to recruit talented Cambodian research staff and to develop ressources in Biostatistics and bioinformatics.

**Transfer Knowledge.** The SAB appreciated great improvement done by IPC in Knowledge Transfer and Knowledge translation to stakeholders. The SAB recommended keeping constant attention to finding mechanisms that allows IPC research to be communicated to local stakeholders and inform national and international policy and practice.

**Public good.** The SAB noted the continuing public good provided by IPC clinical services and public health laboratories, including rabies vaccination center, are excellent contributions to public health in Cambodia. The SAB recommended to target ISO accreditation of the food and water microbiology and chemistry Lab.

## **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 38ieme congrès national de la Santé les 15 et 16 mars 2017, et concernant les structures sous sa supervision

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*The short and medium-term objectives of IPC follow the recommendations presented by HE the Minister of Health of Cambodia during the 38th National Health Congress on 15 and 16 March 2017 and concerning the structures under his supervision.*

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## 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

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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.

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*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

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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 en France. Rendre plus visible cette offre de formation et d'encadrement. Contribuer à l'ouverture du master en Infectiologie de USS – Paris Saclay en 2018. Développer les séminaires hebdomadaires ouverts sur la communauté scientifique et universitaire au Cambodge.

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*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 French 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 2018. Develop weekly seminars open to the Cambodian scientific and academic community.*

### DEVELOPPER LES INFRASTRUCTURES / DEVELOPING INFRASTRUCTURES

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Continuer à améliorer les laboratoires et les infrastructures des différentes unités et services.

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*Continue to improve laboratories and infrastructure for epidemiology, virology, Malaria, entomology, Immunology, LBM and LEFS unit or services.*

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

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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. Donner des responsabilités scientifiques et managériales aux jeunes chercheurs cambodgiens prometteurs. Communiquer sur les possibilités de carrières et les besoins de l'IPC.

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*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. 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

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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.

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*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.*

## 2.1.2 ENJEUX SCIENTIFIQUES / SCIENTIFIC CHALLENGES

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

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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 infections fongiques, 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, grippe, 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.

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*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, fungal infections, 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

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En accord avec le Ministère de la Santé du Cambodge et le MoH CDC, en partenariat avec l'OMS et le Ministère de l'Agriculture, renforcer les recherches sur la rage, développer un plan de communication et d'éducation contre la rage, ouvrir le centre de vaccination antirabique de Battambang avec le PHD, et évaluer l'ouverture d'un autre centre en province.

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*In collaboration with the Ministry of Health of Cambodia, the MoH CDC, the Ministry of Agriculture, the WHO, strengthen research on rabies, develop a communication and education plan against rabies, open the PHD-IPC Battambang center, and assess the feasibility of opening another vaccination center in Province.*

### ETUDIER LES RESISTANCES / STUDYING RESISTANCE

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L'étude, le diagnostic et la gestion des résistances aux médicaments : bactéries, mycobactéries, paludisme, HIV, autres virus, infections fongiques et aux insecticides pour les vecteurs de dengue et de paludisme, seront développés, soutenus et renforcés.

*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.*

#### **ESTIMER LA TRANSMISSION / ESTIMATING TRANSMISSION**

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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, champignons..). L'IPC doit poursuivre son engagement dans cette voie, y compris par des approches de modélisation.

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*Knowledge of the mechanisms of transmission—vectorial, direct, nosocomial—from reservoirs or households; 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, fungal infections). The IPC is set to pursue its commitment on this path, including modeling approaches.*

#### **DEVELOPPER LES PLATES-FORMES / DEVELOPING PLATFORMS**

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Permettre le développement de technologies modernes de diagnostics, d'expérimentations, et d'analyses épidémiologiques en poursuivant le développement et l'équipement des 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 disponible ou à acheter (séquenceurs, cytomètres de flux, trieur de cellules, chromatographie en phase gazeuse - Spectrométrie de masse, Maldi-Tof, etc.).

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*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**

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Renforcer l'IPC dans le domaine de la modélisation, des biostatistiques, de l'entomologie médicale, 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.

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*Strengthen the IPC in the area of modelling, immunology, chemistry, biostatistics, as well as in medical entomology, 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**

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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. Des unités mixtes de recherche (IPC – Université – autres centre Cambodgien de recherche) pourraient être développées, par exemple en bactériologie, virologie, sur les hépatites ou en entomologie médicale.

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*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.*

*Joint research units (IPC - University - other Cambodian research centers) could be developed, for example in bacteriology, virology, hepatitis or medical entomology.*

## **DEVELOPPER LES CAPACITES ET LES COMPETENCES / SKILLS AND CAPACITY BUILDING**

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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 2018 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.

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*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 2018 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 L'IPC / MAKING THE IPC MORE VISIBLE**

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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.

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*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**

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Faire vivre et valoriser le comité Hygiène, Sécurité et Qualité (réactivé en 2015).  
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.

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*Give life to and enhance the Hygiene, Safety and Quality Committee (reactivated in 2015).  
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**

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Continuer à aménager les laboratoires, et les services de soutien et support, ainsi que les services de santé publique (rage, microbiologie alimentaire, unité de biologie médicale), 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.

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*Continue to ensure development of the laboratories, help and support services, as well as public healthcare services (rabies, food microbiology, medical biology unit) 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**

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L'IPC est engagé dans une démarche d'accréditation ISO 15189, dans un premier temps pour l'unité de biologie médicale, devant aboutir en 2018. 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, tests diagnostic rapide paludisme (sera transféré au CNM), rage).

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*The IPC is committed to an ISO 15189 accreditation process, firstly for the medical biology unit, expected to go full circle in 2018. The objective is to gradually extend (over three to four years) this labeling to other services, including food microbiology. Other laboratories are national or international referral laboratories (dengue, avian influenza, rapid detection tests for malaria (to be transferred to CNM), rabies).*

### **2.2.2 ENJEUX SCIENTIFIQUES / SCIENTIFIC CHALLENGES**

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

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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 a été initié (formation, nouveaux responsables, suivie de la qualité, nouveaux laboratoire).

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*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, process 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).*

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

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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 fongiques 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.

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*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.*

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## **AMELIORER LES DIAGNOSTICS / IMPROVING DIAGNOSIS**

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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 biomarqueurs seront poursuivis.

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*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.*

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

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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 initié 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 has initiated 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.*

## **2.3 AUTRE SPECIFICITE / OTHER SPECIFIC FEATURE**

### **2.3.1 DEVELOPPER LA PLATE-FORME REGIONALE DE RECHERCHE ASIE / DEVELOPING THE ASIA REGIONAL RESEARCH PLATFORM**

Les institutions membres d'AVIESAN (Institut Pasteur, INSERM, IMMI, ANRS, IRD, Fondation Mérieux, CIRAD) et l'IPC ont construit une plate-forme régionale de recherche (PRR-Asie) sur le campus de l'Institut Pasteur du Cambodge, dont l'objectif est d'accueillir des projets transdisciplinaires et interinstitutionnels autour des maladies infectieuses, impliquant différents partenaires. Le directeur de l'IPC poursuivra ses efforts pour rendre la PRR attractive et efficiente.

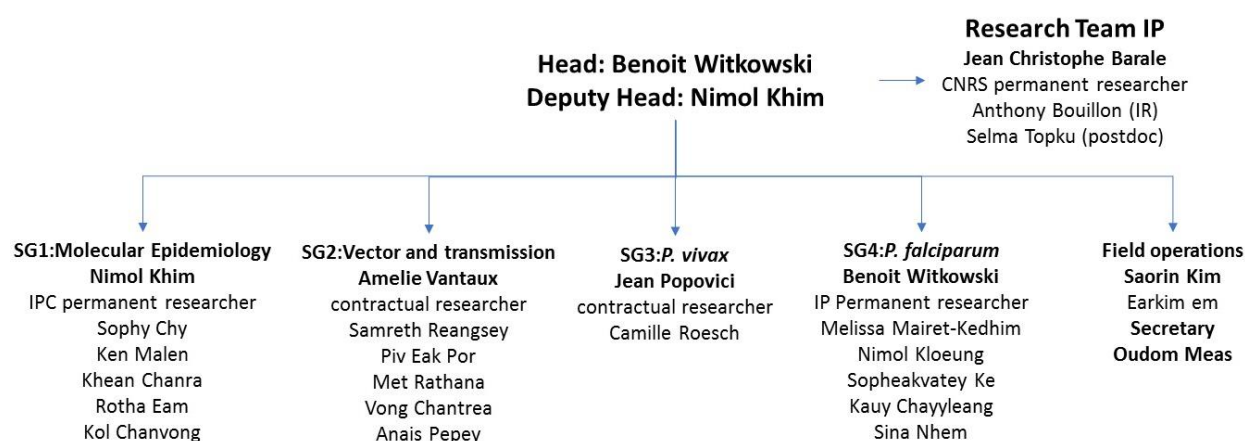
*The AVIESAN member institutions (Institut Pasteur, INSERM, IMMI, ANRS, IRD, Fondation Mérieux, CIRAD) and the IPC have built a regional research platform (PRR-Asia) on the campus of the Institut Pasteur du Cambodge. Its thrust is to take on board multi-partner transdisciplinary and inter-institutional projects focusing on infectious diseases. The IPC director will continue his efforts to make the PRR attractive and efficient.*

## **3 ACTIVITIES IN 2017 AT L'INSTITUT PASTEUR DU CAMBODGE**

### **3.1 MALARIA MOLECULAR EPIDEMIOLOGY**

#### **3.1.1 FUNCTIONAL STRUCTURE OF THE UNIT**

Malaria Molecular Epidemiology Unit (MMEU) was directed by Dr. Didier Menard up to September 2017. Thereafter, Dr. Benoit Witkowski has taken over the Unit direction. The Unit is organized around four thematic: *Plasmodium falciparum* blood stages, *Plasmodium vivax* blood stages, Molecular Epidemiology and Malaria transmission. The Unit is composed (September 2017) 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), two PhD student (Melissa Mairet-Khedim & Camille Roesch) 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	Institut Pasteur
Ministry of Health	Pasteur International Network
Partner for Development (Cambodia)	WHO
ITC	MMV
URC	University of Georgia
UHS	MORU
	UCSF
	Institut Cochin
	MSF
	University of Gottenburg
	INSERM
	CNRS
	TAD
	SANOFI
	NIMPE
	Lao NMCP
	Myanmar NMCP
	WEHI
	CWRU

### 3.1.2 RESEARCH PROGRAMS – MAJOR ACHIEVEMENTS IN 2017

#### i. Drug resistance epidemiology

MMEU was involved in molecular epidemiology programs intending to determine parasites resistance prevalence in Cambodia 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.

## ii. Setup of *P. vivax* liver stage platform

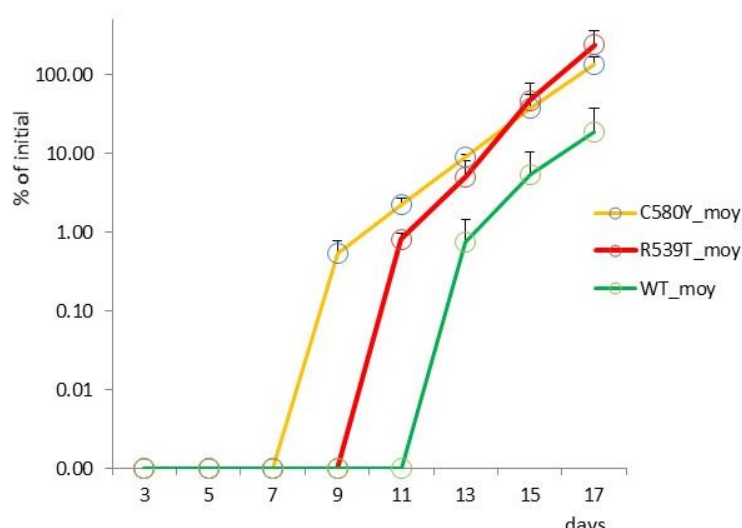
Previously, MMEU investigation range was restricted to parasites blood & vector stages. Such limitation drastically limit the research questions relative to vectors that could be addressed and finding significance. Moreover, *P. vivax* liver stage targeting is a main objective for enable to reach vivax malaria elimination feasibility. In collaboration with University of Georgia, we have implemented such expertise in Cambodia. This is composed to a field entomological facility fully potent to sustain mosquito colonies and carry out vector infection with field isolates collected in Cambodia, completed by a cellular culture facility at IPC able to perform hepatocytes infections with sporozoites isolated from vectors. While this platform is primarily designed to implement sustainable high throughput drug screening proficiency, it will also serves to develop new research thematic. Particularly, our future research will address the questions of the vector infectivity, the determination of parasites genetic recombination in mosquito and the metabolism of hepatocytes and parasites during liver infection.

## iii. *P. falciparum* drug resistance

- Amodiaquine

Amodiaquine-artesunate combination was tested in Cambodia (2016-2017) during WHO/CNM TES campaign. A total of 64 patients were treated with ASAQ and among those, 18% (n=12) have experienced a treatment failure (D28 follow-up, PCR corrected). The 64 isolates were tested with AQSA method. Median survival value was 15.4% [0.9-55.3 IQR] for ACPR group and 63.9% [29.1-77 IQR] for TF group. The difference between the two groups was statistically significant (p=0.007, Mann-Whitney's test). Despite the non-deployment of ASAQ in Cambodia 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.

- Artemisinin

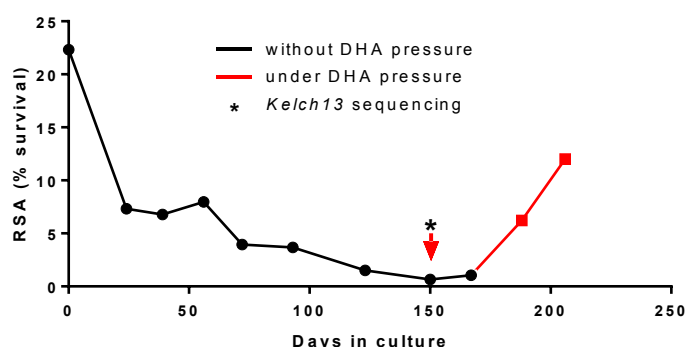


MMEU in collaboration others have demonstrated previously the role of K13 as a marker of artemisinin resistance.

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.

#### iv. Plasmodium vivax invasion biology

*P. vivax* is characterized by its tropism for reticulocyte and by an invasion pathway involving DARC receptor. The prevailing paradigm that makes the interaction between PvDBP and DARC essential for *P. vivax* invasion is questioned since this parasite has been observed in Duffy negative individuals. Understanding how *P. vivax* invasion takes place in individuals lacking the DARC receptor and identifying the molecular actors involved in Duffy negative individuals would be of critical importance to design a universal vaccine against diverse *P. vivax* strains. The interaction between *Plasmodium vivax* Duffy binding protein (PvDBP) and Duffy antigen receptor for chemokines (DARC) has been described as critical for the invasion of *P. vivax* in human Duffy positive reticulocytes. The observation of *P. vivax* infections in Duffy negative reticulocytes questions the ubiquitous role of the PvDBP-DARC interaction. The pathways by which the parasite is able to enter the Duffy negative reticulocytes are unknown but two possible mechanisms have been suggested in the literature: the amplification of the gene coding for PvDBP as well as the involvement of alternate proteins, in particular a newly described Erythrocyte Binding Protein, PvEBP, which is able to bind Duffy negative red blood cells. Here, we analyzed the genomic polymorphisms (copy number variation and sequence polymorphism) of PvDBP and PvEBP in isolates from two different locations: Cambodia where the vast majority of people are Duffy positive and Madagascar where an admixture of Duffy positive and Duffy negative individuals coexists. We show that PvEBP is far more conserved than PvDBP suggesting it plays a critical role in the biology of *P. vivax*. We also show that copy number variation of PvEBP, but not PvDBP, is significantly different between isolates from Cambodia and Madagascar. Those results further strengthen the possible role of PvEBP in the invasion of Duffy negative individuals. This data have been proposed for publication (Roesch *et al. under submission*).

