Institut Pasteur du Cambodge

2019 Scientific Report and 2020 Perspectives

Institut Pasteur du Cambodge

Institut Pasteur International Network

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1. LIST OF ACRONYMS

ACIP	Action Concertée Inter-Pasteurienne (Institut Pasteur)
ACT	Artemisinin combination-based resistance
AFD	Agence Française pour le Développement (French Development Agency)
AFRIMS	Armed Forces Research Institute for Medical Sciences
AIV	Avian Influenza Viruses
AMP	Antimicrobial Peptides
AMR	Antimicrobial Resistance
ANRS	*Agence Nationale de Recherche sur le SIDA et les* Hépatites (French National Agency for AIDS and Hepatitis Research)
ANSES	Agence Nationale de Sécurite Sanitaire (French National Agency for Sanitary Safety)
ARCAHE	Antibiotic resistance at the Human/Animal/Environment interface in a “One Health” Approach in Cambodia – FSPI project
ARTC	Anti-Rabies treatment Center for post-exposure prophylaxis (Service open to public)
ASAQ	Artesunate/amodiaquine
ASIDE	Alerting and Surveillance for Infectious Diseases Epidemics
AQ	Amodiaquine
AQ-R	Amodiaquine Resistant(ce)
AVIEASAN	Alliance Nationale pour les Sciences de le Vie et de la Santé (France)
BSL	Biosafety level
BIRDY	Bacterial Infections and Antibiotic-Resistant Diseases among Young Children in Low Income Countries
CANARIES	Consortium of Animal Networks to Assess Risk of Emerging Infectious Diseases through Enhanced Surveillance
CDC	Centers for Disease Control and Prevention
CEG	Community Epidemiology Group
CIRAD	*Centre de Coopération Internationale en Recherche Agronomique pour le Développement (*French Agricultural Research and International Cooperation Organization*)*
CENAT	National Center for Tuberculosis and Leprosy Control (Cambodia)
CNM	National Center for Parasitology, Entomology, and Malaria Control (Cambodia)
CPE	Carbapenemase Producing Enterobacteria
CWRU	Case Western Reserve University (USA)
CRG	Clinical Research Group
DAA	Direct Acting Antiviral Therapy
DARPA	Defense Advanced Research Projects Agency
DBS	Dried Blood Spot
DENV	Dengue Viruses
DES	Diplôme d’etude spécialisée (specialized diploma for MD or PharmD)
DF	Dengue Fever
DHF	Dengue Hemorrhagic Fever
DNA	Deoxyribonucleic Acid
DSS	Dengue Shock Syndrome
DTRA	Defense Threat Reduction Agency (USA)
DVI	Dengue Vaccine Initiative
EID	Emerging Infectious Disease
EPH	Epidemiology and Public Health Unit of Pasteur Institute of Cambodia
ESBL-E	ESBL-producing Enterobacteriaceae
ETI	Expert Technique Internation (under French Ministry of European and Foreign Affairs)
FAO	Food and Agriculture Organization of the United Nations
FAVN	Fluorescent Antibody Virus Neutralization
FLDs	Fragmented and Loop Primer Ligated dsRNA Sequencing
FRNT	Foci Reduction Neutralization Test
FSPI-MAEA	Solidarity Fund for Innovative Projects, Civil Societies, The French-Speaking World And Human Development, Ministry of European and Foreign Affairs, France
GCRF	Global Challenges Research Fund

GDAPH	Under the Cambodian Ministry for Agriculture, Forestry and Fisheries
GF	Global Fund to Fight AIDS, TB and Malaria
GIS	Geospatial Information System
GISRS	Global Influenza Surveillance and Response System
GLASS	Global Antimicrobial Resistance Surveillance System
GMS	Greater Mekong Subregion
GPS/GSM	Global Positioning System and Global System for Mobile Communicationss
HBV	Hepatitis B Virus
HC	Health Center
HCV	Hepatitis C Virus
HEPAR	Hepatitis E and ARENA Virus Project
HEV	Hepatitis E Virus
HFMD	Hand-Foot-and-Mouth Disease
HIV	Human Immunodeficiency Virus
HLA	Human Leuckocyte Antigen
HS-RDT	High Senstivity Rapid Diagnostic Test
ICEMR	International Center of Excellence in Malaria Research
ILI	Influeza-like Illnesses
INF	Interferon
INSERM	Institut National de la Santé et de la Recherche Médicale (France)
IP	*Institut Pasteur* (Pasteur Institute)
IPC	*Institut Pasteur du Cambodge* (Pasteur Institute of Cambodia)
IPIN	*Institut Pasteur* International Network
IRD	*Institut de Recherche pour le Développement* (Institute for Research and Development-France)
IRIS	Immune reconstitution inflammatory syndrome
ITC	Institute of Technology (Cambodia)
IVI	International Vaccine Initiative
JVE	Japanese Encephalitis Virus
KBH	Kantha Bopha Hospital
LAM	Live Animal Market (LBM might be used for Live Bird Market in the text)
LBM	Laboratoire de Biologie Médicale – Medical Biology Laboratory (Service open to public)
LEFS	Laboratory for Environment and Food Safety Laboratory (Service open to public)
LORV	Loei River Virus
LMI-DRISA	International joint Laboratory “Drug Resistance in South East Asia” (IRD)
LSHTM	London School of Hygiene and Tropical Medicine
MAFF	Ministry of Agriculture, Forestry, and Fisheries (Cambodia)
MDR	Multi-drug resistant(ce)
MEAE	*Ministère de l’Europe et des Affaires Etrangères (*French Ministry for European and Foreign Affairs)
MEF	Ministry of Economy and Finances (Cambodia)
MERS	Middle East Respiratory Syndrome
MESRI	*Ministère de l’Enseignement Superieur, de la Recherche, et de l’Innovation.* (French Ministry for Higher Education, Research and Innovation)
MEYS	Ministry of Education, Youth and Sports (Cambodia)
MiVEGEC	*Maladies Infectieuses et Vecteurs Écologie, Génétiques, Evolution, et Contrôle (IRD, France)*
MinION	DNA sequencing using Oxford Nanopore Technologies
miRNA	Cell-free circulating microRNA biomarkers
MOH	Ministry of Health (Cambodia)
MMEU	Malaria Molecular Epidemiology Unit (Pasteur Institute of Cambodia)
MMV	Medicines for Malaria Venture
MTB	*Mycobacterium tuberculosis*
MSAT	Mass Screening and Treatment
MTCT	Mother to Child Transmission of HIV
MTRU	Malaria Translational Research Unit
NAHPRI	National Animal Health and Production Research Institute

NAMRU	US Naval Army Medical Research Unit
NaVRI	National Veterinary Research Institute
NCHADS	National Center for HIV/AIDS, Dermatology and Sexually Transmitted Diseases (Cambodia)
NDCP	National Dengue Control Program (Cambodia)
NIC	National Influenza Centre
NIH	National Institutes of Health (USA)
NIP	National Immunization Program
NIPH	National Institute for Public Health (Cambodia)
NMCH	National Maternal and Child Health Center
NSSF	National Social Security Fund
OIE	World Organisation for Animal Health
PCR	Polymerase Chain Reaction
PEP	Post Exposure Prophylaxis
PHD	Public Health Department
p.i.	Post infection
PMI	President's Malaria Initiative (USA)
PrEP	Pre-exposure Prophilaxis
PREEMPT	Preventing Emerging Pathogenic Threats
PRR	Platforme Régionale de Recherche (building PRR)
PSI	Population Services International
PTR	Programme Transversal de Recherche (funding mechanism from Institut Pasteur in Paris)
QA/QC	Quality Assurance/Quality Control
QFT	Qantiferon
RABV	Rabies Virus
RDT	Rapid Diagnostic Test
RGC	Royal Government of Cambodia
RUA	Royal University of Agriculture (Cambodia)
RUPP	Royal University of Phnom Penh (Cambodia)
RPC	Rabies Prevention Center
RAS	Resistance-Associated Substitution
SARI	Severe Acute Respiratory Illness
SAB	Scientific Advisory Board
SHCH	Sihanouk Hospital Center of Hope (Cambodia)
SIV	Swine Influenza Virus
STI	Sexually Transmitted infections
SVR	Sustained Virological Response
TB	Tuberculosis
TLR	Toll-like Receptors
TWG	Technical Working Group
UGA	University of Georgia (USA)
UHS	University of Health Sciences (Cambodia)
UMR	*Unité mixte de recherche* (joint research units) France
USAID	United States Agency for International Development
UP	University of Puthisastra (Cambodia)
VEG	Veterinary Epidemiology Group
VIC	Vaccine International Center (Service open to public)
VL	Viral Load
VU	Virology Unit
WCS	Wildlife Conservation Society
WEHI	Walter and Elizabeth Hall Institute (Australia)
WENV	Wēnzhōu virus
WHO	World Health Organization
WHOCC	WHO Collaborating Center on Influenza- Melbourne Australia
WNV	West Nile Virus
ZIKV	Zika Virus

2. EXECUTIVE SUMMARY

Due to the exceptional COVID-19 epidemic workload, the 2019 scientific report will be only produced in English

In 2019, the *Institut Pasteur du Cambodge* (hereinafter abbreviated as IPC or the Institute), a Cambodian institution focusing on infectious diseases), has maintained its three major missions: research, services and training, drawing on the expertise and skills of its 255 employees and 22 or so trainees and visiting scientists. The main highlights are as follows:

- Two changes at the Senior Management level: a new Director (Dr. Laurence Baril replaced Dr. Didier Fontenille in November 2019) and a new Financial and Administrative Director (Mr. Christophe Mousset replaced Mr. Guillaume Daufresnes in September 2019); Dr. Sowath LY remained in the position of Deputy Director;
- Continued diversity of collaborative research projects to inform public health recommendations and policy for infectious disease control improvement and public health. More specifically, in 2019 recommendations were offered in these technical areas:
    - Emerging Diseases: (research supported by the Solidary Fund at the French Ministry of Foreign Affairs (FSPI-MEAE)
    - Malaria: (funding received from United States National Institutes for Health (NIH), the World Health Organization (WHO), the Global Fund to Fight AIDS, TB and Malaria (GF), and Initiative5%-Expertise France);
    - Hepatitis B Ta Prohm: (supported by the French National Agency for AIDS and Hepatitis Research (ANRS);
    - Tuberculosis miRNA and TB Speed (with funding from ANRS, UNITAID and Initiative5%);
    - Avian flu and seasonal flu (supported by United States Department of Health and Human Services (DHHS) through the Alerting and Surveillance for Infectious Disease Epidemics-ASIDE Project- and the Food and Agriculture Organization of the United Nations (FAO), and WHO).
    - Dengue: Through the Ecomore 2 Project, supported by AFD which explores whether integrated vector control in schools can mitigate the epidemic peaks and absenteeism and reduce hospital overcrowding during the dengue epidemic season.
- Development of the Antimicrobial Resistance (AMR) research and search for financial support for this research;
- Support for continuous scientific improvement of the local staff and ongoing capacity-building training activities;
- Published high impact research findings to ensure diffusion and support policy change recommendations;
- Responded to the 2019 rabies crisis by treating more than 78,000 post-exposure prophylaxis patients, by opening a third anti-rabies treatment center in Kampong Cham (following Battambang in 2018), and the appointment of a new head for the vaccine international center and the anti-rabies treatment centers;
- Completed a successful ISO 15189 accreditation audit for the medical biology laboratory;
- Strengthened regional collaboration including institutional partners from Vietnam, China, Thailand, Myanmar, Laos, and Philippines;
- Strengthened existing partnerships with Cambodia's universities (University of Health Science (UHS), the Royal University of Phnom Penh (RUPP), the Royal University of Agriculture (RUA), and the Institute of Technology (ITC), providing mentorship and training Cambodian and international students (about 100 students hosted);
- Successful implementation of the international master's degree in infectious diseases from UHS and University of Paris-Saclay;
- Continued scientific communication through weekly seminars open to the entire Cambodian scientific community;
- Contribution to several infectious disease guidance documents developed by the Ministry of Health (MOH), Ministry of Agriculture, Forestry, and Fisheries (MAFF), WHO, and FAO;
- Continued encouragement and support of career development of Cambodian scientific and administrative staff with new responsibilities in all parts of the institution.

The *Institut Pasteur du Cambodge,* a member of the *Institut Pasteur* International Network (IPIN), is a government-approved non-profit institution. The Institute signed an agreement with the Royal Government of Cambodia (RGC) in 1992 which was renewed by an endorsement in 2013. The Institute is under the senior patronage of the Cambodian Ministry of Health, on behalf of which it carries out life sciences and health research, public health and

services as well as training activities. The Institute has reinforced this mission over the past 15 years in collaboration with national and international institutions. It contributes to the diagnosis, research, and prevention of the priority infectious diseases in Cambodia and the Greater Mekong Sub region (GMS). 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 foremost the *Institut Pasteur* in Paris. It hosts local researchers and international researchers from *Institut Pasteur* (IP), *Institut de Recherche pour le Développement* (IRD) and the French Agricultural Research and International Cooperation Organization (*Centre de Coopération Internationale en Recherche Agronomique Pour le Développement*, CIRAD).

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 (SEA) in the areas of diagnosis and the study of pathogen transmission, analysis of biological and genetic markers for severity or the antimicrobial resistance of microorganisms, prevention and clinical research. The significant scientific advances were published in more than 50 scientific articles (and book chapters) in 2019 have been made possible due to the insight and perseverance of researchers in securing contracts and carrying out research, the key involvement of the IPC's technical and administrative personnel, support from the Cambodian MOH, critical support from the French Ministry for Higher Education, Research and Innovation (MESRI), the French Ministry for European Foreign Affairs (MEAE), and *Institut Pasteur* in Paris. It is important to mention that research projects and service activities make up by far the largest share of IPC resources, comprising 90% of the annual budget and 10% from MESRI.

IPC is intensely involved in university-level training. Students in biology, biomedical sciences, engineering, master's and doctoral students from different universities in Cambodia, France and other countries were hosted by IPC. In 2019, in total17 graduate students had long term training (from several weeks up to 36 months) at IPC (13 Cambodian students, 4 foreign students). IPC strategy focuses on the promotion of careers for young Cambodian scientists by providing them with a competitive, world-class scientific environment. Memoranda of Understanding have been signed with UHS, ITC and renewed with Calmette Hospital. Relationships with RUA, RUPP, and Puthisastra University were reinforced in 2019.

In 2019, IPC had a staff of over 255 comprising 14 nationalities throughout year 2019, including eight local senior scientists, two expatriates at the senior management level (one international technical expert from MEAE and one from IP), ten expatriate scientists (eight from IP, one CIRAD and one IRD), two research engineers from IRD and three from IPC, five PhD students and five post-doctorates.

IPC is composed of an administrative and financial department, several services (medical biology unit, laboratory for environment and food safety (LEFS), vaccine international center, three anti-rabies treatment centers, five research units: immunology, medical and veterinary entomology, virology, malaria, and epidemiology/public health including a team dedicated to clinical research.

Its operating budget is supplemented by research contracts, service delivery agreements, MESRI and MEAE government grants and other resources, as shown in the table below.

Operating Budget: Respective Share by percentage by Three Main Components by Year, 2012-2019								
	2012 (%)	2013 (%)	2014 (%)	2015 (%)	2016 (%)	2017 (%)	2018 (%)	2019 (%)
Research Contracts	40	48	57	58	60	57	57	55
Service Delivery	30	27	21	25	29	32	32	35
MESRI	20	17	14	12	11	11	11	10
Total Share of Budget	90	92	92	95	100	100	100	100

IPC's activities and financial matters are reviewed by the Liaison Council chaired by His Excellency (HE) the Minister of Health, in the presence of HE the Ambassador of France, the President of IP (Paris) and representatives of several national authorities (Ministries of Finances, Ministry of Agriculture and Fisheries and Ministry of Education, Youth and Sports) and international agencies (WHO, UNICEF, etc.). The scientific activities are also assessed every 24 months by a Scientific Advisory Board (SAB), its last session having been held on 6-7 December 2018). The scientific strategy is modified based on the recommendations of those two boards and IPC remains a bridgehead of Pasteur-inspired research on infectious diseases in Cambodia and in Southeast Asia.

IPC activity main results for 2019 are presented in the report organized by units and by services.

3. PERSPECTIVES AND CHALLENGES

The challenges and perspectives are related to the three IPC missions: life sciences and health research activities, 2) public health and services activities, 3) teaching and training activities. The objectives include the recommendations made the previous year by the Liaison Council and the Scientific Advisory Board (SAB).

A transition was made between the two directors, Dr. Didier Fontenille and Dr. Laurence Baril, and between the two administrative and financial directors, Mr. Guillaume Daufresne and Mr. Christophe Mousset. Mr. Mousset assumed his role in September 2019 and Dr. Baril arrived in mid-November 2019. The role of Dr. LY Sowath, the Deputy Director, was key to ensure continuity of activities and the link with the main partners including the Ministry of Health.

A. Major comments from the previous Liaison Council meeting (30 April 2019)

The comments from the previous Liaison Council emphasised the quality and richness of the scientific results obtained by the IPC and their relevance to the needs of the Cambodian people.

Firstly, the distinguished work of Dr. Didier Fontenille during his five-year leading role as IPC Director was recognized and he was strongly acknowledged for his dedication to establish IPC as a leading research institution in Cambodia, and also for his support to public health efforts and strong partnerships with other Cambodian institutions and development partners.

The 2019 recent Liaison Council discussion was mostly dedicated to the response to the rabies crisis in Cambodia. The opening of a third provincial antirabies treatment was useful (Post meeting note:- a total 78,000 subjects received post exposure prophylaxis (PEP) in 2019 across the three anti- rabies treatment centers run by IPC). The three centers, however, will not be sufficient to cover the whole country. It was acknowledged that IPC could play a technical advice role and should continue to perform research on rabies, and the Ministries, with support from development partners and WHO, will have the leadership role to eliminate rabies. The Ministry of Health (MOH) and the Ministry of Agriculture, Forestry and Fisheries (MAFF) are developing a national strategic plan for rabies elimination in Cambodia. The Ministry of Education, Youth and Sports (MEYS) will be involved too.

The Ministry for Economy and Finances (MEF) highlighted the need to have a partnership between the National Social Security Fund (NSSF) and IPC.

The launch in September 2019 of the international master's degree in infectious disease based at UHS with Paris-Saclay University is aligned with the objectives of the Ministry of Health and the Ministry of Education, Youth and Sports to support higher education on life sciences and health. Upon opening, it welcomed students not only from Cambodia, but from other countries as well (France, Ghana, and Thailand in 2019).

B. Major recommendations of the Scientific Advisory Board meeting (December 6 and 7 2018)

The scientific advisory board (SAB) considers that research and service activities being offered by IPC are of good quality and are useful. Publications are deemed satisfactory, and SAB appreciated the restructuring of the medical entomology and immunology units.

Training at IPC is viewed as good. Involvement in the new master's degree with UHS is worthwhile. New research projects like AMR and Aspergillosis have strong value for the surveillance system for Cambodian public health. Several research projects address highly relevant issues for public health, including TB, Rabies, Hepatitis, Dengue, Malaria, among others. Service activities are very relevant, including but not limited to LEFS and rabies PEP. SAB also congratulated IPC for achieving ISO 15189 accreditation for the LBM. New responsibilities for Cambodian staff and scientists is a positive evolution.

Despite the overall positive review by the SAB, it did raise some concerns:

- IPC must secure new grants for 2019-2021,
- IPC lacks a good statistician and bio-information specialist,
- IPC must improve its attractiveness for graduate students,
- Turnover of expatriates is problematic,

- IPC should organize writing/publishing workshops and develop a mentorship program with research leaders,
- SAB recommends building a supportive IPC student/alumni community,
- SAB recommends strengthening communications with the public health authorities, universities and ministries.

3.1 SHORT-TERM OBJECTIVES

The objectives were defined by the new management team and were adapted taking into account the global COVID-19 crisis. The Scienctific Advisory Board meeting was postponed to the end of Year 2020, as have several other important scientific workshops and meetings.

3.1.1 INSTITUTIONAL CHALLENGES

IPC PLAYS A LEADING ROLE IN EMERGNING INFECTIOUS DISEASES RESEARCH AT REGIONAL AND INTERNATIONAL LEVEL

IPC, working under the auspices of the Cambodian MOH, the *Institut Pasteur* (Paris), is a key national component of Cambodian research in life sciences and health with a strong focus on emerging infectious diseases. IPC continues to be integrated in the Cambodian network of basic and applied research on infectious and retains solid partnerships with universities, hospitals, and other governmental and nongovernmental research centers. IPC continues to strengthen its regional and international perspective and partnerships, with the *Institut Pasteur* International Network (IPIN) being an important component of its international visibility. In November 2019, IPIN Directors meeting has identified two main priorities AMR and Rabies control; IPC is fully aligned with those two priorities.

More than 90% of the staff, including senior researchers and managers, is Cambodian but scientists or trainees from seven nationalities were represented in 2019. IPC continues to host senior researchers from different institutions such *as Institut Pasteur* (Paris), CIRAD, for the “one-health” component and IRD. IPC develops highly talented Cambodian scientists and managers and provides them with the opportunity to have leadership roles in the institution.

DEVELOPING SCIENTIFIC CAPABILITIES

IPC contributes at different levels for developing scientific capabilities.

For non IPC staff, IPC continues to contribute to the training and coaching of lab technicians, master’s degree students, engineering students and commits to host doctorate-level students (PhD, Phar.D., DMedSc, doctorate in veterinary medicine, enrolled in Cambodian and foreign universities. It is key to make these training and supervisory and mentoring activities more visible. All scientists based at IPC including colleagues from CIRAD and IRD are contributing to these educational efforts.

IPC contributed significantly to the successful launching of the international master’s degree in infectious disease from UHS - Paris Saclay Universities through teaching activities, a part-time project manager, facilitation of the missions from the foreign lecturers and hosting trainees at Master’s years/levels one and two.

IPC previously hosted weekly seminars open to the Cambodian scientific and academic community but due to the COVID-19 crisis since March 2020 they have been cancelled and will be restarted using the webinar mode in June 2020.

IPC acknowledges that the global COVID-19 crisis will impact the magnitude of all activities for at least the first semester of 2020.

DEVELOPING INFRASTRUCTURE

IPC continues to improve laboratories and infrastructure for epidemiology, virology, malaria, entomology, immunology, LBM, LEFS units and the vaccination centers (Phnom Penh, Battambang and Kampong Cham Rabies vaccination centers). In 2020, a focus will be put on the new equipment for the virology (including bls-3) and immunology units but also the new insectarium for entomology, re-organization of the IT team, a functional BLS-2 in PRR building. To note, the PRR building was constructed in 2014 under the auspice of the French Alliance for Life Sciences and Health, AVIESAN) and hosts the Malaria and Entomology Units. In addition, to better welcome and ensure safety of the visitors, the entrance of IPC will be rebuild (pending evaluation of the construction of the Calmette Hospital new building nearby IPC and external financial support).

DEVELOPING IPC STAFF COMPETENCIES AND MAKING CAREERS AT IPC ATTRACTIVE

An attractive institution is an institution with common culture and goals with respect of roles and responsibilities at all levels, this also includes a particular attention to gender equity.

For IPC staff, IPC facilitates staff member career development through different means: short/medium technical courses (through IPIN or other partner institutions) and longer training to obtain diplomas. Efforts toward e-learning certifications and diplomas will be encouraged at all levels in the institution. This is very important in domains where technologies are evolving so rapidly in areas such as life sciences and health research. Also it is important to identify young talents to help them to later contribute to middle management and leadership management of the institution.

A group training on research management will be held for the scientific staff in June 2020 (pending travel clearance for the training supervisor based in Thailand) as will be implemented individual coaching to develop leadership and managerial skills for highly talented senior scientists.

A managerial training will be developed and implemented for the non-scientific middle and leading staff to ensure a unified and common vision and language in the institution.

A new development career plan format to identify institutional and staff needs will be implemented in 2021 by the human resources team, taking into account gender equity. In addition, assessment for a health insurance security and retirement scheme will be done in 2020.

Altogether, these adjustments and introductions will help to make IPC job opportunities more attractive and will facilitate communication on career opportunities and on the needs of IPC.

KEEPING THE BUDGET BALANCED – IMPROVING INSTITUTIONAL COMMUNICATION

The IPC is a non-profit scientific institution and the operating budget is composed by research projects (55%), the MESRI dotation (10%) and the services (35%, including LBM, LEFS, the vaccine international center and rabies center). A fourth revenue component could be through fundraising and must be developed further. In 2020, an initiative started to give more external visibility to IPC with the constitution of a new communication team. Website, leaflets, and social network use will be revisited and adapted to external public needs and habits.

3.1.2 SCIENTIFIC CHALLENGES

INNOVATION AND EXCELLENCE IN SCIENCE

IPC continues to encourage the ambitious and innovative research projects across the organization and strong external partnerships focusing on health and environment, host-pathogen interactions, drug resistance, entomology, epidemiology and clinical research. The topics are on the major infectious diseases with public health impact: emerging viruses, malaria, encephalitis, dengue, flu, HIV, hepatitis, influenza and other respiratory diseases, rabies, tuberculosis, AMR in people, animals and the environment. The research teams continues to publish their research findings in renowned scientific journals and have them disseminated among the relevant departments of the MOH, MAFF, to WHO and other governmental and nongovernmental national and international partners. The teams take every opportunity to also communicate with the public at large.

REINFORCING ACTIVITIES FOR RABIES CONTROL

IPC continues to strengthen its research on rabies in people and animals using the “one health” approach, clinical research on vaccine schedules, immune responses and serves as rabies referral laboratory for humans and animals. In collaboration with the MOH, WHO and FAO, IPC contributes to the implementation of the national strategic plan for rabies elimination and to advocate on rabies for future access to preventive vaccine in people and animals.

DRUG RESISTANCE AWARENESS WITH ONE HEALTH APPROACH

The diagnosis and management of drug resistance—bacteria, mycobacteria, malaria, HIV, other viral diseases, fungal infections and insecticide resistance for dengue and malaria vectors – should continue to be developed, supported and reinforced. Sharing a common vision across teams and exchanges on know-how, and strong collaboration with hospitals, animal and environmental sectors will be maintained and strengthened. The “one health” approach continues to be promoted with support from CIRAD. In collaboration with the IRD (LMI-DRISA initiative), Doherty Institute in Melbourne (Australia) and *Institut Pasteur* (Paris), IPC strengthens its capacity on AMR for bacteria including bioinformatics approach. IPC also contributes to the WHO GLASS initiative for AMR under the leadership of the MoH. IPC will also assess the best way to focus on more neglected pathogens such as leptospirosis and melioidosis.

ESTIMATING TRANSMISSION AND MODELLING APPROACH (INCLUDING GIS – REMOTE SENSING)

Knowledge of the mechanisms of transmission—vectorial, direct, nosocomial—from reservoirs or transmission foci; symptomatic or asymptomatic, anthropic or wild, is critical for disease risk assessment and disease control. This is a transdisciplinary topic applicable to several pathogens of interest for IPC such as malaria, dengue, Japanese encephalitis, other types of encephalitis, respiratory viruses, rabies, pertussis, leptospirosis, melioidosis, bacterial sepsis, fungal infection. IPC will also assess how to develop stronger partnerships to develop mathematical modelling approach based on such data to better inform the public health decision making process. An important component of infectious disease transmission modelling is the inclusion of geospatial information system (GIS) and remote sensing. A health geographer from IRD is based at IPC since 2018 and will develop a team to ensure that this component will be part of most of the research project as it is a key added value. This new team (GeoHealth) will also make sure some Cambodian staff will be trained on this topic.

DEVELOPING PLATFORMS

IPC encourages the use of the most recent technologies for research and also for near real time diagnosis, data management and data analysis by maintaining the development and equipment of laboratories and knowledge development. For this, IPC promotes sharing knowledge and development of skills and competencies across the units and services. It is an important way of development and innovation for entomology, immunology, bio-banking, bio-informatics, data management, biostatistics, genomics and modelling, as well as through access to shared costly equipment already available or to acquire (sequencing machines, flow cytometers, cell sorter, Maldi-Tof, etc.).

ADDRESSING THE GAPS

Through collaboration and partnership with other research institutions, IPC will continue its effort to develop capacities in the area of mathematical modelling, immunology, chemistry, data management, biostatistics, as well as in genomics, and bioinformatics. These are gaps that have been indentified in 2018 by the SAB and needs special attention from the senior management and from the senior scientists for the years to come.

3.2 MID-TERM OBJECTIVES

3.2.1 INSTITUTIONAL CHALLENGES

STRENGTHENING COLLABORATION AND PARTNERSHIP

The dynamism of the institution is driven by its collaborative mindset and willingness for continuing acquisition of new knowledge. Collaborations are firstly with Cambodian institutions including ministries, educational and research institutions and through the *Institut Pasteur* International Network including *Institut Pasteur* (Paris). IPC also has long lasting collaborations with multiple international partners in the USA, Europe and continues to reinforce the collaborations with institutions based in the Asia Pacific region.

SCIENTIFIC SKILL DEVELOPMENT AND CAPACITY BUILDING

As mentioned above, IPC contributes both to national and international scientific skill development for students and professionals. This contribution is accomplished using different means such as short dedicated courses or lectures (immunology, entomology, biostatistics, clinical trials, GIS, etc.), support to the International of UHS – Paris Saclay Universities, conferences including for the public at large (*Institut Français* du Cambodge).

IPC continues to be involved in the training offered by Cambodia's institutions of higher education (and even in the sub-region), notably the University of Health Sciences, the Royal University of Agriculture, the Royal University of Phnom Penh and the *Institut de Technologie du Cambodge*. IPC hosts Cambodian and international students for short or medium term trainings.

IMPROVING NON-SCIENTIFIC COMMUNICATION

Access to IPC though its website, leaflets, and via social media allow to better communication on the scientific activities and services delivered needs to be reviewed and improved. As mentioned above, a communication team will be organized and communication with traditional media also needs to be reinforced to better explain the significance of the scientific results and the role of IPC in life sciences and health research.

MANAGEMENT AND WELL-BEING AT IPC

As mentioned above, training on managerial skills and competencies at leadership and intermediate management levels in the organization will be performed in 2020. The well-being of the staff is essential to maintain the dynamism of the institution and preserves its capacity to act quickly at the onset of health crises crisis such as the COVID-19 epidemic.

Monthly leadership teams meetings (scientific and non-scientific), weekly meetings at senior management level and within each unit or service are key components for exchanges of information and to improve work systems at IPC.

Also, monthly meeting with personnel representatives to understand the needs at all levels in the organization will be prioritized by the Direction. A strategy will be developed in 2020 regarding the health insurance mechanism and retirement scheme at IPC to be aligned with the governmental vision and decision.

In addition to the managerial component, two bodies: the Hygiene, Safety and Quality Committee and the Biobanking Committee continue their role for the security of the institution, the safety of the staff and the quality of the work delivered.

ACCREDITATION

LBM has obtained the ISO 15189 certification; the last audit in November 2019 helped to continue to improve the quality of services. The objective is to continue the accreditation process with ISO 17025 norms applied by LEFS, metrology, reference laboratories in virology (dengue, rabies, flu and COVID 19). The quality improvement process will take two to three years. The teams will work together to ensure that internal audit capacities are implemented to respect those norms and ways of working.

3.2.2 SCIENTIFIC CHALLENGES

CONTINUE TO DEVELOP THE LEFS

Cambodia, a country on a fast-track economic development path, depends on its tourism and agri-food industries to increase its resources. In both of those sectors, the microbiological and chemical quality of the products (raw or processed agri-food products, swimming pool water, drinking water, meals) require testing and certification. The role of the LEFS is important, and the laboratory will continue its effort towards ISO 17025 accreditation, strengthen its position at national level and develop its partnership with international organizations.

