



2020 Scientific Report and 2021 Perspectives Institut Pasteur du Cambodge



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1. LIST OF ACRONYMS

ACIP	Action Concertée Inter-Pasteurienne (<i>Institut Pasteur</i>)
ACT	Artemisinin combination-based resistance
ADE	Antibody-Dependent Enhancement
AFD	Agence Française pour le Développement (French Development Agency)
AFRIMS	Armed Forces Research Institute for Medical Sciences
AFTC	Anonymous Free Testing Center
AIV	Avian Influenza Viruses
AMP	Antimicrobial Peptides
AMR	Antimicrobial Resistance
ANRS	<i>Agence Nationale de Recherche sur le SIDA et les Hépatites</i> (French National Agency for AIDS and Hepatitis Research)
AQ	Amodiaquine
AQ-R	Amodiaquine Resistant(ce)
ART-R	Artemisinin resistant
ARCAHE	Antibiotic resistance at the Human/Animal/Environment interface in a “One Health” Approach in Cambodia – FSPI project
ASAQ	Artesunate amodiaquine
ASIDE	Alerting and Surveillance for Infectious Diseases Epidemics
AST	Antibiotic Susceptibility Testing
AVIESAN	Alliance Nationale pour les Sciences de la Vie et de la Santé, France (French Alliance for Life Sciences and Health)
Beta-CoVs	Beta Coronaviruses
BP	<i>Burkholderia pseudomallei</i>
BSL	Biosafety level
BIRDY	Bacterial Infections and Antibiotic-Resistant Diseases among Young Children in Low Income Countries
BMGF	Bill and Melinda Gates Foundation
CANARIES	Consortium of Animal Networks to Assess Risk of Emerging Infectious Diseases through Enhanced Surveillance
C-CDC	Cambodian Center for Disease Control and Prevention
CEG	Community Epidemiology Group
CIRAD	<i>Centre de Coopération Internationale en Recherche Agronomique Pour le Développement</i> , CIRAD (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)
CPC	Centre Pasteur du Cameroun
CPE	Carbapenemase Producing Enterobacteria
CWRU	Case Western Reserve University (USA)
CRG	Clinical Research Group
CSS	Cross Sectional Survey
DAA	Direct Acting Antiviral Therapy
DARPA	Defense Advanced Research Projects Agency
DBS	Dried Blood Spot
DENV	Dengue Viruses
DF	Dengue Fever
DHA-PPQ	Dihydroartemisinin Piperaquine
DHF	Dengue Hemorrhagic Fever
DNA	Deoxyribonucleic Acid