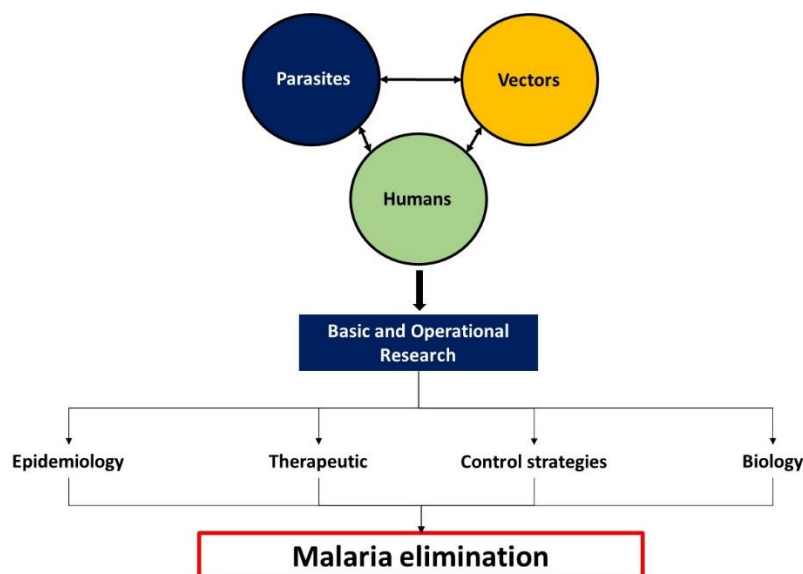
### 3.I.3 RESEARCH PROGRAMS – PROSPECT 2018

#### Research plan overview

It have been officially proposed an elimination of malaria 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 malaria epidemiology in Cambodia**
- b) **Explore the therapeutic options**
- c) **Strategize the malaria control**

#### d) Understand the parasite biology



#### i. Epidemiology

##### **Drug resistance epidemiology.....Funding source 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.

##### **Malaria molecular epidemiology.....Funding source NIH**

MMEU is part of the Asia-Pacific International Centre of Excellence in Malaria Research (Asia-Pacific ICEMR) 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 Mondulhiri 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 is conducted in Cambodia in association between MMEU and Epidemiology Unit of IPC.

##### **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

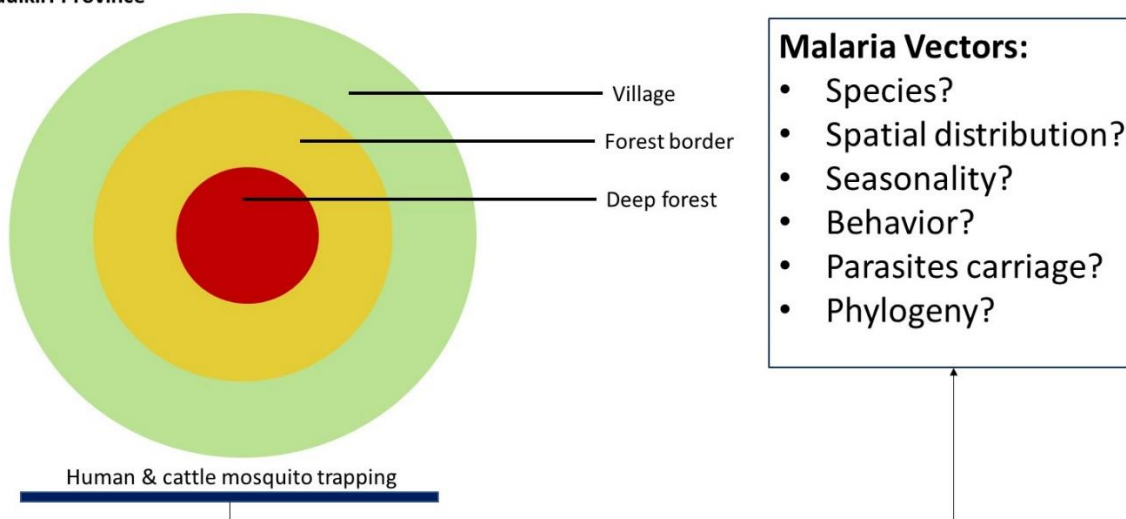


(aSGPs) as markers of exposure. Geospatial information system (GIS) technology and spatial analysis methods will be used to quantify and visualize human-vector contact. The research undertaken as part of this project will enhance our understanding of host and parasite factors that are contributing to 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 challenges inherent in eliminating malaria in the Asia-Pacific.

#### **Malaria vectors epidemiology.....Funding source NIH**

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 regrouped under vector epidemiology. Aspects 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 Mondul Kiri province (high malaria prevalence) and expected outcome will enable design of the further vector control initiatives.

**Mondul Kiri Province**



#### **ii. Therapeutic**

#### **Lead compounds for resistant malaria treatment.....Funding source MMV**

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.

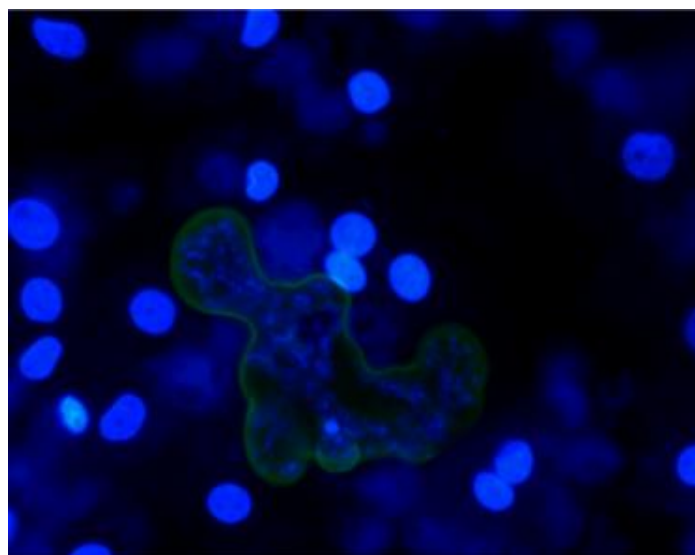
#### **OZ439-FQ phase IIb.....Funding source MMV/Sanofi**

As previously mentioned, there is currently an urgent need in the implementation of novel association that can potentially 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.

### **New treatments against *P. vivax* liver stages.....Funding source UGA/MMV**

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 proposed for publication in the journal "Nature Communication" (under review).

### **iii. Control strategies**

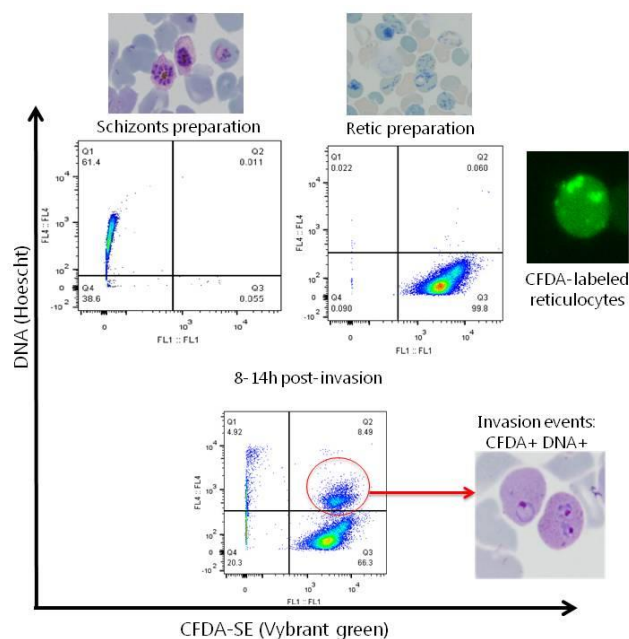
### **Malaria Elimination in Cambodia.....Funding source 5% initiative**

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.

#### iv. Biology

##### ***P. vivax* neutralizing antibodies.....Funding source WEHI**

As mentioned before, *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*.

### 3.I.4 SUPPORT TO NATIONAL AUTHORITIES

MMEU is part, such like all IPC research units, 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

##### **3 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)

##### **1 Master student:**

Anais Pepey: geography of malaria transmission (February 2018)



## ii. Teaching

The Malaria Molecular Epidemiology Unit staff are 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 achieved 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 achieved 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 achieved 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 Mondul Kiri 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.

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## 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 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%, and the Agence Française de Développement (AFD).

Other research projects of interest are being implemented alongside the three main research groups, such as the Southeast Asia encephalitis project.

In 2017, 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. The virology unit of IPC is an essential and longtime partner in the majority of EPH unit projects and activities. In 2017, new operational research projects were developed with the IPC malaria unit and at least one will be implemented in 2018.

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### 3.2.2 RESEARCH PROGRAMS – MAJOR ACHIEVEMENTS IN 2017

#### **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: Patients' recruitment completed and ongoing follow-up. Main partners in Cambodia: MoH

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

## **Dog population dynamic and incidence of dog bites in rural setting in Cambodia**

Rabies is endemic in Cambodia and transmitted to human mainly by dog bites. A better understanding of dog population dynamics and frequency of dog bites would help inform rabies prevention in humans and guide dog vaccination programs or dog population control programs in the future. A cohort of 10 villages in Kandal province, Cambodia, was established in March 2017 after an initial door to door census of dogs and documentation of dog bite injuries. Identification of dogs was done by taking photographs of the animals. A follow up assessment at 12 months is planned in March 2018 to recapture, register new dogs and documents lost dogs. Following the initial survey in 1737 households, we counted 2,207 dogs and 440 cats in 10 villages, giving a population ratio of 1 dog for 4.5 humans. The bite incidence was estimated at 2.2 bites per 100 persons per year.

EPH Unit Team leaders: Sowath Ly, Veronique Chevalier. Financial support: WHO and CIRAD

## **Dog population dynamic in rural setting in Kandal and Battambang province, Cambodia**

An on-going pilot study in Kandal and Battambang provinces aims at recording the demographic parameters of a local dog population. An exhaustive census of the dog population was performed in 10 villages of Kandal district. Each household of each village was visited and demographic information of dog population were recorded using a dedicated questionnaire. Each dog was individually identified, and vaccinated against rabies. A new visit will be implemented 1 and 2 years after the initial census to re-capture the censed dogs, and identify, document and vaccinate the new ones. During these 2 additional visits, each censed dog will be blood sampled, and blood will be tested for anti-rabies antibodies using the fluorescent antibody virus neutralization test (FAVN). Reasons for missing status will be recorded.

EPH Unit Team leaders: Sowath Ly, Veronique Chevalier. Financial support: IPC/CIRAD

## **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 will be followed-up, of which 12 will be randomly allocated the school-based intervention and 12 will be 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 will be performed as effectiveness outcomes.

Financial support: Funded by AFD and PANIC

## **Study on health and economic burden of dengue disease in rural population in Cambodia**

A 2-year study (2015-2016) was implemented to estimate the burden of dengue in Cambodia. Specific objectives included to document the morbidity, mortality and financial costs associated with dengue virus infections in rural settings from two provinces. The study had four components: (1) six serological surveys among 2000 participants aged 1 to 55 years old; (2) a health care utilization survey among 400 randomly selected households that were part of the serological survey; (3) passive facility-based surveillance in 5 hospitals in two study provinces to screen for dengue cases using rapid test (NS1, IgM, IgG) and PCR test at IPC; and (4) cost of illness study to include 200 confirmed dengue cases found at the study hospitals to document the cost for patients and for hospitals. Field data collections ended in 2016 while serology testing and data cleaning ended in October 2017.

EPH Unit Team leaders: Sowath Ly, Arnaud Tarantola. Financial support: Dengue Vaccine Initiative DVI, IVI

### **Zika Alliance**

In 2015-2016, six sequential blood samplings were performed on 2000 participants aged between 1 and 55 years old as part of a DVI study. In the context high regional ZIKV circulation during this period, the collected samples will form a basis of serological testing for ZIKV infection and circulation in Cambodian communities.

Financial support: Funded by Dengue Vaccine Initiative (DVI, IVI) and IP-Paris

### **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 650 patients were included. In the Mekong Region, preliminary results show that unidentified causes of encephalitis remain high (43%) while Japanese encephalitis is the main cause (35%) followed by tuberculosis, scrub typhus and Herpes simplex virus which are of similar proportions (3%). Partners: LOMWRU, Institut Pasteur Paris, National Institute of Epidemiology and Hygiene in Vietnam.

EPH Unit Team leaders: JD Pommier, P.Piola. Financial support: Fondation Total, INSERM, AIRD, IRD, RIIP, CIRAD

### **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 immunosuppressed HIV-infected adults initiating antiretroviral therapy with CD4 cell counts <100/mm<sup>3</sup>) 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/mm<sup>3</sup>): 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 of patients is completed and follow-up is ongoing. Main partners in Cambodia: NCHADS, CENAT, SHCH

Team leader: L. Borand. Financial support: ANRS

### **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, Kampong Cham & Takeo Provincial Hospitals

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

### **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.

### **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 infections was ~8.8/1000 live-births with 6/7 (86%) isolates resistant to at least one antibiotic recommended by the 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 Extended-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. 145 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 53.1% [95%IC: 44.8-61.2] and most frequently detected ESBL-genes were bla-CTX-M-15 (n=42/98 isolates, 42.9%) and bla-CTX-M-55 (n=16, 16.3%). 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.

### **MEAT AND FISH STUDY – 2016/2017**

Particularly high ESBL-producing *Escherichia coli* (ESBL-Ec) prevalence in Southeast Asia suggests widespread exposure. We assessed ESBL-Ec contamination among fish, pork, and chicken sold in two markets in Phnom-Penh and compared genomes to healthy BIRDY women carriage isolates. 93/150

(63%) fish and meat samples were positive for ESBL-Ec, mainly expressing CTX-M-55 genes (62/93). Over 40% of ESBL-Ec from healthy women clustered with food-origin isolates (n=39), and 20 were significantly more likely to encode CTX-M-55 and express chloramphenicol (CHL) resistance ( $p < 0.05$ ). CHL-resistance in ESBL-Ec from healthy women was associated to consumption of sun-dried poultry (aOR:6.1, 95%CI:1.6-23.6). ESBL-Ec from contaminated animal products may be spreading to the community, potentially via consumption.

EPH Unit Team leaders: M. Nadimpalli, A. de Lauzanne. Partners and Funding: Gillings Foundation, Pasteur Foundation U.S., MSDavenir, Monaco Department of International Cooperation, Institut Pasteur.

### **ECOMORE Economic Development, Ecosystem Modifications and Emerging Infectious Diseases Risk Evaluation. 2013-2017 (data Myanmar)**

In Myanmar, Ecomore project was focused on severe acute respiratory syndrome (SARI) in children, responsible for increased mortality worldwide. We conducted a retrospective case-control study among 502 children hospitalized for SARI to assess associated factors to death, particularly the presence of Multidrug Resistant Gram Negative Bacteria (MDR-GNB). Besides malnutrition, severity criteria and infection by *Pneumocystis jiroveci*, being colonized/infected by a MDR-GNB was significantly associated to mortality (aOR: 3.8, 95%CI:1.7-8.2).

EPH Unit Team leaders: Y. Yves Froehlich, A. de Lauzanne. Partners and Funding: National Health Laboratory, Yangon and Yankin Hospitals-Myanmar, IP, IP Cambodge, AFD.

### **Seroprevalence of zoonotic pathogens associated with land-use change in Monduliri province (LACANET)**

The objective was to investigate the risk of exposure to zoonotic pathogens, such as scrub typhus, leptospirosis and Japanese encephalitis virus in residents and workers engaged in extractive industries associated with land-use change. A collaborative effort (human, animal and ecosystem health) investigated the role of land use change in disease dynamics by conducting diseases surveillance in domestic and wild animal reservoirs and assessing the pathogen exposure in humans living in these locations. The project ended in December 2017.

Financial support: European Union

### **Comacross TéléNipah**

The goals are to: (i) Assess the circulation of the Nipah virus (NiV) in the reservoir of the virus by sampling flying foxes (*Pteropus* sp.) (ii) Characterize the interface between flying foxes and humans and identify potential routes of transmission for the virus to spill over (iii) Assess potential former spill overs of the virus by sampling (blood) in humans exposed to the bats based on the telemetry study and testing the sera for NiV Abs by ELISA and Luminex. During the human seroprevalence study conducted on 16-18 May 2016, 122 persons were blood sampled from 27 families living near *Pteropus* roosts. Data analyses are on track.

EPH Unit Team leaders: J. Cappelle. Financial support: CIRAD/CNES

### **ComAcross/SEA (Work Package 3)**

EPH Unit Team leaders: V. Chevalier- R. Duboz

A first compartmental and deterministic model adjusted with (i) serological data collected from 2 pig cohorts in Cambodia (Cappelle et al, 2016) and (ii) data of a national serological study performed in Cambodian pigs (Veasna et al, 2009) corroborates the existence of direct transmission between pigs. We combined this first model with a second model representing typical farrow-to-finish pig farms. We



use this model to assess the impact of pig vaccination, pig herd management and vector control on intra-herd and village transmission. Lastly, we analyzed the feeding behavior of the main JE vectors in a rural setting of Cambodia using baited traps and PCR analysis of available mosquito blood meals.