EMERGING INFECTIOUS DISEASES PREPAREDNESS

Human and animal pathogens emerge or re-emerge in the Asian region with a potential of epidemic/pandemic risk for humans. Preparedness at public health level, including laboratory, is a priority need. The Great Mekong Sub-region (GMS) has been affected by or even been the epicenter of several epidemics such as EV-71, avian flu viruses, chikungunya, Zika, arenaviruses and coronaviruses. The recent COVID-19 pandemic due to a novel coronaviruses reminds us again on the priority of having a network of well-equipped and highly-competent laboratories throughout the region to ensure an early response to new pathogens.

Viruses and spillover mechanism from animals to humans have demonstrated the health and socio-economic risk they pose, but it is also important to continue to consider other type of emergences. The drug resistance is growing from bacteria, viruses and parasites. Research on AMR is a priority for IPC (tuberculosis, enterobacteriaceae, bordetella, STI, malaria...). The changing epidemiology expected within the malaria context due to the expected upcoming elimination of *Plasmodium falciparum* within GMS and the role of *Plasmodium vivax* the coming years is of interest and required ongoing attention. Hepatitis B and C have become a major public health issue and clinical research to improve treatment and clinical management are ongoing. Hepatitis E is an under-estimated problem and field sero-surveillance is needed to understand the magnitude of the infection in rodents and humans. Zika, chikungunya, Nipah, new coronaviruses continue to be a lethal threat as they are emerging.

With its expertise, experience and infrastructure (including its BSL-3 laboratory), IPC has a major role to play in risk detection and scoping, monitoring emergence and public health control measures in Cambodia and in the region. The trans-disciplinary research conduct by IPC with its partners is a key component for success to respond to the emergence of infectious diseases.

TESTING NEW DRUGS AND VACCINES

Many new preventative/therapeutic disease control measures are being developed (new strategies for drugs, vaccines, vector control tools). IPC is already conducting several clinical studies (Phase 4) with partners and its know-how is well recognized for HIV, TB, hepatitis, rabies and malaria. IPC should assess its potential role in the clinical research from Phase 2 to Phase 3 (malaria, dengue, tuberculosis, hepatitis, rabies, EV-71, vector control, new coronavirus, etc.). However, it is up to the Ministry of Health and national regulatory authorities to decide if Cambodia should be involved in the early stages of clinical research. A reflection will be initiated in 2020 with the support from INSERM in France and the APPRISE initiative in Australia to identify the opportunities for the country to initiate clinical research for new drugs and vaccines of use in light of emerging pathogens.

Emerging infectious diseases preparedness 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. IPC conducts research in these fields through international and international collaborations including having researchers from CIRAD based at IPC.

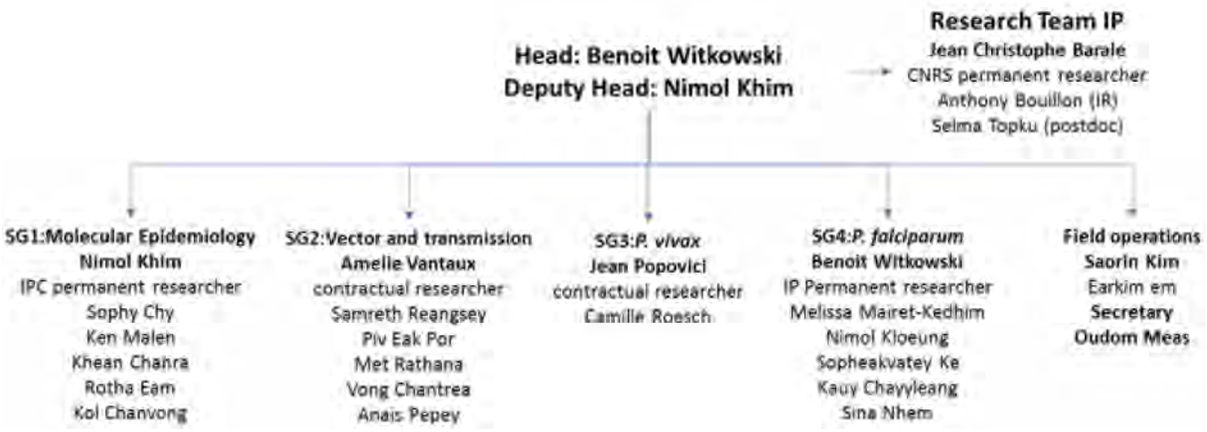
The recent COVID-19 epidemic confirms the importance of the "one health" approach to support the public health response to epidemics and IPC prioritizes the cooperative and multidisciplinary works with medical, veterinary, economic and human sciences sectors.

4. 2019 ACTIVITIES AT INSTITUT PASTEUR DU CAMBODGE

4.1 MALARIA MOLECULAR EPIDEMIOLOGY

4.1.1 FUNCTIONAL STRUCTURE OF THE UNIT

Dr. Benoit Witkowski has directed the Malaria Molecular Epidemiology Unit (MMEU) since September 2017. The unit is organized around four thematic areas: Plasmodium falciparum blood stages, plasmodium vivax blood stages, molecular epidemiology and malaria transmission. The unit is composed of the Head of Unit (B. Witkowski-IP permanent researcher), one Deputy Head (Nimol Khim-IPC permanent researcher), two contractual researchers (Amelie Vantaux & Jean Popovici), four PhD students (Melissa Mairet-Khedim, Camille Roesch, Anais Pepey, Kutub Ashraf) and 17 technical and administrative staff. Since December 2016, the MMEU at IPC is part of an entity called malaria translational research unit (MTRU) based at the Pasteur Institute in Paris under the leadership of Jean Christophe Barale. MTRU is a mixed international research entity between IP and IPC dedicated to malaria.



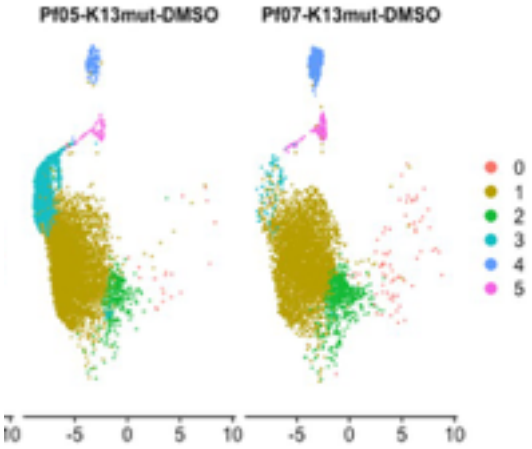
Malaria Molecular Epidemiology Unit collaborator	
national	international
CNM Ministry of Health Partner for development (Cambodia) ITC URC UHS	Institut Pasteur Pasteur International Network WHO MMV University of Georgia MORU UCSF Institut Cochin MSF University of Gottenburg INSERM CNRS TAD SANOFI NIMPE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

4.1.2 RESEARCH PROGRAMS – MAJOR ACHIEVEMENTS IN 2018-2019

a. CHEMOTHERAPY OF MALARIA PARASITES

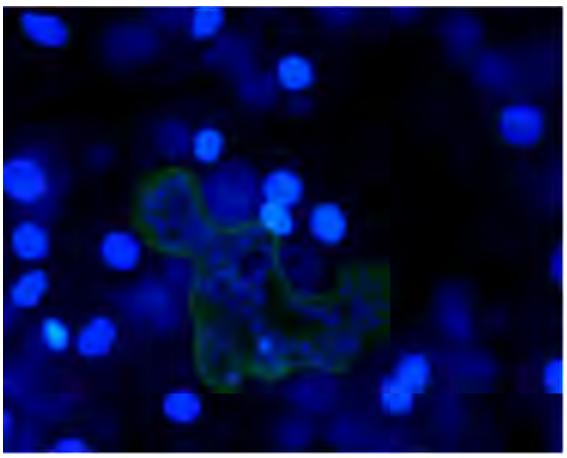
Drug Resistance Epidemiology and Mechanisms

The malaria unit has been collaborating with the National Center for Parasitology, Entomology, and Malaria Control (CNM) and WHO for several years on the question of drug resistance epidemiology and on the therapeutic efficacy of treatments. In 2018-2019 we conducted a large investigation that included the Greater Mekong Subregion (GMS), South Asia, Asia Pacific, the Middle East and African countries. More than 1000 *P. falciparum* samples from various locations were tested for presence of resistance markers. The main focus of our investigation was on gene association with artemisinin combination-based (ACT) resistance. We were also involved in the therapeutic efficacy measurement of ACT drugs. In term of results, we demonstrated the excellent therapeutic efficacy of pyramax in Cambodia and Vietnam. Interestingly we showed that existing resistance patterns were not associated with alteration of therapeutic and in vitro efficacy of pyronaridine. These results were disseminated through 3 publications. In collaboration with WHO and CNM, MMEU is involved in biological investigations of therapeutic efficacy studies. Among results generated we have noted the existence of amodiquine resistance in Cambodia despite the non-deployment of artesunate/amodiaquine (ASAQ) and a putative and limited use of amodiaquine (AQ) in the 80's, the isolates tested have presented a wide range of susceptibility to AQ with upper values corresponding to what is designated as AQ-resistant (AQ-R) in literature. A clear association between AQ susceptibility and clinical outcome was noted through AQSA which enabled us to conclude 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 results have been submitted for publication (Mairet-Khedim et al under review). Finally, we are conducting a longitudinal study involving CNM, WHO and Institut Cochin (France) related to the evolution of chemoresistance in Cambodia in line with the implemented therapeutic strategies. This study started in 2016 and has already explored resistance patterns of more than 1,000 isolates. In addition to basic molecular epidemiology, this study includes phylogenetic investigations and cellular biology. Results are going to be disseminated via a peer-reviewed publication. Our research group within the MTRU framework and in collaboration with others institutions (IP, Walter and Elilzatbeth Hall Institute (WEHI) is involved in the deciphering of the artemisinin resistance mechanism. Our research plan combines whole genome sequencing, single cell analysis and pull down technique. Details of the results cannot be presented yet but we have successfully obtained a clear picture of resistant malaria parasites upon drug exposure. These results will ultimately help in the design of a better resistance diagnostic test but also in the design of new antimalarial drugs. In addition we have investigated mechanisms linked to multi-drug resistance (MDR) in Cambodia. Mainly our research was focused on AQ, PPQ and MQ resistance. We have characterized phenotypic change associated with an unusual profile of multiresistance implicating co-resistance to MQ and PPQ. This study includes proteomic and transcriptomic analysis and will be submitted soon for publication (Mairet Khedim et al.).



Therapeutic Options Against P. Vivax Liver Stages

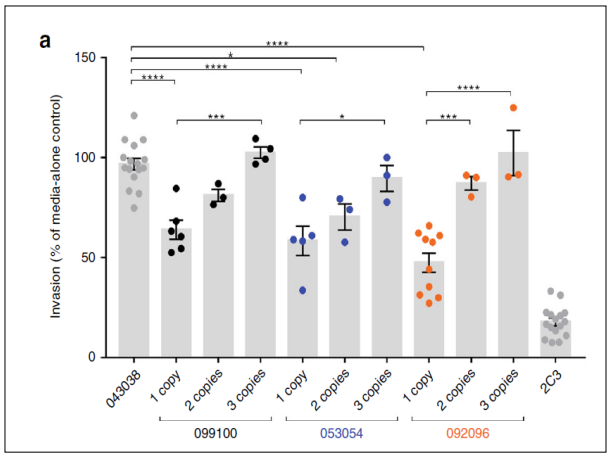
The main characteristic of plasmodium vivax is the development in a patient's liver of specific cellular stages named hypnozoites. These hypnozoites remain silent for a variable duration until they exit their dormancy. Upon awakening the hypnozoites develop to hepatic shizonts that finally lead to blood stage infection, reactivation of symptoms and transmissibility of the disease. Because of this feature, *P. vivax* will be tremendously difficult to eradicate. Very few treatments 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 use worldwide. However, in certain patients presenting glucose-6-phosphate dehydrogenase (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 most widespread. For these patients primaquine usage is unsafe or presents an unfavorable risk/benefit balance. For these reasons the development of a 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 the Medicines for Malaria Venture (MMV) (Brice Campo). Methodologically, we use the blood from *P. vivax* infected patients to feed anopheles mosquitos, following which of the mosquitos develop sporozoites that are used, after vector dissection, for infecting human hepatocytes maintained in vitro. At that time, a variety of drugs can be assessed for their anti-hypnozoite effect. Feasibility of this project was successfully demonstrated in 2017. In 2018 MMEU developed a platform for assessing high throughput drugs screening against *P. vivax* hypnozoites. To date a panel greater than 1500 molecules has been tested. The first results were accepted for publication in the journal ''Nature Communication''.

b. Functional immunity

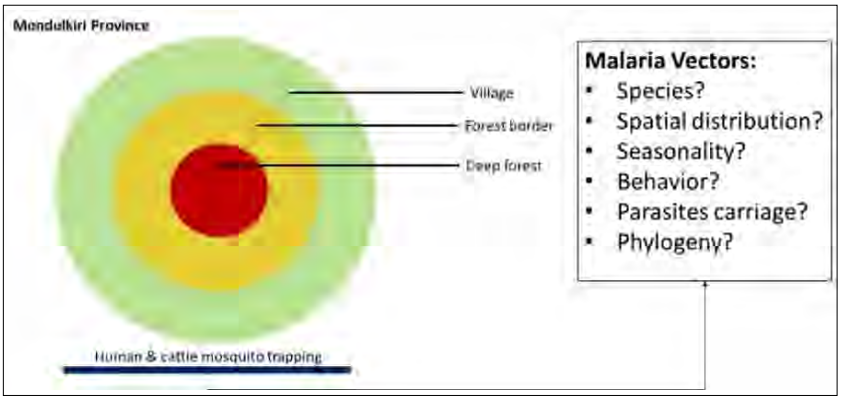
Plasmodium vivax is responsible for chronic infections that drastically limit the disease control operational range, at least with the existing tools. One innovative strategy would be to develop vaccine against *P. vivax*. Among possible targets, development of antibodies directed against blood stage 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 receptors involved in red blood cell invasion. In a collaboration between IPC and CWRU (Pr Chris King) we have evaluated parasite response to monoclonal antibody targeting Pvdbp, a major ligant for parasites invasion.



These investigations have helped to define the more promising HumAbs that could be at the origin of a novel vaccinal approach. These data have been published in Nature Microbiology (Urusova et al. 2019) and Journal of Immunology (Carrias et al 2019). In parallel, we have investigated whether genetic variation in the parasite may alter this response. We have demonstrated that pvdbp amplification alters significantly inhibitory action on the HumAbs on parasites invasion. We also showed a clinical significance between presence of a neutralizing antibody and infection with amplified parasites. Ultimately these results show that vaccinal approach could be jeopardized by parasites' evolution to reach immunoresistance. These data have been published in the journal Nature Communication (Popovici & Roesch et al 2020).

Malaria and Malaria Vectors Epidemiology

MMEU is part of the Asia-Pacific International Centre of Excellence in Malaria Research (Asia-Pacific ICEMR PI I Mueller) which aims at addressing the key challenges to malaria elimination in the Asia-Pacific. A coordinated set of in-depth studies into the epidemiology, entomology and biology of residual malaria transmission in three sites spanning the entire Asia-Pacific transmission gradient from moderately and high transmission in Papua New Guinea to low, highly focal transmission in Cambodia are carried out. In this context a cross sectional study was set up in Mondulkiri province. A census study of 10,393 individuals and 2,351 households was carried out from which a random sample of 4,200 individuals in 1,147 households were screened for malaria infection. This cross-sectional study will enable, beyond epidemiological considerations, to investigate the foci of transmission, the immunology of individuals in Cambodia, to define the parasite population structure and ultimately to rationalize further operational research initiatives. The results have provided a clear snapshot of malaria in Keoseima District (overall prevalence=8.3%). Notably a gradient of prevalence was noticed depending on the proximity of villages with forests. Ultimately, we have determined that prevalence of malaria in Cambodia is localized but not low; the highest rate showed a prevalence of about 40% of parasite carriage, mainly represented by *P. vivax*. In 2019, a cohort study was launched on



951 individuals being followed-up monthly for a year. This study will contribute to the understanding of how the asymptomatic/symptomatic status of the individual evolves. It will inform us regarding what the immunological shift and what the proportion of asymptomatics are in transmission. Ultimately, this study will accurately show the infection dynamic in the population for *P. vivax* and P. *falciparum* and will help to set up better control strategies. The vector is the main denominator of malaria transmission. No malaria elimination initiatives have or will be successful without a strong focus on the vector itself. The datasets 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 with the ICEMR-Asia Pacific Program under thedirection of I. Muller. Aims of this program are to bring a better understanding of vectors in Cambodia. This project addresses several medical entomology questions which can be grouped under vector epidemiology. Among questions being investigated are the spatial distribution of the anopheles, their behavior, their phylogeny, tropism and ultimately their potency to carry human parasites. This project is being conducted in Mondulkiri Province (which has a high malaria prevalence) and the expected outcomes will be the enabling of the design of further vector control initiatives. The first results suggest that anopheline - initially described as the main vector (*A. dirus*)- is not solely responsible for malaria transmission. We found that the malaria parasite prevalence was 3.7% in the vectors, and that 14 out of the 27 anopheles species collected were carrying *Plasmodium sp*. parasites. Additionally, vector behavior investigations have revealed that day biting represents a notable proportion of human-vector contact during daytime (20.23% of anopheles mosquitos collected between 6am and 6pm). Overall, we have identified a gradient in parasite carriage with an epicenter in deep forest areas. These data will be submitted publication early 2020 (*Vantaux et al.*). A second part of the study focused on GPS-tracking of local human populations (360 participants) to better understand human movements and select 37 mosquito collection sites based on human density and land cover types. Quarterly mosquito collection started in October 2019 and will continue for a year.

Intervention Program for Malaria Control

The MMEU is committed to action aiming at the control and elimination of malaria in Cambodia. It is now clear that the forest represents the main area of malaria infection risk and as such, the forest- goers population is the most at risk to contract malaria. As the forest is the key transmission area it is also where resistance to treatment is high and needs urgent attention. Elimination of malaria in areas near the forest there is critical. In collaboration with the IPC public health unit (EPH), our structure is involved in two malaria elimination programs, one funded by the 5% initiative, the second by the Global Fund to Fight AIDS, Tuberculosis, and Malaria (GF). The principal investigators for these interventions are Dr. Patrice Piola, while Dr. Benoit Witkowski is co-principal investigator on the latter. These projects are conducted in Mondulkiri, Stung Treng & Kratie Provinces. The primary objective of the activity in Mondulkiri Province is to better understand epidemiological aspects in and around the forest, while the action in the two other provinces is interventional. The Malaria Unit's specific role is the laboratory element of these initiatives including molecular diagnosis, analyzing and assessing drug resistance, and entomology. A total 1,788 mosquitos were collected in Stung Treng among which 234 were found to be *Anopheles sp.* females. Despite a low number of anopheles samples a high species diversity was observed: 13 different species . *Anopheles dirus* made up 24% of the anopheles mosquitos collected and was sampled in all collection sites except in sectors 6 and 13 of Stung Treng. No *Anopheles minimus* mosquitos were collected, and only two *Anopheles maculatus* and 10 *Anopheles barbirostris* mosquito were found in the samples collected. A total

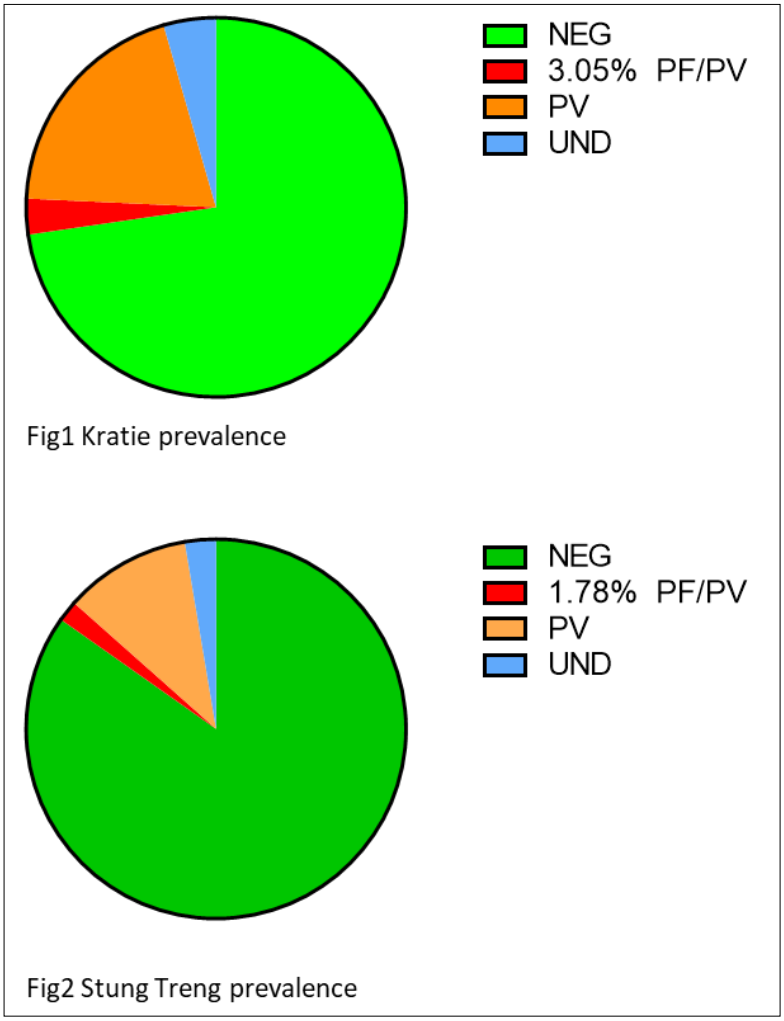


Fig1 Kratie prevalence

Fig2 Stung Treng prevalence

of 908 mosquitos were collected in Kratie Province, among which 104 were *Anopheles sp.* females. A total of eight different species were collected. *Anopheles dirus* made up 74% of the anopheles mosquitos collected and was found in all collection sites in Kratie except sector 1. No *Anopheles minimus* mosquitos were collected, and only 1 *Anopheles maculatus* and 8 *Anopheles barbirostris* mosquitos were collected.

A key preliminary outcome of this project was the finding that individuals with forest-related occupations have a much higher malaria prevalence than do the non-forest related occupational groups. Simultaneously, we have found a major presence of the malaria vector in these areas. Finally we have also demonstrated that malaria in forest-goers is mainly asymptomatic and therefore undetectable with conventional diagnostic tools. This information is critical as it will inform the elimination intervention plan for 2020 and possibly beyond.

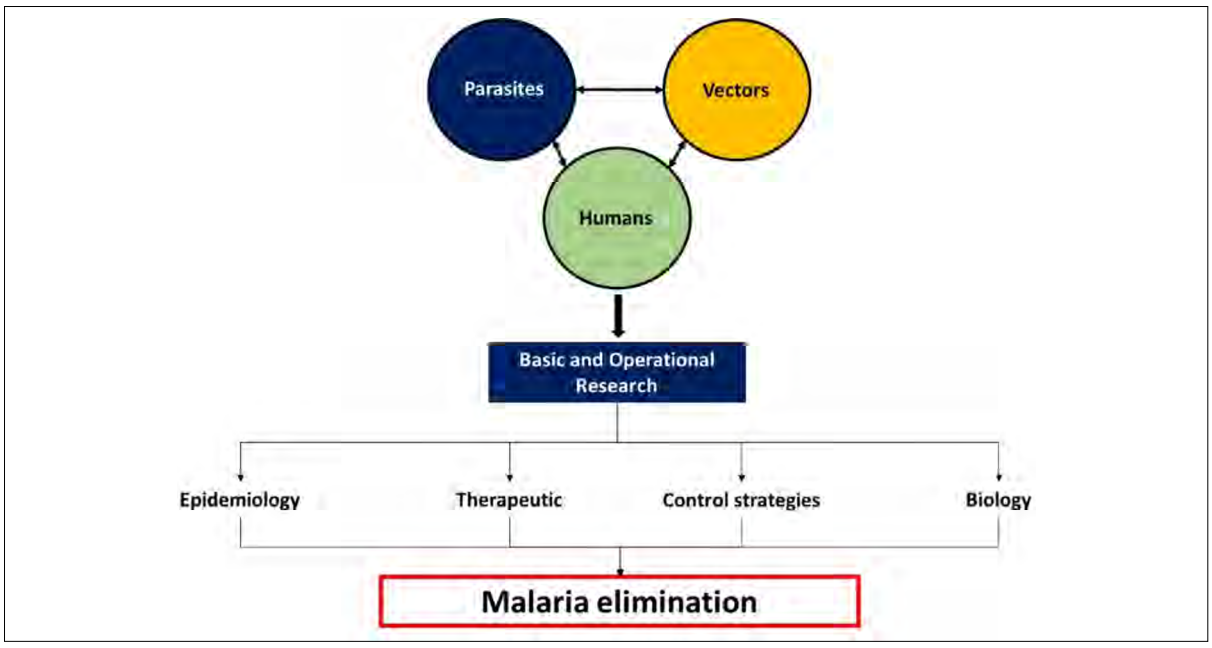
In parallel, we have jointly developed research questions with CNM and the United States Agency for International Development (USAID)/President's Malaria Initiative (PMI) Cambodia Malaria Elimination Project (CMEP) implemented by the University Research Company (URC) to address malaria diagnostics in Cambodia. Malaria diagnostics are essential to elimination efforts. Notably, malaria infection is often asymptomatic with low parasite density. These infections are poorly monitored due to conventional methods' lower sensitivity;such cases may be substantial contributors to malaria transmission. In the context of ongoing malaria pre-elimination activities in Battambang, Pursat and Pailin Districts in Western Cambodia, we have evaluated novel highly sensitive rapid diagnostic tests (HS-RDT) in the detection of asymptomatic malaria. Our results have shown that HS-RDT is not appropriately adapted to the situation in Cambodia, showing 0% sensitivity in our study. This surprising result is nonetheless helpful helpful as it will inform national strategy on managing asymptomatic malaria in Cambodia

4.1.3 RESEARCH PROGRAMS –2020 PLANS

Research Plan Overview

The RGC goal is the elimination of all genuses of malaria in Cambodia by 2025. Malaria imposes a serious burden on Cambodia's public health, and its eradication in Cambodia has global importance as Cambodia is at the forefront of drug resistance and research. Positive outcomes in Cambodia are critical to preventing further emergence and spread of antimalarial resistance worldwide. That said, drug resistance is only one side of the malaria challenge in Cambodia. Several additional questions need to be addressed to reach a more thorough and evidence-based understanding of this disease. Thus, the MMEU research plan uses a holistic approach toward malaria and structured around four main research questions that aims to:

a. Determine the dynamics of malaria in Cambodia;
b. Explore the therapeutic options and drug resistance patterns (multi-centric);
c. Strategize the malaria control approach; and
d. Understand the parasite biology.



a. Malaria Epidemiology in Cambodia

ICEMR Program……..……..……Funding Source NIH

The objective for 2020 is to provide extra information on malaria prevalence in our cohort of inhabitants in Mondulkiri Province. Malaria prevalence measurement will be done on a quarterly basis in 2020. In parallel, we will investigate, in collaboration with IP and WEHI, different biological metrics that may enable better understanding of epidemiology. A serological analysis will be performed to define malaria exposure rates in the population. We will determine whether asymptomatic carriers represent a reservoir for resistant parasites. All these lab-based data will be analyzed against epidemiological information. We will also investigate the parasite population structure using the amplicon sequencing approach. All this information is expect to bring new insights into malaria epidemiology and will enable a better tailoring of specific control measures.

Vector-Human Geospatial Study……..……..……Funding Source NIH

In order to characterize transmission dynamics in Kaev Seima District, we will use amplicon sequencing to determine the population genetics of the parasites. A PhD student was trained on the method in Melbourne, Australia and will transfer the technique to IPC. Based on the parasite samples collected in both the human and the vector populations we will analyze parasite populations to determine transmission routes, the population's connections etc. In addition to molecular detection and genotyping of *Plasmodium sp*. 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. A third aspect we will investigate in this project are the immune characteristics of the human population. A transfer of competencies was done in October 2019 to setup luminex assays to investigate antibodies responses in the cross-sectional samples. Further investigations on the cohort samples will be carried out. 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 research will also help develop or validate methods that could significantly improve the efficiency of malaria control interventions, 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 Region.

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b. Therapeutic and Drug Resistance

Drug Resistance Epidemiology….....FundingSource: WHO

Cambodia is known to be a hotspot for antimalarial drug resistance development. Previous experience has demonstrated that resistance may spread in large part owing to population movement. Thus, efficient tracking of antimalarial drug resistance must 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 to detect resistance spread early. In this context and in collaboration with WHO, MMEU will be in charge of molecular investigation regarding drug resistance in the GMS region. The objective is to characterize the presence or absence of known resistance markers and to determine the rate of treatment failures in selected areas. Additionally, this initiative will be a basis for deciphering putative new resistance markers and for providing new insights into drug resistance mechanisms & epidemiology.

New Therapeutic Options……..……Funding: MMV/UGA, SRC, PTR, MMV, NIH

One of the main targets in drug research will be the liver stage of P. vivax. A collaboration setup in 2017 between MMV/UGA/IPC will be continued and strengthened for the 2020 period. IPC will build capacity and implement high-throughput screening capabilities. The objective will be to identify compounds that could actively target *P. vivax* hypnozoites. The second main target is learning more about the multiresistant strains of *P. falciparum*. The malaria unit has developed expertise in malaria drug resistance determination. It has separated several hundreds of clinical isolates from Cambodia characterized for their chemo-susceptibility and their genotype of resistance. One of the major objectives for next year is the development of new antimalarials able to tackle resistant strains. In this context MMEU has developed a collaboration to investigate the in vitro efficacy of promising preclinical lead compounds against resistant *P. falciparum* strains from Cambodia.

Therapeutic Efficacy of Primaquine…….Funding (undetermined)

To date amino-8-quinolines are the only drug that provide effective antiparasitic response against hypnozoites. Among those, primaquine is the only drug registered in Cambodia for this purpose. So far, there has not been any no rigorous assessment or data on the therapeutic efficacy on the globally recommended regimen in Cambodia. Therefore, we aim to launch a randomized primaquine efficacy trial to determine whether the recommended regimen could support *P. vivax* control in Cambodia. This trial should be launched late 2020 and will include 300 patients. Parallel to this clinical trial we are also aiming to better decipher parasites' relapse chronology and mechanisms.

c. Strategize Malaria Control

Intervention…….Funding: Global Fund 5% initiative

In the first phase of intervention projects that the EPH Unit and the MMEU are jointly implementing, we have demonstrated that a screen and treat strategy is not relevant in Cambodia for malaria elimination. Consequently, these initiatives will now change to a new approach based on intermittent preventive treatment in subjects at highest risk of malaria (forest populations). This approach is likely to be setup in 2020 and will include multiple efficacy metrics (entomological carriage of parasite, prevalence and evolution of drug resistance).

d. Understanding Parasite Biology

G6PD Diagnostics……Funding: PMI (under discussion)

South East Asia is a main focus of human G6PD deletion. This genetic trait increases the likelihood of severe adverse events under certain drug administrations, especially primaquine. To date, no satisfactory test exist for use in Cambodia. We will therefore evaluate a new G6PD field test (quantitative) and determine whether it can safely identify G6PD deficient individuals. Funding for this project is under discussion. This project is being developed and implemented in association with Impact Malaria, CNM, the United States Centers for Disease Control and Prevention (CDC), and Population Services International (PSI) under PMI/USAID funding, and will offer important insight to optimize case management of *P. vivax* in Cambodia.