Financial support: ComAcross (EU)

### **ComAcross – Role Playing Game**

EPH Unit Team leaders: R. Duboz

One core objective of the ComAcross project is the development of methods and tools to ease cross-sectorial collaborations in a One Health framework. Interactions between actors with different knowledge and objectives are supported by participatory techniques leading to the design of innovative solutions. The objective of this case study is to test a participatory technique, Role Playing Game (RPG), for public health purposes. Based on the Japanese Encephalitis (JE) and Nipah case studies, we designed a RPG where farmers play their own daily activities and are exposed to JE and Nipah. The objectives are: the study of JE and Nipah risk perception in local communities; the transfer of epidemiological knowledge to the farmers; and the co-design of innovative surveillance and control measures. Training sessions with collaborators from AVSF and NaVRI taught the techniques. Two game sessions were organized with farmers in Kampot and Kandal provinces. The RPG will be presented to the zoonoses technical working group.

Financial support: ComAcross

### **Understanding, tracking and eliminating malaria transmission in the Asia - Pacific Region (ICEMR)**

Eliminating malaria from the Asia-Pacific will be made much easier if countries have a good understanding not only where people with malaria infections and disease are living but also where they may have acquired infections. A coordinated set of epidemiological and vector studies in Cambodia aim at determining the key reasons where and when people acquire infection and how transmission is maintained. The main objective of the ICEMR project is to better understand host and parasite factors that sustain residual malaria transmission despite intensified control and to apply this knowledge to develop novel ways of accurately identifying and delineating pockets of residual transmission. End of 2017, a census was performed on 10000 persons from 17 villages near Keoseima (Mondulkiri) followed by a cross-sectional study in a subset of 6000 individuals (continuing in 2018) to estimate the prevalence of malaria and set the foundations of a cohort.

EPH Unit Team leaders: P. Piola (IP Paris Ivo Mueller and IPC Malaria Unit). Financial support: NIH

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### **3.2.3 RESEARCH PROGRAMS – PROSPECT 2018**

#### **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)

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



### **Study on remanence immune protection (RESIST 3)**

The objective study is to determine whether the long-term humoral and cellular immune response after 3 sessions is comparable to that after 4 sessions. We propose to compare the duration and intensity of long-term immune responses to rabies post-exposure prophylaxis between 3 sessions (incomplete, non-compliant patients) vs. 4 sessions (complete) among patients bitten by lab confirmed rabid dogs and who received vaccination 5 and 10 years ago.

EPH Unit Team leaders: L. Sowath, A. Tarantola. Financial support: IP Paris

### **RESIST 4: cost effectiveness of PEP against rabies**

IPC has been generating key information on number of dogs, incidence of dog bites, cost of transport and prophylaxis, and characteristics of victims of dog bites at IPC's Rabies Prevention Center (>20,000 patients per year over more than 10 years). These data will be used for mathematical modeling to estimate the cost-effectiveness and number lives spared by comparing the current versus new abbreviated vaccination regimen.

EPH Unit Team leaders: L. Sowath, A. Tarantola. Financial support: Funded by WHO and IPC

### **TB-Speed Research Project (UNITAID/ Global Fund 5% Initiative)**

The majority of children with TB are not diagnosed/not reported and do not benefit from appropriate treatment. The TB-Speed Research Project will carry out activities aiming at reducing childhood mortality from TB by evaluating innovative cost-effective approaches for resource-limited settings. The diagnostic approach will include use of molecular diagnosis assay applied on nasopharyngeal aspirate and stool sample, introduction of digital chest radiography, and training and mentoring of clinicians for screening and diagnosing of paediatric TB. Current status: This research project is currently preparation. Main partners in Cambodia: CENAT, National Pediatric Hospital, Kampong Cham & Takeo Hospital

Team leader: L. Borand – R. Ferhi. Financial support: UNITAID / Global Fund 5% Initiative

### **miRNA as prediction and/or prognostic markers of IRIS in TB-HIV co-infected patients**

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

### **A hospital based case-control study to identify risk factors of leptospirosis 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: E. Chevanne, Y. Froehlich, P. Piola. Funding: AFD

## Zika Surveillance

The Zika pandemic that started in 2015 in the Americas caused thousands of microcephalies in newborns from infected pregnant women. An active surveillance of Zika-like syndromes in pregnant women attending ante-natal clinics will be implemented, including a PCR confirmation by the IPC virology unit. This sentinel surveillance in selected ante-natal clinics will allow to detect an emerging ZIKV circulation in Cambodia, with a focus on the most vulnerable group.

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

## 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 will aim at an in-depth understanding of malaria transmission inside three forests totalling 60km<sup>2</sup> (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. Funding: French Initiative 5%. Partners: Partners for Development, CNM.

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### 3.2.4 SUPPORT TO NATIONAL AUTHORITIES

- 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)
- Training on rabies post-exposure prophylaxis for 6 health officers from the Ministry of Health, August 2017 (Dr LY Sowath)
- 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)
- Participation to the writing of National Guidelines for Rabies Control in Cambodia (OMS, FAO, C-CDC, IPC, GDAPH) (Dr Véronique Chevalier)
- 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)

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### 3.2.5 TEACHING AND TRAINING

- Clinical Research Assistant training at the University of Health Sciences (20-24 March 2017): Dr L. Borand, Dr D. Bunnet, Dr P.Piola
- Lecture for field officers in the Applied Epidemiology Training (AET) program organized by the Ministry of Health and US-CDC. Lesson: scientific communication (IMRAD), August 2017. (Dr LY Sowath)
- Two epidemiology team from IPC gave two sessions on use of GPS devices and mapping using QGIS software for officers of Cambodian Veterinary Applied Epidemiology (CAVET) program organized by the Ministry of Agriculture and US-CDC, June 2017. (Dr LY Sowath)

- Supervision of two officers of Cambodian Veterinary Applied Epidemiology (CAVET) program organized by the Ministry of Agriculture and US-CDC, Jan-December 2017. Activities: (1) Clinical surveillance of canine rabies at dog slaughter houses in Kampot province and (2) KAP surveys related to rabies in Tbong Khmum province. (Dr LY Sowath)
- Sessions of teaching at the University of Health Sciences on "Infectious Agents" for students at foundation year, 2017 (Dr LY Sowath)
- Training to Dry Blood Spot collection in newborn and Hep B vaccination of newborns (Dr Laurence Borand)
- Several training given on clinical research and Good clinical Practices (Dr Laurence Borand)
- Medicine students from UHS internship (Dr Laurence Borand)
- Internship from one student from Poitiers University (Dr Laurence Borand)
- PhD supervisor: "Modelling of JE transmission in Cambodia and optimization of control measures" (Dr Véronique Chevalier)
- Master II student (InterRisk) supervision (Dr Véronique Chevalier)
- "Trophic behavior of JE vectors in Cambodia" (Dr Véronique Chevalier)
- "Dog population structure in a province of Cambodia (Kandal) : consequences for Rabies circulation" (Dr Véronique Chevalier)
- Refresh training for BIRDY Study in National Pediatric Hospital, Kampong Speu hospital for Nurses and Doctors. Jan & June 2017. (Dr Agathe de Lauzanne)
- Training for the BIRDY Team (13 persons): Basics bacteria, viruses, AMR. AMR clinical cases. June 2017. (Dr Agathe de Lauzanne)
- Participation to trainings with DMDP in University of Health Sciences. Module Infectious Disease Training of 4-5-6th years medical students. 7-8th June 2017. (Dr Agathe de Lauzanne)
- Master 1 from Institut de Technologie du Cambodge (ITC). One Student: Sarith Nob. Development of a mobile application to transfer BIRDY lab results to prescriptors. 01-09/2017. (Dr Agathe de Lauzanne)
- Master 1 from National Institute of Public Health (NIPH) – Epidemiology and nutrition. One Student (Lach Siyin) left before finishing. 2017. (Dr Agathe de Lauzanne)
- Master from Public Health CNAM-Pasteur School. Two students - Elsa Foucault – ESBL-E fecal carriage in mothers from the BIRDY cohort and associated factors. Fabio Ghilardi: Ecomore Myanmar: Severe Acute Respiratory Infection (SARI) in 2 pediatric hospitals. Associated factors to MDR-GNB. 05-10/2017. (Dr Agathe de Lauzanne)

### 3.2.6 PUBLICATION LIST

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9. 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.
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## Abstracts

- High Level of Extended Spectrum Beta Lactamase Producing Enterobacteria (ESBL PE) Fecal Carriage in Mothers and Infants: Preliminary Results of the BIRDY Program in Cambodia. Agathe de Lauzanne, Siyin Lach, Alexandra Kerleguer, Veronique Ngo, Sok Touch, Arnaud Tarantola, Laurence Borand, and the BIRDY study group. "2016 Institut Pasteur International Network Symposium", Poster. 31th Nov 2016. (**Dr LY Sowath**)
- 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**)
- 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 29 Nov. 2017. (**Dr A. de Lauzanne**)

## Presentations

- Presentation of BIRDY preliminary Results in National Pediatric Hospital - Medical and paramedical staffs. June 2017. (Dr Agathe de Lauzanne)
- High prevalence of respiratory Non-tuberculous Mycobacteria Respiratory Infection in Children Living with HIV in South-East Asia. Borand L. de Lauzanne A. Laurence BORAND, Inghammar M., Ung V., Cheng S., Pham Thu H., Msellati P., Tejiokem M., Ouedraogo AS. Godreuil S., Delacourt C. Blanche S. Marcy O. for the ANRS 12229 PAANTHER 01 study group. Paris, CROI July 2017.
- Participatory modeling and simulation: a case study on Japanese encephalitis and Nipah in Cambodia. Raphaël Duboz, Aurélie Binot, Panomsak Promburom, Julien Cappelle, Alpha O. Diallo, Veronique Chevalier. Epidemics6 - International Conference on Infectious Disease Dynamics 2017. Sitges, Spain

- Pig-to-pig direct transmission of Japanese encephalitis: its importance in two pig cohorts in Cambodia. Alpha Oumar Il Diallo, Véronique Chevalier, Julien Cappelle, Didier Fontenille, Raphaël Duboz. ModAH. Modeling in Animal Health, Nantes, France

### 3.3 HIV/VIRAL HEPATITIS

#### 3.3.1 FUNCTIONAL STRUCTURE OF THE UNIT

Name	Arrival at IPC	Roles	Graduation	PubMed (n° of papers)	Perspectives (2018-2019)
François Rouet	Jun. 2013	Head	PharmD, PhD	118	-
Janin Nouhin	Mar. 2002	Researcher	PhD, PSRL	22	Post-PhD
Sophearot Prak	Jan. 2015	Monitor	MSc	3	Ext. training
Chhairat Leang	Jan. 2018	Lab. Tech.	Lab. Tech.	-	-

#### 3.3.2 RESEARCH PROGRAMMES – MAJOR ACHIEVEMENTS IN 2017

##### 3.3.2.1 HEPATITIS C INFECTION

##### **Performance characteristics of a new commercial assay for HCV RNA viral load quantification in plasma: Preliminary results in Cambodia**

**Context:** 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.

**Methods:** The evaluation was conducted on 101 plasma specimens 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.

**Main results:** All 76 (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 median difference of -0.4 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 25 plasma samples having undetectable VL with the Roche test.

**Scientific communication:** An abstract has been accepted as poster exhibition in the IAS Conference (Paris, 23-26 July 2017) - Title: Performance characteristics of a new commercial assay for HCV RNA viral load quantification in plasma: Preliminary results in Cambodia (abstract # MOPED1179)

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

**Financial support:** IPC.

## **In-depth phylogenetic analysis and molecular epidemiology of HCV genotypes circulating in Cambodia**

**Context:** 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.

**Objective:** 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 is to identify risk factors associated with the emergence of a predominant genotype.

**Main results:** 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.

### **3.3.2.2 HEPATITIS D INFECTION**

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

**Context:** 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.

**Objective:** To establish the seroprevalence of HDV and to detect HDV RNA, in case of positive anti-Delta antibody.

**Methods:** A total of 300 samples positive for HBsAg will be tested for total anti-Delta antibodies using ELISA. The National Ethics Committee of Cambodia approved the study on November 1st, 2017.

**Main results:** One out of 178 samples was positive for total anti-Delta antibodies leading to a prevalence of 0.6%. The remaining samples will be assessed in 2018.

**Partners:** A. Kerleguer, S Heng (Medical Biology Unit, IPC), E. Tuaillon (UMR Inserm 1058 Montpellier).

**Financial support:** UMR Inserm 1058 Montpellier.

### **3.3.2.3 HEPATITIS E INFECTION**

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#### **Seroprevalence of HEV in Cambodia, 1996 – 2017**

**Context:** 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.

**Methods:** 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.

**Main results:** 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)

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### 3.3.3 RESEARCH PROGRAMMES – PROSPECT 2018

#### 3.3.3.1 NOSOCOMIAL OUTBREAK OF HIV AND HCV IN ROKA – ANRS 12352

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**HIV and HCV infections in 2 communes from the Battambang province, Cambodia: prevalence rates, viral strains, and unsafe injection practices**

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 protocole of the study was approved by the National Ethics Committee for Health Research on May 2nd, 2017. The study will start by the end of March 2018.

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

**Financial support:** ANRS

#### 3.3.3.2 TA PROHM STUDY - TOPIC 2: ANRS 12345

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**Test and treat strategy using rapid test and tenofovir treatment to prevent 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 for around 300 of them who will test positive for HBeAg (“test and treat” strategy). The first woman was enrolled in the study in October 2017. By March 1<sup>st</sup>, 2018, 161 women were already included in the study.

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

**Financial support:** ANRS

#### 3.3.3.3 OTHERS STUDIES

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**Estimation of Hepatitis E Virus (HEV) emergence in Cambodia, Thailand and Vietnam and identification of risk factors associated to blood and/or food and/or environmental and/or zoonotic exposure**



This project has been accepted by the end of 2016 by the scientific committee of the PRR Asia platform. The total budget is 75,000€. Studies are starting from human specimens (in Thailand and Vietnam), and swine liver, water and wild life reservoirs (for Cambodia).

### HCV Direct Acting Antiviral Resistance 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 likely propose 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. This work will be done in collaboration with Dr Edouard Tuaillon (UMR Inserm 1058 Montpellier) and Dr Stéphane Chevalliez (Reference National Center for Hepatitis B, C, and D, Hôpital Henri Mondor, Paris, France). We are likely submitting the proposal for research grant to the ANRS by September 2018.

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### 3.3.4 SUPPORT TO NATIONAL AUTHORITIES

Follow up of the HIV – Hepatitis Roka Outbreak

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### 3.3.5 TEACHING AND TRAINING

François Rouet and Janin Nouhin participated to 9<sup>th</sup> IAS Conference on HIV science, Paris France (July 23<sup>rd</sup> – 26<sup>th</sup>, 2017). Sophearot Prak attended Technical training on QGIS "Data from the field to an interactive web map", Bangkok, Thailand (January 16<sup>th</sup> – 19<sup>th</sup>, 2017) and Technical training on QGIS "Analysis of spatialized data with R" Bangkok, Thailand (November 12<sup>th</sup> - 17<sup>th</sup> Nov 2017).

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### 3.3.6 PUBLICATION LIST – 2017

2016

1. Nouhin J, Prak S, Madec Y, Barennes H, Weissel R, Hok K, Pavio N, Rouet F. Hepatitis E virus antibody prevalence, RNA frequency, and genotype among blood donors in Cambodia (Southeast Asia). *Transfusion*. 2016; 56(10):2597-2601.
2. Vun MC, Galang RR, Fujita M, Killam W, Gokhale R, Pitman J, Selenic D, Mam S, Mom C, Fontenille D, Rouet F, Vonthanak S; Roka Cluster Investigation Team. 2016. Cluster of HIV Infections Attributed to Unsafe Injection Practices - Cambodia, December 1, 2014-February 28, 2015. *MMWR Morb Mortal Wkly Rep*. 2016; 65(6):142-5.
3. Barennes H, Virak K, Rouet F, Buisson Y, Strobel M, Vibol U. 2016. Factors associated with the failure of first and second-line antiretroviral therapies, a case control study in Cambodian HIV-1 infected children. *BMC Res Notes*. 2016; 9:69.

2017

1. 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*. 2017; doi: 10.1093/cid/cix1071
2. 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.
3. 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.



## 3.4 IMMUNOLOGY PLATFORM

### 3.4.1 FUNCTIONAL STRUCTURE OF THE UNIT

The team is composed of one laboratory technician and a residential scientist (team leader: Pean Polidy). The laboratory is equipped with state-of-the-art flow cytometers, cell culture system, elisa and elispot system. The research topic is focus on the immunological mechanism of HIV co-infection and biomarkers discovery associated with HIV co-infection particularly with tuberculosis and viral hepatitis.

### 3.4.2 RESEARCH PROGRAMS-MAJOR ACHIVMENT IN 2017

**Theme 1 - MicroRNA (miRNAs) as prediction and/or prognostic markers of IRIS (immune reconstitution inflammatory syndrome) in TB/HIV co-infected patient – ANRS No 12358**

Coordinators: Dr. Daniel Scott-Algara (IP Paris), Pean Polidy (IPC), Dr Laurence Borand (IPC)

Supported by ANRS (Agent National de Recherche sur le VIH/SIDA et les Hépatites virales)

Duration: 24 months (2016-2018). Budget: 105.7K

MicroRNAs (miRNAs) are small, typically 22 nucleotides, non-coding (nc), endogenous, single-stranded RNAs. Recently, the explosion of research in this area has established miRNAs as powerful regulators of gene expression and 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 common biomarkers have not been identified so far. However, the studies of miRNA in acute HIV infection and co-infections like tuberculosis are lacking. In addition, the demand of using plasma miRNA as biomarkers in clinical application is high. 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, sequencing technique). In contrast, 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.

In this study, we propose to evaluate by flow cytometry whether circulating miRNA pattern might be applicable as potential biomarkers in HIV disease. For HIV disease, we propose to study the miRNA expression profile in a cohort of patients with a HIV infection and tuberculosis and correlate it with their clinical evolution and the occurrence of IRIS.

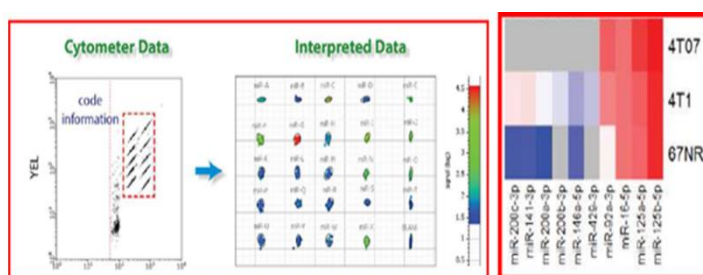


Figure 3. Circulating microRNA flow cytometry data decode in microRNA microarray

Milestone (Table 1) and prospective (Table 2) of the study are shown below:

Table 1

Working achievement	
Submission to ANRS call 2016 - 1	Sep-2015
Re-submission to ANRS call 2016 - 2	Mar-2016
Approval by the ANRS	Jul-2016
Presentation to association of the patients	Jul-2016
Submission to Clinical Research Committee in Pasteur Paris (CoRC)	Feb-2017
Approval NECHR (ref 103 NECHR)	Apr-2017
Approval NECHR for protocol amendment ( ref. 159 NECHR)	Jun-2017
ANRS agreement using CAMELIA biobank	Jun-2017
Flow cytometry set-up validation	Jul-2017
Validation circulating microRNA by PCR and Thermocycler	Feb-2018
Patient-control enrolment	Feb-2018

Table 2

Perspective	Tentative date
Running first samples	Mars-2018
Data analysis and microarrays generation	Jun-2018
Generation of whole data and statistical analysis	Oct-2018

## Theme 2- Immunity to tuberculosis in highly immunosuppressed HIV infected and uninfected patient: Immune correlates study of survival and ART timing in HIV patients with tuberculosis patients.

Coordinator: Anne Goldfeld (Boston Children Hospital, US) and Pean Polidy (IPC)

Supported by US-NIH

Duration: 24 months (06/2016-05/2018), Budget: 21.6K

The CAMELIA clinical trial demonstrated a significant survival benefit when antiretroviral therapy was initiated early (2 weeks) versus late (8 weeks) after start tuberculosis in highly immunosuppressed HIV+ patients with newly diagnosed tuberculosis. We tested the hypothesis that earlier antiretroviral in CAMELIA led to immune-correlated linked to enhanced survival.

Blood was prospectively collected at six timepoints from 175 TB+/HIV+ (CAMELIA) patients and at four timepoints from 36 TB-/HIV+ highly immunosuppressed patients between antiretroviral treatment initiation and 32 weeks, and at matched timepoints from HIV-negative (TB+ and TB-) control patients. T cell phenotypes, serum biomarkers and T cell receptors were examined. TB co-infection in the CAMELIA patients resulted in significant greater frequencies of activated CD4+ and CD8+ T cells ( $p < 0.001$ ) and significantly higher viral loads ( $p < 0.0001$ ) compared to levels in the TB-/HIV+ patients group. Among CAMELIA patients, higher central memory CD4+ T cell frequencies at antiretroviral initiation was significantly associated with mortality ( $p = 0.038$ ) (figure 1) and late antiretroviral initiation ( $p = 0.025$ ). Late ART also associated with higher frequencies of regulatoryCD25<sup>+</sup>CD127<sup>low</sup> T cells ( $p = 0.003$ ) and decreased T cell clonal expansion. The paper was submitted on Jan, 2018.

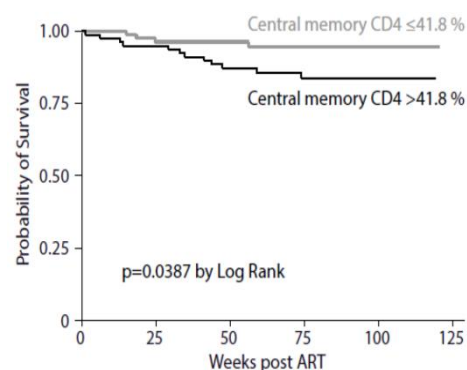


Figure 1 : high CM CD4 T cell of CAMELIA (> 42%) associated with mortality

## Theme 3 - Interleukin-1 receptor antagonist, a biomarker of response to anti-TB treatment in HIV/TB co-infected patients.

Coordinators: Prof. Laurence Weiss (IP Paris), Pean Polidy (IPC)

Supported by ARI (Association pour la Recherche en immunopathologie) and IPC

Duration: 6 months

Despite the high frequency of tuberculosis-associated immune reconstitution inflammatory syndrome (TB-IRIS) in human immunodeficiency virus (HIV)/TB co-infected patients, no diagnostic test is available. Here, we investigated whether monocyte/macrophage activation markers can predict TB-IRIS occurrence and if they are modulated by anti-TB treatment. Frozen plasma was obtained from 127 HIV/TB co-infected adults naïve for antiretroviral therapy, enrolled in the CAMELIA trial, 36 of whom developed TB-IRIS. Concentrations of IL-1Ra (competitive inhibitor of IL-1), sCD14 (glycophosphatidy inositol anchored protein), and sCD163 (hemoglobin scavenger receptor) were measured at anti-TB treatment onset (baseline), after 8 weeks of anti-TB treatment and at TB-IRIS time.

At baseline, IL-1Ra and sCD14 concentrations were similar in TB-IRIS and non-IRIS patients. sCD163 concentrations, although significantly higher in TB-IRIS patients, did not remain associated with TB-IRIS occurrence in multivariate analysis. At the time of TB-IRIS, patients displayed higher concentrations of IL-1Ra ( $p = 0.002$ ) and sCD14 ( $p < 0.001$ ). The most striking result was the significant decrease in IL-1Ra after 8 weeks of anti-TB treatment (median reduction: -63% ( $p < 0.0001$ )) (figure 2)

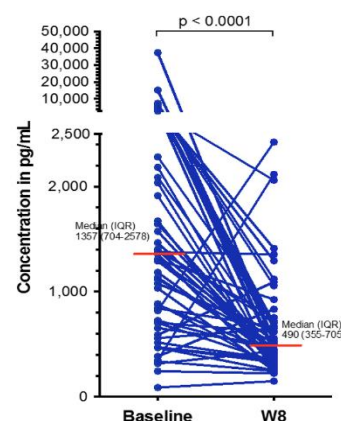


Figure 2. Dramatic decline in IL-1Ra levels between baseline and W8 of TB treatment (-63%)

None of the biomarkers tested was associated with TB-IRIS occurrence. However, repeated measurement of IL-1Ra could help for the diagnosis of TB-IRIS. The substantial reduction of IL-1Ra under treatment suggests that IL-1Ra could be a surrogate biomarker of anti-TB treatment response in HIV-infected patients.

Publication: N. Janin et al. 2017, *Journal infection*, May, 2017

### 3.4.3 RESEARCH PROGRAMS –PROSPECT 2018

#### Biomarker for monitoring tuberculosis treatment response:

Diagnosis of pulmonary tuberculosis currently based on assessment of clinical signs, chest X-ray and detection of *Mycobacterium tuberculosis* (Mtb) in sputum. Microscopic detection of Mtb in sputum smears is the most routinely used for diagnosing pulmonary tuberculosis and therapeutic response monitoring. However, this approach is poorly sensitive for diagnosing tuberculosis, thus numerous cases of tuberculosis remain undiagnosed. Empirical TB treatment initiated on the basis of highly clinical suspicion of tuberculosis without bacteriological confirmation is a frequent choice for clinicians in developing countries, especially in severely immunosuppressed HIV –infected patients. Thus, the additional tool are urgently needed not only to help diagnose TB, but also to evaluate the response to anti-tuberculosis drug treatment, specifically in patient treated empirically, so that the clinicians could decide whether or not such empirical treatment could be continued or stopped safely. In a pilot study with Cambodian patients from the CAMELIA clinical trial, we found that plasma IL-1RA level dropped dramatically after 2 months of TB treatment (Janin et al 2017). Activated macrophages in tuberculosis infection released numerous soluble form of receptors including hemoglobin scavenger receptors (CD163) and IL-1RA. In response to the rapid decline of mycobacterial load following anti-tuberculosis drugs treatment, the macrophages will be less activated and consequently the levels of activation markers of macrophages will be rapidly decreases. We aim of this proof concept study is to demonstrate that IL-1RA concentrations significantly decline within two weeks following prescription of anti-tuberculosis drugs in HIV and/or TB patient cohort in Ivory Coast and in Cambodia's. The project will submit to ANRS on March 2018.

#### Teaching and student supervising

- Innate immunity of infectious diseases course (Coordinator: Dr Daniel Scott-Algara and Prof. Jean Marc Cavaillon) funded by Pasteur International network which will be held on October 2018.

- International joint Master of infectiology between Paris Saclay University, Pasteur Institute in Paris, Pasteur Institute in Cambodia and University of Health Science which will be held on mid-2018.
- Master of medical biology of the University of Health Sciences of Cambodia

Through these training program, we expect to select a qualified student for thesis/internship training at Pasteur Institute of Cambodia.

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### 3.4.4 SUPPORT TO NATIONAL AUTHORITIES

Mr. Pean has provided the support in thesis evaluation of Dr Choeung Rithy, student of master in medical biology (formerly DES medical biology) of the faculty of pharmacy-University of Health Science (USH). Dr Choeung has defended successfully his thesis on 16 October 2017 under the supervision of Dr Philippe Dussart (head of virology unit of IPC).

Mr Pean has also contributed annually in reviewing and updating the teaching curriculum in medical basic sciences of the USH.

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### 3.4.5 TEACHING AND TRAINING

In October 2017, Mr. Pean has taught in immunology for 20 students of master of medical biology of the University of Health Sciences. He spend 10 hours of teaching on the role of innate immune receptors (TLR, NOD, RLR, CLR) in inflammation and infection.

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### 3.4.6 PUBLICATION LIST

2017

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2. Crabol Y, Pean P, Mey C, Duong V, Richner B, Laurent D, Santy K, Sothy H, Dussart P, Tarantola A, et al. A prospective, comparative study of severe neurological and uncomplicated hand, foot and mouth forms of paediatric enterovirus 71 infections. Int J Infect Dis. **2017** Jun

## 3.5 IMMUNOLOGY G4

### 3.5.1 FUNCTIONAL STRUCTURE OF THE UNIT

- Tineke Cantaert, PhD, Head of the Group
- Thi My Hoa Vo, PhD, Postdoctoral Researcher
- Vinit Upasani, Msc, PhD student
- Sivlin Ung, MSc, Laboratory Manager

### 3.5.2 RESEARCH PROGRAMS – MAJOR ACHIEVEMENTS IN 2017

#### Introduction:

Dengue viruses (DENV) infect up to 390 million individuals each year, of which 500 000 cases require hospitalization (1, 2). 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 (3). The mosquito vectors, *Aedes aegypti* and *Aedes albopictus* both thrive well in populated urbanized areas, contributing to the spread of DENV. Costs for dengue treatment are substantially and include both medical care and non-medical costs such as lost working days and preventive measurements and can be estimated to 15-20 USD per person per year (4, 5). In Cambodia, during the 2006-2008 epidemic, total costs reached between 0.03-0.17 of the Gross Domestic Product and was mainly carried by patients themselves (6, 7).

DENV is a member of the family *Flaviviridae*, and consists out of 4 related serotypes (DENV-1 to DENV-4) (8, 9). 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). Dengue shock syndrome is characterized by significant loss of intravascular plasma volume leading to hypovolemic shock, usually between the 4<sup>th</sup> and 6<sup>th</sup> day of fever onset, and is the most common life-threatening complication of dengue (2, 10).

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 (11-14). It remains to be investigated to what extent these mechanisms contribute to human pathogenesis. 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 hampered (15, 16).

#### 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. 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).

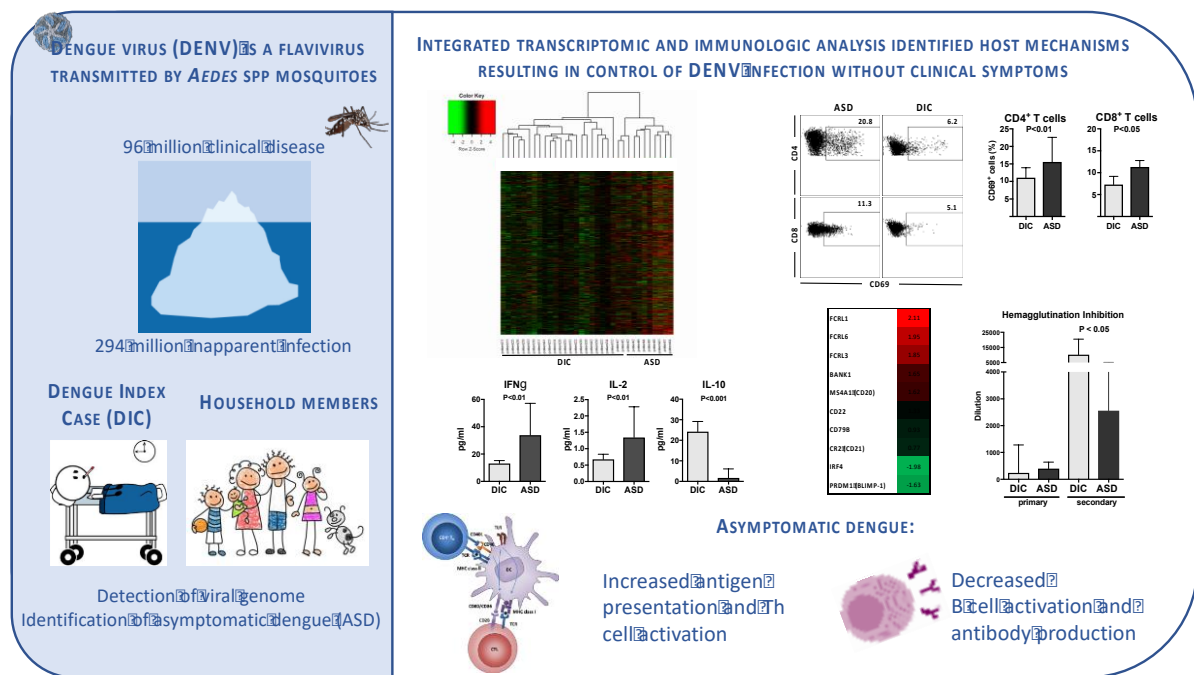


Figure 1: Graphical abstract of Simon-Loriere, Duong *et al*, Scie Transl Med, 2017

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.

#### AIM2: Setup of a cohort of well characterized, early DENV-infected patients.

Children admitted to the Kantha Bopha hospital in Phnom Penh,  $\geq 24$  months of age suspected of DENV infection, of which clinical symptoms initiated less than 96 hours are included in the study. The inclusions have started in July 2016 and will continue until November 2017. Blood samples are sent to the Virology Unit at IP Cambodia for dengue diagnostics. We screen for dengue infection using the rapid diagnostic (combo test for NS1 and IgM/IgG detection, SD Bioline Dengue Duo kits from Standard Diagnostics – Kyonggi-do, Korea) and we perform DENV serotyping, viral load and DENV serology (IgM and IgG) (17). DENV positive cases were defined as individuals positive for NS1 and/or positive RT-PCR and individuals showing IgM seroconversion between day of hospitalization and day of discharge from the hospital. Inhibition hemagglutination assay is performed at admission and at discharge in order to discriminate between primary and secondary infection. During hospitalization, patients are carefully monitored and all clinical data and diagnosis are recorded in a dedicated anonymized case report form for subsequent WHO classification (2). All patients or legal guardians are informed by the clinician and give their consent before inclusion in the study. The protocol has been approved by the National Ethics Committee for Health Research in Cambodia and Clinical Research Department at IP in Paris. This setup reflects the real-life setting where a biomarker predicting development of severe disease will be extremely useful.