4.1.4 SUPPORT TO NATIONAL AUTHORITIES

MMEU works under the umbrella of the Cambodian Ministry of Health, as do all IPC research units. Specifically, MMEU is a main collaborator and a main technical support of the Cambodia National Malaria Control Program at CNM. In particular, MMEU provides its support to drug efficacy studies that are conducted yearly in Cambodia. Additionally, MMEU is involved in quality assurance and quality control (QA/QC) for malaria screening among Cambodian UN peacekeepers sent on international missions abroad.

4.1.5 TEACHING AND TRAINING

Students

PhD students:

Melissa Mairet-Khedim: P. falciparum resistance (since January 2017)

Camille Roesch: biology of P. vivax invasion (since January 2017)

Kutub Ashraf: P. vivax liver stage (since April 2018)

Anais Pepey: Ecology of malaria transmission (since November 2018)

Interns

Beatrice Tappy (February-July 2019) Synergy of antimalarial drugs

Daniel Calixto (March-July 2019) Novel antimalarial drugs

IPIN researcher Mobility: Sandrine Nsango (March-May 2019) DHA-PPQ resistance in Cameroon

4.1.6 TEACHING

The Malaria Molecular Epidemiology Unit staff are involved in the international master's degree in infectious disease at the University of Health Science Phnom Penh and the University of Paris Saclay. Benoit Witkowski teaches a 2.5h course on malaria parasites evolution; Amélie Vantaux teaches a 2.5h course on host manipulation by parasites, while Nimol Khim teaches a 4h bioinformatics course.

4.1.7 PUBLICATION LIST 2019

2019

1. **In vitro activity of ferroquine against artemisinin-based combination therapy (ACT)-resistant Plasmodium falciparum isolates from Cambodia**. Mairet-Khedim M, Nardella F, Khim N, Kim S, Kloeung N, Ke S, Kauy C, Eam R, Khean C, Pellet A, Leboulleux D, Leang R, Ringwald P, Barale JC, Leroy D, Menard D, Witkowski B. J Antimicrob Chemother. 2019 Nov 1;74(11):3240-3244. doi: 10.1093/jac/dkz340.PMID:31518407
2. **Efficacy and Safety of Pyronaridine-Artesunate plus Single-Dose Primaquine for the Treatment of Malaria in Western Cambodia.** Leang R, Khim N, Chea H, Huy R, Mairet-Khedim M, Mey Bouth D, Dorina Bustos M, Ringwald P, Witkowski B. Antimicrob Agents Chemother. 2019 Sep 23;63(10). pii: e01273-19. doi: 10.1128/AAC.01273-19. Print 2019 Oct. PMID: 31358594
3. **High therapeutic efficacy of artemether-lumefantrine and dihydroartemisinin-piperaquine for the treatment of uncomplicated falciparum malaria in Somalia**. Warsame M, Hassan AM, Hassan AH, Jibril AM, Khim N, Arale AM, Gomey AH, Nur ZS, Osman SM, Mohamed MS, Abdulrahman A, Yusuf FE, Amran JGH, Witkowski B, Ringwald P.Malar J. 2019 Jul 11;18(1):231. doi: 10.1186/s12936-019-2864-1.PMID: 31296223
4. **Pyronaridine-artesunate efficacy and safety in uncomplicated Plasmodium falciparum malaria in areas of artemisinin-resistant falciparum in Viet Nam (2017-2018).** Bui Quang P, Huynh Hong Q, Tran Thanh D, Le Thanh D, Nguyen Quang T, Truong Van H, Khim N, Witkowski B, Cong DT, Bustos MD, Ringwald P, Ta Thi T. Clin Infect Dis. 2019 Jun 28. pii: ciz580. doi: 10.1093/cid/ciz580. [Epub ahead of print] PMID: 31251812
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4.2 EPIDEMIOLOGY & PUBLIC HEALTH

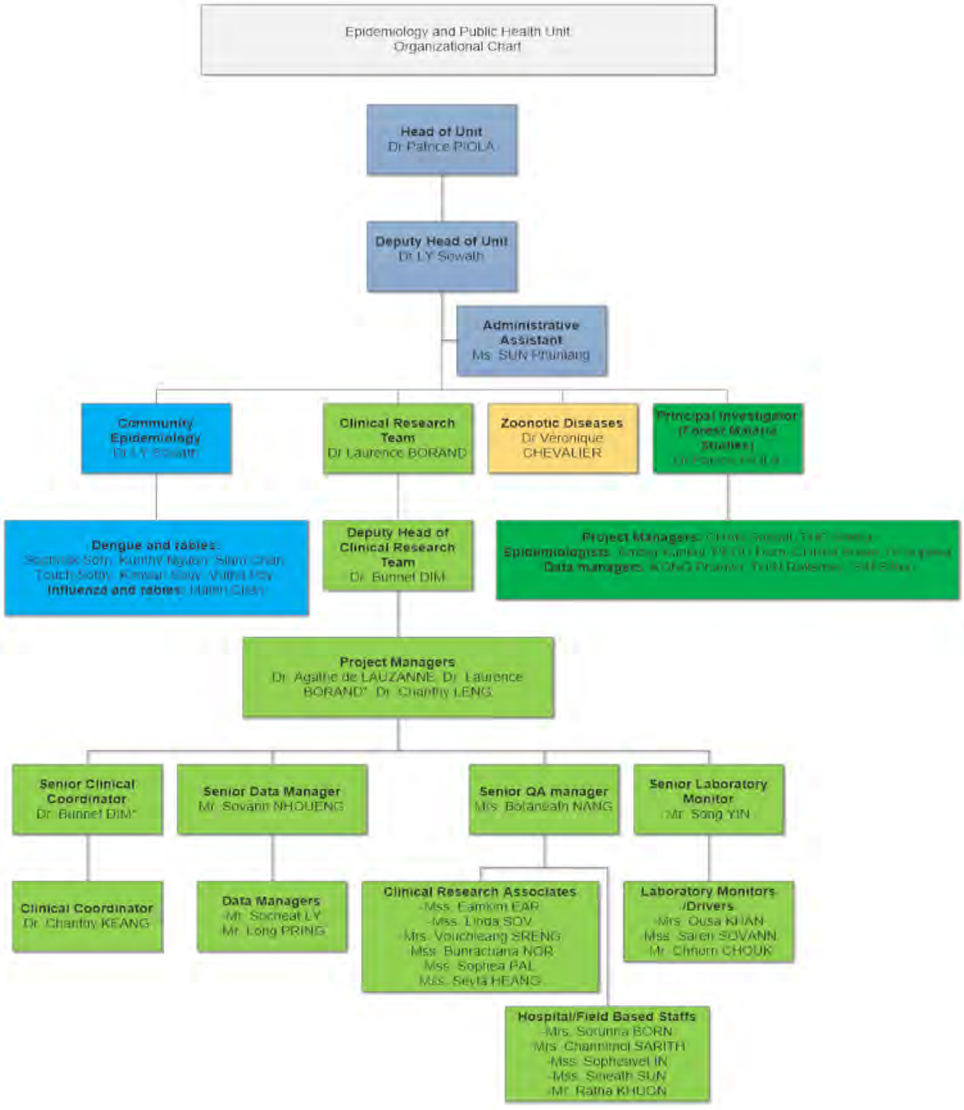
4.2.1 FUNCTIONAL STRUCTURE OF THE UNIT

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.

Three rabies prevention centers (one in Phnom Penh and two in provinces) and the International Vaccination Center of the *Institut Pasteur du Cambodge* are also part of the EPH and under the direct responsibility of the deputy head of the EPH, Dr. Sowath LY. However, in May 2019, these services became part of the newly created vaccination unit under the leadership of Dr. Yiksing PENG.

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, and avian flu and outbreak investigations.
- The Clinical Research Group (CRG), led by Dr. Laurence Borand, has a long history of guideline-influencing 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 CIRAD and IPC. Its main focus is on zoonotic diseases with a strong modeling component. Diseases addressed by 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 EPH rely on close collaborations with the IPC laboratory units as well as the MOH and its component units including National Center for HIV/AIDS, Dermatology and Sexually Transmitted Diseases (NCHADS), the National Center for Tuberculosis and Leprosy Control (CENAT), the National Immunization Program (NIP), the National Veterinary Research Institute (NaVRI), the Council for the Development of Cambodia (CDC), and CNM to a name a few. The unit's projects would not be possible without the interest and contribution of several reference hospitals in Phnom Penh and across the country, including the Calmette Hospital, the National Maternal Child Health Center (NMCH), Kompong Cham and Takeo Provincial Hospitals, Sihanouk Hospital Center of Hope (SHCH), Jayavarman VII Hospital, and The National Pediatric 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*, Dengue Vaccine Initiative , the International Vaccine Initiative (IVI), the European Union, *Fondation Total*, *Institut National de la Santé et de la Recherche Médicale* (INSERM), *L'Agence inter-établissements de recherche pour le développement International* (AIRD), the International Division of Pasteur Institutes, *Institut Pasteur* in Paris, CIRAD, Pasteur Foundation, MSD Avenir, Gillings Public Health Fellowship, the World Health Organization, UNITAID, Global Fund Initiative 5% (Expertise France), and the *Agence Française de Développement* (AFD).

Two important grant proposals on clinical research were accepted by the ANRS in 2019: the DATURA- ANRS 12424 clinical trial "Determination of Adequate Tuberculosis Regimen in Adults and Adolescents Hospitalized With HIV Associated Severe Immune Suppression (CD4 ≤ 100 cells/µL) and the "Pilot Therapeutic Study of Children and Adolescents with Active HCV Infection Born from HIV/HCV Coinfected Women in Cambodia: an Additional Step to Reach Elimination of Hepatitis C within the HIV National Program.

4.2.2 RESEARCH PROGRAMS IN 2019

4.2.2.1 VIRAL DISEASES

a. Rabies

Follow up of patients receiving the WHO 2018 recommended rabies PEP using intradermal vaccination protocol

Based on the previous study, WHO endorsed 2018 guidelines to provide three post-exposure prophylaxis (PEP) sessions of two-site intradermal 0.1 mL vaccine doses each at days 0, 3 and 7 is the first one-week PEP regimen to be recommended. To evaluate the protective antibody response survival time, we aim to follow a cohort of around 170 patients at day 14, at 6 months and at 1 year after the first session of the vaccine schedule using the vaccination protocol described above. Both the EPH and immunology units, IPC, are involved in this work (2019-2020).

Team leaders: Sowath LY, Laurence Borand

Funding: *Institut Pasteur du Cambodge*

Rabies PEP immune response study (RESIST 2)

Data is available showing that the immune response at Day 14 after a one-week protocol of three intradermal injections at days 0, 3 and 7 is comparable to a 5-dose regimen. The number of doses administered in the one-week, three session protocol, however remains high at 12 intradermal doses. Clinical, epidemiological and biological data are being gathered to estimate whether the existing intramuscular and intradermal protocol can be shortened (three-session, one-week regimen; without Day 28 session) and the number of doses reduced (6 instead of 8) in PrEP and in PEP protocols, at no risk to patients. Current status: Publication in the Lancet Infectious Diseases Journal. Main partners in Cambodia: MoH

Team leaders: Sowath LY, Laurence Borand, Arnaud Tarantola

Funding: International Division

Rabies - One Health

The purpose of this project is to create a “One Health” network for rabies research and control in dogs and human populations in Cambodia, Lao PDR and Vietnam. This three-year project is organized into three research packages and includes several activities: 1) research and working visits between partners, 2) joint seminars and workshops aimed at expert opinions, knowledge exchange and mutual learning, and 3) field surveys (dog demography, vaccination and vaccine coverage follow-up in Kandal Province). A workshop on FAVN diagnostics was organized by IPC in 2018 in collaboration with the Nancy Laboratory for Rabies and Wildlife (ANSES). Demographic parameters, bite incidence risk factors, human/dog relationships, bite management behavior are available: a publication is on track. Serological analyses of post-vaccination sera have been achieved. A second paper is under preparation.

Team leaders: Véronique Chevalier, Sowath Ly

Funding: Swedish Research Council

PTR field tests for rabies diagnostics

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

Team leader: V.Chevalier

Funding: *Institut Pasteur Paris*

Control of rabies in Battambang Province, Cambodia

The global objective of this project is to reduce rabies induced mortality in Battambang Province, through 5 main activities: (1) information, education and communication; (2) improvement of case monitoring and management (3) estimation of demographic parameters of the dog population (4) evaluation of the vaccination efficacy in dogs, both at the individual and population level (5) recommendation on vaccination strategies based on demographic parameters, owner acceptability and feasibility. Communication tools, such as leaflets or web communication, have been agreed upon. A second PEP center was opened by IPC in September 2018. Three thousand dogs are being monitored and vaccinated, with a sub-batch of 800 dogs being sampled every 6 months. Demographic parameters, bite incidence risk factors, human/dog relationships, bite management behavior are available: a publication is on track. Serological analyses of post-vaccination sera have been achieved. A second paper is under preparation.

Team leaders: Véronique Chevalier, Sowath Ly

Funding: *Région Occitanie (France)*

b. Dengue and Zika

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

A clustered randomized controlled trial (2017-2019) covering two dengue seasons was initiated 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 neighboring villages where students from the school reside. Interventions consisted of 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) educational method. In March 2018, a total of 24 clusters were followed-up, of which twelve were randomly allocated the school-based intervention and twelve were controls. In all 24 study clusters, active community-based surveillance of dengue-like illnesses among children aged 5-15 years old and saliva serological follow-up among school children of same age were performed as effectiveness outcomes. Results consolidation for preparation of a manuscript are in process.

Team Leaders: Sowath LY, Patrice Piola

Funding: Funded by AFD

Surveillance of Zika-like syndromes and microcephaly

The Zika pandemic that started in 2015 in the Americas caused thousands of microcephalies in newborns from infected pregnant women. From mid-2018 to mid-2019, an active surveillance of Zika-like syndromes in pregnant women attending the antenatal clinic of Calmette Hospital was initiated. All pregnant women with a WHO case definition of Zika-like syndrome were tested by PCR by the IPC virology unit for confirmation. Mothers of newborns with microcephalies in Calmette Hospital were serologically tested from Zika. This light sentinel surveillance were done to detect emerging ZIKV circulation in Cambodia, with a focus on the most vulnerable group. Few pregnant mothers presented Zika Like syndromes, and none had a Zika infection confirmed by PCR.

Team Leader: Patrice Piola

Funding: French Ministry of European and Foreign Affairs

c. HIV AND/OR TUBERCULOSIS INFECTION

STATIS ANRS 12290

Despite the initiation of, highly active antiretroviral therapy (HAART) many patients die of tuberculosis within the first month of treatment. The "STATIS (Systematic vs. Test- tuberculosis guided Anti TB Treatment Impact in Severely Immuno-suppressed HIV-infected Adults Initiating Antiretroviral Therapy With CD4 Cell Counts <100/mm3)' is a multicentric randomized controlled trial aiming to compare two experimental strategies to reduce the mortality and occurrence of severe bacterial infections (incl. tuberculosis) at six months in severely immunodeficient adults infected with HIV (CD4 < 100/mm3): 1) a strategy for intensive screening and repeated tuberculosis testing through workable tests during the day (Xpert *Mycobacterium tuberculosis* (MTB) / RIF, LAM urinary, chest radiography); and, 2) a strategy of systematic empirical anti-tuberculosis treatment initiated two weeks before the start of HAART. Recruitment and follow-up of patients is completed 199 patients included in Cambodia within a total 1050 included. Main partners in Cambodia are NCHADS, CENAT, and SHCH.

Team leader: Laurence Borand

Funding: ANRS

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

The role of miRNAs in HIV disease and tuberculosis is yet to be completely defined. The objectives of this study are to identify the miRNA expression profile as potential novel predictive and prognostic biomarker for IRIS, and to identify the miRNA expression profile in TB patients and HIV/TB co-infected patients. Patient recruitment and follow-up ongoing are completed, analysis is ongoing. The main partner in Cambodia is the Sihanouk Hospital Center of Hope.

Team leader: Laurence Borand

Funding: ANRS

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

In a pilot study previously done with Cambodian patients from the CAMELIA clinical trial, we found that IL-1Ra plasma concentrations dropped dramatically after two months of TB treatment. The objective of this current proof-of-concept study is to demonstrate that IL-1Ra concentrations significantly decrease within two weeks following TB treatment initiation in adults with documented TB. This study is **currently under preparation, and the main partner in Cambodia is the Sihanouk Hospital Center of Hope.**

Team leaders: Laurence Borand

Funding: ANRS

TB-Speed research project

The majority of children with TB are not diagnosed or reported and do not benefit from appropriate treatment. TB-Speed is a multicentre (seven countries) research project, aiming at improving the diagnosis of childhood tuberculosis through decentralization of TB diagnosis and systematic tuberculosis diagnostic in vulnerable children. Registration and follow-up of patients in the study are ongoing. The TB-Speed decentralization component is also ongoing. This research project is currently under preparation, CENAT, the National Pediatric Hospital, the Kampong Cham and Takeo Hospitals, and Batheay and Ang rokar Districts' health facilities are the main partners in Cambodia.

Team Leader: Laurence Borand.

Funding: UNITAID/5% Initiative.

OPTICAM: Optimizing Latent TB treatment among people living with HIV in Cambodia

The aim of the project is to improve Latent Tuberculosis Infection (LTBI) treatment uptake in people living with HIV in Cambodia by addressing the barriers and assessing the impact of the short-course, 3 drug 3HP use in the LTBI treatment uptake and completion as part of a comprehensive intervention. Phase 1 study preparation is completed and ongoing activities are currently in process. Main partners in Cambodia are NCHADS, CENAT, and the Clinton Health Access Initiative (CHAI).

Team Leader: Laurence Borand

Funding: Global Fund 5% Initiative

d. HEPATITIS

Ta Prohm - ANRS 12345: Tenofovir as Prevention of Hepatitis B Transmission for Mothers

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 is completed and follow-up is ongoing. Main partners in Cambodia: Calmette Hospital, NMCH, Jayavarman VII Hospital, National Pediatric Hospital, Kampong Cham and Takeo Provincial Hospitals and seven health centers from Kampong Cham and Takeo Provinces.

Team Leaders: Laurence Borand, Patrice Piola

Funding: ANRS

Pilot therapeutic study of DAA treatment for children and adolescents with active HCV infection born from HIV/HCV coinfected women in Cambodia – ANRS 12420

Transmission from mother to child is the main route of acquisition of HCV mono-infection and of HCV/HIV co-infection in children. Approximately 25% of HCV-infected children spontaneously clear the virus but clearance rate seems to decrease for HIV/HCV co-infected children. Advanced liver diseases with cirrhosis occur for less than 5% of children but the proportion of patients with bridging fibrosis/cirrhosis, evaluated by liver biopsies, was reported to increase from 11% to 20% in a median time of 5.8 years. For HIV/HCV co-infected children, data are scarce. The objective is to evaluate the effectiveness of daclatasvir/sofosbuvir combination for children aged more than 6 years old and adolescents with active HCV infection. Secondary objectives notably include the evaluation of plasma exposures of sofosbuvir, GS-331007 (predominant circulating metabolite of sofosbuvir), and daclatasvir Current status: the study is under preparation. Main partners in Cambodia: NCHADS and OI/ART sites.

Team Leader: Laurence Borand

Funding: ANRS

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 resistance in pediatrics and developing countries. Antimicrobial resistance (AMR) is one of the biggest threats worldwide. BIRDY is an international (Madagascar, Senegal, Cambodia) multicentric and prospective cohort study aiming to estimate bacterial infections and AMR incidence among neonates and young children from rural and urban community settings, to describe and characterize pathogenic and colonizing bacteria, and assess the burden of AMR. In Cambodia, among 815 mothers, group-B streptococcus vaginal carriage was low: <1%, digestive carriage of *Klebsiella pneumoniae* was high: 68% and digestive carriage of ESBL-producing enterobacteria was extremely high: 75%. Neonatal mortality was low compared to national data, 6.2 vs 14.0/1000 live-births respectively. Incidence of neonatal sepsis was ~5.2/1000 live-births with 3/4 (75%) isolates resistant to antibiotic recommended by WHO. A national results dissemination meeting was held in April 2019. There are ongoing validation activities. Partners are the Cambodian CDC, two health centers, and 3 hospitals.

Team leaders: A. de Lauzanne, Laurence Borand

Funding: *Institut Pasteur* 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 determine early prevalence of ESBL-E fecal carriage in newborns, to follow acquisition during the first year of life, and identify risk factors and investigate ESBL genes in Cambodia. One hundred forty-seven newborns from two urban and rural community settings were enrolled and followed for one year. Preliminary results: At day three of life, the prevalence of ESBL-E fecal carriage among newborns was 53% [95%IC: 45-61], remaining stable up to 12 months of life: 52% [95%IC: 44-60]. Most frequently detected ESBL-genes were $bla_{CTXM-15}$ bla_{CTXM} -55 and $bla_{CTXM-27}$. Being in an 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. A presentation of study results was made at the national result dissemination meeting of BIRDY. Ongoing sharing activities continue. The main partner for this activity is the French National Research Committee for carbapenem resistance.

Team Leader: A. de Lauzanne

Funding: BIRDY project, Lund University-Sweden.

Pertussis immunization programs in low income countries (PERILIC)

Infection by Bordetella pertussis or Bordetella Para pertussis 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. That said, 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 3-15 years old (WP2). Regarding WP1, participant recruitment and follow-up are completed, while for WP, participant recruitment is completed. Main partners in Cambodia are NIP, NIPH, Several provincial hospitals, private clinics and health centers.

Team leader: Laurence Borand

Funding: *Fondation Total*.

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

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

Team leader: Patrice Piola

Funding: AFD

4.2.2.3 PARASITOLOGICAL DISEASES

Understanding of malaria epidemiology and malaria elimination inside forests

Malaria elimination is a priority in Cambodia, where P.*falciparum* strains are resistant to artemisinin derivatives and to nearly all partner drugs. However, the main reservoirs of parasites in Cambodia are inside its forests. While 2017 saw a doubling of malaria cases, there is still a very limited understanding of malaria epidemiology and transmission inside forests; hence no malaria elimination strategies specific to this environment. A study done in collaboration with MMEU aims at an in-depth understanding of malaria transmission inside three forests totalling 200km² (Year 1: 2019-2020) followed by an intervention (Year 2: 2020-2021) 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 them and in their surrounding villages. Partnering with IPC in this research are CNM and Partners for Development.

Team leaders: Patrice Piola, Sophea IV, Amber Kunkel

Funding: Global Fund Initiative 5%.

Effectiveness of malaria elimination inside forests

The overarching objective of this study is to eliminate malaria infections inside forests (and consequently in surrounding villages) within a year (August 2019 to August 2020), achieving this through quarterly in-forest active mass screening and treatment (MSATs with HS-RDTs) and continuous passive detection to efficiently treat all malaria infections and provide them with a vector control kit. Estimates of the malaria incidence from all the health centers (HCs) neighboring intervention forests will be compared to the incidences of approximately 100 HCs neighboring other non-intervention forests in Cambodia (control forests) in similar transmission areas. A significant drop in malaria notifications among HCs surrounding intervention forests compared to control forests would strongly suggest the effectiveness of the abovementioned interventions inside forests, Cambodia's main reservoirs. This would open new perspectives in malaria elimination strategies in South East Asia. Forest goers are a mobile community accustomed to the harsh working condition inside forests and are the best placed to become a new type of malaria worker- forest malaria workers (FMWs). A tailored Android application will be developed to support FMWs across all components of this project. The data uploaded from this MHealth application will be used in near real-time to monitor, track (GPS) and evaluate all field activities and malaria indicators. Main collaborators are Partners for Development, Malaria Consortium, ITC, and CNM.

Team leaders: Patrice Piola, Amber Kunkel

Funding: Resistance to Artemisinin Initiative (Global Fund).

4.2.2.4 ZOONOTIC DISEASES

Influenza Surveillance in Cambodia

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

EPH Unit team: Malen Chan

Funding: FAO, USAID

4.2.3 RESEARCH PROGRAMS-2020 PLANS

ARCAHE

This activity is led by the LBM and LMI DRISA.The objective of this project is twofold: to identify the sources of emergence and spread of resistant bacteria in Cambodia using a “One Health” approach; to evaluate if the MinION technology could be used as diagnostic tool. The expected results will represent the baseline for the setting up of a surveillance system, will allow stakeholders to implement efficient control strategies and will help determine the capacity of MinION technology to be used as diagnostic tool. This activity should start mid 2020

Team Leaders: Véronique Chevalier, Patrice Piola

Funding : FSPI - MEAE

4.2.4 SUPPORT TO NATIONAL AUTHORITIES

The following summarizes key support to Cambodian National Authorities during 2019 as part of our ongoing programs and projects.

- Opening of IPC-PHD Rabies Prevention Center at Kampong Cham Province in March 2019 (Epidemiology and Public Health Unit, IPC)
- Contribution to the development of “Rabies Surveillance and Response Guidelines” for Cambodia. This work was coordinated by WHO, FAO, MAFF and MOH (Dr. LY Sowath)
- Participation in the writing of “National Guidelines for Rabies Control in Cambodia” (WHO, FAO, Cambodian CDC, IPC, GDAPH) (Dr. Véronique Chevalier)
- Participation in the Zoonotic Technical Working Group
- Membership in the Cambodian TB/HIV technical working group from January 2019
- Participation in the Joint Program Review (June 2019) for National TB Program in Cambodia in charge of the research section
- Contribution to the guidelines writing on Latent Tuberculosis Infection Management in July 2019
- Participation in the preparation of the Cambodian TB National Strategic Plan in November 2019
- Contribution to the guidelines writing on Elimination Prevention of Mother to Child Transmission of HIV/AIDS, Syphilis and Hepatitis B in December 2019
- Participation on the Zoonosis Working Group (Drs. Ly Sowath, Véronique Chevalier, Patrice Piola)

4.2.5 TEACHING AND TRAINING

- Master’s degree in clinical trials at the London School for Hygiene and Tropical Medicine, funded by the ANRS for Dr. Dim Bunnet
- Several trainings given on clinical research and good clinical practices (Clinical Research Group)
- Pediatric chest-X ray interpretation training (Clinical Research Group)
- Pediatric tuberculosis (Clinical Research Group)
- Focus Group Discussions and Qualitative Interviews (Clinical Research Group)
- Master II (InterRisk) supervision, student Sorn Sopheak, graduated in 2019 (Dr. Sowath Ly)
- Lecture sessions at Applied Epidemiology Training program (Dr. Sowath Ly)
- Supervision of two students in Cambodian Veterinarian Applied Epidemiology Training (CAVET) (Dr. Sowath Ly)
- Lecture sessions in training on Outbreak Investigation and Response, March 2019 (5 Lecturers from EPH Unit)
- Training of students in 2nd and 3rd years of the Bachelor Degree of Arts, Faculty of Social Science and Humanities, Major in Geography and Land-management from Royal University of Phnom Penh (RUPP)
- Public Health Bachelor Degree students from UHS internship (Dr. Laurence Borand)

4.2.6 PUBLICATION LIST 2019 AND PLANNED PUBLICATIONS 2020

2019

1. **"Meat and Fish as Sources of Extended-Spectrum β-Lactamase-Producing Escherichia coli, Cambodia."** Nadimpalli M, Vuthy Y, de Lauzanne A, Fabre L, Criscuolo A, Gouali M, Huynh BT, Naas T, Phe T, Borand L, Jacobs J, Kerléguer A, Piola P, Guillemot D, Le Hello S, Delarocque-Astagneau E; BIRDY study group. Emerg Infect Dis. 2019 Jan; 25(1).
2. **"CTX-M-55-type ESBL-producing *Salmonella* enterica are emerging among retail meats in Phnom Penh, Cambodia."** Nadimpalli M, Fabre L, Yith V, Sem N, Gouali M, Delarocque-Astagneau E, Sreng N, Le Hello S; BIRDY study group. J Antimicrob Chemother. 2019 Feb 1; 74(2):342-348.
3. **"A 1-week intradermal dose-sparing regimen for rabies post-exposure prophylaxis (RESIST-2): an observational cohort study"**. Tineke Cantaert*, Laurence Borand*, Lauriane Kergoat, Chanthy Leng, Sivlin Ung, Sotheary In, Yiksing Peng, Chandara Phoeun, Chanthy Hing, Chun Navy Taing, Manil Saman, Sivuth Ong, Channa Mey, Rithy Choeung, Sowath Ly, Philippe Dussart, Hervé Bourhy*, Arnaud Tarantola*. Lancet Infectious Diseases September 27, 2019.Doi:https://doi.org/10.1016/S1473-3099(19)30311-1
4. **"A Treatment-Decision Score for HIV-Infected Children with Suspected Tuberculosis"**. Olivier Marcy, Laurence Borand, Vibol Ung, Philippe Msellati, Mathurin Tejiokem, Khanh Truong Huu, Viet Do Chau, Duong Ngoc Tran, Francis Ateba-Ndongo, Suzie Tetang-Ndiang, Boubacar Nacro, Bintou Sanogo, Leakhena Neou, Sophie Goyet, Bunnet Dim, Polidy Pean, Catherine Quillet, Isabelle Fournier, Laureline Berteloot, Guislaine Carcelain, Sylvain Godreuil, Stéphane Blanche, Christophe Delacourt, ANRS 12229 PAANTHER 01 STUDY GROUP. Pediatrics. 2019 Aug 27. pii: e20182065. doi: 10.1542/peds.2018-2065.
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2020

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2019

17. 50th Union World Conference on Lung Health, 30 October to 02 November 2019, Hyderabad, India. **Childhood TB diagnostic capacities in primary health care facilities in high TB burden countries: results from the TB-Speed cross-sectional descriptive survey**. E Wobudeya, S Niangoran, L Borand, TE Mao, J-V Taguebue, R Moh, C Khosa, J Mwanga Amumpaire, M Bonnet, O Marcy, TB-Speed Study Group

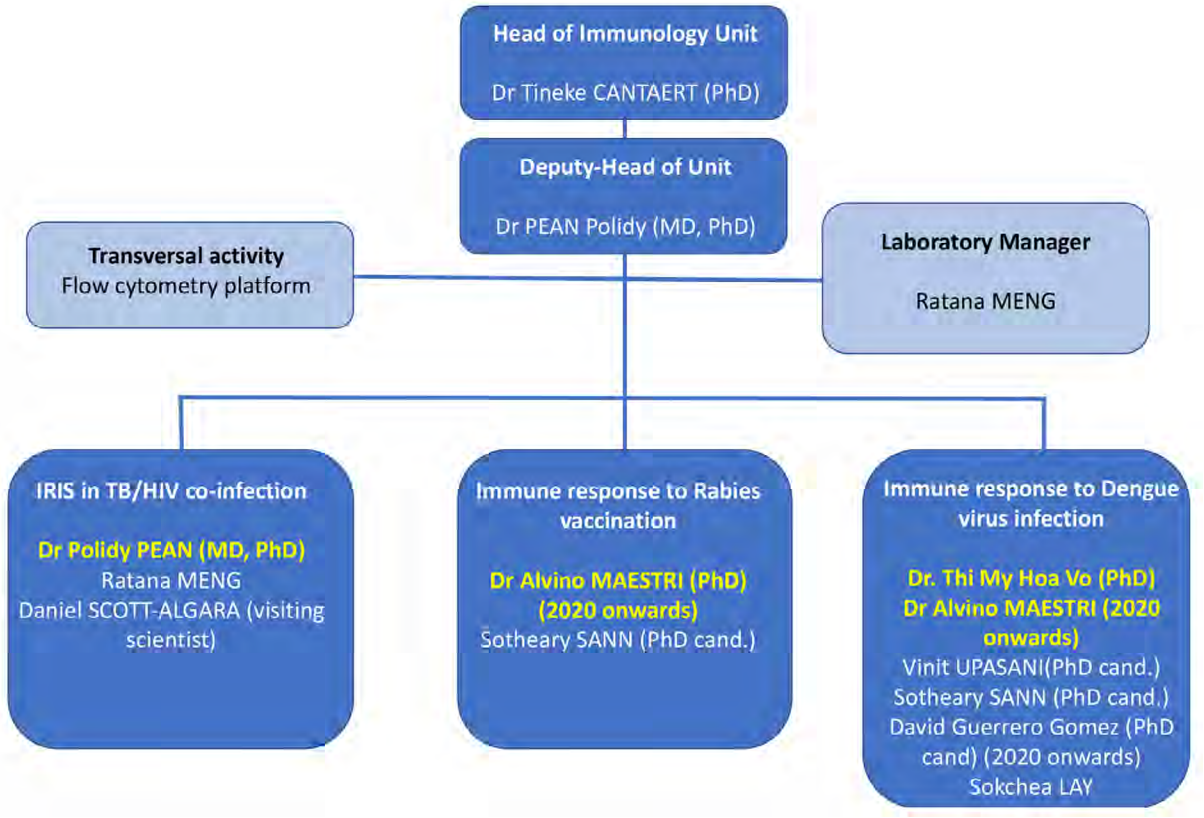
Presentations

18. **Rabies in Cambodia at 4th Cambodia Pediatric Updates Symposium**. Sowath Ly for rabies working group, IPC. May 2019, Siem Reap, Cambodia.