End 2017, we have included 21 dengue-like fever control patients, 50 DF patients and 31 DHF/DSS patients. Inclusions will continue in 2018.

Project Ongoing

Collaboration with: Virology Unit, IP Cambodia



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### 3.5.3 RESEARCH PROGRAMS – PROSPECT 2018

#### AIM3: characterization of adaptive immune responses in asymptomatic DENV infected individuals

During the 2018 dengue transmission season, we will setup a new collection of asymptomatic dengue-infected individuals as secondary objective in the ECOMORE II project, funded by the French Development Agency. The availability of PBMC from asymptomatic individuals will allow us to further characterize in depth by novel cutting edge techniques the cellular B and T cell responses in these individuals such as antigen specificity(18, 19), Thelper functionality and detailed B cell subset analysis. As we observe a difference in quantity of anti-DENV antibodies between asymptomatic cases and dengue index cases (Figure 1), is there also a difference in the quality of the antibody response? Therefore, we will investigate the affinity and specificity of serum antibodies from clinical and asymptomatic dengue-infected cases for a whole array of DENV-derived antigens (obtained in collaboration with Institut Pasteur Paris). In addition, crossreactivity between different serotypes will be measured by serum depletion assays and neutralization will be measured by foci-reduction neutralization assay. In addition, we will analyze the detailed glycosylation pattern of antigen-specific antibodies by nanoLC-MS/MS in asymptomatic individuals and dengue index cases (20).

Collaboration with: Virology Unit and Epidemiology Unit, IP Cambodia; Structural Virology Unit, IP Paris; Rockefeller University, NY, USA

Funding: HHMI-Wellcome Trust International Research Scholars Program

#### AIM4: Identification of immunologic markers for the prediction of severe dengue: a hypothesis driven approach.

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 a new biomarker predictive for the development of severe dengue within 72 hours after onset of symptoms in a clinically relevant setting, based on the results of our previous study on asymptomatic dengue patients and investigating our cohort of well described early DENV-infected individuals. (see AIM1 and AIM2).

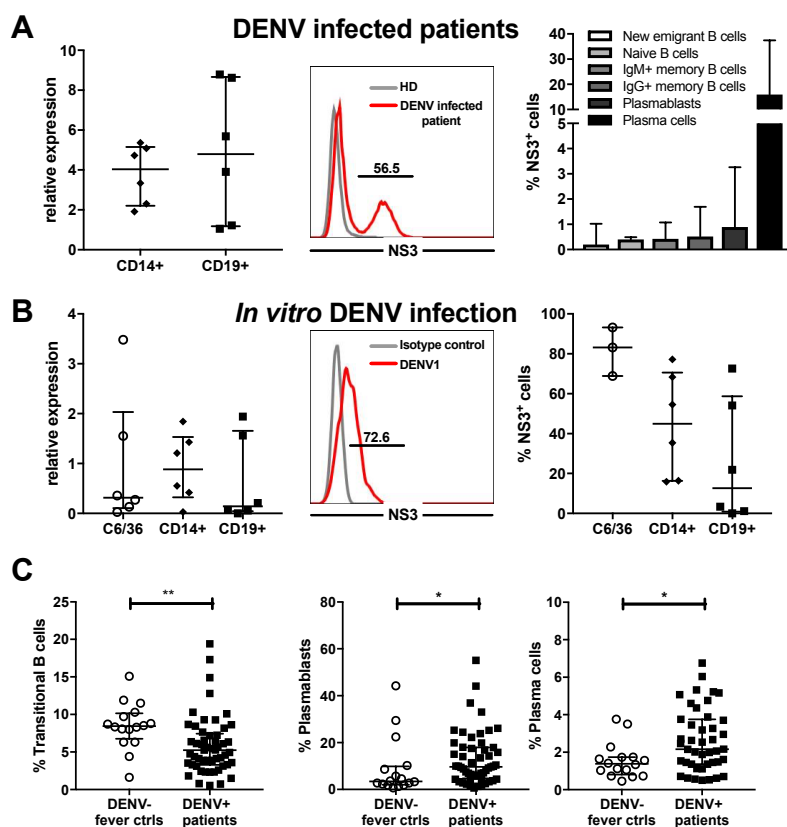
Project Ongoing

Collaboration with: Virology Unit, IP Cambodia; Instituto Pasteur Rome; Groningen University

Funding: HHMI-Wellcome Trust International Research Scholars Program

#### AIM5: Molecular and functional characterization of B cell responses during DENV infection

In the blood, DENV is tropic for monocytes and dendritic cells (21-23) and a few reports have suggested that B cells support viral replication (24-26). 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 (27-29). 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 2). 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 2: B cells are susceptible to DENV infection in vivo and in vitro.** A. DENV infection in monocytes (CD14+) and B cells (CD19+) isolated from DENV infected patients by real time PCR and FACS. NS3 measures intracellular dengue derived non-structural protein 3, indicating infection of the cells. B. In vitro infection of C6/36 insect cells, CD14+ monocytes and CD19+ B cells measured by real time PCR and FACS. C. DENV infection alters the composition of the B cell compartment compared to control patients with undifferentiated fever. Median and interquartile ranges are shown. P values are obtained by Mann-Whitney test

Project Ongoing

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

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

### 3.5.4 SUPPORT TO NATIONAL AUTHORITIES

Research done in this unit is basic research, and there is no direct support to national authorities. However (see below) this group contributes to capacity building and teaching in Cambodia.

### 3.5.5 TEACHING AND TRAINING

- 10 hours in the Immunology Module, Master Medical Biology, University of Health Sciences
- Member of the Steering Committee International Master Infectious Diseases and coordinator of the Immunology Module.
- 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 students:  
Thimoro CHENG, Northern Illinois University, Bachelor Biochemistry (June-August 2017 (will return in summer 2018)

### 3.5.6 PUBLICATION LIST

#### Awards 2017

HHMI-Wellcome International Research Scholar to Tineke Cantaert

Early Career Research Prize in Vaccinology R&D, International Union of Immunological Societies to Tineke Cantaert

#### Publications

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##### 2017

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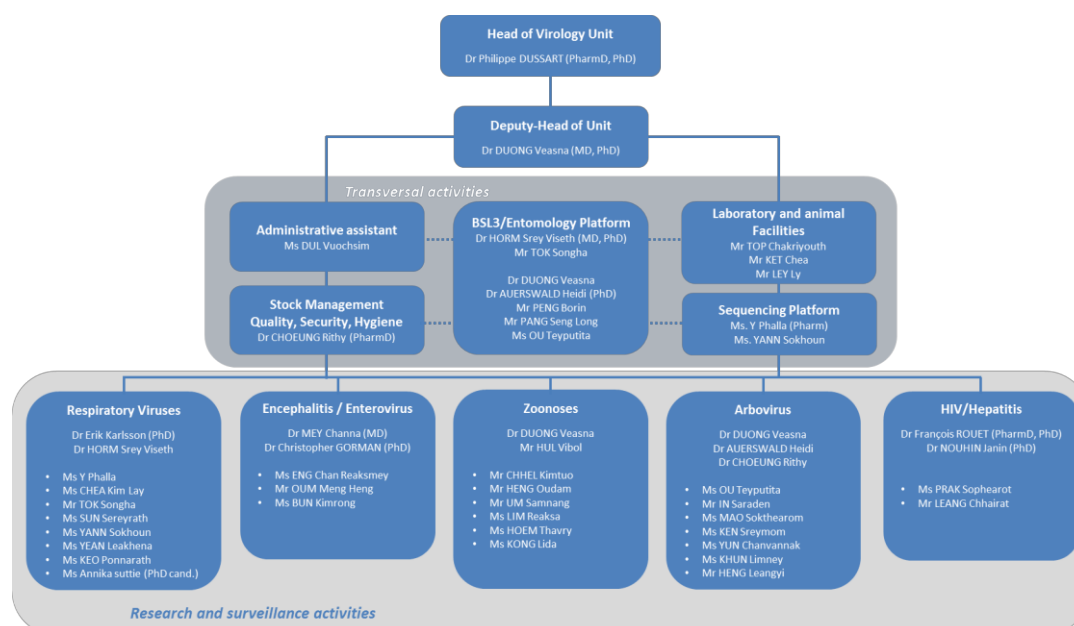
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6. R. Huy *et al.*, Cost of dengue and other febrile illnesses to households in rural Cambodia: a prospective community-based case-control study. *BMC public health* 9, 155 (2009).
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## 3.6 VIROLOGY

### 3.6.1 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 four main components: (1) arboviruses (ex: dengue, Zika, chikungunya and Japanese encephalitis), (2) respiratory syndromes (mainly seasonal and avian influenza), (3) viral encephalitis (Japanese encephalitis, EV-A71), and (4) 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).



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## 3.6.2 RESEARCH PROGRAMMES – MAJOR ACHIEVEMENTS IN 2017

### 3.6.2.1 ARBOVIRAL DISEASES

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#### **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 a mosquito-specific virus. More sequencing is needed to confirm this preliminary observation.

**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:

- Characterize recent DENV genotype diversity and evolutionary dynamics in Cambodia and New Caledonia in relation with the epidemiological profile,
- Evaluate the potential role of vector-driven selection in DENV genotype replacements by investigating vector-virus interactions *in vivo*,
- Measure the relative ability of DENV genotypes to replicate in mammalian cells *in vitro* and to produce subgenomic flavivirus RNAs (sfRNAs)

**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

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 continents. 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).

**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 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* and *Culex*). After establishing standardized protocols for vector competence studies with other partners of the consortium in 2017, mosquitoes collected from different Cambodian areas (urban versus rural areas) will be tested in 2018.

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

**Financial support:** European Union



## Chikungunya

**Virology Unit Team leader:** P. Dussart

Chikungunya virus (CHIKV) is an alphavirus that has been reported in Asia since the 1950s, constituting the Asian genotype. Since 2005, strains from the Eastern, Central, and Southern African (ECSA) genotype have caused several outbreaks across Asia. Viruses from the ECSA genotype were detected in Cambodia in late 2011 preceding an outbreak in a rural community in 2012. A former investigation from 2012 found a higher risk of infection in people younger than 40 years old, suggesting pre-existing herd immunity in the older Cambodian population due to previous infection with an Asian genotype. In 2016, we returned to the site of the 2012 study and collected serum from individuals born before 1975 and born after 1980. We analyzed the resulting 154 serum samples from 2016 for neutralization against the Cambodian ECSA isolate and three strains belonging to the Asian genotype. This revealed that 22.5% (18/80) of the younger study participants had no CHIKV antibodies whereas only 5.4% (4/74) of the older population remained naïve. Study participants infected during the Cambodian ECSA outbreak had twofold neutralizing titers against the Cambodian ECSA strain and the oldest Asian genotype virus included in the study (Thailand 1958) compared to two other newer Asian genotype viruses. This neutralization data supports the hypothesis that the older population was exposed to an Asian genotype virus during the 1960s. The observed cross-reactivity confirms that the CHIKV strains we investigated belong to a single serotype despite the emergence of novel ECSA genotype viruses and supports the importance of development of a Chikungunya vaccine (Auerswald et al., *Emerg Microbes Infect*, 2018).

**Financial support:** IPC.

### 3.6.2.2 AVIAN INFLUENZA VIRUSES

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**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 2017, there were six (6) outbreaks of avian influenza – one A(H7N3) and five 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(H7N3) and A(H5N1) and sequence analysis of the outbreak strains showed that the viruses were closely related to strains circulating in live bird markets between 2015 and 2017. A publication has been recently accepted in *Emerging Infectious Diseases* describing the A(H7N3) outbreak.

- ***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 HA and NA gene sequences have also been completed for a number of other A(H5N1) strains from 2017. 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. This component of the research has been delayed and is expected to be completed in 2018.

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

Since 2015, we have conducted LBM surveillance in Orussey market in Phnom Penh to determine the circulation characteristics of avian influenza in Cambodia. To date, our team have successfully isolated 45 A(H5N1) viruses, 68 LPAI viruses and 22 viruses from mixed infections from LBM samples collected in 2015 and 2016. In 2017, these isolates were 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. Samples from 2017 are currently being isolated and surveillance will continue into 2018.

- ***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.

- ***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, 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 and analysis will continue and the study is projected to be completed in 2018.

- ***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, we collected 1,098 poultry samples and 544 swine samples with 19.6% and 6.3% influenza positivity respectively. We also collected 415 human serum samples. Data collection and analysis will continue and the study is projected to be completed in 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. A publication detailing this study has been submitted.

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

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

### 3.6.2.3 NEUROTROPIC INFECTION

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#### **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. Regionally, we found Japanese encephalitis virus (JEV) is the most common etiology, followed by herpes simplex virus 1 (HSV1), Dengue virus and enterovirus A71 (EV-A71). Of the known etiologies, 34% of pathogens were preventable and 10% curable. The outcome of the patients recruited were: 16% death, 49% sequelae and 35% recovery. 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 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.

**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 2017, the IPC virology unit, in collaboration with the Kantha Bopha hospitals, continued surveillance of hospitalized patients with non-severe or severe HFMD Syndrome. During the first 10 months of the year, an upsurge of suspected cases of severe HFMD was observed, mainly during the first trimester of 2017. In total, 55 patients with positive detection of enteroviruses (56% EV-A71, 44% other HEV) presented: isolated HFMD syndrome (n=4), HFMD syndrome associated with encephalitis (n=39), HFMD syndrome with encephalitis and cardiopulmonary involvement (n=12). Most of the EV-A71 viruses detected clustered with sub-genotype C4 previously detected in the country.

EV-A71 strains have been phylogenetically classified based on their capsid coding region into genogroups: A to G. whereas genogroup A includes the historical prototype strain, genogroups B and C have been reported worldwide in major outbreaks, and represent the two canonical circulating EV-A71 subtypes. New genogroups E and F were recently identified in Africa and Madagascar, respectively. With colleagues from Institut Pasteur in Paris, we compared antigenic and immunogenic features of EV-A71 strains, which belong to the canonical (B-C) and the new (E-F) genogroups. By using collections of human sera from Cambodian patients with neutralizing antibodies against EV-A71 genogroup C, we evaluated the epidemiological risk of a population affected by a canonical EV-A71

genogroup from being protected against the new genogroups E and F. All human sera showed rather similar cross-neutralization activities between isolates of genogroups B, C, E and F. These results indicate that the antigenic features of all tested genogroups are quite similar among the serotype EV-A71. They also suggest that the neutralizing antibody response induced by strains of the canonical genogroups B and C is likely to be protective against the new genogroups E and F. Importantly, our findings provides valuable information in terms of public health and EV-A71 vaccine development.

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

**Financial support:** Total Foundation.

### 3.6.2.4 ZOONOSES

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#### PREDICT 2

Virology Unit Team leader: V. Duong

The PREDICT 2 project has been carried out since 2014 and is now entering its fourth year of surveillance in wild animals (bat and rodents), domestic animals and humans in 2018. In 2017, 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. A selection of samples have been tested at IPC for Alphavirus, Coronavirus, Filoviridae, Flavivirus, Hantavirus, Influenza A virus, Rhabdoviridae, Paramyxoviridae and Bunyaviridae. 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](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 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. In Year 3, a total of 323 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 project intends to build capacity for surveillance and field investigation for zoonotic diseases, enhance laboratory capacity to detect zoonotic diseases, improve national and regional cross-sectorial collaborations by establishing a One Health (OH) surveillance and laboratory network and conduct strategic research on two important drivers of disease emergence – wildlife trade and land-use change.

During year 3 of the project (2017), we have succeeded in establishing wildlife and human disease surveillance systems in both countries, with the collection and analysis of 6,192 mosquitoes, as well as 3,001 samples from 245 rodents associated with disease outbreaks/mortalities. Our wildlife disease surveillance system has made significant progress in highlighting OH issues in the region, while ensuring capacity building at the national and provincial levels, as exemplified by the coordinated wildlife

farming surveillance activities initiated and led by NAHPRI in year 3. Innovative research projects to investigate the role of land-use change and wildlife trade in zoonotic disease emergence are discovering important information about priority pathogens, vector distributions and the risks to humans and livestock. The Cambodia and Lao PDR field teams have been very active during year 3, both spending >100 days in the field, and >200 days analysing collected samples in the laboratories. The project also provides opportunities for training (both field and laboratory) of staff from all three OH sectors and improving collaboration. The project has also contributed to the inclusion of the environmental/wildlife sector into the OH strategy, which has allowed significant progress in the establishment of a OH disease surveillance network in Cambodia and Laos.

**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: H. Auerswald

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 conducted in 2018.

**Financial support:** Institut Pasteur, Paris – IPC

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## **3.6.3 RESEARCH PROGRAMMES – PROSPECT 2018**

### **3.6.3.1 ARBOVIRAL DISEASES**

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The various studies in progress on dengue, Chikungunya and Zika virus will be continued in 2018.

#### **Dengue virus and mosquito specific virus interaction**

The preliminary results obtained in 2017 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 viruses as the presence of these mosquito viruses in nature might 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) will be conducted for the ACIP project and on three ZIKV strains from Africa (Senegal), Asia (Malaysia) and South America (Martinique) for ZikAlliance using *Aedes aegypti* 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.

## **Chikungunya**

We observed similar neutralization potency against the recent ECSA IOL CHIKV strain from Cambodia and the oldest CHIKV strain of the Asian genotype (Auerswald et al, 2018). This mirrors the phylogeny of the two genotypes as the ECSA lineage is proposed to have originated from the Asian lineage. Since we have serum samples remaining from the seroprevalence study, we would like to investigate the specific epitopes responsible from cross reaction to both genotypes. We aim to establish a collaboration with colleagues from Ben-Gurion University of the Negev, Israel to utilize an antigen microarray tiled with overlapping 20-mer peptides to analyze cross reaction the Cambodian serum samples. We aim to identify common epitopes shared by the different genotypes which will have an important implications for vaccine development.

### **3.6.3.2 SEASONAL AND AVIAN INFLUENZA VIRUSES**

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#### **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 will be recruited to monitor background levels of respiratory carriage.

#### **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 have been able to isolate these viruses and, in 2018, plan to commence full risk assessment on these isolates including phylogenetics/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.

#### **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, 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.



### 3.6.3.3 NEUROTROPIC INFECTION

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#### **South East Asia Encephalitis Project – SEAE**

In 2018, retrospective laboratory testing will continue, including testing for auto-immune encephalitis markers and tuberculosis. Furthermore, samples from patients of interest will be submitted for pathogen discovery. A final meeting of stakeholders and partner institutes is scheduled for 2018 in Phnom Penh, where results, analysis strategies and future plans will be discussed.

#### **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.

### 3.6.3.4 ZOONOSSES

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#### **PREDICT 2**

IPC's Virology Unit will continue to collaborate with the Cambodian authorities to conduct field missions to collect samples twice a year (during the dry and the rainy seasons) in Kandal province, to continue documenting the interface between humans, livestock and rats, and in Kampong Cham province, to study the interface between humans, livestock and bats. All field work will be finished by the end of September 2018 and the final year (year 5) of PREDICT 2 will be dedicated to laboratory testing and analysis of results. Positive samples detected from bats and rodents in this project will be subjected for further characterization in order to understand potential for crossing species barriers. Full genome sequencing of previously detected viruses will be performed either at UC Davis or with other collaborators. Human and domestic animal samples are currently being tested and the results may provide more insight into the circulation of viruses between different hosts within the same community.

#### **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 aim to have a Cambodian Ph.D. student working in the Virology Unit on this topic starting in 2018.

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## 3.6.4 SUPPORT TO NATIONAL AUTHORITIES

#### **Participation to National Technical Working groups**

Virology Unit is an active member of the zoonotic technical working group with monthly meeting, sometime at IPC, involving major partners and collaborators from Ministry of Health and Ministry of Agriculture, fisheries and forestry.

## 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 2017, 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.). In contrast to previous years, where DENV-1 was the main dengue serotype detected from 2011 to 2015, 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. Only few DENV-3 cases have been detected and sporadic DENV-4 cases have been diagnosed. Overall, circulation of dengue viruses in the country was low in 2017. Half the number of dengue-suspected cases and one-third fewer samples were received by IPC compared to 2016. From the 5,902 hospitalised cases in 2017, only 3 deaths were reported within the whole country. DENV-2 circulation should be carefully monitored in the coming years as a low herd immunity in the Cambodian population could be a risk factor for disease emergence, which could lead to a large dengue outbreak in the near future.

## 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, Monduliri, 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).

During this period influenza A/H3N2 viruses was the most commonly detected (n=163), followed by A/H1N1pdm09 virus (n=70), Influenza B (n=34) (27 B/Yamagata and 7 B/Victoria) and 4 A/H3 (NA subtype not available). Classical Influenza A/H1N1 seasonal viruses were 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.

- ***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). From 01 January to 31 December 2017, 142 samples were received from the Kantha Bopha Foundation hospitals 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=100) and Kantha Bopha Hospital in Phnom Penh (n=42). The samples were all negative for A/H5N1, A/H7N9 and MERS-CoV; however, 28 (20%) SARI cases were positive for A/H1N1pdm09 influenza viruses and 12 (8%) were positive for A/H3N2 viruses (Figure 2).

Similar to the influenza season in 2016 (rainy season in Cambodia), a large spike in A/H1N1pdm09 positive SARI patients were detected starting in June 2017 with a high mortality rate (~50%) and mortality continued to increase when the dominant subtype switched to A/H3N2 in October of 2017. In addition to the seasonal ILI samples, we have also sent samples from 11 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 has 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 195 brain samples from dogs, an increase of 24% compared to 2016. The positivity rate also increased to 60.9% (n=117) of dog brain samples found to be infected by rabies virus in 2017 compared to 2016 (50.3%, n=79). Average percentage of positive dog heads observed from 2002 to 2017 currently stands at 50.6%.

## Contribution to Rabies national Guidelines

IPC's Virology and Epidemiology Units have been involved in the writing of Rabies Cambodian Guidelines, with other bodies from Ministry of Health and Ministry of Agriculture / Animal Health. Objectives is to eliminate rabies by 2025 – 2030.

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### 3.6.5 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 2017, more than 30 IPC staff (Virology Unit staff representing the majority) followed different types of training, courses and/or workshops organized at IPC by IPC scientists 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 three foreign students (one Master and two Ph.D. students), as well as eight Cambodian students (two Master's, four veterinary and two lab technicians' students). In 2018, we plan to register one virology staff in Ph.D. program in the field of virology.

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13. Lacroix A, Duong V, Hul V, Sorn S, Holl D, Omaliss K, Chea S, Hassanin A, Theppangna W, Silithammavong S, Khammavong K, Singhalath S, Afelt A, Fine A, Goldstein T, Olson S, Joly D, Groatorex A, Keatts L, Dussart P, Frutos R, Buchy P. Diversity of bat astroviruses in Lao PDR and Cambodia. *Infect Genet Evol*. 2016; 47:41-50.
14. Cappelle J, Duong V, Pring L, Kong L, Yakovlev M, Prasetyo DB, Peng B, Choeung R, Duboz R, Ong S, Sorn S, Dussart P, Tarantola A, Buchy P, Chevalier V. Intensive circulation of Japanese Encephalitis virus in peri-urban sentinel pigs near Phnom Penh, Cambodia. *PLoS Negl Trop Dis*. 2016; 10(12):e0005149.
15. Duong V, Blasdel KR, Eloit M, Chretien F, Ly S, Hul V, Deubel V, Morand S, Buchy P. Evidence of human infection by a new mammarenavirus endemic to Southeastern Asia. *Elife*. 2016;5. e13135.

## 3.7 MEDICAL ENTOMOLOGY PLATFORM

### 3.7.1 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). The platform collaborates with other scientists working on mosquitoes or vectors from the Malaria Molecular Epidemiology Unit (Amélie VANTAUX) and from Virology Unit (Veasna DUONG).

In 2016, this Unit had only two staff: **Sebastien BOYER** (PhD), head of Unit, and **Sony YEAN**, Master 1 student and laboratory technician. The Medical Entomology Platform recruited 3 new technicians in 2017 with Ecomore 2 and Zikalliance projects. **CHHUM Moeun**, **SUOR Kimhuor** and **CHHUOY Kalyan** were recruited as technicians of Medical Entomology, and Sony YEAN continued in Master 2.

In 2017, the Unit has 5 members.

### 3.7.2 RESEARCH PROGRAMS – MAJOR ACHIEVEMENTS IN 2017

#### **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).

This year, we mainly focused on exploiting the results the diversity, dynamics and implication of potential vectors in JEV transmission (mainly *Culex*). The article is under progress. An article is under progress and should be submitted this year (Peng Borin et al. Density and dynamic of Japanese encephalitis virus's vector in peri-urban and rural in Cambodia).

Moreover, we also developed a protocol to study the behavior of the JEV vector species. Japanese Encephalitis virus (JEV) remains the most important cause of acute encephalitis in Eastern and Southern Asia. *Culex tritaeniorhynchus* is the primary vector of JE in rural settings, followed by *Culex vishnui* and *Culex gelidus*, whereas anthropophilic species like *Culex quinquefasciatus* are probably more involved in urban and peri-urban JE transmission. Pigs are the main amplifying hosts and Ardeid birds the main wild reservoir. However, a recent study has shown that young chicks and ducklings experimentally developed a viremia high enough to allow virus transmission to mosquitoes. Even though JE is still considered a rural disease, several studies conducted in Cambodia, Thailand and Vietnam showed that JEV and its vectors can be found in peri-urban areas. There is still a lot to learn concerning JE's complex epidemiology Mosquito feeding behavior is a key parameter to improve our understanding of this epidemiology. The goal of this work is to analyze the feeding behavior of the main JE vectors in a rural and a peri urban setting of Cambodia using two methodologies, ie baited traps and PCR analysis of available mosquito blood meals.

The two expected outcomes are (1) the analysis of the feeding behaviour of the main JE vector species in rural and peri urban area of Cambodia, and (2) assumptions on the respective epidemiological role of these mosquito species for JE transmission, and on the involvement of domestic birds in the epidemiological cycle of JE in Cambodia.

The experiment last all 2017 and finished in December. The results will be exploited in 2018.

#### **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 against main dengue vectors: *Aedes aegypti* and *Aedes albopictus* 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.

In 2017, we begun the entomological inventory in 24 schools in 2 provinces. Entomological inventories were done in May, August and December 2017. It will be continued in February 2018. Then, we will begin the integrated vector control in March 2018 in 12 schools. After that the entomological inventories will continue in May, August, November 2018 and February 2019.

#### **Resistance to insecticides.**

In 2016, we collaborated with Malaria Consortium, Ministry of Health and NAMRU to study the resistance of *Aedes aegypti* to insecticides. In 2016, these results were presented in 4 different conferences (see last report for the listing).

These results were presented in 2017 in a conference at Pasteur Institute. The corresponding article is accepted under minor revisions. It will be published in 2018.

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*, In Press

### **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 build 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 this project we have 2 main objectives: (1) Resistance to insecticides and (2) Back to the wild. For the first objective, we made insecticide resistance tests but with other partners on 8 pops of *Aedes aegypti* (with a strong resistance); see above. That said, if a global paper of insecticide resistance is to come out, we will share these results obviously, knowing that I sent mosquitoes to Jean-Philippe David for molecular characterization in the Zikalliance consortium.

For the 2nd objective, we faced (unfortunately) administrative issues and we finally obtained by the End of the Year (December) the authorization to have access for sampling in the natural areas (primary forests). Field missions are planned next year (2018).

Nevertheless, the Zikalliance consortium allowed to collaborate with other scientists from the IP network and allowed to write a review that is programmed for publication:

Boyer S, Calvez E, Chouin-Carneiro T, Diallo D, Failloux AB. 2018. An overview of mosquito vectors of Zika virus. *Microbes and Infection*. In Press.

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### **3.7.3 RESEARCH PROGRAMS – PROSPECT 2018**

#### **Asia-Pacific IPIN - Threat and Risk Assessment of Yellow Fever in Asia**

Yellow fever (YF), transmitted by the mosquito vector *Aedes aegypti* and *Ae. albopictus* is a potentially deadly disease with symptoms including jaundice, enlargement of the liver, and haemorrhage. It is caused by YFV (Flavivirus, Flaviviridae), a virus that was first isolated in West Africa in 1927. Globally, the heaviest burden of YF is in Africa where the endemic area covers 34 countries and concerns ca 500 million people. Recently, as stated in Chinese centers for Disease Control and Prevention (CDC), a total of 11 imported cases were reported in China by the end of April 2017. The Asia Pacific IPIN decided to assess the potential threat on introduction of YF in the Asia-Pacific region.



## Dynamics of mosquito species 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.

## ZIKV-DENV competition study

We want to develop a project with the Virology Unit to understand the interactions between co-circulating ZIKV and DENV strains and field-collected mosquito vector species. We are currently writing the project to be submitted.

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### 3.7.4 SUPPORT TO NATIONAL AUTHORITIES

The article describing the resistance to insecticides of *Aedes aegypti* populations was presented to the Ministry of Health recommending to use a novel insecticide strategy. Trainings for technicians from CNM, RUPP, RUA and IPC have been organized.

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### 3.7.5 TEACHING AND TRAINING

#### Teaching.

Two Master 2 students were hosted in Medical Entomology Platform in 2017.

**Sony YEAN** is a Cambodian Master 2 student at Royal University of Phnom Penh in Master of Science in Biodiversity Conservation. She is working on the biodiversity and relative density of *Aedes aegypti* in treated and untreated schools, within Ecomore 2 project. Its thesis is supervised by Sebastien BOYER

**Kshitiz SHRESTHA** is a Nepalese Master 2 student at Kastesart University (Bangkok, Thailand) in InterRisk master. He will work on the feeding behavior of Japanese Encephalitis vectors in rural and peri urban area of Cambodia. Its internship is supervised by Veronique CHEVALIER and Sebastien BOYER.

#### Training.

In 2017, the Medical Entomology Platform managed to obtain a training fund from World Health Organization in order to develop systematics of mosquitoes in Cambodia. Professor Paul Rueda from Smithsonian Institute will normally come to train Cambodian technicians and scientists. The invited/involved partners are the Ministry of Health (CNM), the Royal University of Phnom Penh (RUPP), Malaria Consortium, NAMRU and IPC.

In 2017 again, a training was organized at IPC at the End of the Year (November 2017) on the mosquito systematics and taxonomy, with the financial support of the Ambassade de France. Partners such as Ministry of Health (CNM), RUPP and Royal University of Agriculture (RUA) also participated to the training.

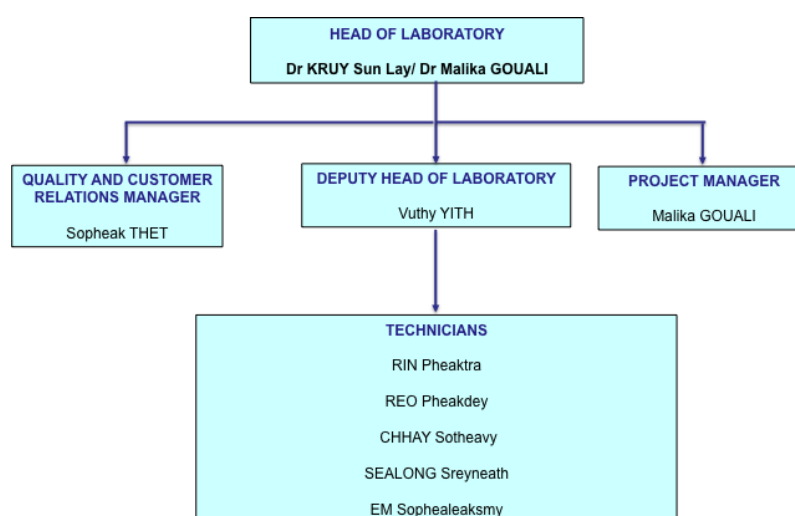
Moreover, Sony YEAN participated to a special PCR training for blood feeding hosts determination in IRD, Montpellier in April 2017, and also followed courses of Statistics organized by the IPC in November-December 2017.

### 3.7.6 PUBLICATION LIST (2017)

1. Randriamaherijaona S, Raharinjatovo J, Boyer S. Durability monitoring of Long-Lasting Insecticidal (mosquito) Nets (LLINs) in Madagascar: 1- physical integrity and insecticidal activity. *Parasites & Vectors*, 2017, 10:564.
2. Jean Jose Nepomichene TN, Andrianaivolambo L, Boyer S, Bourgouin C. Efficient method for establishing F1 progeny from wild populations of Anopheles mosquito. *Malaria Journal*, 2017, 16:21.