4.3 IMMUNOLOGY UNIT

4.3.1 FUNCTIONAL STRUCTURE OF THE UNIT

Tineke Cantaert, PhD, Head of Unit
Polidy Pean, PhD, Deputy Head of Unit
Thi My Hoa Vo, PhD, Postdoctoral Researcher
Alvino Maestri, PhD, Postdoctoral Researcher (2020 onwards)
Vinit Upasani, MSc, PhD student
Sotheary Sann, MSc, PhD student
David Guerrero Gomez, PhD student (2020 onwards)
Ratana Meng, Laboratory Manager
Sokchea Lay, Laboratory Technician



4.3.2 RESEARCH PROGRAMS-MAJOR ACHIEVEMENTS IN 2019

a. Dengue viruses: adaptive immune responses during Flavivirus infection

Dengue viruses (DENV) infect up to 390 million individuals each year, of which 500,000 cases require hospitalization. Since 2012, dengue is the most important vector-borne viral disease of humans and likely more important than malaria globally in terms of morbidity and economic impact. The mosquito vectors, *Aedes aegypti* and *Aedes albopictus* both thrive well in populated urbanized areas, contributing to the spread of DENV. DENV is a member of the family *Flaviviridae*, and consists out of 4 related serotypes (DENV-1 to DENV-4). Dengue virus infection results in a range of clinical outcomes, from asymptomatic infection, to classic dengue fever (DF), to dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). Most primary infections are mild and probably provide lifelong protection against the infecting serotype. In contrast, secondary infection with a heterologous DENV serotype can result in more severe dengue, suggesting that primary DENV infection triggers a host memory immune response that can result in either protection or enhancement of subsequent infection. Due to the incomplete understanding of the relevant adaptive immune responses leading to protection or enhancement of disease in secondary infection and the absence of conclusive biomarkers for protection, vaccine development has been severely hampered. Our previous work comparing the immune response in asymptomatic acute infected individuals with hospitalized patients revealed profound differences in the adaptive immune response profile associated with a different clinical outcome to infection (Simon-Loriere et al, Scie Transl Med, 2017).

Determination of antibody-independent b-cell functions during acute DENV infection

Antibodies are produced by terminally differentiated B cells, plasmablasts and plasma cells. However, besides antibody production, B cells have diverse functions. For example, B cells with regulatory functions, termed Bregs, have important roles in maintenance of tolerance and homeostasis through the production of immunosuppressive cytokines IL-10 and TGF-β. In the context of DENV infection, not much is known about the antibody-independent B cell responses. We detected decreased frequencies of Bregs during acute DENV infection in patients with severe dengue compared to patients with mild disease, which was associated to decrease CD40L plasma concentrations and decreased platelet counts in these patients. B cells from dengue patients were refractory to toll-like receptors (TLR) stimulation *in vitro* resulting in decreased B-cell specific IL-10 cytokine production, which is paralleled by an increased expression of inhibitory FcγR in vivo in DENV-infected patients. Collectively, our results indicate that a defective B cell response in dengue patients may contribute to the pathogenesis of dengue during the early phase of infection (Upasani et al, Frontiers Immunol 2019).

Discovery of novel biomarkers for severity at hospital admittance

Early detection of severe cases will help to identify patients that benefit from intensive therapy. Currently, no prognostic marker has been identified and early diagnosis relies on multi-parameter interpretation by the health care provider. We aim to identify novel biomarkers predictive for the development of severe dengue within 72 hours after onset of symptoms in a clinically relevant setting. We have an ongoing collaboration with Kantha Bopha Hospital, Phnom Penh. Blood samples of DENV-suspected pediatric cases are sent to the Virology Unit at IP Cambodia for dengue diagnostics. Currently, we have included 430 well-characterized DENV-infected children. After careful transcriptomic profile analysis, we have identified an 18-gene RNA signature that can detect severe cases among secondary-infected dengue patients at hospital presence. This signature can yield new pointers into the underlying pathogenesis of severe disease (Nikolayeva et al, J Infect Dis, 2018). In a separate study, we could show that enhanced TLR2 expression on circulating monocytes is associated with severe disease at the early stages of disease development. In addition, increased protein expression of TLR2, an innate sentinel, generally associated with bacterial infections, leads to an increase in DENV-infected cell mass. In addition, our functional analyses uncover the ability of TLR2 to sense DENV infection and to fuel infection-mediated inflammatory responses leading to activation of the human vascular endothelium (Aguilar-Briseno et al, submitted).

Novel vaccine development for DENV prevention

In humans, both CD4+ and CD8+ T cells contribute to protection against DENV with CD8+ T cells preferentially targeting non-structural proteins NS3, NS4B and NS5. Hence, a minimal DENV antigen has been designed from conserved and highly antigenic T cell epitopes (DENV1-NS). DNA immunization with a plasmid encoding DENV1-NS in mice expressing different human HLA class I molecules confirmed the induction of a strong CD8 T cell response against peptides derived from these NS regions. Using this strategy, and with the intention to develop an effective T cell-based vaccine, we show that a prime-boost immunization of human HLA class I transgenic mice with low dose of a modified mRNA encoding DENV1-NS induces a strong T cell immunity, with a significant protection against DENV1 infection, after transient blockade of the IFN type I receptor in the absence of neutralizing or sub-neutralizing anti-DENV antibodies (Roth et al, Frontiers Immunol 2019).

b. INNATE RESPONSES TO HIV/TB CO-INFECTION

ANRS No12358: "MicroRNA (miRNAs) as prediction and/or prognostic markers of IRIS (Immune Reconstitution Inflammatory Syndrome) in TB/HIV co-infected patient (Mirbio)".

MiRNAs are reported as powerful regulators of post-translational gene expression and can act as biomarkers in several infectious diseases. Host miRNAs target certain HIV genes, affecting HIV replication thus thereby participating in viral control. In HIV elite controllers, a set of expressed miRNA can characterize this clinical phenotype. Several studies reported the characterization of miRNA expression profile in tuberculosis (TB) patients, but evaluation of miRNA expression in co-infections such as TB/HIV are lacking. Hence, we aim to evaluate by flow cytometry whether a circulating miRNA pattern might be used as potential biomarkers in HIV/TB coinfection and to correlate the miRNA expression profile of 28 selected miRNAs with the clinical evolution and the occurrence of IRIS. Interestingly, we identified two miRNA candidates (miR-150 and miR-145) that could discriminate between HIV-TB co-infection from HIV, TB mono infection and healthy controls. As a next step, we will analyze exomiR, miRNA in the plasma-secreted exosome. The statistical analysis and manuscript preparation will be done in 2020.

c. Immune responses to rabies virus (RABV) vaccination

The international health authorities are backing an effort to eliminate canine-mediated rabies in humans by 2030. This will require improving access to adequate and timely post-exposure rabies prophylaxis. All individuals were referred to the rabies prevention clinic at IP Cambodia and received two-dose intradermal PEP at days 0, 3, 7 and 28. We showed that all individuals demonstrated rabies virus neutralizing antibody titers considered protective (≥ 0.5 IU/ml) at day 28, immediately before the last injection. Protective titers were reached notwithstanding eRIG use, age, sex, nutrition status or dog infective status (Cantaert T, Borand L et al, Lancet Infect dis 2019). Hence, we provide evidence that rabies PEP can be abridged to a two-dose, three-sessions, one week (D0, D3, D7 regimen. Based on these results the WHO endorsed changes in its April 2018 guidelines. This "IPC protocol" is the first one-week PEP regimen to be recommended and the shortest and most vaccine-sparing rabies PEP protocol endorsed.

4.3.3 RESEARCH PROGRAMS –2020 PLANS

a. Dengue viruses: adaptive immune responses during flavivirus infection

Unravel adaptive immune responses in asymptomatic DENV-infected individuals

In 2018, we conducted a field study in order to collect a new cohort of asymptomatic individuals with acute infection and a cohort of hospitalized patients in collaboration with the virology unit and epidemiology and public health units, IP Cambodia. Biological specimen and associated data were obtained at days zero 0, 3, 5, 10, and 60 after confirmed DENV-infection in both hospitalized and asymptomatic DENV-infected individuals. This longitudinal patient cohort provides us with a unique opportunity to study the immune responses initiated after dengue infection that provide protection from disease, but also clear the virus. In previous work we revealed that these individuals show an increased activation of the adaptive immune compartment regulated by proper feedback mechanisms, leading to elimination of viral infection without excessive immune activation (Simon-Loriere et al, Scie Transl Med, 2017). Here, we demonstrated that B cell activation and plasma cell development pathways are differentially regulated between the two groups of individuals. We postulate that this will shape the antibody repertoire differentially in asymptomatic individuals compared to hospitalized patients. Therefore, we aim to understand the mechanism of the generation and protection of the humoral immune response in these individuals on a single cell level. We will evaluate the development, quality and quantity of the humoral responses in detail in a cohort of acute infected, asymptomatic Cambodian children and compare them to that of hospitalized children. To accomplish this goal, we work on the following specific aims: 1) Generation of DENV-derived antigens from clinical field isolates ; 2) Determination of antibody affinity for four key-DENV antigens in individual plasmablasts by droplet microfluidics to define on a single cell level the cross-reactivity and epitope specificity of antibody secreting cells; 3) Investigation of the clonality and affinity maturation profiles of the B-cell receptor of DENV-specific B-cells by sc-RNAseq in asymptomatic individuals to gain insight into the generation of B cell responses associated to protection after infection ; 4) Detailed investigation of the polyclonal anti-DENV serum response of asymptomatic individuals to uncover novel correlates of protection. Partners in this effort were the IPC virology unit, the structural virology and the antibodies in therapy and pathology units at IP Paris (P. Bruhns), and CB UTECHS, IP Paris (M. Hasan).

Team Leader: Tineke Cantaert

Funding: Janssen Horizon grant 2019-2022.

Determination of antibody-independent B cell functions during acute DENV infection

In the blood, DENV is tropic for monocytes and dendritic cells, and a few reports have suggested that B cells support viral replication. We are able to detect DENV-infected B cells in a patient cohort of Cambodian children. However, the consequence of direct B cell infection by DENV on both antibody dependent and antibody-independent B cell functions remains unknown. Therefore, 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. Partners to the virology unit, IP Cambodia are University of Groningen, virology department.

Funding: Calmette-Yersin RIIP PhD program (2017-2019)

Antibody effector functions during DENV infection

The two recent phase III trials with CYD-TDV have demonstrated a critical discordance between seroneutralization as measured by *in vitro* assays and *in vivo* protection from infection and hospitalization. Indeed, the gold-standard *in vitro* neutralization assay only measures the capacity of antibodies to neutralize virus, preventing direct infection. However, many more functions are attributed to antigen-specific antibodies in protection against viral infections, which are critically dependent on the formation of immune complexes, the Fc portion of IgG and interaction with Fcγ receptors and downstream effector functions. Indeed, antibody-dependent enhancement (ADE) has been observed during heterotypic DENV infection. Here, the antibody-virus complexes bind to FcγR on antigen presenting cells, thereby facilitating virus internalization through FcγR or other associated receptors resulting in infection of permissive cells such as monocytes and dendritic cells. Effector functions of IgG are critically dependent on glycosylation of the Asn297 of the heavy chain. Our preliminary data generated in collaboration with Dr. Ravetch show altered glycosylation profiles in asymptomatic individuals. In more detail, whereas the abundance of afucosylated IgG1 is increased in hospitalized patients undergoing secondary infection, this is not the case in asymptomatic individuals. In addition, both during primary and secondary infection sialylated IgG1 is decreased in asymptomatic individuals. This altered glycosilation profile could modulate antibody-effector functions and/or affinity maturation and the production of neutralizing antibodies conferring protection from severe disease (manuscript in preparation).

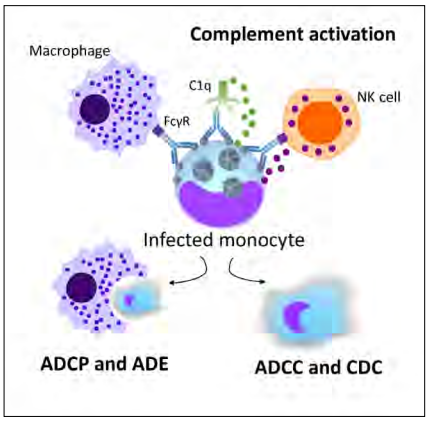


Figure 1: Cell based assays for antibody effector functions analysis.

Four *in vitro* cell-based assays have been optimized in the immunology unit to evaluate antibody-effector functions of IgG generated during DENV infection: antibody-dependent enhancement assay (ADE), antibody dependent cytotoxicity assay (ADCC), antibody dependent phagocytosis assays (ADCP), and complement dependent cytotoxicity (CDC). (Figure). All four assay results will be correlated to clinical outcome and other parameters such as viral load, DENV IgG titers, platelet count, haematocrit and duration and severity of symptomatic infection. Protection and risk signatures will be uncovered using multivariate analysis methods. This research is done in partnership with Calmette-Yersin RIIP PhD program (2017-2019), Rockefeller University, Laboratory of Molecular Genetics and Immunology.

Team leader: Tineke Cantaert

Funding: HHMI/Wellcome International Research Scholars Program (2017-2021).

Discovery of Novel Biomarkers for Severity at Hospital Admittance

Type I IFN plays an important role in the early innate defense pathways after DENV infection. Indeed, through opsonization by sub neutralizing or non-neutralizing antibodies, DENV infection can suppress innate cell immunity and type I IFN production to facilitate viral replication. Type I IFN are mainly produced by plasmacytoid dendritic cells (pDC) in response to viral infection. However, it remains to be investigated if the type of IFN production and/or responses of specific DC cell subsets and functions early in the course of disease might be associated to disease outcome after DENV infection. Here, we will leverage our patient cohort of early admitted patients which has been bio banked in collaboration with Kantha Bopha Hospital, Phnom Penh (see above, completed work Determination of Antibody-Independent B Cell Functions during Acute DENV Infection.). We aim to investigate: 1) the gene expression profile in PBMC of type I IFN subtypes (e.g. IFNα, IFNβ, IFNω and IFNε) and IFN-related pathways (Interferon stimulated genes, ISG), which have been shown to be important during in vitro DENV infection and 2) protein levels of IFN-I subtypes using ultrasensitive quantification by Simoa digital ELISA in plasma samples (13 IFNα subtypes, IFNα2, IFNβ) and 3) different DC cell subsets in peripheral blood by flow cytometry. All parameters will be correlated to disease outcome, clinical parameters of the patients such as platelet counts

and viral load. Partners comprise the virology unit at IP Cambodia, Pasteur Cenci-Bolognetti Foundation, IP Roma, Immunobiology of Dendritic Cells Unit, IP Paris.

Team Leader: Tineke Canteart

Funding: ACIP 2019-2021

Immunity to aedes mosquito saliva

DENV is transmitted by mosquitos of the *Aedes spp*, mainly *Aedes Aegypti* and *Aedes Albopictus*. When a mosquito inserts its proboscis and probes for blood, the mosquito ejects a salivary mix of vasodilators, anticoagulants, and other anti-hemostatic components into both the epidermis and the dermis. In addition, mosquito saliva also contains proteins that modulate both innate and adaptive immune responses (reviewed in Cantaert T and Manning J, Vaccines, 2019). However, little is known about skin immunity to mosquito saliva. Hence, characterization of the immune responses to vector saliva and pathogens will be important in order to identify critical aspects of the innate and adaptive immune responses after an infected vector bite. Therefore, we aim to: 1) Unravel the local early and late immune signatures of *Aedes Aegypti* bitten and unbitten skin. 2) To investigate the effect of mosquito saliva on the immune response to DENV. We will compare DENV replication in primary immune cells (dendritic cells and keratinocytes) in presence or absence of *Aedes aegypti* salivary gland homogenate and we will identify changes in immune responses of primary immune cells (DC and keratinocytes) to DENV in the presence of *Aedes aegypti* saliva (eg. type I IFN responses). Partners include Malaria and Vector Research Laboratory, United States NIH National Institute of Allergy and Infectious Diseases NIH, MiVEGEC Unit, IRD, UR224 Entomology Unit, IP Cambodia.

Funding: Calmette-Yersin RIIP PhD program (2020-2022); NIH collaborative agreement

b. Innate responses to HIV/TB co-infection

Micro-RNA as surrogate markers for tuberculosis diseases in HIV infected children

We have observed that miR-150 and miR-145 could discriminate TB/HIV co-infection from HIV, TB mono-infection and adult healthy donors (preliminary data of ANRS 12358, see above). Moreover, in the ANRS 12229 (PAANTHER 01) patient cohort, our collaborators have shown a scoring algorithm by combining GenXpert testing, clinical signs and contact history, the chest radiograph, together with ultrasound and QFT that can be used for TB diagnosis with sensitivity of 88.6% and a specificity of 61.2% (Marcy et al, *Paediatrics*, 2019). Based on the availability of the plasma biobank of the PAANTHER 01 study, we will propose an associated study to assess the circulating microRNA expression in this cohort. We expect to identify a microRNA candidate that could be used as biomarkers and/or combination biomarkers to aid TB diagnosis. The project proposal will be submitted to INSERM-ANRS for funding in 2020. Partners: Bordeaux Population Health Centre U1219, *Université de Bordeaux*, Epidemiology and Public Health Unit, IP Cambodia Lymphocyte Cell Biology.

Team Leader: PEAN Polidy

Funding: undetermined

ANRS N° 12394: "Lowering Inter Leukin-1 receptor Antagonist Concentrations after TB Treatment Onset: A Proof of Concept Study in Cambodia and Ivory Coast (LILAC-TB)"

The Immunology Unit (Dr PEAN Polidy) is supporting the EPH Unit, the project is coordinated by Dr Laurence Borand.

Vaccine Responses to Rabies Virus (RABV) Vaccination

The WHO endorsed a new shortened protocol of PEP in the April 2018 guidelines. This IPC protocol of three PEP sessions of two-site intradermal 0,1 mL vaccine doses each at days 0, 3 and 7 is the first one-week PEP regimen to be recommended (Cantaert T, Borand L et al, Lancet Infect dis 2019). The "IPC protocol" is to date the shortest and most vaccine-sparing rabies PEP protocol approved by the WHO. An evaluation of the protection and antibody responses of this newly WHO-endorsed vaccination protocol is necessary. Therefore, we aim to monitor safety, efficacy, and protection (as measured by anti-rabies virus neutralizing antibody responses) in patients up to one year after the newly WHO-endorsed IPC protocol vaccination regimen. We are establishing a biobank in the framework of this study. All samples and viral strains collected in this biobank will be used for further in depth analysis of the immune response, such as T cell responses and antibody affinity responses. Partners include the virology unit, and the Epidemiology and Public Health Units and the vaccination center at IP Cambodia.

Team Leader: LY Sowath

Funding: Internal to IPC

Platform: Flow Cytometry

The flow cytometry platform will be extended with the purchase of a FACS-ARIA III cell sorter funded 50% by a Wellcome Trust multi-user equipment grant. The ability to perform cell sorting on site will strengthen all immunology unit projects and will allow us to perform more mechanistic and functional studies. Research and collaborative projects with MMEU and VU will be performed with the equipment. In addition, external collaborative projects for research (NIH) and teaching (UHS) are strengthened.

4.3.4 SUPPORT TO NATIONAL AUTHORITIES

- Drs. Tineke Cantaert and Pean Polidy are members of the steering committee for the international master's degree in infectious disease and coordinator of immunology module in master's year 1 (University of Health Sciences, Phnom Penh, Cambodia and *Universite Paris*-Saclay, Paris, France).

- Based on the results discussed in IP Cambodia's Vaccine responses to rabies Post-Exposure Prophylaxis research, WHO endorsed changes in its April 2018 guidelines. This IPC protocol is the first one-week PEP regimen to be recommended and the shortest and most vaccine-sparing rabies PEP protocol endorsed. This PEP-vaccination scheme has been implemented at the rabies vaccination centers of *Institute Pasteur du Cambodge*.

4.3.5 TEACHING AND TRAINING

Drs. Tineke Cantaert and Polidy Pean also undertook the following:

- Ten 10 hours/year each of teaching in the immunology module within the master's degree program in medical biology, UHS;

- Served as members of the steering committee for the international master's degree in infectious disease, and coordinators of the immunology module.

PhD student:

- Vinit UPASANI was supported by a Calmette-Yersin grant from IPIN. The student has been enrolled at the University of Groningen, the Netherlands since 2017
- Sotheary SANN is enrolled at the University of Hasselt, Belgium in 2019 Visits to UHasselt are covered by a BOF/BILA grant of the Flemish government.

The list below includes internship thesis students:

- Axelle VANDERLINDEN: University of Antwerp, Belgium: Master's thesis in biomedical sciences: November 2018-June 2019
- David GUERRERO GOMEZ: University of Antwerp, Belgium: Master's thesis biomedical sciences: November 2018-June 2019'
- Sokchea LAY: University of Health Sciences, Phnom Penh, Cambodia: Master's thesis biomedical sciences: October 2018-May 2019
- Sara VAN DE KERKHOVE: University of Antwerp, Belgium: Master's thesis biomedical sciences: November 2019-June 2020
- Mao Sowathiro, Bart University, UK: Bachelor Biological science: June-September 2019

4.3.6 PUBLICATION LIST 2019

Awards and Grants Approved in 2019

1. 2019-2025 Wellcome Trust Multi-user Equipment Grant: **Advancing flow cytometry for the on-site study of tropical infectious diseases.** PI: Tineke Cantaert
2. 2019-2022 Janssen Horizon: **Understanding of host immunity against dengue virus at single cell resolution.** PI: Tineke Cantaert
3. 2019-2021 Transversal research Program Institut Pasteur: **Towards new vaccine strategies for dengue virus infection: identification of protective humoral immune responses in asymptomatic dengue-infected individuals**. PI: Cantaert Tineke
4. 2019-2021 Inter-Institut Pasteur Concerted Actions: **Dengue virus-mosquito-host interactions: assessing type I interferon mediated immune processes and immunological effects of mosquito salivary glands on Dengue infection**. PI: Carolina Scagnolari, Co-investigator: Tineke Cantaert
5. 2019-2021 Inter-Institut Pasteur Concerted Actions: **Determinants of the expansion of dengue minority serotypes.** PI: Arnaud Tarantola, Co-investigator: Tineke Cantaert

Publication list 2019

6. Upasani V, Vo HTM, Ung S, Heng S, Laurent D, Choeung R, Duong V, Sorn S, Ly S, Rodenhuis-Zybert I, Dussart P, Cantaert T**. Impaired Antibody-Independent Immune Response of B Cells in Patients With Acute Dengue Infection.** Frontiers in Immunology 2019;
7. Manning J, Cantaert T. **Time to Micromanage the Pathogen-Host-Vector Interface: Considerations for Vaccine Development.** Vaccines 2019
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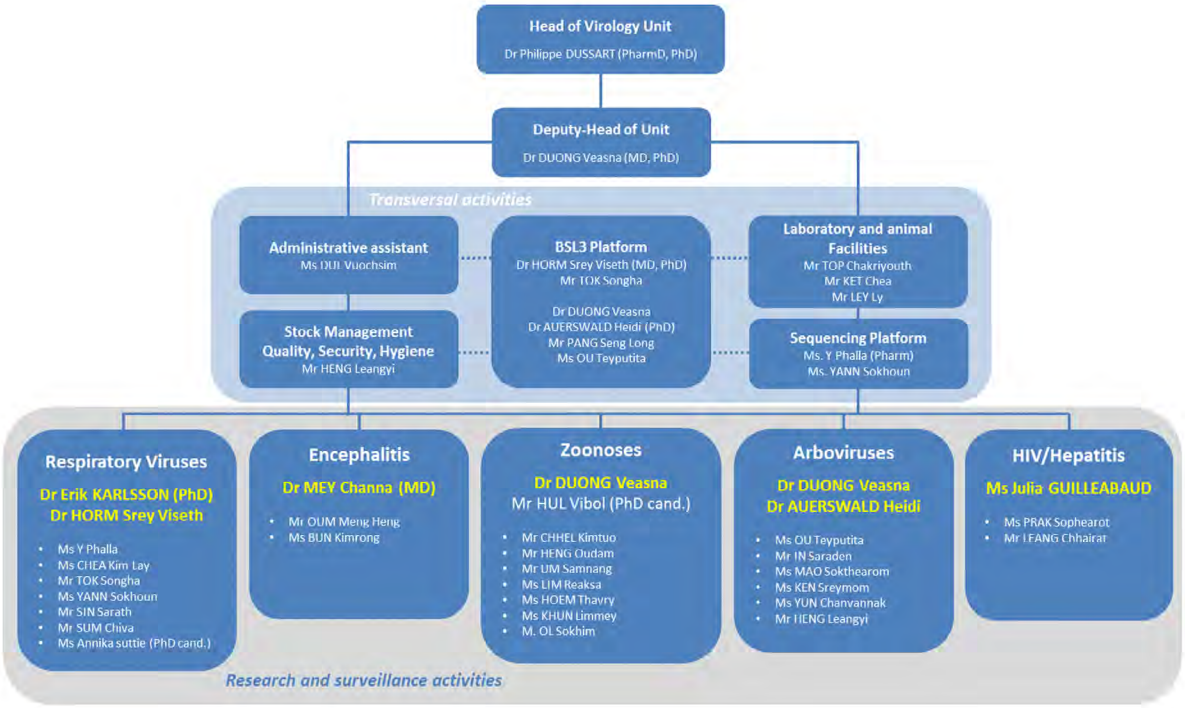
4.4 VIROLOGY

4.4.1 FUNCTIONAL STRUCTURE OF THE UNIT

The activities of IPC's virology unit are directed towards biomedical research and the surveillance/monitoring of infectious diseases. They can be divided into five main components: 1) arboviruses (ex: dengue, Zika, chikungunya and Japanese encephalitis), 2) respiratory syndromes (mainly seasonal and avian influenza), 3) HIV and viral hepatitis, 4) viral encephalitis (Japanese encephalitis, EV-A71), and 5) zoonotic and emerging pathogens (ex: coronaviruses and Nipah virus). Six researchers are involved in these surveillance and research activities with approximately 30 persons working in the 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).



4.4.2 RESEARCH PROGRAMMES – MAJOR ACHIEVEMENTS IN 2019

4.4.2.1 ARBOVIRAL DISEASES

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

Under team leader Veasna Duong, with M. Dupont-Rouzeyrol (IPNC) and L. Lambrechts (IP, Paris),

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.

Considering dengue serotypes circulating in Cambodia, we chose to work on DENV-1 and looked the replacement of genotypes and/or lineages in the past 10-20 years. We observed that genotype 1 of DENV-1 was the only genotype circulating in Cambodia since 2000. However, we often saw lineage replacement and at least five lineages were found co-circulating in the country. For this study, 27 DENV-1 isolates were selected among the three lineages of interest (lineage 3, 2005-2007, lineage 4, 2005-2016 and lineage 5, 2007-2016) and sent to IP Paris for full genome sequencing. Isolates from each of the three lineages (lineage 3, 4 and 5) have been selected for competition vector competence study using 50/50 virus ratio in blood feeding. RT-qPCR primers and probes were

designed to detect and differentiate the virus from the three lineages in mosquitos. The mosquito experiment was conducted with two DENV-1 strains from lineage 3 and from lineage 4 as the RT-qPCR assay could not differentiate DENV-1 from lineage 4 and lineage 5.

Our findings show that the percentage of mosquitos infected with lineage 4 (red color bar) increased through time post blood feeding and was significantly higher than mosquitos infected with lineage 3 virus (pink color bar) at day 14 (Fig. 1). The difference of infection was more noticeable in mosquito saliva when compared between DENV-1 strains from lineage 3 and DENV-1 from lineage 4b (Figure).

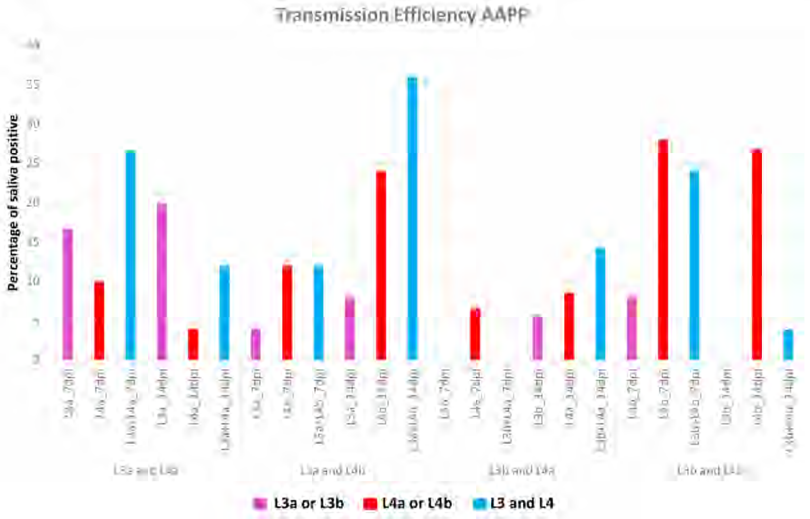


Figure 1: Transmission efficiency of mosquitos infected with DENV-1 from lineage 3 and lineage 4.

This research was undertaken in partnership with IPNC and IP Paris.

Team Leader: Veasna Duong

Funding: *Institut Pasteur*, Paris.

Dengue vaccine initiative (DVI): Zika virus foci reduction neutralization test (FRNT) analysis within DVI study panel

Zika virus (ZIKV) is an emerging mosquito-borne virus and member of the family *Flaviviridae*, which also includes dengue virus (DENV), and Japanese encephalitis virus (JEV). So far, the detected prevalence for ZIKV in Cambodia is low. However, as a specific ZIKV surveillance program is not in place, the extent of ZIKV infections in Cambodia is unknown. Serological studies on ZIKV are challenging due to its high cross-reactivity to DENV and JEV. The most specific assay is a virus neutralization test that measures the amount of protective (neutralizing) antibodies.

Samples collected during a community-based study that originally aimed to investigate the seroprevalence for DENV (DVI study) were collected in September 2016. A high titer of neutralizing antibodies against a certain virus indicates a recent infection with this virus. Based on the neutralizing antibodies, we identified 29 samples (26.1%) as ZIKV positive, 13 samples (11.7%) as DENV positive, and two samples (1.8%) as JEV positive. The remaining 67 samples showed similar titers of neutralizing antibodies against several viruses and therefore a precise identification of the last infection was not possible. As previously observed, these findings suggest ZIKV has circulated in Cambodia and has had low-level impact on public health.

Team Leader: Philippe Dussart

Funding: DVI – ZIKAlliance Consortium.

Replication variance of African and Asian lineage Zika virus strains in different cell lines, mosquitos and mice

Zika virus has long been known to cause only mild febrile illness. The epidemic that started in 2007 on Yap Island and spread through the Pacific Islands and into the Americas changed that perception as neurological disorders – including congenital microcephaly and GuillainBarré syndrome – were associated with ZIKV infections. ZIKV strains are divided into two major lineages: the African lineage and the Asian/American lineage with the strains that caused the recent outbreaks.

In this study led by V. Vuong in collaboration M. Dupont-Rouzeyrol (IPNC), O. Faye, M. Grandadam (IPL), VM Cao-Lormeau (*Institut Louis Malardé*), and AB Failloux (IP, Paris) we aimed to investigate the replication of African and Asian ZIKV strains in vitro and in vivo to reveal phenotypic differences. In addition, we investigated the vector competence of Cambodian *Aedes aegypti* mosquitos for these ZIKV strains. We observed a significant higher pathogenicity of the African ZIKV strain in vitro (cytopathic effect in mosquito and mammalian cells), and in vivo in both *Ae. aegypti* and mice (reduced survival). Both rural and urban mosquito populations were competent to

transmit ZIKV as early as 7 days p.i. depending on the population and the ZIKV strain. We observed the highest transmission efficiency for the African ZIKV isolate (80% 10 days p.i., 93.3% 14 days p.i.) and for the Cambodian ZIKV isolate (80% 14 days p.i.). *Ae. aegypti* mosquitos from a rural habitat showed significant higher transmission and survival rates than those from urban Phnom Penh. Overall, our results highlight the phenotypic differences of the ZIKV lineages and the potential risk of ZIKV transmission by *Ae. aegypti* mosquitos. Further investigations of Cambodian mosquito species and ZIKVspecific surveillance in humans is necessary to improve the local risk assessment. Partners in this effort are the IP New Caledonia, IP Senegal, IP Laos, *Institut Louis Malardé and Institut Pasteur Paris (*ACIP*).*

Team Leader: Veasna Duong

Funding: *Institut Pasteur, Paris (*ACIP*)*

Improved detection of dengue and Zika viruses using multiplex RT-Qpcr assays

Dengue virus (DENV) and Zika virus are important viral pathogens, known to cause human infections with similar symptoms. They are transmitted by common vectors and co-circulate in intertropical regions. Moreover, dengue fever results from infection with one of four different serotypes of dengue virus. Considering the recent ZIKV emergence, multiplex and up-to-date assays are more preferable for detection of both viruses in a single reaction. This study aimed to develop: 1) a one-step duplex real-time reverse transcription polymerase chain reaction (RT-qPCR) assay to efficiently and simultaneously detect and quantify DENV and ZIKV; 2) a fourplex RT-qPCR to differentiate and quantify the four DENV serotypes. The detection limit of the duplex assay was 0.028 and 0.065 FFU (focus forming unit)/ml for DENV and ZIKV respectively. The lower limit of analytical sensitivity of fourplex assay was 0.01 FFU/ml for DENV-1 and 0.1 FFU/ml for DENV-2,-3 and -4. The assessment of specificity indicated both assays were highly specific to targeted viruses with negative results for other *Flaviviridae* such as Japanese encephalitis, West Nile, yellow fever or hepatitis C viruses. The newly developed RT-qPCRs were shown to be more sensitive than a previously described assay in detecting DENV in clinical samples and are suitable for routine diagnosis. The effort includes partners CNM and MOH.

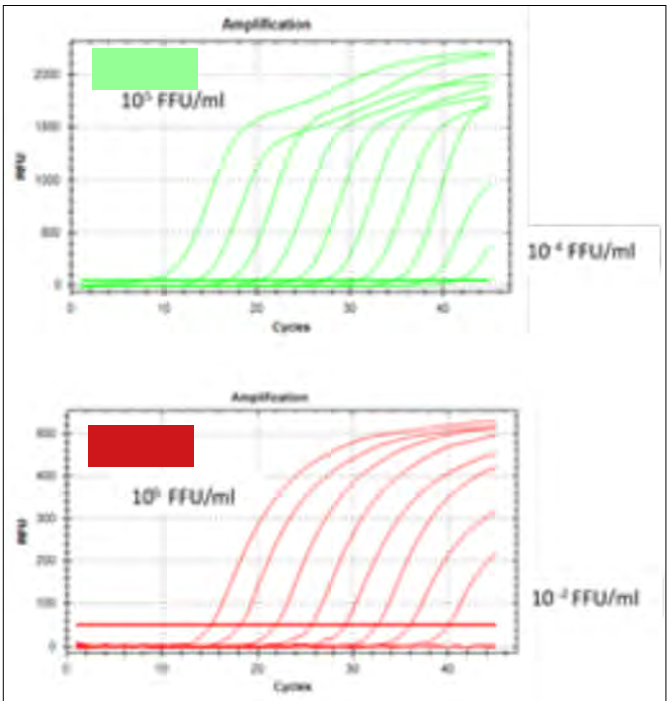


Figure 2: Amplification plots and linear ranges of different dilutions of the duplex real-time RT-PCR assay displayed by each fluorophore.

Team Leader: V. Vuong

Funding: *Institut Pasteur du Cambodge*.

PREEMPT – Understanding arbovirus emergence and changing the approach to intervention

Arboviruses are viruses that are transmitted from an animal reservoir to humans via an arthropod vector. These viruses result in a large burden of disease worldwide. The Preventing Emerging Pathogenic Threats (PREEMPT) program targets viral pathogens within the animal reservoirs and insect vectors where many diseases originate before they spill over into humans. The program combines bio surveillance and modeling with novel technologies for treating or containing high-risk pathogens at their source to prevent the emergence and reemergence of human-pathogenic threats.

As part of the *Institut Pasteur* Arbovirus Challenge (IPAC) team, IPC's entomology unit periodically collects mosquitos from various field sites to map mosquito densities and dynamics. This effort was led by Philippe Dussart from IPC in partnership with S. Boyer (also IPC) and C. Saleh and C. Koh from IP Paris. Some of the mosquitos collected were shipped to IP Paris for next generation sequencing for the identification of the mosquito species and the virome of the single mosquitos. Special emphasis lies in the investigation of insect-specific viruses. Additionally

to the sequencing efforts, some samples were processed directly at IPC for virus isolation on mosquito cells. Single mosquitos were homogenized and used for inoculation of *Aedes albopictus* C6/36 cells. Culture samples that showed signs of infection (cytopathic effect, CPE) were further analyzed for the presence of known arboviruses like dengue, Zika or chikungunya virus. Furthermore, RT-PCRs for the detection of JEV, WNV and USUV were set-up. Culture samples with observed CPE but absence of known arboviruses were send to IP Paris for further investigation and sequencing. The entomology unit of IPC and IP Paris partnered in this activity.

Team Leader: Philippe Dussart

Funding: US Defense Advanced Research Projects Agency (DARPA)

Serological investigation of domestic animals for antibodies against Japanese encephalitis virus and related viruses

Japanese encephalitis virus (JEV) is the most common cause of viral encephalitis cases in humans and is widely distributed in Asia. The natural hosts are wild ardeid birds (like herons) and *Culex* mosquitos act as the transmitting vectors. Swine are also known to be amplification hosts for the virus. Experimental studies suggest that young ducks and chickens could be infected with JEV. However, the role of poultry and other domestic animals in the epidemiological cycle as potential secondary reservoirs is unknown. Additionally, serological studies of JEV and other flaviviruses are difficult due to the intense cross-reactivity of their antibodies. This effort was done in partnership with Véronique Chevalier from CIRAD based in EPH Unit at IPC.

We aimed to investigate the prevalence of antibodies against JEV in several domestic animal species. Hence, we used samples from poultry, swine and dogs collected in various studies conducted in cooperation with IPC's epidemiology and public health unit. We determined the flavivirus seroprevalence using a hemagglutination inhibition assay (HIA). Due to the high cross-reactivity of JEV antibodies to related viruses like West Nile virus (WNV) and Dengue virus (DENV), we used the more specific foci reduction neutralization test (FRNT) to investigate the immune response further.

We found a significantly higher JEV seroprevalence in ducks (24%) than in chickens (13%). In pigs and dogs, the JEV prevalence was even higher (34% and 40%, respectively). However, the results of the HIA were difficult to interpret as they are often affected by the high cross-reactivity between JEV and DENV. The serological results of dogs were outstanding, as they showed a high degree of virus-specific antibodies that could be detected by HIA and FRNT. Based on the FRNT we also found seven poultry birds and three dogs with WNV antibodies, which is the first serological evidence for WNV presence in Southeast Asia for decades. These results and the easier acquisition of samples from dogs compared to the other investigated animals makes them better sentinel animals for JEV surveillance programs.

Team Leader: Philippe Dussart

Funding: ANSES

4.4.2.2 AVIAN INFLUENZA VIRUSES

The team leader for these activities is Erik 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 bird markets (LBMs)

As the Cambodian National Influenza Centre (NIC) and WHO H5 Reference Laboratory (H5RL), IPC supports the Cambodian Ministry of and Ministry of Agriculture, Forestry and Fisheries in the confirmation of influenza infections in humans and animals. In 2019, Cambodia had no reported outbreaks of avian influenza. In agreement with the National Animal Health and Production Research Institute (NAHPRI), all influenza positive samples are sent to IPC for confirmation and viral characterisation. In past years, IPC laboratory testing confirmed the detection of outbreaks, mainly of subtype A/H5N1, and sequence analysis of the outbreak strains showed that the viruses were closely related to strains circulating in live bird markets in 2015, 2017 and 2018. Further phylogenetic, antigenic, and molecular risk assessment analyses are ongoing and a manuscript detailing connections between seasonality, environmental factors, sociological factors, outbreaks and market samples is in preparation and expected by mid-2020.

Avian influenza surveillance in Cambodia border regions

In 2017, in collaboration with the FAO and NAHPRI, IPC sought to establish avian influenza virus surveillance in Cambodian border regions to obtain a greater understanding of the dynamics of cross-border movements of AVIs into Cambodia and to obtain their molecular profiles. During the 2018- 2019 study period, we collected 5,120 poultry samples from 2,560 birds (paired tracheal and cloacal swabs) with 23.6% and 6.1% influenza positivity respectively. Overall, combining information from both tracheal and cloacal swabs on a “per bird” basis, avian influenza virus was detected in 27.8% (n=712) total birds.

Analyses is complete on both 2017-2018 and 2018-2019 periods and several manuscripts are in preparation One manuscript detailing the 2017-2018 efforts was published. This collaboration continues for the 2020-2021 season.

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

Since 2011, IPC has conducted LBM surveillance in Orussey Market in Phnom Penh to determine the circulation characteristics of avian influenza in Cambodia. Isolates from 2015 to 2019 have been transferred to the WHOCC in Melbourne for full genome sequencing using next generation sequences (NGS) and have been completed. These analyses will reveal important information about the rate of reassortant events occurring in LBMs and the risk of emergence of novel AIV strains. Final samples from mid-2019 have just been sequenced and phylogenetic, antigenic, and molecular risk assessment are underway as below:

Subtype A/H5

Since 2014, the majority of A/H5 samples detected have the N1 subtype NA and fall into clade 2.3.2.1c. Cambodian A/H5N1 viruses all cluster with previously identified viruses and are closely related to other viruses in the Mekong Delta from 2012-2018.

While A/H5N6 viruses have been circulating in Southeast Asia since 2013, the first detection of avian influenza subtype A/H5N6 in Cambodia occurred in February 2019. Indeed, detection of these clade 2.3.4.4 viruses represent the first detection of a new clade in Cambodia since the 2.3.2.1c A/H5N1 lineage reassorted with and replaced the former 1.1.2 A/H5N1 lineage in 2014. The detection of these novel lineage viruses, association with outbreaks in poultry throughout Southeast Asia, their zoonotic potential, co-circulation with other AIV of concern, and the ability of these A/H5Nx viruses to evolve rapidly make it imperative to maintain constant, rigorous, and vigilant surveillance for AIV in Cambodian poultry populations. Further phylogenetic, antigenic, and molecular risk assessment analyses are ongoing.

Subtype A/H7

A/H7 viruses are of particular concern as they have been a leading cause of zoonotic infections over the past two decades, with human cases due to independent H7-lineages being detected across multiple continents. While the A/Anhui/1/2013-lineage H7N9 viruses have not been detected outside of China, A/H7 AIVs have been detected infrequently in the Greater Mekong Sub region since 2009. In Cambodia, active surveillance in 2015 detected a few A/H7 viruses in ducks (A/H7N3, A/H7N7, A/H7Nx), whereas in January 2017, A/H7N3 was detected in association with a duck mortality event in Kampong Thom Province. That same year, A/H7N7 was detected in a live market in Takeo Province in September.

Starting in February 2018, two months after the first A/H7N4 human case in Jiangsu, China, A/H7N4 was detected in ducks in Cambodia for the first time and frequency of detection increased in March and April of the same year and has continued to be detected sporadically in 2019 in the country. These A/H7 viruses contain at least one gene segment with high genetic similarity and common evolutionary origins to the Jiangsu A/H7N4 samples and continue to reassert in the region.

Subtype A/H9

Subtype A/H9 AIVs circulate globally in wild avian species and are endemic in domestic poultry in many Asian, Middle Eastern and African countries. A/H9 AIVs also commonly donate internal protein genomic segments to non-A/H9 viruses through reassortment, increasing zoonotic potential.

In 2013, surveillance efforts in Cambodia expanded to encompass A/H9 viruses. It is now evident that A/H9 LPAI viruses circulate endemically in Cambodian poultry, similarly to Bangladesh, China and Vietnam and LBM workers are exposed to these viruses. All A/H9 viruses identified thus far in Cambodia are classified as having an N2 subtype NA and sequencing indicates all of these viruses fall into Clade 4.2 (BJ94/Y280-like). The majority of viruses are similar to those circulating in Vietnam and Indonesia from 2014 to 2017. The majority of Cambodian A/H9N2 viruses

detected after 2015 belong to two genotypes, P and V. Overall, two distinct groups of viruses appear to be circulating concurrently and require further investigation. It is currently unclear how these two groups were introduced to the country; however, both cross-border poultry trade and wild bird migration may play a role.

Longitudinal Serological Surveillance for AIV Infection in Poultry Workers

Following a serosurvey in 2015, and similar to what has been conducted in the 2017-2018 surveillance, longitudinal human serum sampling was conducted twice in 2018 (once at the start of the study, once in-between Pchum Ben and Bon Om Touk) and twice in 2019 (between Lunar New Year and Khmer New Year and at the end of the study) by the public health and epidemiology unit at IPC in conjunction with NAHPRI in 4 provinces.

Regarding serological analyses, the majority of hemagluttination inhibition assays (HAIs) have been performed for the 2015, 2017-2018 and 2018-2019 LBM worker samples against all 4 human seasonal strains for given time period, as well as appropriate A/H5N1, A/H5N6, A/H7Nx, and A/H9N2 strains. Correlation between seroconversion and poultry handling risks as well as other sociodemographic data are being analyzed and a publication is expected to be conjoined with the A/H9N2 physolgenetics and antigenic charactertistics (as described above) in late 2020.

Risk of AIV exposure in other high-risk groups in Cambodia outside the LBM

AIV circulation occurs also outside and as a function of the LBM network. AIVs pose a significant risk for zoonotic transmission to humans and animals due to exposure to infected poultry or contaminated poultry products. Indeed, several cases of zoonotic transmission of AIV to wild, domestic and peri-domestic animals have been reported. Often times, these zoonotic infections occurred from animals being housed near or with wild bird species or poultry or by being fed foodstuffs consisting of or in contact with raw poultry products. In all cases, exact diagnosis was unknown until post mortem exam and animals were manipulated with minimal biosecurity, causing a risk of contamination. Cambodia has a rich array of wildlife with 212 mammal species, 536 bird species and 176 reptile species, many of which are under threat, endangered or critically endangered. Therefore, Cambodia has a large cohort of conservation groups and facilities working with and for these species. In addition, a thriving pet market, especially in Phnom Penh, houses numerous types of animals together without regard for biosecurity. These animals can include anything from domestic dogs and cats to small rodent species, small birds and even large poultry (chickens, turkeys). All of these animal species have been shown to be susceptible to AIV infection. Like LBM workers, pet store workers, animal handlers, zookeepers, ornithologists, hunters, wildlife rehabilitators, scientific collectors and field biologists are all at risk from the same zoonotic infections with AIV, many of which can be subclinical. In 2019, IPC began to survey individuals who work in close contact with wild and captive animals to determine risk exposure and studies are ongoing and will continue into 2020.

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

	Test	Timing of follow up	Total number of samples received	Number of test performed	Accomplished %
Pregnant women / Mother	HBV VL	Inclusion	1193	1193	100%
		Week 4 post-partum	255	200	78%
		Delivery	919	7623	83%
		Week 24 post-partum	643	453	70%
		Extra inclusions	15	15	100%
Baby	HbsAg ELISA	Birth	891	711	80

During our 2018-2019 study, in collaboration with national and provincial officials of NAHPRI, we continued to identify, enroll and track middlemen between Takeo market and Phnom Penh as well as collect poultry samples (oral and cloacal swabs) from middlemen's homes, provincial or district markets and/or previously identified novel stock houses to the central live bird market located in Phnom Penh. Focus was placed on middlemen previously enrolled in the 2017-2018 study and on trying to enroll those who collect and transport from the Vietnam border. As the peak in avian influenza virus circulation in poultry markets in Phnom Penh is usually associated with the four main festival periods (Lunar New Year, Khmer New Year, Pchum Ben and Water Festival), similar to the previous year, we focused our investigation and sampling over the three weeks associated with the festival periods as well as three weeks in non-festival periods. In addition to NAHPRI, this study was designed and conducted in the field in collaboration with IPC's Epidemiology and Public Health Unit. Tracking, poultry movement data, and risk assessment for the 2019 season was started in January 2019 with 7 middlemen out of Takeo market in Takeo Province and Orussey market in Phnom Penh. Tracking continued through the end of April 2019 with all middlemen

participating until the end of the study. Unfortunately, despite numerous active efforts being made to identify further middlemen to bring into the study, no middlemen agreed to participate that represented linkage between Cambodia and Vietnam. Data from the 2018-2019 tracking is being analyzed and assessed for quality and attempts are being made to confirm points of interest and novel hotspots, including those identified in the 2017-2018 study. This study was done in partnership with the MOH, NAHPRI, FAO, WHOCC, US-CDC, NIPH, Kantha Bopha Hospital, and the United States Naval Army Medical Research Unit (NAMRU)-

Team leaders: Erik Karlsson, Sowath LY, Malen Chan

Funding: US-DHHS, USAID, FAO, WHO, IPC

4.4.2.3 HIV AND VIRAL HEPATITIS

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

This prospective study aims to evaluate the effectiveness of a strategy based on the use of RDT for the diagnosis of HBV infection in pregnant women in Cambodia and the use of a treatment by tenofovir ("test and treat" strategy) to prevent HBV mother-to-child transmission. The study plans to include around 300 women positive for HBe antigen (HBeAg) receiving tenofovir and 600 women HBeAg negative without tenofovir treatment.

Our team is involved in quantification of HBV DNA viral load (VL) at inclusion and during the follow-up of mothers at different timings up to six months post-partum. Additionally, Hbs antigen (HbsAg) detection with serology methods is performed from dried-blood spot (DBS) samples from babies at delivery timing. This will allow us to correlate HBV VL results from both serum and DBS samples type.

Enrolments started in October 2017. By 23rd of March, 2020, 1,193 women were included in the study and had an HBV VL performed. HbsAg ELISA was performed for 711 babies at delivery timing on DBS with a positivity rate of 10.8% (77/711). This study is being done in partnership with the EPH (L. Borand), ANRS (O. Segeral) and Calmette Hospital (C. Chhun).

Table below presents the progress of HBV VL and HbsAg analysis:

	Test	Timing of follow-up	Total number of samples received	Number of tests performed	Accomplished %
Pregnant women/ Mother	HBV VL	Inclusion	1193	1193	100%
		Week 4 post-partum	255	200	78%
		Delivery	919	762	83%
		Week 24 post-partum	643	453	70%
		Extra inclusions	15	15	100%
Baby	HbsAg ELISA	Birth	891	711	80%

Table below present the progress of analysis performed at the Virology Unit as part of the TA PROHM study.

	Test	Timing of follow-up	Total number of samples received	Number of tests performed	Accomplished %
Pregnant women/ Mother	HBV VL	Inclusion	1193	1193	100%
		Week 4 post-partum	255	200	78%
		Delivery	919	762	83%
		Week 24 post-partum	643	453	70%
		Extra inclusions	15	15	100%
Baby	HbsAg ELISA	Birth	891	711	80%

Team Leader: Julia Guillebaud (in Virology)

Funding: ANRS

Prevalence and phylogenetic analysis of hepatitis b in captive and wild-living Pileated gibbons (Hylobates pileatus) in Cambodia

Hepatitis B virus (HBV) is a public health problem worldwide. Apart from infecting humans, HBV has been detected in nonhuman primates including captive gibbons. The aim of this study was to determine the prevalence of HBV in wild-living gibbon populations in western and eastern provinces of Cambodia and compare HBV DNA strains collected from these animals with samples from captive-gibbons at the Phnom Tamao Wildlife Rescue Center, Cambodia. HBV infection was screened from blood and stool samples from captive pileated gibbons (Hylobates pileatus), and stool samples from wild gibbons. All samples with detectable HBV DNA viral load were subjected to sequencing in the HBV Reverse Transcriptase (RT) coding region with overlapping of S-gene. Overall, 45.8% of wild-living pileated gibbons sampled from west Cambodia were positive for HBV. Phylogenetic analysis of HBV DNA sequenced from wild-living gibbons and captive-gibbons revealed that gibbon strains were closely related to each other and different to human viral strains. Our findings support previous assumptions that 1) the strain of HBV identified in gibbons is species-specific, 2) wild-born captive gibbons positive for HBV became infected prior to captivity, and 3) it might be appropriate to release captive gibbons that test positive for specific serological markers of HBV provided they are otherwise suitable candidates for reintroduction. The analysis is done in collaboration with the Wildlife Alliance (N. Marx).

Team Leader: Philippe Dussart

Financial support: Wildlife Alliance, Institut Pasteur du Cambodge.

Clinical utility of resistance-associated substitutions characterization in patients infected with HCV genotype 6 failing direct-acting antivirals treatment in Cambodia

Direct-acting antivirals (DAAs) based treatment targeting multiple regions of HCV have proven remarkably efficient, with high rates of sustained virological response (SVR) defined as having undetectable HCV RNA viral load (< 15 IU/mL) for 12 – 24 weeks after the end of treatment (SVR12 or SVR24). Despite the efficacy of DAAs, a small proportion of treated patients experience virological failure associated with resistance of HCV to DAAs, which is caused by emergence of DAA resistance-associated substitutions (RASs) in HCV genome. Many RASs have been described among patients infected with HCV genotype non-6. For HCV genotype 6, which is predominantly circulating in Cambodia and other countries in South East Asia, very few data on RASs are available. Therefore, the knowledge of RASs profile presenting in HCV genotype 6 strains and their clinical impacts is urgently needed.

The main objective of this study is to examine the presence and clinical impact of RASs in patients infected with HCV genotype 6 and failing DAA treatment in Cambodia. The specific goals are 1) to study whether DAA treatment failure is associated with the emergence of DAA RAS 2) to characterize the pattern of RASs viral diversity among patients failing DAA treatment using whole genome sequencing approach 3) to investigate whether the emergence of post-treatment RASs is associated with the selection of pre-existing substitutions among minor viral variant. HCV full genomes of 21 pairs of serum samples from patients that did not respond to HCV treatment have been obtained (genotype 6, n=20; genotype 1, n=1). Further analyses looking for RASs in pre-treatment samples and among low frequency variants is ongoing to understand the origin of the resistance. Partners in the study are *Médecins Sans Frontières* (JP. Dousset and M. LePaih, and IP Paris (E. Simon- Lorière).

Team Leader: Philippe Dussart

Funding : Médecins Sans Frontières, Institut Pasteur du Cambodge.

4.4.2.4 NEUROTROPIC INFECTION

Surveillance of enterovirus in Cambodian paediatric patients and analysis of risk factors for severe disease

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 2019, the IPC virology unit, in collaboration with the Kantha Bopha Hospitals, continued surveillance of hospitalized patients with non-severe or severe HFMD syndrome with objective to better understand circulation dynamic of EV-A71 strains at a regional level. As expected, consistent with previous observation of a cyclical pattern of EV-A71 outbreaks observed every 2-3 years and upsurge of severe HFMD cases observed in early 2017, four times more patients (n=83) were sampled in 2019 until mid-September with a more intense detection of enteroviruses during the first semester. In total, 36% (n=30) of patients had HFMD syndrome associated with encephalitis and 64% (n=53) had HFMD syndrome with encephalitis and cardiopulmonary involvement. Among these patients, 64% (n=53) had positive detection of enteroviruses (70% EV-A71, n=37; 30% other HEV, n=16).

The EV-A71 detected in 2018 clustered with other Cambodian EV-A71 within subgenogroup C4. We also were able to sequence the 5 HEV: two were negative and three were positive for Coxackievirus A6 (n=1) and Echovirus E21 (n=2). We used pastml software developed by C3BI at *Institut Pasteur* in Paris to try to reconstruct the ancestral country in order to document from which country the EV-A71 derived. The result showed that Cambodia EV-A71 were most probably introduced from Vietnam with the furthest ancestor in China. Further virological and molecular characterization of EV-A71 and other HEV strains detected in 2019 will be conducted during the upcoming year of the project aiming to provide recommendation to Cambodian MOH in terms of enterovirus surveillance. Kantha Bopha Hospitals (D. Laurent) and IP Shanghai (F. Arenzana) partner in this effort.

Team Leader: Veasna Duong

Funding: *Fondation Total*

4.4.2.5 ZOONOSES

PREDICT 2

During the PREDICT-2 Project (2015-2020), in partnership with the University of California, Davis, we focused surveillance at two high-risk interfaces based on knowledge gained during PREDICT-1. These two locations focused on 1) intensifying animal production in the bat guano trade at Varinh, Kampong Cham Province and 2) the market value chain in the rodent trade at Chrey Thom, Kandal Province. We also performed syndromic surveillance of people at three clinics that serve these two high-risk communities. We performed "One Health" concurrent longitudinal surveillance of people, their animals and wildlife at these communities and also surveyed the people sampled about their daily activities and occupations to better understand the risk of spillover and to inform on behavioral change to decrease risk. Viral family testing was performed for seven viral families/genera at *Institut Pasteur du Cambodge* with concurrent training and transferring of protocols to the national animal and human laboratories.

In total, samples were collected from 1,803 people, 2,715 domestic animals and 5,890 wild animals, including 875 rodents and 5,006 bats. PCR screening detected 20 known and 11 PREDICT-1 viruses and five new viruses. Our testing has expanded our understanding of viruses circulating in people, leading to the detection of viruses not routinely tested for in people with Influenza-like illness (ILI), sudden, acute, respiratory infection (SARI), fever of unknown origin (FUO), hemorrhagic fever and encephalitis. Full genome sequencing was performed to further characterize seven viruses detected in animals. PREDICT-2 also supported ""One Health" initiatives for effective collaboration across disciplines and government sectors, including engagement with the Cambodian Zoonotic Technical Working Group and veterinary and human health university faculties.

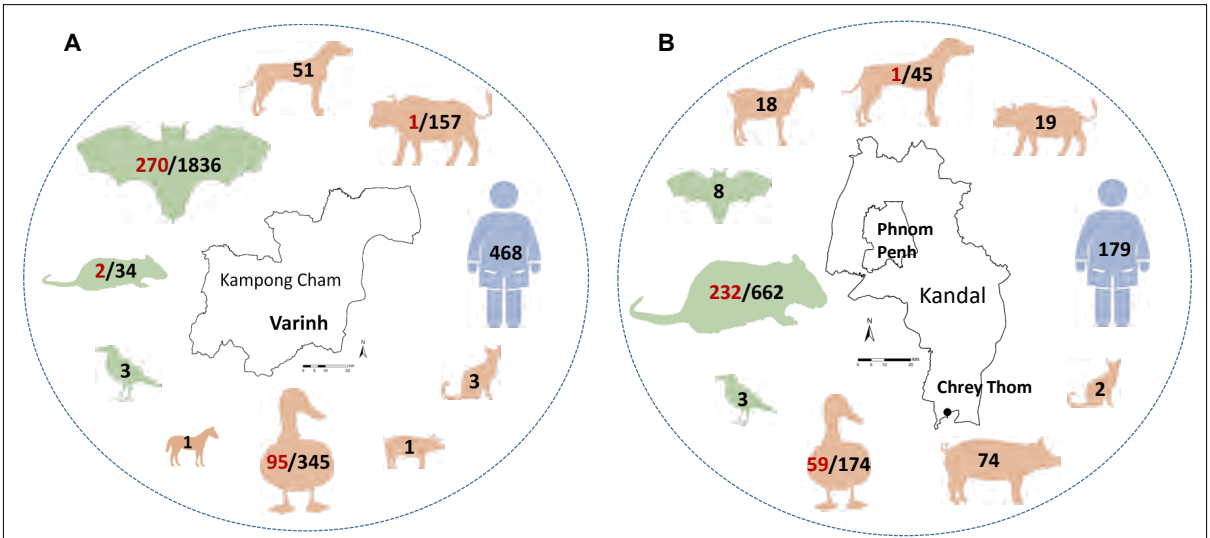


Figure: A: Number of positives by taxa group of the samples tested (A) from bat farms in Varinh, Kampong Cham; (B) from Rodent Trade at Chrey Thom, Kandal.

Major achievements- 2019

PREDICT-2 performed the first ever concurrent sampling of wildlife, domestic animals and people in Cambodia, with excellent collaboration from the human, animal and forestry sectors of the government. These multi-disciplinary teams were trained in "One Health" implementation from the field to the laboratory.

Thirty-three students from the schools of veterinary medicine (8), medicine (2) and epidemiology (23), 13 of whom were women, were trained in PREDICT laboratory and field sampling protocols. Two of these students have since been hired as permanent staff at IPC, and one has received a master's program scholarship for the "One Health" -focused InterRisk program at Kasetsart University in Thailand (www.onehealthsea.org/interrisk). These personal successes reflect the significance and workforce impacts of the project in Cambodia.

PREDICT PCR protocols have been adopted for use in outbreaks by the Cambodian Government. Two great examples include the detection of Influenza A (subtyped as H5N1) in a hairy-nosed otter at the Phnom Tamao Zoo, and the detection of Ursid gammaherpes virus after a disease outbreak in bears, also at the zoo. All of this work was done in collaboration with the Wildlife Conversation Society (WCS), the University of California Davis, NAHPRI, CDC Cambodia, and the MOH.

Team leader: Veasna Duong

Financial support: USAID

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

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 (Blasdell K et al., 2016). One of the discovered viruses is a variant of the Wēnzhōu virus (WENV), formerly isolated in Eastern China (Li K et al., 2015) and other designates as Loei River Virus (LORV) from the north of Thailand. This project initially focused on the cultivation of this Cambodian variants of WENV and LORV. Several trials under diverse cultivation conditions were conducted using simian, canine and rodent cell lines. Both viruses were successfully cultivated in macrophage and fibroblast cell lines from *Rattus norvegicus*, the same rat species that was found as host for WENV first in China and in Cambodia. However, these cells are not suitable for use in cell-based assays as they barely exhibit a cytopathic effect after infection even with high concentration of virus. In 2019, the Ph.D. work of a staff member of the virology unit in collaboration with the University of Aix-Marseille began developing specific antigens for Enzyme-Linked Immunosorbent Assay (ELISA) and molecular assays like quantitative Polymerase Chain Reaction (qPCR) to evaluate the prevalence of mammarenaviruses in Cambodia in retrospective and prospective samples. This tool development was in partnership with Aix-Marseille University.