## 3.8 ENVIRONMENT AND FOOD SAFETY LABORATORY ANALYSIS LABORATORY (LEFS)

### 3.8.1 FUNCTIONAL STRUCTURE OF THE UNIT



Functional and nominative organization chart of LEFS

Updated on 1<sup>st</sup> November 2017

In 2017, different changes have occurred in the team:

- In July 2017, the head of laboratory, Dr KRUY Sun Lay retired and Dr Malika GOUALI was appointed interim head of the laboratory pending identification and training of the future Cambodia head of Laboratory.
- The technical capacity of the LEFS has been strengthened with the recruitment of 2 new technicians respectively in April and November 2017.
- From July to November 2017, LEFS hosted Mrs SENG Navin, PharmD, PhD, as a half time trainee. She was recruited in September 2017 at IPC as scientific searcher and identified as the future head of Laboratory after the departure of Dr Malika GOUALI at the end of April 2018.
- On 2017, December 1<sup>st</sup>, Mr YITH Vuthy was appointed as the deputy head of the laboratory.

### 3.8.2 ROUTINE ACTIVITY 2017

In 2017, the laboratory has tested 5611 samples comprising 1674 samples of food, 2256 water samples for microbiology testing and 1681 water samples for chemical testing.

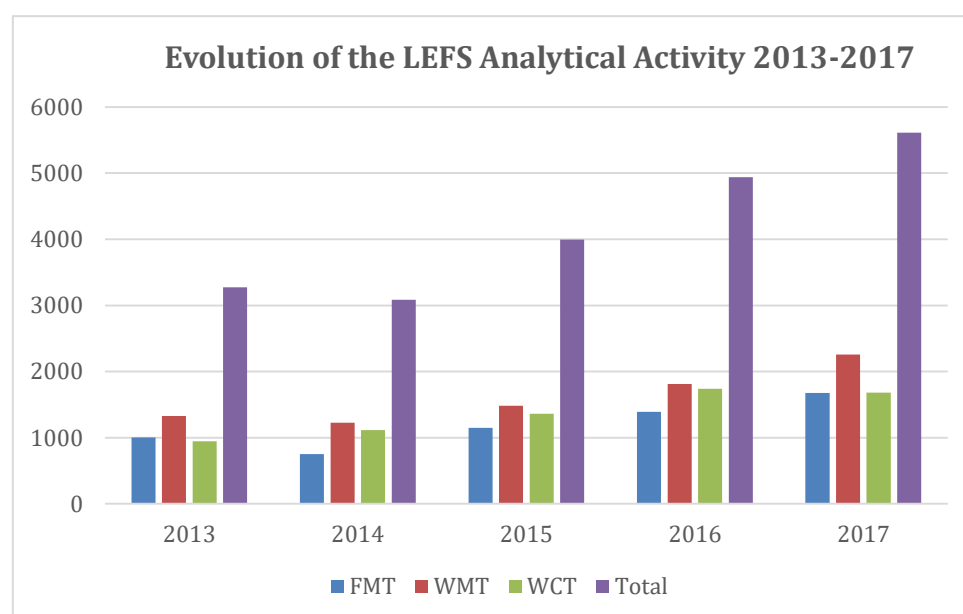
Comparatively to 2016, for microbiology testing, the number of samples increased respectively for food and water by 26,2% and 25%. For chemical testing, we note a decrease of 3,3%.

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				
	2013	2014	2015	2016	2017
Food microbiology testing	1001	750	1147	1390	1674
Water microbiology testing	1326	1224	1485	1810	2256
Water chemical testing	946	1113	1362	1738	1681
<b>Total</b>	<b>3273</b>	<b>3087</b>	<b>3994</b>	<b>4938</b>	<b>5611</b>

**Figure 1**



**FMT: food microbiological testing, WMT: water microbiological testing, WCT: water chemical testing**

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

- 1,6% of food samples (48/1674) were not satisfied, 27 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 and Moringa.
- 13,1% of water samples (296/2256) were not satisfied and therefore were unfit for human consumption because of a faecal contamination.
- 27% of ice cubes samples (53/197) served in the restaurants and bars of Phnom Penh and bars are contaminated by faecal bacteria as coliforms, *E. coli* and intestinal enterococci.
- 6,5 % of water samples (108/1681) tested for chemical are not satisfied.

In terms of development, several important changes occurred in 2017 in the laboratory as:

- Implementation of international standard methods in microbiology to be in accordance with NF EN ISO 17025 requirements in case of a project to get an accreditation in the future.
- Setting up of a dedicated reception and welcome office for the customers
- Improvement of laboratory visibility : regular update of the website, presentation flyer of LEFS 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)
- Strengthening of laboratory team with 2 additional technicians recruited respectively in April and November 2017
- Finally, move of the activities to a new laboratory 3,5 times bigger than the old one and responding to international standards.

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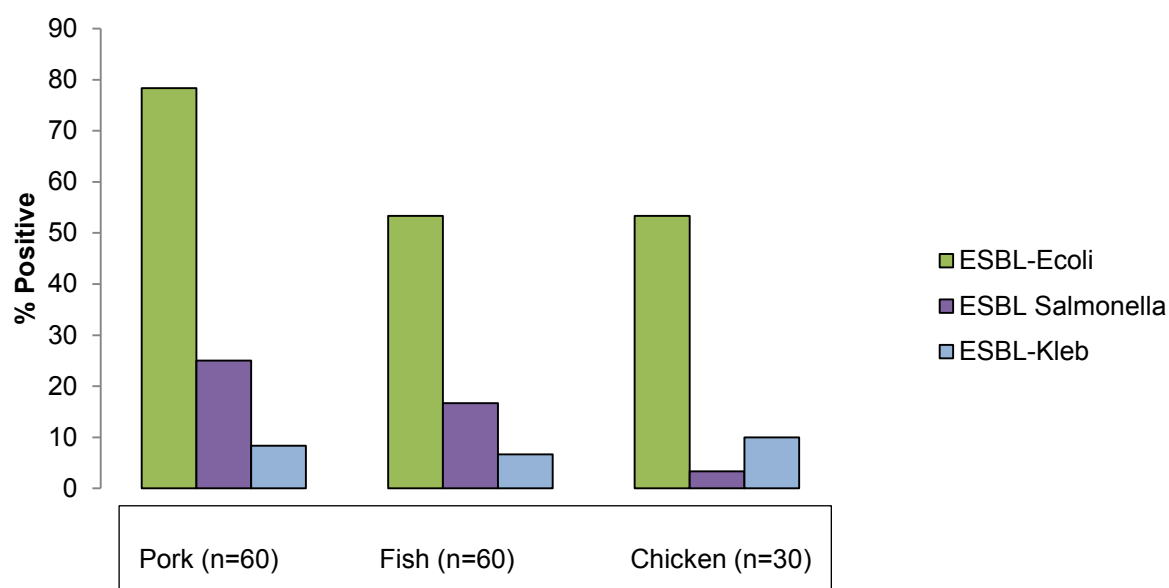
### 3.8.3 RESEARCH PROGRAMS - MAJOR ACHIEVEMENTS IN 2017

#### **Ancillary Birdy project 2016-2017**

In Cambodia, overuse of 3rd generation cephalosporins (e.g. cefotaxime, ceftriaxone, cefatazidime) has contributed to a rapid increase in extended-spectrum beta-lactamase (ESBL)-producing enterobacteriaceae. In this prospective study conducted by Maya Nadimpalli, a post-doctoral searcher from Institut Pasteur (Paris) hosted in the laboratory for 6 months and Vuthy Yith, deputy of laboratory, we examined whether meat and fish could be a possible source of exposure to ESBL-producing bacteria among healthy women living in Phnom Penh. From September to December 2016, 150 samples (chicken, fish and pork samples) were collected from two markets in Stung Meanchey, Phnom Penh and assessed for the presence of ESBL producing, enterobacteriaceae.

We detected ESBL-*Ec* among 93/150 fish and meat samples (63%), comprising 32/60 fish (53%), 45/60 pork (75%), and 16/30 chicken (53%). All ESBL phenotypes were encoded by CTX-M-type genes, of which CTX-M-55 was most common. CTX-M-55 was detected in 23/32 ESBL-*Ec* from fish (72%), 27/45 from pork (60%), and 12/16 (75%) from chicken. We identified three carbapenem-resistant *E. coli* (all OXA-type) from two pork and one fish.

**Figure 2: Prevalence of ESBL on Meat and Fish**

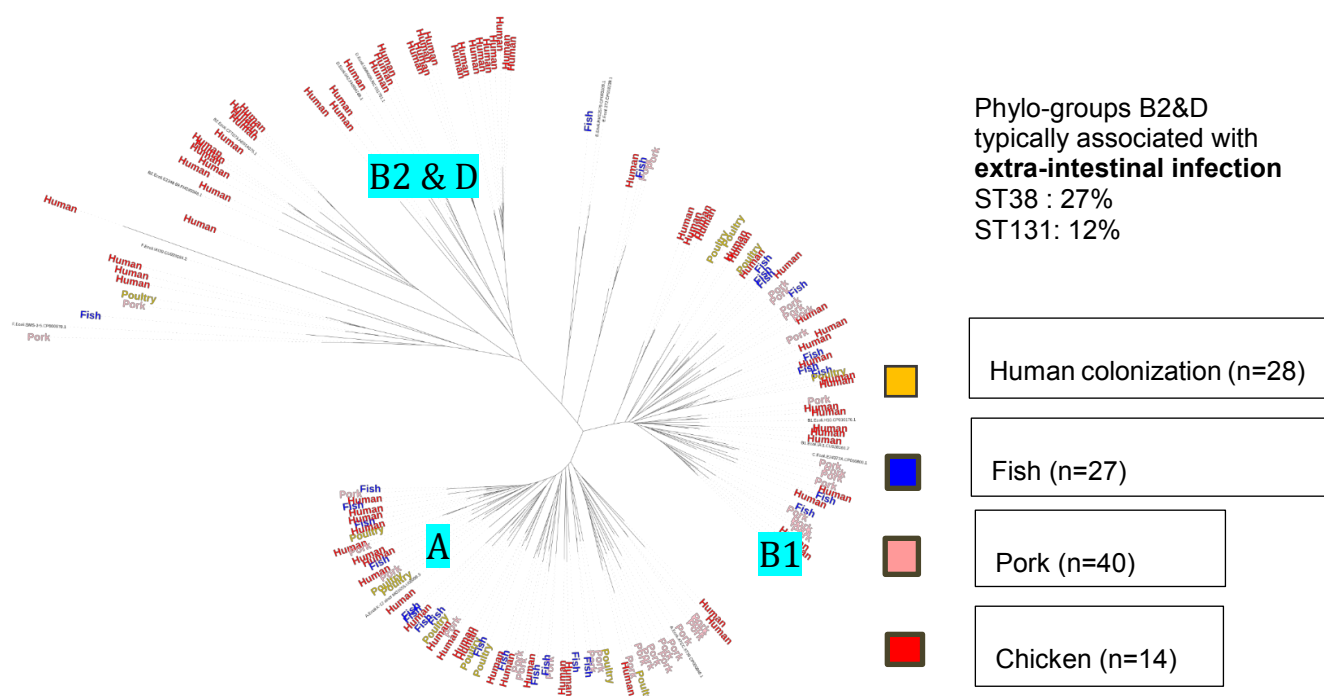


The second aim of the study which was conducted in 2017, was to compare these ESBL-*Ec* with those isolated from healthy human carriers and patients with clinical infections, in order to examine the potential contribution of meat consumption to local transmission of ESBL-*Ec*.

For this, we included ESBL-*Ec* from healthy humans living in the same neighborhood of Phnom Penh in which meat and fish were sampled. Fecal swabs were collected as part of the BIRDY program (an ongoing surveillance program of bacterial infections among young children in low-income countries) from healthy women at the time of delivery or up to ten days after. We included ESBL-*Ec* from women who had been sampled up to one year before meat and fish sample collection began, i.e. from Sept 2015 through December 2016.

Genomic comparisons of ESBL-*Ec* isolates revealed three clans : one (B2 D) composed almost exclusively of human-origin ESBL-*Ec*, and two (A and B1) comprising both human- and food-origin isolates. ESBL-*Ec* from healthy women that clustered with food-origin isolates (n=48) were significantly more likely to encode CTX-M-55 ( $p<0.01$ ). Among them, a subset (n=23) were more likely to express chloramphenicol (CHL) resistance ( $p<0.05$ ). Women colonized with CHL-resistant (versus susceptible) ESBL-*Ec* more frequently reported eating dry poultry (adjusted odds ratio: 6.5, 95% confidence interval: 1.7, 24.9).

## Genomic comparisons of ESBL-Ec isolates (2017)



### 3.8.4 RESEARCH PROGRAMS - PROSPECT 2018

In 2018, with its new direction, 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.

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### 3.8.5 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 2017, 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 142 food products for microbiology testing and 40 for chemical testing (27 for borax, 7 for sodium hydrochlorite and 3 for formalin).

This collaboration was renewed in 2018 and the Ministry of Health plans to send us this year 400 samples of street food for the same parameters for an average of 50 samples a month since January 2018.

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.

LEFS is a member of the workgroup involved in Safe Food Fair Food (SFFF) project which is coordinated by National Animal Health and Production Research Institute (NAHPRI), Department of Animal Health and Production, Phnom Penh, Cambodia in collaboration with the International Livestock Research Institute (ILRI) and the Centre for Livestock and Agriculture Development (CeAgrid).

The objective of this project is to reduce in Cambodia the burden of foodborne disease in informal, emerging formal, and niche markets and targeting small and medium scale producers.

### 3.8.6 TEACHING AND TRAINING

During 2017, The Head of Laboratory, Dr KRUY Sun Lay delivered classes in Immunology at University Of Health Sciences and Military University of Medecine.

In terms of training, the laboratory, as every year, has supervised 9 trainees coming from different universities of Cambodia for an internship of 1-3 months.

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

**Table 2:**

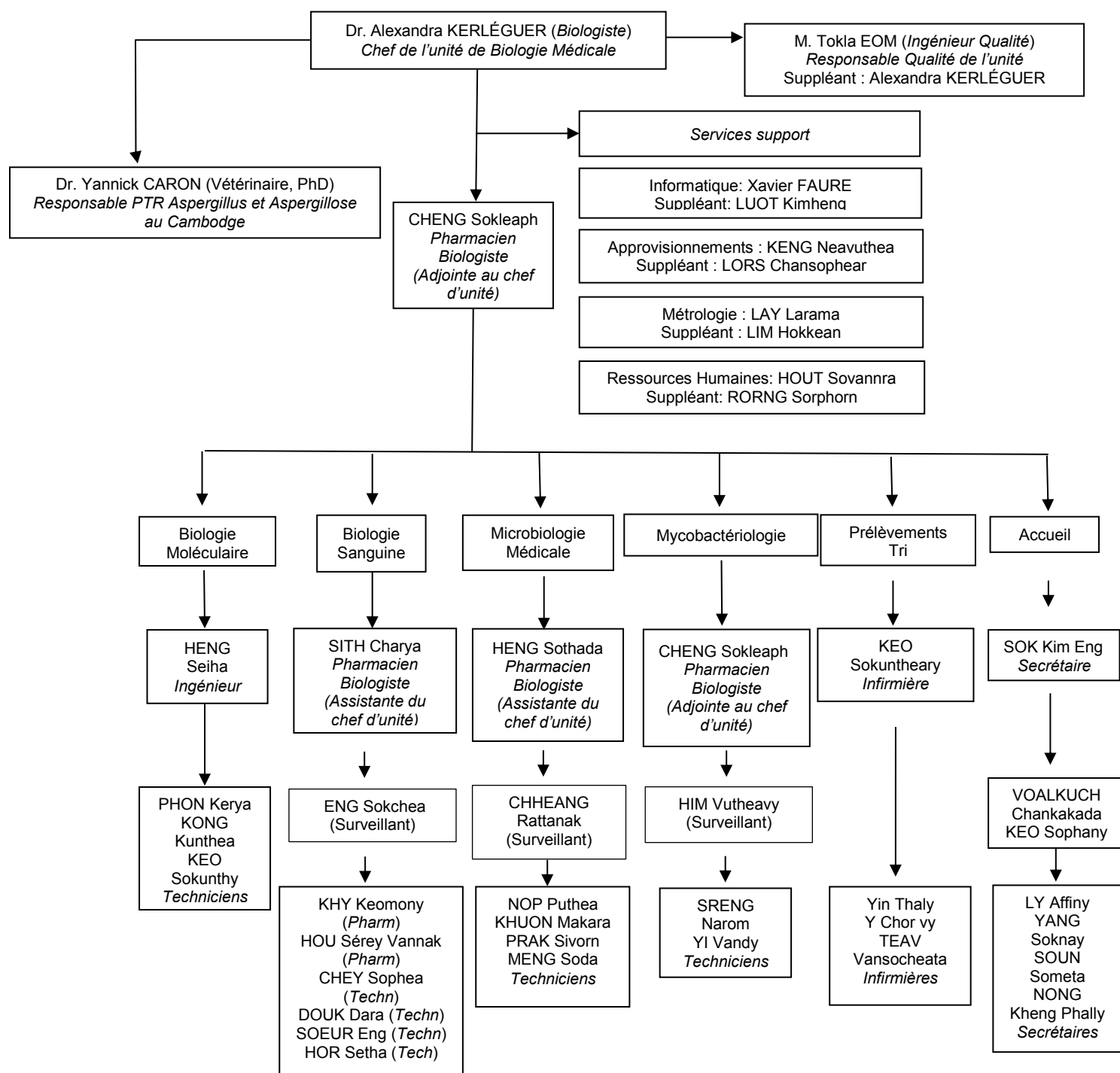
UNIVERSITY	Number of STUDENTS	PERIOD	DATES
Royal University of Agriculture (RUA)	1	3 months	26/10/2016-26/01/2017
University of Health and Sciences (UHS)	6	2 months	17/01/2017 - 03/03/2017 25/04/2017- 15/06/2017 25/04/2017- 15/06/2017 20/06/2017 - 10/08/2017 28/11/2017 - 18/01/2018 28/11/2017 - 18/01/2018
TSMC (Technical School for Medical Care)	2	2 months	07/08/2017 - 26/09/2017 27/09/2017 - 18/11/2017