Team Leaders: Heidi Auerswald, Veasna Duong

Funding: *Institut Pasteur du Cambodge*, Campus France

Investigation of rabies neutralizing antibodies in dogs for monitoring vaccination effect using fluorescent antibody virus neutralizationttest (FAVNT)

Rabies is a viral disease that can affect all mammals, but infected domestic dogs cause the majority of estimated 59,000 human annual cases globally, and estimated 800 cases annually in Cambodia. Neutralizing antibodies are considered a correlate for protection from the lethal disease. One of the serological assays recommended by OIE and WHO for the detection of these is the fluorescent antibody virus neutralization test (FAVNT).

With the assistance of ANSES (OIE Reference Laboratory for Rabies, Malzeville, France), we conducted an international workshop on FAVNT training including people from Faculty of Agriculture, National University of Laos in Vientiane and National Institute of Hygiene and Epidemiology in Hanoi. Afterwards, the FAVNT was set-up at IPC and was used first for the monitoring of neutralizing antibodies in a dog vaccination study conducted by IPC's Epidemiology and Public Health Unit.

The investigation of the vaccine campaign in Kandal revealed that more than 80% of the dogs had neutralizing antibodies even one year after their vaccination. The vaccination success was further elevated by an additional booster vaccination, as then 99% of the dogs were protected. The FAVNT is an important tool for future rabies vaccination studies and will be used next for the investigation of a canine vaccination study in Battambang. Partners were ANSES (M. Wasniewski and F. Cliquet), CIRAD (Véronique Chevalier), and the EPH Unit of IPC (Sowath LY)

Team Leader: Heidi Auerswald

Funding: Swedish Research Council

Establishment of the CANARIES Network

The Consortium of Animal Networks to Assess Risk of Emerging Infectious Diseases through Enhanced Surveillance (CANARIES) came together for the first time 12-14 June 2019 in Phnom Penh, Cambodia. The inaugural meeting, hosted by the IPC with sponsorship from the Defense Threat Reduction Agency (DTRA), Cooperative Threat Reduction, Biological Threat Reduction Program and the UK Global Challenges Research Fund (GCRF), brought together representatives from Cambodia, Egypt, Israel and Chile as well as experts from the UK, Australia and the USA. CANARIES was envisioned as a network of previously established and connected formal and informal global human and animal influenza surveillance networks to apply a multisectoral, multi-level approach to integrating programs, policies, legislation, and research to achieve better ""One Health" " outcomes.

The opening day of the event set the scene for a realized CANARIES, providing background on "One Health" and Emerging Infectious Disease (EID), and an immersive live bird market (LBM) experience. The second day provided a platform for participants to share knowledge and expertise. Presentations focused on the current state of surveillance efforts in Live Animal Markets (LAMs) were delivered by researchers, as well as stakeholders from the Cambodian government and international bodies (WHO, U.S. CDC, FAO, and OIE). Panel and open discussions allowed participant-driven information sharing, covering surveillance in animals, agriculture and humans in LAMs in South and Southeast Asia, South America, West/Central Africa and the Middle East. The day wrapped up with a brainstorming activity to identify "One Health" gaps and needs in the LAM ecosystem, helping shape the CANARIES objectives and goals that were defined on the third day. The consortium is actively writing manuscripts, grants, and other collaborative efforts, and a steering committee meeting and a second consortium meeting are being planned for 2020.

Team Leader: Erik Karlsson

Funding: DTRA (USA) – United Kingdom GCRF.

4.4.3 RESEARCH PROGRAMMES – 2020 PLANS

4.4.3.1 ARBOVIRAL DISEASES

Emerging Infectious Diseases Research Center (EIDRC)

The main goal of this project is to take part in an Emerging Infectious Diseases Research Center in Southeast Asia and West/Central Africa with an inter-continental "One Health" approach designed to improve the capacity to respond rapidly and effectively to outbreaks.

The objectives are: to 1) improve the capacity to respond to outbreaks, 2) identify factors influencing emergence of RNA viruses at virus, vector and reservoir levels, 3) study host adaptive immune responses to RNA viruses, and 4) enhance surveillance, detection and understanding of transmission dynamics of RNA viruses (in Africa, Rift Valley Fever Virus and Crimean Congo Hemorrhagic Fever Virus; in South-East Asia, Dengue virus).

In Cambodia, IP, in partnership with IP Paris, IP Dakar (Senegal), and CP Cameroon, aims to increase surveillance of dengue and dengue-like illness within the country via passive and active surveillance in hospitals and field sites, to characterize the mosquito population and associated viruses from ecologically classified environments and to study host immune response to flavivirus infection.

Team leader: Veasna Duong

Funding: NIH – National Institute of Allergy and Infectious Diseases

4.4.3.2 SEASONAL AND AVIAN INFLUENZA VIRUSES

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

In collaboration with Kantha Bopha Hospital, 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 (Figure 3). Additionally, in 2016, we received an increased number of SARI cases positive for seasonal human influenza (pdmH1N1) 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. Increased morbidity and mortality was also high in the 2017 season.

To analyze samples between 2014 and 2016, we used FTD33 kits (Fast Track Diagnostics) to identify respiratory pathogen prevalence for samples of unknown etiology from all SARI samples obtained between 2014 and 2019 as well as confirmed previous influenza diagnosis as an internal positive control. Numerous viruses and bacteria were identified in these samples. In addition, further work is ongoing on investigating coinfection etiology and prevalence in these samples as a function of year. In addition, a cohort of healthy Cambodian children was collected in 2017-2018 to further understand normal colonization and chances of nosocomial infection. In addition, once all data has been analyzed the overall goal is to correlate with demographic and clinical data and to determine levels of SARI risk in subsets of Cambodian children. Attempts are being made to sequence and phylogenetically classify viruses of interest, especially RSV, Coronaviruses, and Paramyxoviruses. Analysis of database is ongoing and we are actively collaborating with epidemiologists and biostatisticians for the best way to analyze and present the data. A manuscript describing the 2014-2017 data between SARI and Healthy children as well as reasons for increased mortality from influenza between 2016 and 2017 compared to 2014-2015 is in preparation and is projected to be submitted mid-2020.

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

Through various passive surveillance systems, IPC has detected a number of zoonotic transmission events in mammals, especially in captive populations. One such event occurred in 2015 and another in 2017. In 2017, we were able to isolate these viruses and, in 2018, have commenced full risk assessment on these isolates including phylologenetics/molecular analysis, antigenic testing and mammalian studies in conjunction with Dr. Stacey Schultz-Cherry at St Jude Children's Hospital in Memphis, TN as part of the Centers for Excellence in Influenza Research and Surveillance (CEIRS) network. Sequencing, molecular, and in vitro work has been completed. In vivo work was completed mid-2019 and the full risk assessment is projected to be completed and a manuscript submitted by mid-2020.

Surveillance of avian influenza and identification of hotspots of spill over 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 2019, in collaboration with the Wildlife Conservation Society (WCS), NAHPRI and other international partners, we seek to start a project looking at influenza prevalence in wild birds in the Mekong Delta region to gain an understanding of basic prevalence and to identify potential hotspots of spillover from wild populations to domestic poultry. Discussions between DTRA, NAHPRI and WCS have commenced and sites have been selected for this study. Final funding approval is pending from DTRA; however, preliminary sampling commenced in mid-2020.

4.4.3.3 ZOONOSES

HEPAR Project: Rodents as reservoirs for Hepatitis E Virus (HEV) Arenavirus and other rodent-borne viruses and risk assessment of infection in human in Cambodia

Context: Rodents are known to host several pathogens that might infect human including bacteria (leptospirosis, rickettsiosis, etc.) and viruses (arenavirus, Hepatitis E virus, Hantavirus, etc.). Zoonotic cases of Hepatitis E virus (HEV) infection are caused by genotypes HEV-3 and HEV-4. However, zoonotic potential of HEV-C hosted in rodents has been suggested recently. Two novel arenavirus species have been identified in southern Cambodia and in Thailand in two peridomestic Rattus species and zoonotic infection in human has been lately reported.

Main objective: To investigate HEV, Arenavirus and other rodent-borne virus infections in rodents and estimate the seroprevalence of those rodent-borne virus infection in human in both urban and rural environments.

Methods: A cross-sectional prospective study in selected open markets of the capital Phnom Penh and rural villages in Kampong Cham, Preah Sihanouk and Mondulkiri provinces during dry and rainy seasons will be carried out.

Expected results: 1) to document the presence of HEV, Arenavirus and other rodent-borne viruses in urban and rural settings in Cambodia, 2) to provide relevant and updated information to public health on this rodent-borne viruses seroprevalence in at-risk population, and 3) to contribute to a better understanding of the risk of occurrence of these zoonotic viruses.

4.4.4 SUPPORT TO NATIONAL AUTHORITIES

National dengue surveillance in Cambodia

As part of a collaboration with the WHO and Cambodia's National Dengue Control Program (NDCP) and within the framework of a national programme on outbreak missions, the virology unit's laboratory received, over 2019, samples from six provincial hospitals and the National Pediatric Hospital in Phnom Penh and from clinical laboratory from IPC. These surveillance sites in Cambodian provinces are located within high risk areas of dengue haemorrhagic fever (high population density, presence of the vector, history of dengue in the region). Results from the monitoring of haemorrhagic syndromes are reported weekly to the various monitoring programme participants (Director of the NDCP, hospital physicians, etc.).

DENV-1 was the main dengue serotype detected from 2011 to 2015, and 2016 was marked by an increased detection of DENV-2 in Cambodia. In 2017, we continued to detect mainly DENV-2, while DENV-1 was still present in the country with a low circulation of dengue viruses. The recrudescence of dengue in 2018 was much higher compared to previous years, and 2019 was marked by a dengue outbreak with more than 68,000 hospitalized cases and 48 deaths reported countrywide. For comparison, in 2018, 24,684 cases and 23 deaths were reported. DENV-1 was the main serotype detected in 2019 (67%) then DENV-2 (29%), while DENV-4 was sporadically detected (4%). The previous huge dengue outbreak occurred in 2007 with around 40,000 hospitalized cases and was caused by DENV-3 serotype.

Cambodian National Influenza Centre and H5 Reference Laboratory

Seasonal Human Influenza Viruses Surveillance

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

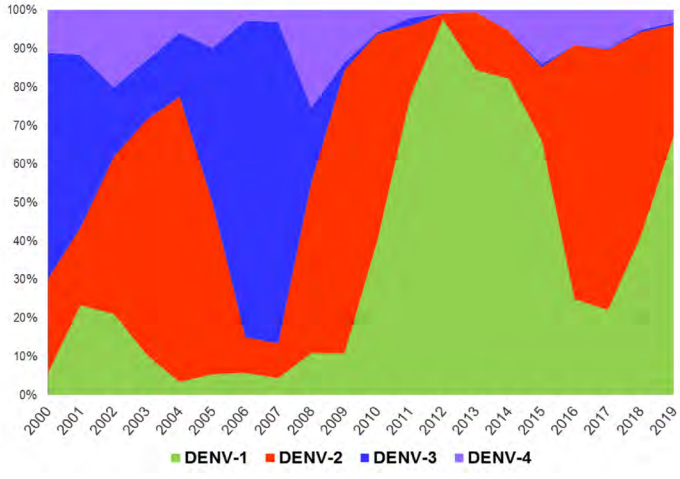


Figure : Proportion of dengue serotype detected by dengue laboratory surveillance in Cambodia from 2000 to 2019.

In 2019, 350 samples were received through the national surveillance system that had previously tested positive by the NIPH and AFRIMS referral laboratories. These samples were tested for influenza viruses and subtyped using the PCR methods described above. These results also closely agreed with the results from the NIPH laboratory; however, n=3 samples

previously tested positive at referral labs were negative when testing at IPC. We also received 99 negative samples for quality control purposes. All of the samples were confirmed as negative in exact concordance with the referral laboratories.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 the predominant one circulated in this period. Cell-culture isolation of influenza viruses on MDCK-SIAT cells was attempted for 343 positive specimens in this reporting period. Of all attempts at virus isolation, influenza viruses were successfully isolated from 90.4% (n=310) of samples. Isolation was not successful from 20 strains subtyped as A/H1N1pdm09, 7 strain of B/Victoria, 5 A/H3N2 and 1 B/Yamagata strain.

During this period human seasonal influenza subtype A/H1N1pdm09 (n=167, 47.4%) predominated, followed by B/Victoria (n=111, 31.7%), A/H3N2 (n=64, 18.3%) and B/Yamagata (n=8, 2.3%), respectively. Influenza A/H1N1 seasonal viruses was not detected.

Subtyping of influenza A viruses was achieved through targeted testing using HA1pdm09, HA3, NA1pdm09 and NA2 real-time RT-PCR assays. Analysis of the influenza B viruses revealed that influenza B/Yamagata lineage was the predominant one circulated in this period. Cell-culture isolation of influenza viruses on MDCK-SIAT cells was attempted for 343 positive specimens in this reporting period. Of all attempts at virus isolation, influenza viruses were successfully isolated from 90.4% (n=310) of samples. Isolation was not successful from 20 strains subtyped as A/H1N1pdm09, 7 strain of B/Victoria, 5 A/H3N2 and 1 B/Yamagata strain.

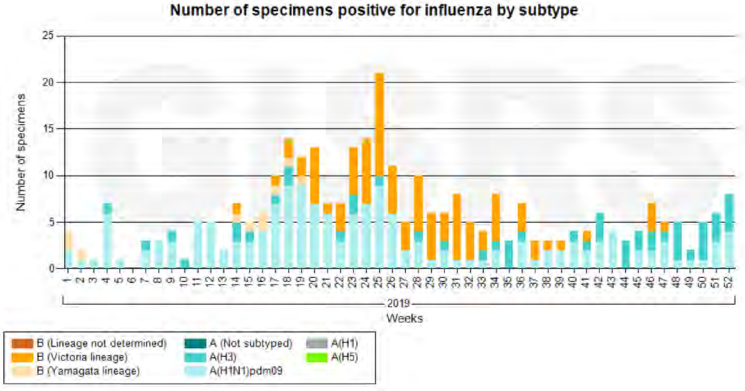


Figure : Positive influenza samples from ILI surveillance in Cambodia, 2019. Data source: FluNet (www.who.int/flunet), GISRS.

In total, 180 virus isolates were antigenically characterized using the hemagglutination inhibition (HAI) assay: 95 A/H1N1pdm09 were A/Michigan/45/2015-like virus, 65 B/Victoria were B/Colorado/6/2017-like (with 7 were low reactors), 14 A/H3N2 were A/Switzerland/8060/2017-like virus (with 4 were low reactors) and 6 B/Yamagata were B/Phuket/3073/2013-like viruses.

Neuraminidase inhibition analysis (SARI) of selected 71 A/H1N1pdm09, 56 B/Victoria, 35 A/H3N2 and 3 B/Yamagata strains isolated in this period have been done to determine the sensibility to the drug oseltamivir and zanamivir to these viruses. All viruses tested are sensitive to these antivirals. Additionally, 47 and 50 of the viruses collected, isolated and analyzed during this period were sent in July 2019 and in January 2020 respectively to the WHOCC in Melbourne, Australia for further analysis. All isolates were of similar antigenic lineage to those tested at IPC.

Severe acute respiratory illness (SARI) surveillance in humans

Nasopharyngeal and throat swab samples are collected from patients presenting to Cambodian hospitals from the KBH hospital system with severe acute respiratory infections. In 2019, 94 samples were received from KBH and were screened for influenza A, influenza A/H5N1, influenza A/H7N9 and MERS-CoV. Samples were received from Jayavarman Hospital in Siem Reap (n=73) and Kantha Bopha Hospital in Phnom Penh (n=21). The samples were all negative for A/H5N1, A/H7N9 and MERS-CoV but 33 (35%) SARI were positive for A/H1N1pdm09 influenza viruses, 4 (4%) were positive for influenza B/Victoria viruses, 2 (2%) were positive for A/H3N2 influenza viruses and 1 (1%) were positive for influenza B/Yamagata virus. In addition to the seasonal ILI samples, we have also sent samples from 16 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. A manuscript detailing human seasonal influenza circulation between 2016 and 2019 is being finalized and will be submitted for publication in early to mid-2020.

Diagnostics for rabies infections

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 is also available at the Virology Unit.

During 2018, the Virology Unit received 173 brain samples from dogs, a decrease of 10% compared to 2017. The positivity rate also increased to 67.6% (n=117) of dog brain tested in 2018 compared to 2017 (60.9%, n=117). From February to June 2019, IPC's rabies prevention center had to face a an upsurge of people coming to receive post exposure vaccination following a Facebook post of a child with clinical symptoms compatible with a rabies infection. During this period, we stopped receiving animal heads for logistics reason. In total, in 2019, the number of dog brains tested (n=125) decreased as we received 28% fewer samples than in 2018. However, the number of samples testing positive for rabies remained high at 68.8% (n=86). The average percentage of positive dog heads observed from 2002 to 2019 is currently 51.1%.

4.4.5 TEACHING AND TRAINING

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

In 2019, the virology unit received one foreign student (Resident in medical biology from *Agence de Santé* -île de France, as well as seven Cambodian students (one bachelor of pharmacy and six lab technician students). Moreover, two staff from National Health Laboratory from Yangon, Myanmar (one microbiologist and one lab technician) were trained to improve their laboratory capacities in rabies diagnosis.

Additionally, two virology staff were trained at *Institut Pasteur* in Paris in bio-informatics/bio-statistics (mathematical modelling of infectious diseases) and NGS data analysis (C3BI: The Center of Bioinformatics, Biostatistics and Integrative Biology) for seven and five weeks, respectively. Also, one virology staff in PhD program in the field of virology at Aix Marseille University spent 4 months in Xavier de Lamballerie Lab (first year of his PhD).

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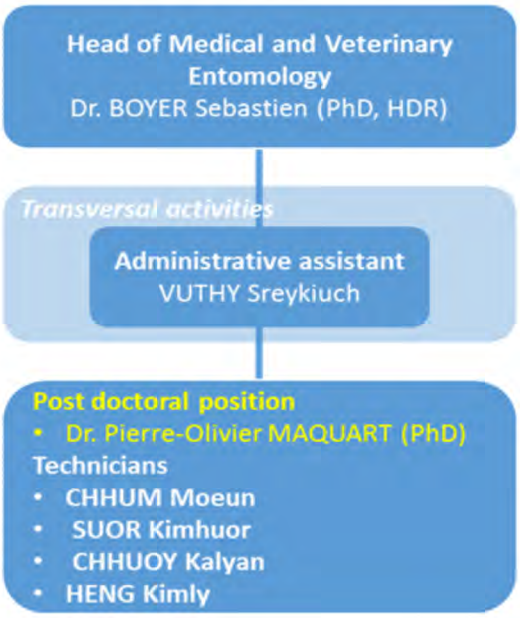
4.5 MEDICAL ENTOMOLOGY

4.5.1 FUNCTIONAL STRUCTURE OF THE UNIT

The Medical Entomology Unit was officially created on the October 1 2018 (N/Réf: N°413/IPC/DIR/2018) at which time Sebastien Boye was recruited by *Institut Pasteur* in Paris for a permanent position. In March 2020, the unit has seven members:

- Sebastien BOYER (Ph.D., HDR), head of Unit,
- Pierre-Olivier MAQUART (PhD.), post-doc,
- 4 technicians in Medical Entomology: CHHUM Moeun, SUOR Kimhuor, CHHUOY Kalyan, HENG Kimly
- VUTHY Sreykhiuch, secretary of the Unit.



4.5.2 RESEARCH PROGRAMS – MAJOR ACHIEVEMENTS IN 2019

Field missions in 2019

Regarding the following projects, in 2019 the Medical and Veterinary Units had 26 field missions in Cambodia and 78 sampling missions in Phnom Penh city. As cumulative days of missions, technicians spent 500 days in the field, (representing, for each, between 72 and 102 days). To this number, we can add 45 field days for the Head of Unit. Finally, another 41 days can be taken into account for 7 missions outside the country for meeting, workshops and congress. Therefore, the grand total of field mission is 586 days for the medical entomology team.

Ecomore 2 project

The Ecomore 2 project is funded by the while *PANIC project* is financed by the European Union. The objective of the project was to determine if a successful integrated vector management (IVM) in localized areas could decrease the incidence of Dengue virus in communities in rural and peri-urban areas. In schools, the IVM was divided in 4 distinct interventions: 1) elimination of breeding sites with the help of students and their professors, 2) scientific animation in the school with education and sensitization of children, 3) the use of a bacterio insecticide (*Bacillus thuringiensis israelensis*) used as a larvicide in water jars and big reservoirs of water, and 4) use of an auto-dissemination insecticide (Pyriproxyfen) for the dissemination of insect growth regulator larvicide and spores of *Beauveria bassiana* for slowly killing adults. The principal expected outcome of the study was the number of dengue-like fever cases in the villages around schools.

We were able to observe if the focalized vector control methods were efficient. Entomological preliminary data during the second year, following interventions, showed a decrease of 50% of *Aedes aegypti* relative abundance in treated clusters compared to untreated ones. The latter showed an average relative abundance of *Ae. aegypti* similar to the first year. The difference for *Ae. albopictus* relative abundance was not significant between the two areas. Epidemiological data acquisition are still in progress: between May and 15 August 2018, 485 dengue-like syndromes were detected-320 in the control area and 165 in the treated area.

The results were shared with the MOH, Ministry of Education, Youth and Sport, and Ministry of Agriculture, Forestry and Fisheries in 2018, 2019 and 2020. The aim of the project is to propose an appropriate intervention strategy to the MOH. The project is scheduled to end in 2020.

DARPA-PREEMPT project

The PREEMPT project aims to determine the mosquito species and virus families present in conserved biodiversity areas. The objectives will be to sample different areas, such as wildlife parks, high conservation areas, and nature parks in order to determine the presence of potential viruses. This work is coordinated with the Ministry of Environment and World Conservation Society in Cambodia.

In 2019, three sites were sampled during both dry and rainy seasons. Prek Taol Wildlife Sanctuary, located in Battambang Province is recognized as a main place for migratory birds in Cambodia. Peam Krasaop Wildlife Sanctuary, located in Koh Kong province, is a mangrove sanctuary. Virachey National Park located in Ratanakiri Province, is described as a primary forest hosting several monkey (macaca, gibbons) and large mammals species (bears, elephant, muntjac deer). An important diversity of mosquito species was observed in the three sampling sites with more than 32 mosquito species representing also a first description of their biodiversity in these areas, including the discovery of new mosquito species for the country. Finally, the mosquitos collected during Year 1 were sent to Institut Pasteur in Paris to determine the presence of virus in the different species.

FSPI project

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

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

In 2019, 12 missions were realized for FSPI projects aiming 5 different areas in 5 different provinces, *ie* Pailin, Preah Vihear, Kampong Som, Battambang and Kampong Thom.

Mosquito species and dynamics in Phnom Penh

The diversity, distribution and seasonality of mosquito species in Phnom Penh is unknown. My team proposed to carry out a weekly follow-up during one year to fill this knowledge gap. We planned to study 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 pagodas.

The field missions in 40 sample points in Phnom Penh began in 2019. The sampling is realized twice a week in Phnom Penh. The objective will be to evaluate the dynamics, and the risk associated with the potential presence of mosquito vector species.

This work is realized in collaboration with the Ministry of Cults and Religion.

Communication and workshops

The second steering committee of the project Ecomore 2 was held in Hanoi (14-17 January 2019), and the 1st national Stakeholders meeting was held in Lipa city in Philippines (27 February 2019). An international meeting with IP Laos, IP Hong-Kong, IP Shanghai on outbreak preparedness and readiness in the Lancang Mekong cooperation region was organized in Kunming, China (May 2019). An exploratory mission was realized in Myanmar in 2019 to meet the NHL partner for entomological collaborations. The 3rd steering committee of Ecomore2 was in Vientiane

(Lao PDR), end of November 2019. Finally, a Leishmaniosis meeting for developing an international consortium was organized by IP Paris in Bangkok in November 2019.

4.5.3 RESEARCH PROGRAMS – 2020 PLANS

DARPA-PREEMPT project

The DARPA-PREEMPT missions will continue during project year 2 with six field missions, three during the dry season and 3 during the rainy season. The three selected sites for 2020 are Phnom Kulen National Park, Phnom Tnout Wildlife Sanctuary and Phnom Aural Wildlife Sanctuary. Mosquitos will be identified and then sent to IP Paris for virus screening.

FSPI project

The FSPI project is also scheduled to end in 2020 and 20 more missions will be undertaken by this time. One postdoctoral scientist arrived in January 2020 to take in charge this project. Within this year, two trainings will be organized within the framework this project with one training on mosquito taxonomy and one training on molecular entomology related to the detection of specific viruses.

Mosquito species and dynamics in Phnom Penh

The characterization of the mosquito dynamics in Phnom Penh will be done in 2020 and 2021. The field missions are expected to end in April 2020. Thereafter, a second year master's degree student from RUPP will analyze the data. A collaboration regarding these data will be developed with IRD scientists. The objective of this collaboration is to develop population dynamics models for *Aedes aegypti* and *Aedes albopictus* mosquitos in Phnom Penh. These models are very useful for testing control and monitoring methods, assessing the effectiveness over time of vector control methods and identifying trends in mosquito populations in the context of global changes.

Resistance to insecticides

The resistance to temephos (larvicide), permethrin and deltamethrin (adulticide) that are used in Cambodia were tested on the main dengue vector species. We demonstrated that *Aedes aegypti* species (four populations) were resistant to the three currently used insecticide in Cambodia. The results were shared to the Ministry of Health-CNM. These results were also presented in six national and four international conferences. The activities continue and should be completed in 2020 with the test of new larvicides and adulticides to be able to propose new alternatives for the Ministry of Health. We will test 15 adulticides and three larvicides.

Vector competence

The study of vector competence of *Aedes aegypti* and *Aedes albopictus* for several viruses (DENV, chinkungunya virus, ZIKV) is performed at IPC Virology Unit PSL3 security laboratory. The objectives are to understand the role of mosquito populations in the transmission, and the adaptation of the virus to the different population, species of *Aedes* species (mainly *Stegomyia* subgenus).

Molecular Entomology

Based on the samples from the different field missions, and in the frame of the development of the unit including new methods for determining mosquito species, the molecular entomology must be developed. The development of this activity is also a necessity related to the development of one specialized taxonomist in the team. The objectives will be to describe the history of different species and the characterization of existing or novel species in the country.

MALDI TOF

The current methods for mosquito identification include both morphological and molecular method. Identification by morphology is skill-dependent and is time-consuming while the identification by PCR is expensive. The matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (maldi-tof ms) technology, now routinely used for bacterial identification, has recently emerged in the field of entomology. The aim of this study will be to use maldi-tof ms to identify mosquitos from Cambodia and to create a useful tool for the ministry of Health and also for our current partner in Southeast Asia.

Veterinary Entomology

As for the medical entomology that was absent in the Cambodian research landscape, I want to develop the veterinary entomology. Based on a common request of the Ministry of Agriculture's General Directorate of Animal Health and Production and the Royal University of Agriculture (RUA), the unit plans to propose a developmental and research project in 2020 based on babesiosis and trypanosomiasis as requested by the two national partners. It could be the first step towards further studies on ticks, responsible of animal and human pathogens' transmission.

4.5.4 SUPPORT TO NATIONAL AUTHORITIES

The medical and veterinary entomology unit works with five different ministries: Ministry of Health, Ministry of Education, Youth and Sport, and Ministry of Agriculture, Forestry and Fisheries, Ministry of Environment and Ministry of Cults and Religion

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

In June 2019, the unit organized an information meeting on the preliminary results of the entomological field study for the directors of schools involved in the ECOMORE 2 project, the four Provincial Directors of the Ministries of Education, of Youth and Sport and the Ministry of Health of the two provinces and for the working group members. Currently, the directors of the 24 selected schools participate actively in the ECOMORE operational phase by facilitating the entomological surveys. This feedback meeting was important, well perceived and very dynamic and participatory.

In July 2019, 11,000 notebooks were distributed and instructions explained to all school children from the 24 schools of the Ecomore 2 project. The Provincial Directors of Ministry of Education, Youth and Sport in Tboung Khmum and Kampong Cham Provinces were enthusiastic and suggested to provide the notebook to all schoolchildren in their provinces. Finally, technicians and officials from the Ministry of Health, specifically the CNM, participated to the Thailand International Cooperation Agency (TICA) training (see Teaching and Training section below) organized at IPC.

4.5.5 TEACHING AND TRAINING

Teaching

In 2019, Mr. Iva Song, a Cambodian second year Royal University of Phnom Penh master's degree student in the science of biodiversity for conservation, did his internship at the medical and veterinary entomology unit. His work was on the spatial distribution and dynamics of mosquitos at 40 pagodas in Phnom Penh City, Cambodia.

In 2019, Ms. Sylvaine Jego, a French second year master's student, did her internship at the same unit. Enrolled at the University of *Bretagne Sud*, France, she is specializing in statistical models as part of her studies in data science. Her specific work is focused on the determination of environmental factors explaining mosquito species' distribution with satellite data. She was co-supervised by Vincent Herbreteau (IRD).

In 2020, two additional second year master's degree students from the master's of science in biodiversity for conservation at RUPP are expected to undertake further training in the unit.

Trainings

In 2019, Vincent Herbreteau supervised one training on global information systems (GIS) for technicians. The objective was to build the competence of the technicians on QGis software in the framework of medical entomology studies.

In 2019, TICA, in the framework of the AFD-funded Ecomore 2 project organized a training on mosquito systematics in Cambodia at IPC in August 2019. Technicians and scientists from CNM and Malaria Unit (IPC) also participated.

In December 2019, a unit technician was trained for a week on Maldi Tof in the medical biology laboratory of IPC. In 2020, two taxonomy (TICA, FSPI) and one molecular entomology (FSPI) trainings are planned.

4.5.6 PUBLICATION LIST 2019

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2. Pezzi L, Rodriguez-Morales AJ, Reusken CB, Ribeiro GS, LaBeaud AD, Lourenço-de-Oliveira R, Brasil P, Lecuit M, Failloux AB, Gallian P, Jaenisch T, Simon F, Siqueira AM, Rosa-Freitas MG, Vega Rua A, Weaver SC, Drexler JF, Vasilakis N, de Lamballerie X, Boyer S, Busch M, Diallo M, Diamond MS, Drebot MA, Kohl A, Neyts J, Ng LFP, Rios M, Sall A, Simmons G. **GloPID-R report on chikungunya, o'nyong-nyong and Mayaro virus, part 3: Epidemiological distribution of Mayaro virus**. Antiviral Research, 2019, 172:104610
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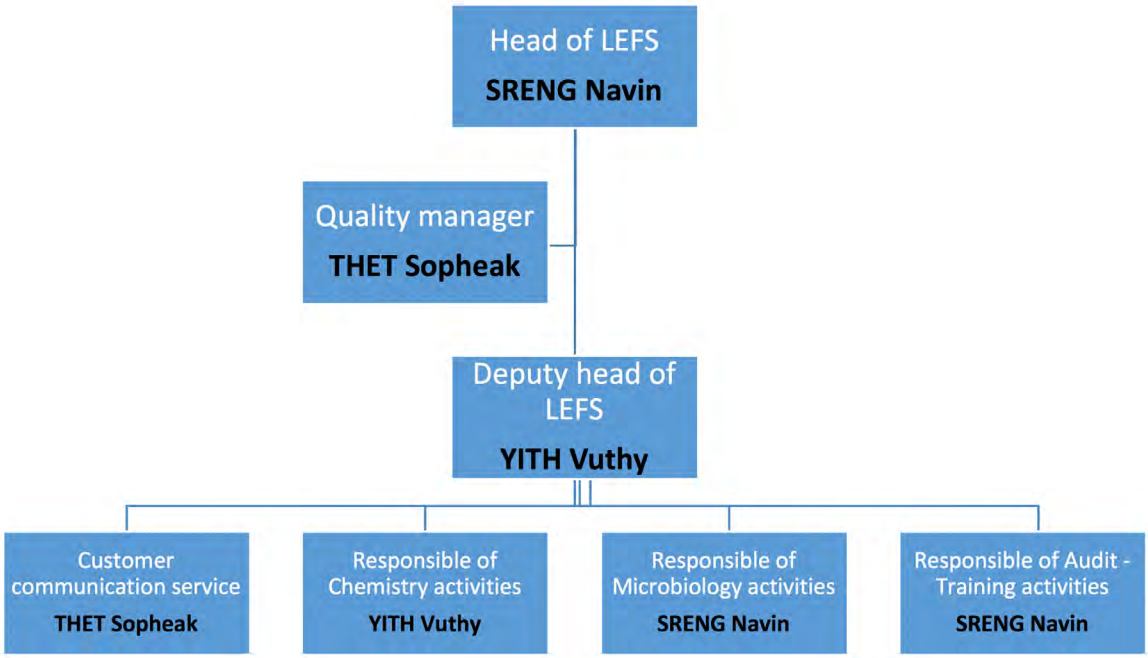
5. Moutailler S, Yousfi L, Mousson L, Devillers E, Vazeille M, Vega-Rúa A, Perrin Y, Jourdain F, Chandre F, Cannet A, Chantilly S, Restrepo J, Guidez A, Dusfour I, Vieira Santos de Abreu F, Pereira dos Santos T, Jiolle D, Visser TM,. Koenraadt CJM, Wongsokarijo M, Diallo M, Diallo D, Gaye A, Boyer S, Duong V, Piorkowski G, Paupy C, Lourenco deOliveira R, de Lamballerie X, Failloux AB. **A new high-throughput tool to screen mosquito-borne viruses in Zika virus endemic/epidemic areas**. Viruses. 2019
6. Tantely ML, Randrianambinintsoa F, Woog F, Raharininrina M, Ratsimbazafy J, Boyer S, Girod R. **Horizontal and vertical distribution of mosquitos (Diptera: Culicidae) in the rainforest of Maromizaha, Madagascar: implications for pathogen transmission to humans and animal**s. Austral Entomology, 2019, in Press.
7. Miarinjara A, Rahelinirina S, Razafimahatratra NL, Girod R, Rajerison M, Boyer S**. Field assessment of insecticide dusting and bait station treatment impact against rodent flea and house flea species in the Madagascar plague context.** PLoS NTD, 2019, e0007604.

4.6 LABORATORY FOR ENVIRONMENT AND FOOD SAFETY (LEFS)

4.6.1 FUNCTIONAL STRUCTURE OF THE UNIT



Poll of engineers, technicians for all activities

Lab technicians:

1. RIN Pheaktra
2. KEO Pheakdey
3. SEALONG Sreyneath
4. SOVANN Chakriya
5. SEM Nita
6. PROEUNG Seyha

In 2019, different changes have occurred in the team: two news staff members were recruited in November 2019. One replaced a staff member who resigned and another one was hired for sampling outside the lab and to assist with lab activities.

4.6.2 ROUTINE ACTIVITIES 2019

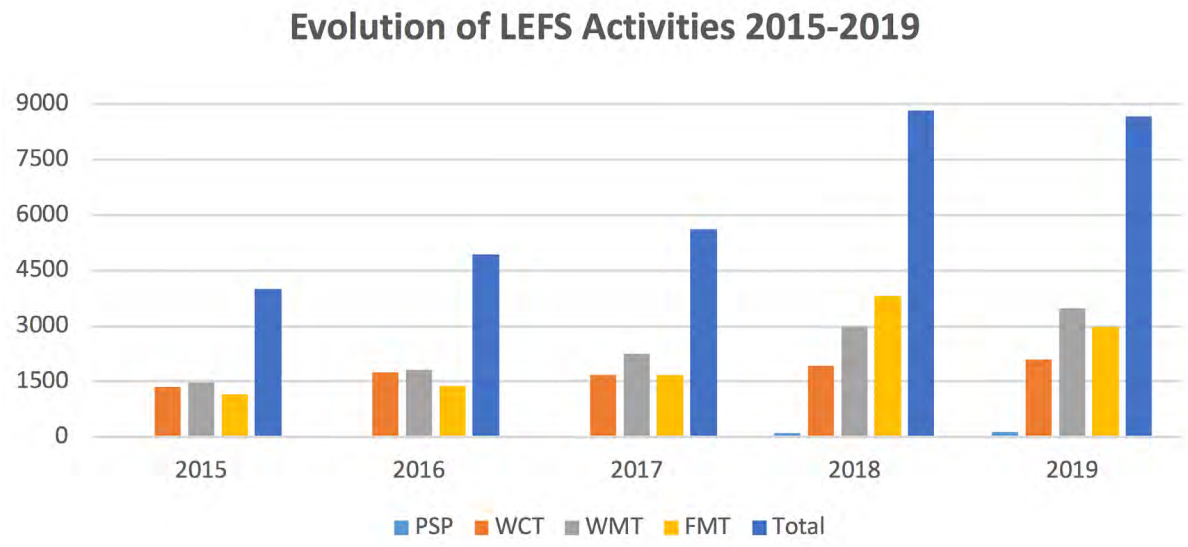
In 2019, the laboratory tested 8,666 samples comprising 2,984 samples of food, 3,477 water samples for microbiology testing, 2,082 water samples for chemical testing and 123 samples for pesticide sample preparation. We provided three trainings on personal hygiene and food safety to our customers.

Compared to 2018, the number of food samples decreased 22%, perhaps due to the absence of the street food project of the Ministry of Health (2018). However, the number of water samples increased respectively for

microbiological and chemical test by 14% and 7%. This positive progression of analytical activities over the last five years is shown in the table and figure below:

Table: Evolution of LEFS activities from 2015 to 2019

Analytical activity	Samples/Year				
	2015	**2016**	**2017**	**2018**	**2019**
Food microbiology testing	1,147	1,390	1,674	3,817	2,984
Water microbiology testing	1,485	1,810	2,256	2,985	3,477
Water chemical testing	1,362	1,738	1,681	1,930	2,082
Pesticide sample preparation	-	-	-	98	123
Total	**3,994**	4,938	**5,611**	**8,830**	**8,666**



PSP: Pesticide sample preparation, WCT: Water chemical testing, WMT: Water microbiological testing, FMT: Food microbiological testing

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

- 68.1% of food samples (2,031/2,984) were reported with conclusion whose 3.6% (73/2031) were not conclusive.
- The unsatisfactory results were due to *Salmonella* contamination (40%), and high levels of hygiene indicators such as enterobacteriaceae (21%), total coliforms (21%) and *E.coli* (14%). More than 50% of *Salmonella* positive food samples were meat products and fresh vegetables. The other products include salads, cooked food, and raw ready to eat food.
- 16% of unsatisfactory food samples were co-contaminated with at least two different germs.
- 75% of water samples for microbiology testing (2,612/3,477) for which test results were acceptable, 22.9% (597/2,612) were unfit for human consumption because of a fecal contamination.
- 29.1% of ice cube samples (171/588) served in the restaurants and bars were found to be contaminated by fecal bacteria as coliforms, *E.coli* and intestinal enterococci.
- 64.6% of water samples undergoing chemical testing and for which results were acceptable, (1,345/2,082), 5.7% (77/1345) were found not conclusive.

The following important changes occurred in 2019 in the laboratory:

- Implementation of international standard methods in microbiology to be in accordance with NF EN ISO 17025 requirements to receive an accreditation in 2022;
- Establish quality management system to ensure the integrity and the quality of our services;
- Establish systems for documentation and traceability;
- 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 requirements from the customers;
- Updating of the laboratory equipment;
- Maintenance of a biannual external verification of microbiological testing (RAEMA)
- Setting up new parameters of analysis such as *Cronobacter spp* and *Listeria monocytogenes* according to ISO/TS 22964 and NF EN ISO 11290-2, respectively;
- Renewal of customer contract in accordance with NF EN ISO 17025 requirement;
- Setting up the sampling and sample collection service for customers;
- Implementation of different quality and technical procedures according to international standards;
- Implementation of culture media production and control according to NF EN ISO 11133.

4.6.3 RESEARCH PROGRAMS - MAJOR ACHIEVEMENTS IN 2019

In 2019, the LEFS focused its activities to reinforce the laboratory competencies and develop new research projects in microbiology.

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

Between 2016 and 2017, we isolated 134 strains of *Salmonella* in different kind of food products (meat products, sea food, ready to eat food, vegetables etc.).

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 in studying the AMR of *Salmonella* strains isolated in routine in LEFS and evaluating 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 aim to determine the antibiotic resistance profile and genotypic characteristic of multi-drug resistant isolates and study the serotype distribution of *Salmonella* among different kind of food products.

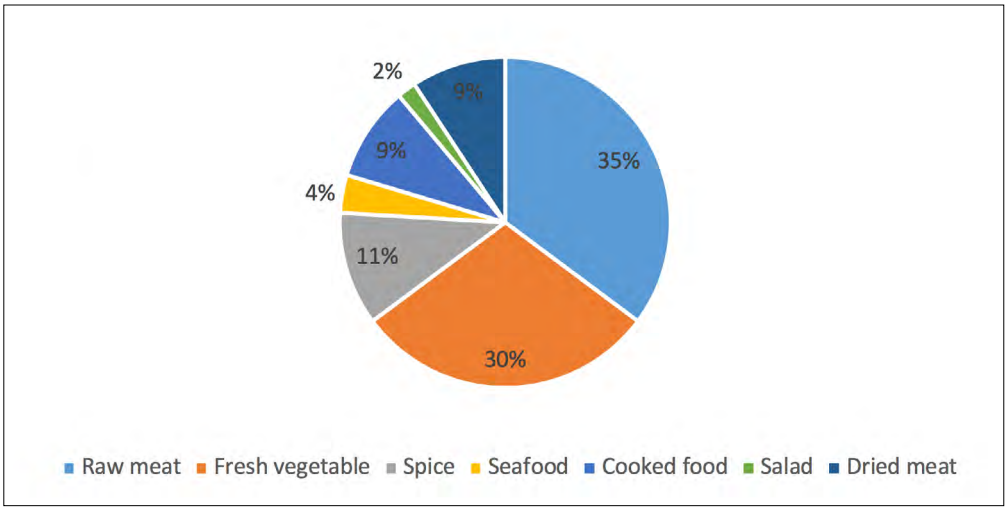


Figure: Different food type contaminated with *Salmonella*

Currently, 54 strains of *Salmonella* have been analyzed. Looking at the food type, raw meats and fresh vegetables are the most contaminated products with *Salmonella* (Figure above). The serotyping results show the enrichment of the circulating *Salmonella* serotype in Cambodia (23 different serotypes) (Figure below).

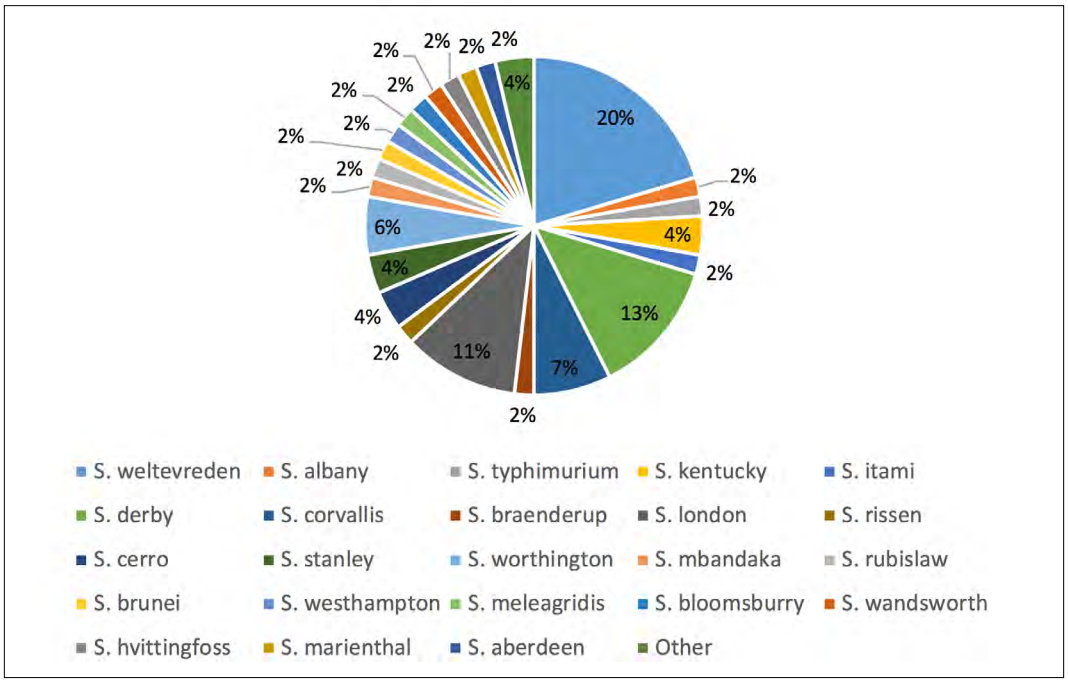


Figure: Serotype distribution of *Salmonella*

In order to study antibiotic resistance, 31 antibiotic discs from different groups were tested. Preliminary results show that 60% of strains of *Salmonella* are sensitive to all antibiotics. Eighty-two percent of resistant strains are multi-drug resistance. The strains considered multi-drug resistant are strains that are resistant to at least three different groups of antibiotics. The table below describes the serotype of multi-drug resistance *Salmonella* with their origin and the concerned antibiotics. These results show that raw meats are the most contaminated with multi-drug resistance strains. It is interesting to note that among the 54 strains tested, no ESBL strain was found.

Table: Multi-drug resistance of *Salmonella* strain

Sample	Serotype	B-lactame						Aminoside		Quinolone			Macrolide	Phenicol	Cyclines				
		Ampicilline	Ticarcilline	Piperacilline	Mecillinam	Amoxicilline + Ac clav	Tircarcilline + Ac clav	Gentamicine	Streptomycine	Nalidixic acid	Ciprofloxacine	Pefloxacin	Azithromycine	Chloramphenicol	Tetracycline	Tigecycline	Trimethoprime	Sulfamides	Trim +Sulfa
Salad	*S. albany*	■	■	■		■	■		■	■		■						■	
Cooked food	*S. typhimurium*	■	■					■	■	■		■		■	■	■		■	
Seafood	*S. kentucky*	■	■	■	■	■	■			■	■	■			■			■	
Cooked food	*Salmonella spp*	■	■	■								■		■			■	■	■
Spice	*S. kentucky*	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Raw meat	*S. derby*												■					■	
Raw meat	*S. derby*	■	■	■			■		■			■			■		■	■	■
Raw meat	*S. derby*	■	■	■			■	■	■			■			■				
Raw meat	*S. rissen*	■	■	■			■								■		■	■	■
Dried meat	*S. worthington*	■	■	■					■						■		■		
Dried meat	*S. worthington*	■	■						■								■		
Raw meat	*S. worthington*	■	■	■					■								■		
Raw meat	*S. derby*				■										■		■		
Raw meat	*S. derby*	■	■	■	■	■						■		■	■		■	■	■
Fresh vegetable	*S. meleagridis*	■	■	■		■	■					■		■	■		■	■	■

Note: Gray Square indicates resistance to an antibiotic

4.6.4 RESEARCH PROGRAMS - 2020 PLANS

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 the Cambodian Center for Study and Development of Agriculture (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 Cambodian farmers. A small study in Cambodia using qualitative methods revealed that untrained sources such as neighbors 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 moderately to extremely hazardous to human health (WHO class Ia, Ib, II). Therefore, 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 research project in cooperation with ITC we plan to study the contamination by pesticides residues in commonly consumed vegetables purchased from farms and local markets in Cambodia.

4.6.5 SUPPORT TO NATIONAL AUTHORITIES

For several years, IPC has supported different laboratories in Cambodia, including the National Public Health Laboratory, the Food and Drug Laboratory of Ministry of Health, Ministry of Industry and Handicraft, Cam Control Laboratory, Ministry of Commerce, National Animal Health and Production Research Institute (NAHPRI), Ministry of Agriculture, Forestry and Fisheries and within the private sector.

In 2019, as part of a national monitoring program, the Ministry of Health sent IPC 503 samples through sampling campaigns of industrial foods imported from different countries.

4.6.6 TEACHING AND TRAINING

In terms of training, the laboratory supervised 13 trainees coming from different universities in Cambodia for internships lasting between one and 2 months.

The details of these are described below.

Table: Internship students at LEFS in 2019

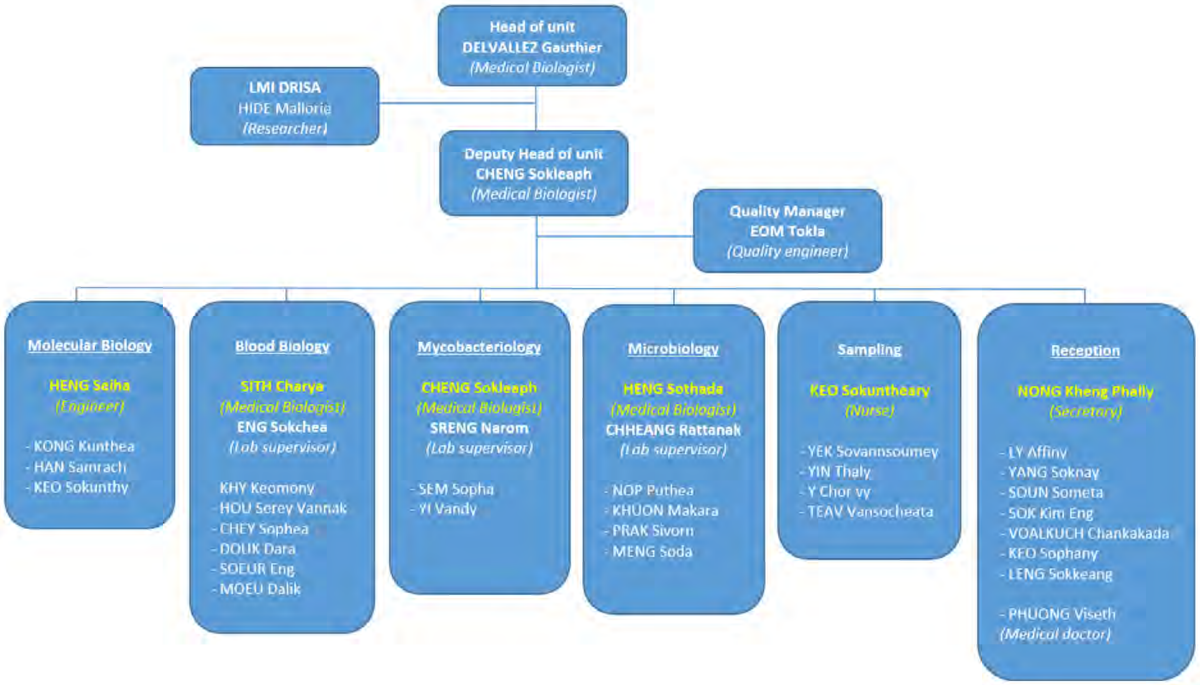
University	Number of students	Scholar Year	Period (month)	Date
University of Health and Sciences (UHS)	9	Year 3	1.5	29/11/2018 - 08/01/2019 29/11/2018 - 08/01/2019 09/01/2019 - 21/02/2019 09/01/2019 - 21/02/2019 02/04/2019 - 30/05/2019 02/04/2019 - 30/05/2019 04/06/2019 - 25/07/2019 04/06/2019 - 25/07/2019 06/11/2019 - 21/12/2019
University of Puthisastra	1	Year 3	1	04/09/2019 - 12/10/2019
International University	1	Year 3	2	01/09/2019 - 31/10/2019
RUPP (Royal University of Phnom Penh)	2	Year 3	1	01/07/2019 - 01/08/2019 01/07/2019 - 01/08/2019

4.6.7 PUBLICATION LIST 2019

1. Maya Nadimpalli, Laetitia Fabre, Vuthy Yith, Nita Sem, Malika Gouali, Elisabeth Delarocque-Astagneau, Navin Sreng, Simon Le Hello. **CTX-M-55-type ESBL-producing Salmonella enterica are emerging among retail meats in Phnom Penh, Cambodia.** J Antimicrob Chemother 2019; 74: 342–348
2. Nadimpalli M, Vuthy Y, de Lauzanne A, Fabre L, Criscuolo A, Gouali M, Huynh BT, Naas T, Phe T, Borand L, Jacobs J, Kerléguer A, Piola P, Guillemot D, Le Hello S, Delarocque-Astagneau E; **BIRDY study group. Meat and fish as sources of extended-spectrum B-lactamase-producing Escherichia coli, Cambodia.** Emerg Infect Dis. 2019 Jan;25(1):126-131
3. Hendriksen RS, Munk P, Njage P, van Bunnik B, McNally L, Lukjancenko O, Röder T, Nieuwenhuijse D, Pedersen SK, Kjeldgaard J, Kaas RS, Clausen PTLC, Vogt JK, Leekitcharoenphon P, van de Schans MGM, Zuidema T, de Roda Husman AM, Rasmussen S, Petersen B; Global Sewage Surveillance project consortium, Amid C, Cochrane G, Sicheritz-Ponten T, Schmitt H, Alvarez JRM, Aidara-Kane A, Pamp SJ, Lund O, Hald T, Woolhouse M, Koopmans MP, Vigre H, Petersen TN, Aarestrup FM. **Global monitoring of antimicrobial resistance based on metagenomics analyses of urban sewage.** Nat Commun. 2019 Mar 8;10(1):1124

4.7 MEDICAL BIOLOGY LABORATORY

4.7.1 FUNCTIONAL STRUCTURE OF THE UNIT



Thirty-nine employees work in the medical biology unit (LBM): four medical biologists (including the Head of Unit and the Deputy Head of Unit), one quality manager, 1 engineer (Molecular Biology platform), 3 Lab supervisor, 15 technicians, 8 secretaries, 5 nurses and 1 medical doctor. The LBM also hosts an IRD engineer in molecular epidemiology in the frame of the LMI DRISA (International joint Laboratory “Drug Resistance in South East Asia”).

4.7.2 ROUTINE ACTIVITY 2019

4.7.2.1 GENERAL ACTIVITY

The main part of the activity consists of medical biology routine analysis (private patients, hospitals, private clinics, non-governmental organizations (NGOs), and anonymous free testing center).

In December 2018, the medical biology laboratory of Institut Pasteur du Cambodge became the first laboratory in Cambodia awarded ISO 15189.

During 2019, the laboratory renewed its accreditation after its external audit in November and added five analyses to its accreditation scope (Syphilis serology, Magnesium, Lipase, Haptoglobin, LDH), bringing the total number of laboratory-accredited analysis to 70). Furthermore, 106,437 out of 136,416 analyses performed in 2019 are part of the scope of accreditation, which represents 78% of the laboratory activity.

Activity in 2019 increased by 8.7% compared to 2018, mainly in the blood biology laboratory (Hematology and Biochemistry).

Laboratory	Number of analysis		
	2017	2018	2019
Hematology	11,407	13,464	14,981
Biochemistry	46,305	61,299	71,617
Immuno-serology	34,026	35,177	33,311
Microbiology	8,276	7,206	7,686
Molecular Biology	10,151	1,786	2,080
Mycobacteriology	7,372	6,529	6,741
TOTAL	117,537	125,461	136,416
Balance		+ 6.7%	+ 8.7%

4,7,2,2 TUBERCULOSIS

The number of cultures for *Mycobacterium tuberculosis* (*M.tb*) diagnosis decreases slowly over the years but this is due to the increase of using the rapid molecular biology test (GeneXpert) for direct detection of complex *M.tb*. On the other hand, we can observe a slight increase in the positivity rate in the recent years (10.2% in 2018, 12.1% in 2019). In 2019, the Mycobacteriology laboratory performed 1059 GeneXpert for direct research of complex *M.tb* and Rifampicin resistant (increase of 70%).

Tuberculosis	2017	2018	2019
Number of culture	2,872	2,493	2,289
Culture MTB positive	245 (8.5%)	255 (10.2%)	277 (12.1%)
Number of GeneXpert	550	624	1,059

Over 26% of GeneXpert test research for complex *M.tb* were positive. This includes 1.8% with a positive detection for Rifampicin resistance. A weak trace of complex *M.tb* was detected in 11% of GeneXpert tests. The bacterial load were too low in those sample, make the detection of Rifampicin resistance impossible.

GeneXpert	Rifampicin resistance			TOTAL
	Positive	Negative	Indeterminate	
Positive	5 (1.8%)	273	0	**278 (26.3%)**
Trace	----	----	117	117
Negative	----	----	----	664
				1,059

The number of antibiogram requests for the tuberculosis decrease over the years.

Antibiotic resistance	2017	2018	2019
Isoniazide	8 (16%)	5 (12%)	2 (5.7%)
Rifampicine	3 (6%)	2 (4.8%)	0
Ethambutol	3 (6%)	1 (2.4%)	0
Streptomycine	8 (16%)	9 (21.4%)	7 (20%)
TOTAL	**50**	**42**	**35**

4.7.2.3 MELIOIDOSIS

25 strains of *Burkholderia pseudomallei* were isolated in the microbiology laboratory, representing a total of 22 patients. Fifty-nine % of the isolated strains come from blood cultures.

Year	Total isolates	Total cases	Blood	Respiratory	Pleural	Abscess
2017	19	17	4	6	0	9
2018	29	25	20	4	0	5
2019	25	22	13	4	2	6

4.7.2.4 ANTIMICROBIAL RESISTANCE

During 2019, the microbiology laboratory isolated 26 strains of Carbapenemase producing enterobacteria (all NDM or OXA-48), 230 strains of enterobacteria producing Extended Spectrum Beta-Lactamase (ESBL) and 68 strains of Methicillin Resistant Staphylococcus Aureus (MR.

Since May 2019, the medical biology laboratory has used the new analyzer ADAGIO for antibiotics susceptibility testing. This analyzer allows precise measurement of the diameter of antibiotic discs, standardization of the method and the no limit keeping of antibiogram picture.

In the six months between July and the end of December 2019 the microbiology laboratory of performed 647 antibiotics susceptibility tests.

July 1 – 31 December 2019	
Bacterial species	**Number of AST**
Escherichia coli	185
Staphylococcus aureus	107
Other bacteria species	82
Klebsiella pneumoniae	77
Pseudomonas aeruginosa	43
Other Enterobacteria species	39
Burkholderia pseudomallei	21
Haemophilus influenzae	20
Acinetobacter baumanii	18
Streptococcus pneumoniae	16
Moraxella cattharalis	16
Salmonella typhi / paratyphi	11
Salmonella sp.	6
Neisseria gonorrhoeae	6
TOTAL	647

Regarding enterobacteria, the percentage of ESBL during this period was 39%, with a clear predominance of *Escherichia coli*, which represents 79 % of ESBL. In addition, 53% of *Escherichia coli* strains tested in the laboratory have an ESBL phenotype. Just over four percent of enterobacteria have a carbapenemase phenotype. *Escherichia coli* represents almost all CPE strains (84.6%). All carbapenemases are NDM or OXA-48 (3 strains have both).

July – 31 December 2019			
Enterobacteria species	**Number of AST**	**Number of ESBL**	**Number of CPE**
Escherichia coli	185	98	11
Klebsiella pneumoniae	77	18	1
Salmonella typhi / paratyphi	11	1	0
Salmonella sp.	6	0	0
Other Enterobacteria species	39	7	1
TOTAL	318	124 (39%)	13 (4.1%)

During this same period, for non-fermenting gram-negative bacilli, 72.2% of *Acinetobacter baumanii* secreted a Carbapenemase, all OXA-23. The two strains of *Pseudomonas aeruginosa*, which secrete Carbapenemase, are NDM.

July 1 – December 31 2019		
Acinetobacter baumanii	18	13 (72.2%)
Pseudomonas aeruginosa	43	2

Over thirty-four percent of *Staphylococcus aureus* are resistant to methicillin.

1st July – 31th December 2019		
Staphylococcus aureus	107	37 (34.6%)

4.7.2.5 HIV SCREENING

ANONYMOUS FREE TESTING CENTER

533 patients consulted the AFTC in 2019 and benefited of a free HIV serology. The HIV-positive population represents 12.6 % of the total effective of the AFTC consultant. We can observe a high increase of HIV positive in 2018-2019, which correlates with a significant increase of homosexual prostitutes in our patients (IPC recommended by NGO?).

Status	Male	Female	TOTAL
HIV +	58	9	67
HIV -	342	124	466

HIV AFTC	2017	2018	2019
Number of HIV serology	636	680	533
HIV positive serology	34 (5.3%)	129 (19.0%)	67 (12. 6%)

HIV NOMINATIVE SEROLOGY

In 2019, the rate of seropositivity of nominative patients stayed stable with a rate at 3.9 %. Prevalence may be overestimated compared to the general population as many patients come to IPC to confirm HIV positive status from other laboratories.

HIV LABM	2017	2018	2019
Number of HIV serology	3531	3566	3306
HIV positive	133	128	129
HIV positive serology	3.8%	3.6%	3.9%

4.7.3 RESEARCH PROGRAMS – MAJOR ACHIEVEMENTS IN 2019

PTR on aspergillosis and aspergillus in Cambodia

This PTR received ethical approval from the Cambodian National Ethics Committee for Health Research on December 26, 2017. It is worth noting that due to ethical, financial, clinical and practical constraints, some adaptations were necessary and a focus on Aspergilloma was decided (new acronym: "AspA" for aspergilloma in Cambodia). A sponsorship transfer from IP Paris to *Institut Pasteur* Cambodia was accepted. The main objective of the project was the setup of the first mycology lab in the Pasteur Institute International Network. This objective was fully accomplished. Furthermore a clinical research assistant (Mala Sim) and a technician (Sreyroth Lim) were recruited to help the patient inclusion process, the monitoring of the study and lab experiments. The patient recruitment was finalized in early October 2018 with 645 included patients. Of these, 53.2% were men (mean age of 57.65 years +/- 16.18) and 46.8% were women (mean age 59.15 years +/- 15.59). Patelia ELISA® (gold standard) was positive for 14.54% of the patients tested (81/557). Eight recombinant proteins (Gel1, Crf1, Cat1, and 18Kd, Sod1, A97, H41 and H70) were tested for diagnosis purposes and only two allowed the differentiation between positive and negative serum control. The recombinant protein A97 showed promising results with a relative sensitivity of 72% and a specificity of 93%.The direct examination of the sputum was positive for 37.7% (210/557) of the patient. Four hundred and eight strains were isolated from patient and 63 from the hospitals environment.