### 3.8.7 PUBLICATIONS LIST2017

1. Yith Vuthy, Kruiy 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

## 3.9 MEDICAL LABORATORY

### 3.9.1 FUNCTIONAL STRUCTURE OF THE UNIT



### 3.9.2 ROUTINE ACTIVITY 2017

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

Number of patient's files: 47,447 (transmitter: 23,671; private: 21,776 // Cambodian patients: 91.5%; Foreigner patients: 8.5%)

Number of total analyses: 123,565

Number of analyses in the accreditation scope: 84,770

Number of analyses transmitted to CERBA (analyses not performed in the lab): 229 (0.18%)

Number of analyses transmitted to CALMETTE HOSPITAL (anatomopathology not performed at IPC): 102 (0.08%)

For elderly patients or those who cannot move to the lab, we propose to draw samples at their home: 23 this year

2°) Focal point

- Accreditation according NF/EN/ISO 15189 European/French standard
- Antimicrobial resistance

a) Extended spectrum beta-lactamase producing Enterobacteriaceae (ESBLE)

	2015	2016	2017
Total enterobacteriaceae	406	505	657
ESBLE (n)	156	166	209
ESBLE (%)	39	33	32

After a sharp increase from 2012 to 215, maybe a start of decrease?

b) Extended spectrum beta-lactamase Enterobacteriaceae carriage

The intestinal carriage of ESBLs is stable from year to year: 47.5% in 2016; 49.6% in 2017

c) Carbapenemase producing

	2014-2016	2017
Enterobacteriaceae	27/1262 (2.20%)	27-657 (4%)
Non-fermenting gram negative bacilli	11/165 (6.5%)	6/81 (7.4%)

The first carbapenemase-producing bacteria appeared in the lab in 2014 and are increasing

- HIV

A HIV positive cases declaration is sent to NCHADS every three months

- HIV weakly positive: 16 cases
- HIV highly positive: 149 cases

As, HIV screening test are very sensitive to avoid false negative, weakly positive must be controlled after 15 days, to discriminate false positive and primo-infection. Eleven patients have been controlled negative, despite the convocations, 5 patients did not come to the laboratory.

Among 149 highly positive samples, only 136 were drawn at IPC laboratory and only 18 did not know their serology status, 1 was a child, 6 month old.

For the first determination, we perform for free, confirmation test with immune-blotting assay. All the 18 patients were confirmed positive and supported by the CDAG for advice and treatment initiation.

As the child was a new-born, viral load has been performed to eliminate maternal antibodies, who persist for 6-18 months. The child was declared seronegative

For identity problems, we do not perform immunoblots on samples that are not collected in the laboratory.

### 3.9.3 RESEARCH PROGRAMS – MAJOR ACHIEVEMENTS IN 2017

#### HCV Genotyping and viral load: MSF

##### Project BIRDY mother ESBL-E fecal carriage

Aim: To describe main types of resistances among ESBL-E carried by pregnant women in Cambodia

- 1°) Training in IPC to implement new techniques
- 2°) ESBL-E was evidenced in 353/445 fecal samples of mothers (79.3%).
- 3°) Analysis and sequencing of these strains

#### 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. In 2017, a total of 204 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, 75 were sensitive to FLDs and 129 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). Fifteen isolates were excluded from the analysis; 2 (drug resistant isolates) were unable to regrow on

subculture, 1 with negative PCR for almost all the study markers and 12 had multiple MIRU genotypes. A total of 189 were included in the analysis. Sequencing of drug resistant genes were performed on 127 drug resistant (DR) isolates and genotyping method were performed on all MTB isolates. Data is being analyzed for publication.

Training: This project data will be used one PhD thesis and at least 3 DES/Master 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

### **TB Reference Materials: Collection, Storage & Distribution**

Collaboration: Foundation for Innovative New Diagnostics (FIND) and Calmette Hospital

The Foundation for Innovative New Diagnostics (FIND) is a non-profit organization dedicated solely to the development of diagnostic tools for poverty-related diseases. By fostering the transformation of proven biological principles into effective products with impact on TB control and by facilitating appropriate use of and ensuring access to these products by public health systems, FIND will fundamentally make an important contribution to the decrease in global health inequities. The purpose of this specimen collection project is to provide clinical reference materials to FIND's partners to facilitate the development of new TB diagnostics suitable for use in high-burden countries. A hundred subjects were enrolled in the study between February 2017 and January 2018. For each participant, approximately 20 ml of urine, 25ml of whole blood and 2 times 5 ml of sputum were collected and processed for TB diagnosis (smear, culture and GeneXpert, LTBI (Quantiferon) and HIV testing. Among 100 participants, 47 were diagnosed for TB disease by having at least one culture or one Xpert positive and received TB treatment. A clinical follow up period of 8 weeks were conducted for 29 out of 53 non-TB patients (no bacteriological confirmation of TB), of which 7 have received TB treatment by having clinical and chest X-ray results presumptive of having TB. All the collected sample from 71 patients with a clear diagnosis (TB or without TB) were shipped to FIND biobank.

### **MIRU-VNTR typing and Spoligotyping of 20 *Mycobacterium tuberculosis* isolates from the Malayan sun bears**

Collaboration: Free the Bears

Since 2009, 19 cases of pulmonary tuberculosis have been diagnosed in sun bears at Phnom Tamao Wildlife Rescue Center and Zoological Gardens (PTWRC). This work aims to illustrate the molecular feature of *M. tuberculosis* isolates from both the sun bears and the in-contact human population using a combination of spoligotyping and MIRU-VNTR typing. The information from this project will be used to develop a risk assessment and management strategy for tuberculosis in the sun bear population at PTWRC. An article for this data is being prepared.

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## **3.9.4 RESEARCH PROGRAMS – PROSPECT 2018**

### **3.9.4.1 PROJECT SPECIFIC TO LBM**

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#### **Collaboration with the “Antimicrobial” laboratory of NIHE, headed by the Dr Tran Huy Hoang**

1°) Training in NIHE

- Simplex or multiplex PCR to detect genes coded for ESBL (blaTEM, blaSHV, blaCTX, ...)
- Characterization of plasmids carrying ESBLs (CTX-M, SHV...)



- Pulse-Field gel electrophoresis to characterize the ESBL-E and determine their relationships between hospitals and communities settings and between countries

2°) Preliminary molecular analyses of a first sampling selected in our ESBL-E collection (*E. coli* and *K. pneumonia*) by PFGE, MLST and Southern-blotting

### **PTR on Aspergillosis and aspergillus in Cambodia (Yannick CARON)**

The beginning of this PTR involved to get ethical approval from the Cambodian national ethical committee (NECHR). As a first step, the submission of the complete clinical protocol to get CoRC (IP) recommendation with the help of the CRT-CC was necessary. This steps explain in part the long delay to begin patient inclusion as it was largely underestimated in term of time in the initial PTR version. 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 acronym: “AspA” for aspergilloma in Cambodia). Indeed, to facilitate ethical approval, it was important to set up an observational study versus interventional study.

The patient inclusion are ongoing since the beginning of January 2018 and the Mycology laboratory is fully operational. Since the collection of blood and sputum required the ethical approval (obtained 25/12/2017), few progress has been realized in workpackage 1, 2 and 3. Nevertheless, the activity of the Laboratory of Medical Analysis of the IPC permits to isolate *Aspergillus* strains. This material was used to set up some of the different techniques that will be used in the PTR (direct examination, mycological culture, DNA extraction, PCR, ELISA). Furthermore a clinical research assistant (Mala SIM) was recruited (10 January to 9 January 2019) to help the patient inclusion process and the monitoring of the study. To date, about 70 patients have been included since the beginning of this year (sputum and sera have been recovered) and 28 *Aspergillus* strains has been isolated from the environment. The analysis of these samples are ongoing.

### **Molecular characterization of ESBL-E and Carbapenemase producing bacteriae, of the strains isolated in LBM**

#### **PTR on Tuberculosis: Discovering the *Mycobacterium tuberculosis* strains causing Tuberculosis in Cambodia and understanding their pathogenicity**

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, bringing together highly-skilled teams from IP Paris (IPP) and IP Cambodia (IPC), is to combine complementary skills, ranging from clinical to fundamental research, with cutting-edge technologies in different scientific fields (such as molecular biology, genetic, host-pathogen interaction) in order to characterize and identify the mycobacterial strains causing tuberculosis in Cambodia and to get deeper insight into factors that govern the intracellular fate of those clinical isolates strains and the response of the host. To solve it, a combination of efficient genomic and molecular biology technologies will be used: genotyping, spoligotyping and whole-sequencing genome. Then, the host-pathogen interaction studies of the characterized strains will be performed through in vitro and in vivo experiments.

#### **3.9.4.2 PROJECT IN PARTNERSHIP WITH OTHER UNITS OR ORGANIZATION**

##### **PERILIC: Pertusis Immunization programs in Low and middle Income Countries**

Improve knowledge

- Of pertusis disease in infants with pertusis syndrome (proportion, origin of contamination, genetic variability of circulating strains)
- On the duration of protection induced by pertussis vaccination for the population of children under 15 years and contact cases

## Studies with the Malaria Unit

G6PD deficiency, artemisinin resistance

## HCV genotyping: MSF

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### 3.9.5 SUPPORT TO NATIONAL AUTHORITIES

- With MOH and WHO: modernization of laboratories, modification of the national laboratory policy and surveillance of Multi-Drug Resistant Bacteria.
- With the CENAT, to develop guidelines for the diagnosis, treatment and monitoring of patients with multi-drug resistant tuberculosis and to improve diagnosis capacity of the National TB Reference Laboratory.
- 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.

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### 3.9.6 TEACHING AND TRAINING

#### 1°) Internship

Biologists / Pharmacists: 2 months internship -18 students

Laboratory Technicians: 3 months internship-10 students

#### 2°) Thesis supervision

FIVE Thesis (1 finished and 4 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
- Genetic diversity of drug resistant *Mycobacterium tuberculosis* isolates circulating in Cambodia between 2013 and 2015
- Étude des mutations des gènes liées à la résistance de *Mycobacterium tuberculosis* isolées des patients au Cambodge
- Hepatitis E virus (HEV) infection in Cambodia: Temporal HEV IgG rates trend in humans, defended in March 2017.

#### 3°) Course

Master 2 pharmacologie clinique, Antimicrobial resistance: 2hours

#### 4°) Other

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

### 3.9.7 PUBLICATION LIST

2016

1. Chheang R, Nop P, Heng S, Tarantola A, Kerléguer A. 2016. Bêta-lactam resistance among enterobacteriaceae: Follow-up 7 years. *International Journal of Infectious Diseases*, 45, 85.
2. Marcy O, Ung V, Goyet S, Borand L, Msellati P, Tejiokem M, Nguyen TN, Nacro B, Cheng S, Eyangoh S, Pham TH, Ouedraogo AS, Tarantola A, Godreuil S, Blanche S, Delacourt C. 2016. Performance of Xpert MTB/RIF and Alternative Specimen Collection Methods for the Diagnosis of Tuberculosis in HIV-Infected Children. *Clin Infect Dis*, 62, 1161-1168.
3. Huynh BT, Padget M, Garin B, Delarocque-Astagneau E, Guillemot D, BIRDY study group. 2016. Bacterial neonatal sepsis and antibiotic resistance in low-income countries. *The Lancet*, 387, 533-534.
4. Chauillac N, Brochard N, Payet C, EGEE (Étude Gatekeepers en EHPAD) study group, Duclos A, Terra JL. 2016. How does gatekeeper training improve suicide prevention for elderly people in nursing homes? A controlled study in 24 centres. *Eur Psychiatry*, 37, 56-62.

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. 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 latrogenic Outbreak of Human Immunodeficiency Virus Type 1 in Rural Cambodia, 2014-2015. *Clin Infect Dis*.
5. 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.

#### ORAL/POSTER PRESENTATION

1. High prevalence of respiratory Nontuberculous Mycobacteria Respiratory Infections in Children Living with HIV in South-East Asia. 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, ANRS 12229 PAANTHER 01 Study Group. MOAB0205, The 9th IAS Conference on HIV Science, 23-26 July 2017 Paris, France
2. Comparative performance of "Loopamp™ MTBC Detection" kit for rapid detection of complex Mycobacterium tuberculosis: a retrospective study. Cheng S, Heng S, Him V, Sreng N and Kerléguer A. The 6th Conference of IUATLD Asia Pacific Region, 22-25 March 2017.

## 3.10 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.

### 3.10.1 RABIES PREVENTION CENTER FOR 2017

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 2017 US\$12 for a protocol of 2 x4 injections). In 2015, the Rabies Prevention Center at Institut Pasteur du Cambodge 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, the Rabies Prevention Center at 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, the Rabies Prevention Center at 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.

Selection and implementation of the satellite PHD – IPC Rabies Prevention Center in Battambang, close to Provincial Hospital and MoH PHD, has been initiated for an expected opening in April 2018. A memorandum of understanding has been signed on 25<sup>th</sup> of December, 2017, between Battambang Provincial Health department and Institut Pasteur du Cambodge.

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### 3.10.2 INTERNATIONAL VACCINATION CENTER FOR 2017

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 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.

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### 3.10.3 VOLUNTARY COUNSELING AND FREE TESTING CENTER (VCTC) FOR 2017

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.

During 2017, the VCTC of the Institut Pasteur du Cambodge handled pre-test consultations of which 633 (647 in 2016) were followed up by post-test counseling (647 pre-test consultations of which 645 were followed up by post-test counseling in 2016). Previous year it was 622 pre-test consultations of which 618 were followed up by post-test counselling in 2015). A total of 74.4 % of the consultants live in Phnom Penh (70.9 in 2016, 66.1% in 2015). The grounds for appeal was a risk ratio or practice in 72.8 % of the cases (69.1 % in 2016, 68.2% in 2015). With 34 cases confirmed in 2017 (33 in 2016, 36 in 2015), the prevalence established was 5.3 % (5.1 % in 2016, 5.8% in 2015) among the overall patient cohort, 6.3 % among persons who had never taken a test (5.5 % in 2016, 6.8% in 2015) and 5.1 % among the consultants who came back for their results and who were directed to the public health care system (5.8% in 2016, 5.6% in 2015). No indeterminate result has been rendered since implementation of the Western blot test.

## 4 CONCLUSION

L'Institut Pasteur du Cambodge, avec plus de 230 personnes, dont plus de 50 cadres scientifiques cambodgiens, français, et d'autres nationalités, et près de 25 étudiants en DES, master et thèse, est parmi les principaux instituts de recherche en santé en Asie du Sud-Est.

Fort de ses près de 60 contrats sur convention, de ses 45 articles scientifiques ou chapitres de livre publiés en 2017, 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 sport du Cambodge, Institut Pasteur et Ministères de la Recherche et des Affaires Etrangères de France, et avec la confiance des partenaires nationaux et internationaux.

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*The Institut Pasteur du Cambodge, with a staff of 230 persons, including over 50 scientific supervisors from Cambodia, France and other countries and with about 25 post-graduate students, ranks among the leading health research institutes in Southeast Asia.*

*With 60 research contracts, 45 scientific articles or book chapters published in 2017, 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: 26 January 2018