Most of the strains belonged to the *A. niger* / *A. tubingensis* clade then *A. fumigatus* and *A. flavus*. Interestingly, the mutation TR34/L98H/T364A was recorded in Cyp51A gene of one *A. fumigatus* strain. Antifungal assays showed this mutation is responsible for a 16-fold increase of the minimal inhibitory concentration for itraconazole and thus indicate a high resistance phenotype (MIC > 2 µg/ml instead of 0.12-1 µg/ml for *Aspergillus fumigatus* wild type KU80). Descriptive statistics of the included patient reveal a high proportion of productive cough longer than one month (78%), weight loss (74.4%), dyspnea longer than one month (68%) and hemoptysis (47.9%). A comparison between the whole sample, TB negative patients and TB negative and Platelia positive patients highlight no statistical difference for diagnosis based on direct examination in the sputum and fungal culture but a tendency to have more positive fungal culture when TB negative and Platelia positive. Concerning these three populations, no statistical difference was found for Platelia and Ct-scan results. In the same way, no statistical difference was found between Platelia negative and positive patients nor Ct-scan positive and Ct-scan negative patients. The main limitations of this study were the poor quality of the sputum (58% of the sputum below the Murray-Washington class 3, the high number of patients with unknown tuberculosis status (24.4%) and the exclusion of patients due to absent Ct-scan (Platelia positive). We hope these first results on aspergilloma diagnosis will help physicians to improve health care of patient and particularly concerning the management of tuberculosis sequelea. An « Aspa day » was organized near the close of the project for scientific restitution and acknowledgment and recognition of all collaborators. The PTR was closed at the end of June 2019 following a last no-cost extension. The project was jointly implemented by three Phnom Penh Hospitals- Calmette, Khmer-Soviet Friendship, and Kossamak. An oral presentation was done in Paris by Dr. Chhorn Sotheary in January 2020 during the 24th Pneumology Congress. The publication is in progress and will be submitted in an international scientific journal as soon as possible.

Team leader: Yannnick Caron

Funding: IP - PTR

Outbreak of trichinellosis in September 2017 in Kampong Thom Province

On September 26, 2017, an important outbreak (33 cases including 8 deaths) of trichinellosis (*Trichinella* spp.) in Central Kampong Thom Province Hospital was reported. Trichinellosis was then diagnosed in Phnom Penh in two hospitals: Calmette and Preah Ket Mealea Hospitals. A proposal to investigate the parasite with the help of the Trichinella Reference Laboratory (Cochin Hospital, Paris, France) to the communicable disease control department of the MOH was accepted. We accessed to the complete medical records (clinical symptoms, diagnosis, medical history, treatment, outcome etc.) of each hospitalized patient in the third hospital experiencing this outbreak. Very few scientific data exists in Cambodia on this zoonotic disease and the molecular characterization of the strain involved could be interesting for the management of this important public health issue. A publication entitled "First description of *Trichinella papuae* involved in a human outbreak in central Kampong Thom Province in Cambodia » was submitted to the journal "Emerging Infectious Disease"; the review process is ongoing.

Team leader: Yannick Caron

Funding: IP Cambodia

International joint laboratory «Drug Resistance in Southeast Asia» (LMI DRISA)

In collaboration with the IRD, IPC has hosted an engineer specialized in molecular biology since August 2018. Dr. Mallorie HIDE will be based in LBM for at least three years within the LMI DRISA framework. She works on molecular epidemiology and drug resistance in *Mycobacterium tuberculosis* and other bacteria in Cambodia as well as on the application of antimicrobial peptides as alternatives to antibiotics.

Laboratory on bacteriology research (LRB)

Within the LBM, an analytic platform dedicated to research in bacteriology has been set up by M. Hide thanks to the financial support provided by IPC and LMI DRISA. For both routine activity and research purpose, IPC bought a Bruker biotyper Maldi-tof, currently available in the LRB. For the molecular characterization of tuberculosis isolates, the automated spoligotyping has been set up using Luminex hardware available on the PRR -ASIA platform.

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

The overall objective of this project is to understand the emergence, spread and evolution of antibiotic resistance in *Mycobacterium tuberculosis* in Cambodia by using genetic characterization of MTB isolates. This is a three year study started in September 2016. By 2018, a total of 473 MTB isolates with known drug susceptibility testing (DST) for fragmented and loop primer ligated dsRNA sequencing (FLDs)(Isoniazid, rifampicin, streptomycin and ethambutol), collected between 2012 and 2015 were included in the study. Among them, 143 were sensitive to FLDs and 326 were resistant to at least one FLD. These isolates were genetically characterized using sequencing of genes involving in the resistance of MTB to first and second line anti-TB drugs and genotyping method. In 2019, the genetic data were obtained for 404 isolates and included in the analysis. Of these 278 were resistant to at least one FLD and 126 were FLD sensitive. Of drug resistant isolates, 113 (28%) were mono-resistant (resistant to only one FLDs), 44 (10.9%) were poly-resistant (resistant to more than one FLDs but not to isoniazide and rifampicine at the same time) and 115 (41.2%) were MDR. The resistance to INH and STM were the most commonly found among our isolates, representing 52.0% (210/404) and 42.3% (171/404), respectively. Rifampicin resistance was detected in 140 (34.7%) isolates, of them 17 (4.2%) were mono-rifampicin resistant. Ethambutol resistance was less common, observed mostly among MDR isolates (48/121). The isolates resistant to all four FLDs, defined as “quadruple resistant”, were found in 9.9% of all isolates. The demographic and clinical data linked to the 404 MTB clinical isolates were collected from the patient recorded. The median age was 44.7 and ranged from 15 to 86 years old, with 63% being male. The majority of patients (97.5%, 318/326) had pulmonary TB. Among 163 patients with known TB treatment history, most study subjects were re-treatment TB cases 134 (82.2%) and 7 (4.3%) patients were people living with HIV. At the time samples were collected for culture, 65.8% (206/313) were smear positive.

43-spacers spoligotyping results were available for 403 isolates and revealed eight families and 22 sub-families. Beijing and EAI families were predominant, accounting for 46.4% and 43.4%, respectively. The 24 loci MIRU-VNTR typing of 404 *M.tb* isolates allowed to distinguished 360 patterns, including 307 unique patterns, 34 patterns shared by 78 isolates (ranging from 2 to 4 isolates per pattern) and 19 mixed genotype patterns (double alleles in at least one locus). A dendrogram was constructed based on both spoligotyping and MIRU results of 384 *M.tb* isolates and 31 clusters were identified which suggest a recent transmission among patients. In deep analysis are being conducted and two publications are in preparation (one focus of highly resistant isolates and other focus on genetic diversity and their link to drug resistance among all isolates). The data from this project is being used in one PhD thesis and one resident in medical biology for a thesis, and will be used in a second DES thesis. The project is a collaboration with CENAT the Ministry of Education, Youth, and Sport, and the PHC Lotus Project of NIHE.

Team leader: Sokleaph CHENG

Funding: LMI-DRISA, IRD, French Embassy in Cambodia

In 2019, IPC submitted five projects to different calls for application of which was accepted for funding by the *Fonds de solidarité pour les projets innovants* (FSPI), Embassy of France in Cambodia.

4.7.4 RESEARCH PROGRAMS AND IMPLEMENTATION ACTIVITIES – 2020 PLANS

Antibiotic resistance in the human, animal and environment interface in an intervention in Cambodia: ARCAHE

In recent years, antibiotic resistance rate has become critical since multiple and extended resistant bacteria have become more prevalent. Even if we know that the current antibiotic resistance crisis is due to the massive use, overuse and misuse of these drugs, in many countries including Cambodia that have significant weakness in antibiotic usage regulation, still very little data are available and no surveillance system exists. With this background, we submitted a project that aims to identify the sources of emergence and spread of resistant bacteria in Cambodia using a "One Health" approach. This project will have a huge impact since for the first time, it will explore the spread of antibiotic resistance in humans, animals and environment in Cambodia. The expected results will represent the baseline for the setting up of a surveillance system, will allow stakeholders to implement efficient control strategies. The initiative will be done with IRD, Calmette, and Battambang Hospitals.

Team leader: Sokleaph CHENG

Funding: FSPI, French Embassy in Cambodia

AVIESAN- South initiative on antimicrobial resistance:

The objective of this activity is to setting up a research network in countries in the south (Sub-Saharan Africa and South-East Asia) to develop research projects related to antimicrobial resistance. Four countries were identified (Cambodia, Madagascar, Ivory Coast and Burkina Faso) and the visit of the Aviesan south delegation to those countries were started since late 2019. The priorities research themes on AMR were will be identified by each countries and will be presented to Aviesan SUD. One or two common research themes will be selected, pilot studies will be conducted to test its feasibility of each research project and the full projects will be applied for funding.

Team leader: Sokleaph CHENG

Funding: Aviesan SUD (Aviesan south)

***Pertussis* genotyping from nasopharyngeal samples collected in Cambodia (Perilic WP1 – ancillary study)**

The objective of this ancillary study being done in partnership with IP Paris is to characterize the main relevant genes carried by *B. pertussis* strains collected in Cambodia as part of the PERILIC study, directly using the primary nasopharyngeal samples. In particular, macrolides resistance and pertactin expression will be assessed and virulence gene alleles (including *prn*, *ptxa*, *ptxc*, *ptxp*, *fim2*, *fim3*) will be determined by genotyping.

Team leader: Sokleaph CHENG

Funding*: Institut Pasteur- Paris*

New AntiMicrobial Peptides (AMP):

The general objective of the study is to evaluate the antibacterial activity of a new set of eight synthetic AMP against clinical isolates of relevant bacteria identified by GLASS. The antibacterial activity of AMP against relevant bacteria involved in human infections in Cambodia (*Acinetobacter* spp., *Escherichia coli, Klebsiella pneumoniae, Salmonella* spp., *Shigella* spp., *Staphylococcus aureus)* will be evaluated. Experiments will start in May 2020 by a master's of medical biology student).

The specific objectives are to set up antibacterial activity testing using broth microdilution method for the eight AMP on *E. coli* reference strain (ATCC 25922), and to measure the susceptibility of relevant resistant bacteria identified by GLASS against the eight new AMP by comparing with sensitive bacterial strain. Partners include LMI-DRISA and MIVEGEC.

Team leader*:* Mallorie HIDE (IRD – LMI DRISA)

Funding: *LMI-DRISA, IPC*

Potential colistin resistance in multidrug-resistant *Enterobacteriaceae*

The current antibiotic of last resort for the treatment of infections in humans caused by multidrug-resistant enterobacteria is often colistin. It belongs to the antimicrobial class designated polymyxins which originates from the organism *Paenibacillus polymyxa.* In Cambodia, colistin is one of the most commonly used antibiotics on small-scale urban pig farms and is used on poultry farms in a packaged amoxicillin-colistin mixture for respiratory and gastrointestinal infections. On pig farms, 20% of phenotypic resistance to colistin have been identified. A few colistin resistance genes *(mcr like, pmrA, pmrB, phoP, phoQ, mgrB,* and *pmrD* genes*)* have been detected from both multidrug-resistant *Escherichia coli* (*EC*) and *Klebsiella pneumonia* (*KP*) isolated from humans, poultry and pigs. Despite this, colistin resistance in multidrug-resistant *Enterobacteriaceae* isolated from patient is poorly known in Cambodia. With this background, our main objective is to explore colistin resistance among both carbapenemase and extended-spectrum Beta-Lactamase (ESBL) isolated from humans.

Experiments (in vitro testing, sequencing) will start in May 2020, working with a master's in life sciences and international health student specializing in infectiology-biology of infectious diseases. Implementation will be done in collaboration with LMI DRISA and MIVEGEC.

Team leader*:* Mallorie HIDE (IRD – LMI DRISA)

Funding: LMI-DRISA, IPC

Genetic diversity and resistance status of *Burkholderia pseudomallei* strains responsible for melioidosis in Cambodia

Burkholderia pseudomallei is an environmental gram-negative bacteria, transmitted to humans by contaminated soil and surface water (percutaneous inoculation, inhalation, or ingestion) and most of cases occur during rainy and humid months. Melioidosis, due to *B.pseudomallei*, is endemic in Cambodia and the majority of cases are in children (>60%) with head and neck infections whereas adults mostly present pneumonia and/or sepsis with increase factors such as diabetes mellitus, renal diseases, chronic lung disease, thalassemia, alcohol consumption, male sex and occupational exposure. Concerning antibiotic resistance, most *Burkholderia* contain a modified lipopolysaccharide that causes intrinsic polymyxin resistance and *B. pseudomallei* exhibits resistance to diverse antibiotics including first and second generation cephalosporins, penicillins, macrolides, and aminoglycosides. Ceftazidime and clavulanic acid-resistant strains have been described and others continue to be identified. No genetic data are available on *Burkholderia* in Cambodia and in this context, our objective is to explore the genetic diversity and resistance status of *Burkholderia* strains responsible for melioidosis, isolated and cryoconserved at the Medical Laboratory since 2011 (n=100). All the bacterial samples will be cultured for identification using Bruker Maldi Biotyper and sequence to explore both genetic diversity and resistance genes.

Experiments (Maldi-tof identification, AST, genetics) will start in May 2020. A Master's of live sciences and international health student specializing in the biology of infectious diseases will work on this study under supervision of IPC. Partners in this work will be LMI-DRISA, and MIVEGEC

Team leader*:* Mallorie HIDE (IRD – LMI DRISA)

Funding*:* LMI DRISA, IPC

Establishing an AMR bacteriology research focus in IPC

During his postdoctoral position, K. Vandelannote will define genomic characteristics and population structure of pathogen specific outbreaks using whole genome sequencing and model transmission networks using clinical and genomic epidemiological data.

4.7.5 SUPPORT TO NATIONAL AUTHORITIES

- With NCHADS, follow-up of HIV seropositives
- Member of CENAT's laboratory TWG for development of technical procedures guideline
- Member of CENAT's TWG on multi-drug resistant tuberculosis.
- Member of TWG on AMR with MOH
- Provide technical support on quality with MOH

4.7.6 TEACHING AND TRAINING

a. Continuing professional development training for staff

- DU Qualité, accréditation et Audit, Université de Lille-II
- Roche Quality Day 2019
- Norme NF EN ISO 15189
- “Breakthrough in Medical Technology” Congress
- Massive open online course (MOOC), viruses and human cancers
- MOOC, Quality Management of Medical Biology Laboratories
- MOOC,The Role of Diagnostics in the Antimicrobial Resistance Response
- MOOC, Epigenetics
- Chemical and Biological Risk, Kalitech
- Course in Antibiotic Resistance
- Effective use of Kalidoc (document management tool)
- Effective use of Maldi-TOF, Bruker
- Cobas e411 refresher training
- Introductory course in applied microbial genomics for public health and antimicrobial resistance

b. Internships

One of IPC’s main missions is to contribute to teaching and training activities. The medical laboratory has been proactive in the training of laboratory technicians and pharmacists from partner institutions in the fields of medical biology including haematology, immune-serology, biochemistry, microbiology and molecular biology. In 2019, the medical biology unit received 38 students for internship:

- Pharmacists from UHS: 20
- Laboratory Technicians from UHS & UP: 12
- Kampong Cham Veterinary School: 6

c. Thesis supervision

- One PhD candidate in health biology, University of Montpellier, studying the genetic determinants and evolution of drug resistance in Mycobacterium tuberculosis in Cambodia, a high tuberculosis burden country

d. Thesis supervision

Supervising of students for their thesis project for the degree of doctor of pharmacy specializing in medical biology from UHS Cambodia.

- Study of enterobacteriaceae producing extended-spectrum beta-lactamase at the *Institut Pasteur* in Cambodia *(*Étude des entérobactéries productrices de bêta-lactamase à spectre étendu à l’Institut Pasteur du Cambodge. Defended on August 2019.
- Carbapenemase-producing bacteria, evolutionary monitoring at the *Institut Pasteur* in Cambodia (*Bactéries productrices de carbapénémases, suivi évolutif à l’Institut Pasteur du Cambodge). Thesis is ongoing.*
- Study of mutations in genes linked to the resistance of Mycobacterium tuberculosis isolated from patients in Cambodia (Étude des mutations des gènes liés à la résistance de *Mycobacterium tuberculosis* isolées des patients au Cambodge). Thesis is ongoing.

Jury member to evaluate a thesis for the degree of doctor of pharmacy specializing in medical biology from UHS.

- Surveillance of the presence of leptospire and Hantavirus carried by rodents in four provinces of Cambodia. Defended on March 2019. *(Surveillance de la présence de leptospire et d'hantavirus portés par les rongeurs dans quatre provinces du Cambodge)*. Defended on March 2019.

e. Participation in the setting up of master's course at UHS

- Co-coordinator of bacteriology module for Master's years one and two in life sciences and international track in infectious disease studies in Cambodia. This degree offered by UHS Cambodia, the University of Paris-Saclay and IPC, Cambodia is an advanced level program and is intended as a continuation of the French bachelor's degree in life science.
- Co-coordinator of bacteriology module for master's in medical biology, UHS.

4.7.7 PUBLICATION LIST 2019

1. Borand L, de Lauzanne A, Nguyen NL, Cheng S, Pham TH, Eyangoh S, Ouedraogo AS, Ung V, Msellati P, Tejiokem M, Nacro B, Inghammar M, Dim B, Delacourt C, Godreuil S, Blanche S, Marcy O; Pediatric Asian African Network for Tuberculosis and HIV Research (PAANTHER) Study Group. **Isolation of Nontuberculous Mycobacteria in Southeast Asian and African Human Immunodeficiency Virus-infected Children with Suspected Tuberculosis**. Clinical Infectious Disease. 2019 May 2;68(10):1750-1753. doi: 10.1093/cid/ciy897.
2. Nouhin J, Iwamoto M, Prak S, Dousset JP, Phon K, Heng S, Kerleguer A, Paih M, Dussart P, Maman D and Rouet F. Molecular epidemiology of hepatitis C virus in Cambodia during 2016–2017. 2019. Scientific Reports. 9. 10.1038/s41598-019-43785-4.
3. Taylor WRJ, Kheng S, Muth S, Tor P, Kim S, Bjorge S, Topps N, Kosal K, Sothea K, Souy P, Char CM, Vanna C, Ly P, Khieu V, Christophel E, Kerleguer A, Pantaleo A, Mukaka M, Menard D, Baird JK. Hemolytic Dynamics of Weekly Primaquine **Antirelapse Therapy among Cambodians with Acute Plasmodium vivax Malaria with or without Glucose-6-Phosphate Dehydrogenase Deficiency**, The Journal of Infectious Diseases, Volume 220, Issue 11, 1 December 2019, Pages 1750–1760, https://doi.org/10.1093/infdis/jiz313.
4. Nadimpalli M, Vuthy Y, de Lauzanne A, Fabre L, Criscuolo A, Gouali M, Huynh BT, Naas T, Phe T, Borand L, Jacobs J, Kerléguer A, Piola P, Guillemot D, Le Hello S, Delarocque-Astagneau E; BIRDY study group**. Meat and Fish as Sources of Extended-Spectrum β-Lactamase-Producing *Escherichia coli*, Cambodia.** Emerg Infect Dis. 2019 Jan;25(1). doi: 10.3201/eid2501.180534.

Conferences:

K Officer, S Heng, S Cheng. **Zoonosis in reverse: Mycobacterium tuberculosis in captive sun bears**. Accepted for poster presentation in The 50th Union World Conference on Lung Health in Hyderabad, India

4.8 OTHER SERVICES PROVIDED BY THE INSTITUT PASTEUR DU CAMBODGE

The medical activities performed by *Institut Pasteur du Cambodge*, commissioned by the Royal Government of Cambodia in the agreement with IPC (described as service activities or public health care activities at *Institut Pasteur*) also provide direct access and services to clients. In May 2019, the vaccination service was created under the leadership of Dr.Yiksing Peng. The International Vaccination Center and three rabies prevention centers of IPC have been established as part of this new service from the epidemiology and public health unit.

4.8.1 RABIES PREVENTION CENTER HIGHLIGHTS FOR 2019

Set up under the terms of Article 7 of the 27 August 1992 convention between the Royal Government of Cambodia and *Institut Pasteur*, the rabies prevention center of the *Institut Pasteur du Cambodge* is the largest rabies prevention center in Cambodia. It has a medical team of 16 full-time equivalents placed under the responsibility of a medical doctor (Dr Yiksing Peng.). This team offers rabies post-exposure prophylaxis protocol as per WHO recommendations at an affordable price, as the treatment is subsidized by IPC. In 2019 the prices was US$15 for a protocol of two-site x three ID sessions, following the new WHO recommended protocol. The vaccine we use is Verorab produced by Sanofi.

In 2019, the rabies prevention center IPC provided rabies PEP to 49,703 people in Phnom Penh, 14,032 people in Battambang, and 10,245 people in Kampong Cham, most of whom were bitten by dogs. Furthermore, a total of 134 animal heads were tested by immunofluorescence for rabies virus at the virology unit, IPC, amount those samples 92 (69%) were positive for rabies, all were from dogs (tested). This information is regularly communicated to MOH and MOH CDC, FAO and WHO.

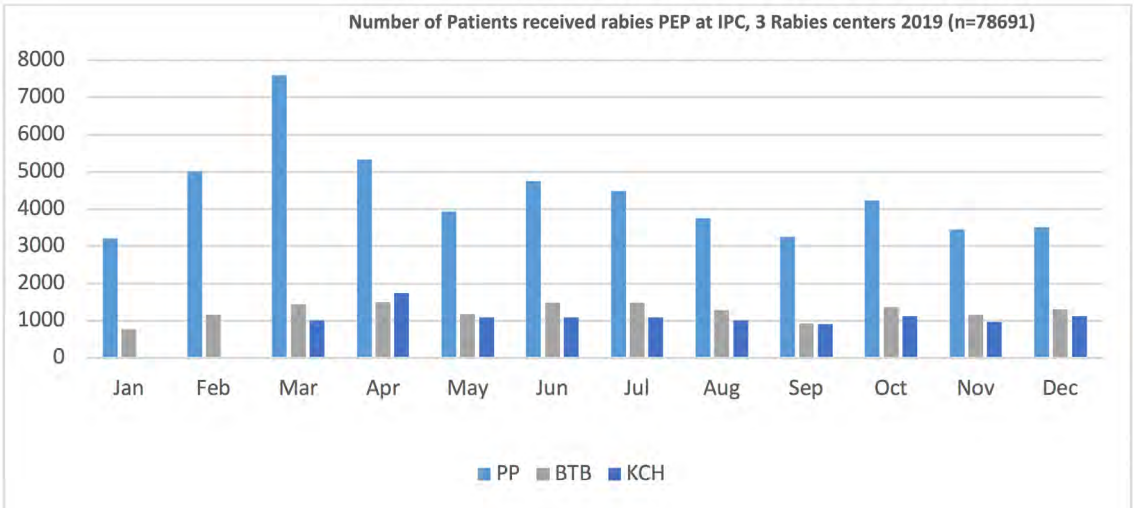
In 2018, the RPC provided PEP to 25,081 people in Phnom Penh, and 1,201 in Battambang, most of the time (82.3%) bitten by dogs. In total 176 animal heads were tested by immunofluorescence for rabies virus at the virology unit, IPC, and of these 121 (68.8%) were positive for rabies (120 dogs, 1 cat, tested).

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

In 2016, RPC IPC had an intake of 21,664 patients for post-exposure management. A total of 157 heads of biting animals were examined at the virology laboratory, of which 79 (50.3%) tested positive for rabies under immunofluorescence.

In 2015, the RPC IPC had an intake of 21,301 patients for post-exposure management. A total of 185 heads of biting animals were examined at the virology laboratory, of which 94 (50.8%) tested positive for rabies under immunofluorescence.

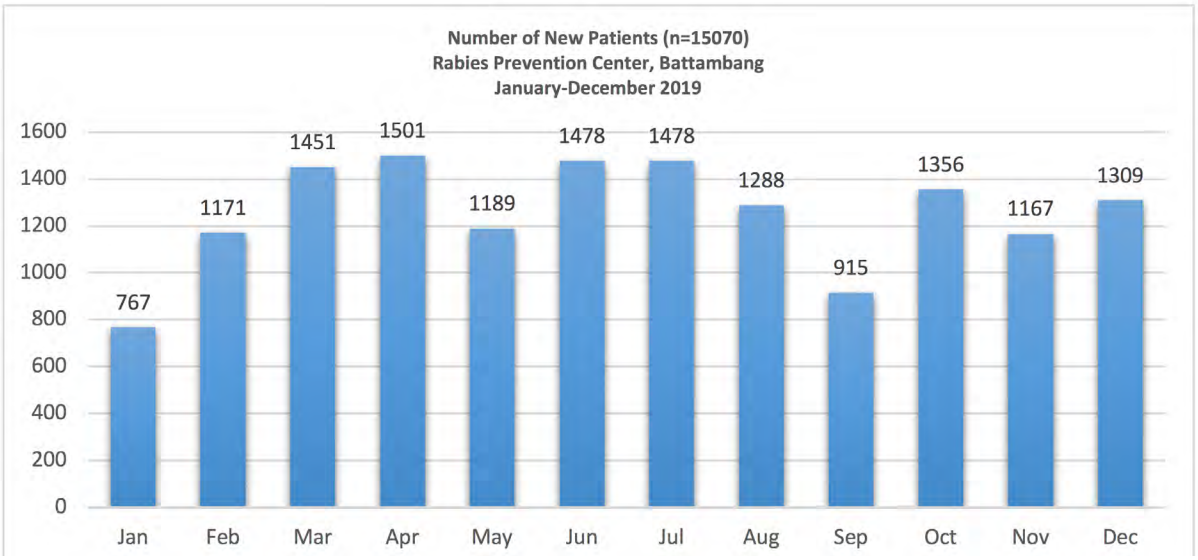
The opening of the satellite rabies prevention center in Battambang, close to the Battambang Provincial Hospital and the MOH's provincial health department, happened in July 2018. A memorandum of understanding was



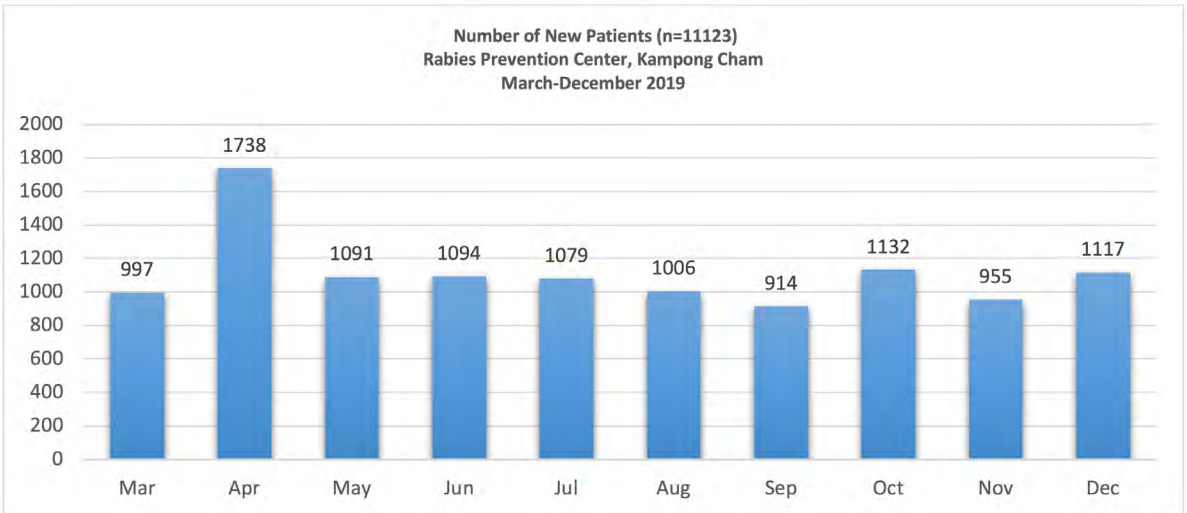
signed on December 25, 2017 between the Battambang Provincial Health Department and *Institut Pasteur* du Cambodge. The center was inaugurated by Her Excellency OR Vandine, General Director for Health and His Excellency the Deputy Governor of Battambang Province, on September 28, 2018.

The Steering Committee for the Rabies Prevention Center in Battambang comprises following members:

- Director of Battambang Provincial Health Department
- Deputy Director of Battambang Provincial Health Department
- Director of *Institut Pasteur* du Cambodge
- Deputy Director of IPC
- Director of Administrative and Finance Unit, IPC
- Head of Vaccination Service, IPC



A second satellite PHD-IPC rabies prevention center in Kampong Cham Province within the provincial hospital was opened on 7 March, 2019. The building used for this center is still a temporary one. IPC and Kampong Cham PHD will work together to seek a permanent home facility during 2020.



4.8.2 VACCINE INTERNATIONAL CENTER FOR 2019

Set up under the terms of Article 7 of the 27 August 1992 convention between the RGC and *Institut Pasteur*, the International Vaccination Center at the *Institut Pasteur du Cambodge* has a medical team of four full-time equivalents under the responsibility of Dr. Yiksing Peng. It offers vaccines to the public from the extended vaccination program and other vaccines or immunoglobulins six days per 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.

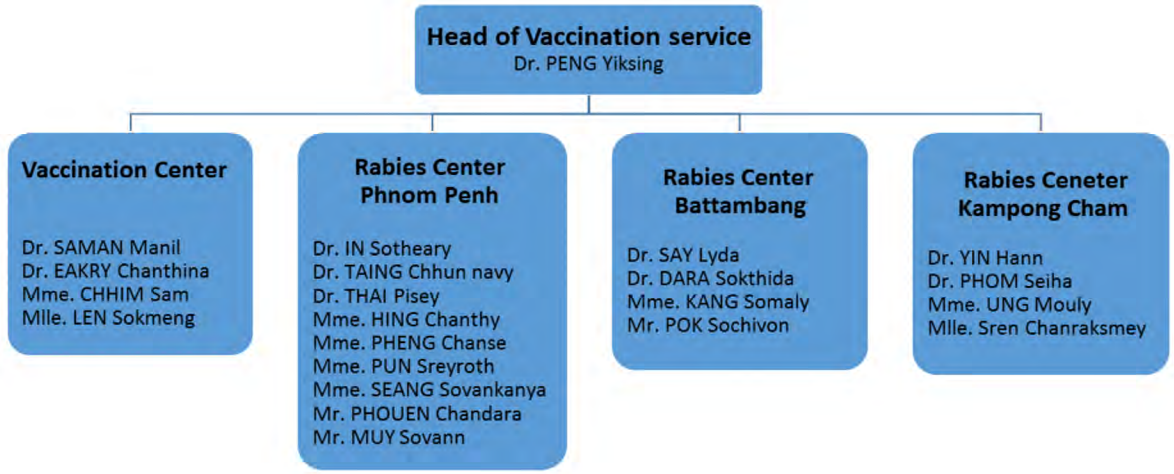
In 2019, 32,673 injections (including the immunoglobulins) were delivered in the framework of 16,673 vaccine protocols, a notable increase of 5.6% compared with 30,915 injections in 2018.

In 2018, the medical team delivered 30,915 injections (including the immunoglobulins) in the framework of 18,947 vaccine protocols.

In 2017, the medical team delivered 28,020 injections (including the immunoglobulins) in the framework of 19,121 vaccine protocols.

In 2016, the medical team delivered 27,043 injections (including the immunoglobulins) in the framework of 16,472 vaccine protocols.

In 2015, the medical team delivered 25,255 injections (including the immunoglobulins) in the framework of 14,148 vaccine protocols.



5. CONCLUSION

The *Institut Pasteur du Cambodge* (IPC) continues to focus on research in emerging, neglected and major endemic infectious diseases. IPC also contributes to the national public health effort and provides health services (vaccinations, medical biology laboratory, laboratory for environment and food safety). IPC is a responsive institution that can rapidly react to any communicable disease health crisis as it notably did in 2019 for rabies and in 2020 for COVID-19. It actively contributes to capacity building and educational training of the Cambodian and international students and professionals in collaboration with Cambodian universities. Its working model is consistent with the missions and values of the other institutions belonging to the *Institut Pasteur International Network* including gender equity. IPC will continue to develop high quality research, provide direct services, and offer teaching and training in life sciences and health research of critical importance to Cambodia and beyond. By working towards these missions, IPC strives to improve the quality of life of the Cambodian population.

Institut Pasteur du Cambodge