DRC	Democratic Republic of Congo
DSS	Dengue Shock Syndrome
DTRA	Defense Threat Reduction Agency (USA)
DVI	Dengue Vaccine Initiative
EC	<i>Escherichia coli</i>
EID	Emerging Infectious Disease
EPH	Epidemiology and Public Health Unit of Pasteur Institute of Cambodia
ERIG	Equine Rabies Immunoglobulins
ESBL-E	ESBL-producing Enterobacteriaceae
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	Solidarity Fund for Innovative Projects
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 Communications
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
HS-RDT	High Sensitivity Rapid Diagnostic Test
HLA	Human Leukocyte Antigen
ICEMR	International Center of Excellence in Malaria Research
ILI	Influenza-like Illnesses
INF	Interferon
INSERM	Institut National de la Santé et de la Recherche Médicale (France)
IP	<i>Institut Pasteur</i> (Pasteur Institute)
IPC	<i>Institut Pasteur du Cambodge</i>
IPIN	<i>Institut Pasteur International Network</i>
IPL	<i>Institut Pasteur du Laos</i>
IRD	<i>Institut de Recherche pour le Développement</i> (Institute for Research and Development-France)
IRIS	Immune reconstitution inflammatory syndrome
ITC	Institute of Technology (Cambodia)
IVI	International Vaccine initiative
JEV	Japanese Encephalitis Virus
KBH	Kantha Bopha Hospital
KP	<i>Klebsiella pneumoniae</i>
LAM	Live Animal Market
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
LTBI	Latent Tuberculosis Infection
MAFF	Ministry of Agriculture, Forestry, and Fisheries (Cambodia)
MDR	Multi-drug resistant(ce)
MEAE	<i>Ministère de l'Europe et des Affaires Etrangères</i> (French Ministry for European and Foreign Affairs)
MEF	Ministry of Economy and Finances (Cambodia)
MERS	Middle East Respiratory Syndrome
MESRI	<i>Ministère de l'Enseignement Supérieur, de la Recherche, et de l'Innovation.</i> (French Ministry for Higher Education, Research and Innovation)
MEYS	Ministry of Education, Youth and Sports (Cambodia)
MIE	Maladie Infectieuse Émergente (Emerging Infectious Disease)
MiVEGEC	<i>Maladies Infectieuses et Vecteurs Écologie, Génétiques, Evolution, et Contrôle (IRD, France)</i>
MinION	DNA sequencing using Oxford Nanopore Technologies
miRNA	Cell-free circulating microRNA biomarkers
MLST	Multilocus Sequence Typing
MoA	Memorandum of Agreement
MOH	Ministry of Health (Cambodia)
MOOC	Massive open online course
MORU	Mahidol Oxford Tropical Medicine Research Unit
MoU	Memorandum of Understanding
MMEU	Malaria Molecular Epidemiology Unit (Pasteur Institute of Cambodia)
MMV	Medicines for Malaria Venture
MTB	Mycobacterium tuberculosis
MSAT	Mass Screening and Treatment
MSM	Men who have Sex with Men
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
NPHL	National Public Health Laboratory
NSSF	National Social Security Fund
NUCS	Northeastern University College of Science
OIE	World Organisation for Animal Health
PCR	Polymerase Chain Reaction
PEP	Post Exposure Prophylaxis
PHC	Primary Health Care
PHD	Public Health Department
PMI	President’s Malaria Initiative (USA)
POC	Point of Care
POCTs	Point of Care Test
PPR	Programme Prioritaire de Recherche

PREEMPT	Preventing Emerging Pathogenic Threats
PrEP	Pre-exposure Prophylaxis
PRR	Plateforme Régionale de Recherche (building PRR)
PSI	Population Services International
PTR	Programme Transversal de Recherche (funding mechanism from <i>Institut Pasteur</i> in Paris)
PVDBP	<i>P. vivax</i> Duffy binding protein
QA/QC	Quality Assurance/Quality Control
QFT	Quantiferon
RABV	Rabies Virus
RAI3	rRegional artemisinin resistance initiative
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
SMRU	Shoklo Malaria Research Unit
SNP	Single Nucleotide Polymorphism
STI	Sexually Transmitted infections
SVR	Sustained Virological Response
TB	Tuberculosis
TGW	transgender women
TLR	Toll-like Receptors
TWG	Technical Working Group
UGA	University of Georgia (USA)
UHS	University of Health Sciences (Cambodia)
UMR	<i>Unité mixte de recherche</i> (joint research units) France
USAID	United States Agency for International Development
UP	University of Puthisastra (Cambodia)
US CDC	American Center for Disease Control and Prevention
UTI	Urinary Tract Infections
VEG	Veterinary Epidemiology Group
VIC	Vaccination International Center (Service open to public)
VL	Viral Load
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

In 2020, the *Institut Pasteur du Cambodge* (hereinafter abbreviated as IPC or the Institute), a Cambodian non-profit research institution dedicated to infectious diseases and emerging pathogens, has maintained its three major missions: scientific research, public health and health services as well as training and education. IPC benefits from the expertise and skills of its almost 300 employees (including 160 permanent staff) plus trainees and visiting scientists. The Institute is working under the senior oversight of the Cambodian Ministry of Health (President of the Liaison Council: His Excellency (HE) Minister of Health, Professor MAM Bunheng) and the *Institut Pasteur* (Paris, France) co-chair of the Liaison Council: The General Director, Professor Stewart COLE). The Institute is one of the 33 members of the *Institut Pasteur International Network* (IPIN).

The 2020 main highlights are as follows:

- Instrumental contribution of the Institute to the public health response against COVID-19 in Cambodia and in the Greater Mekong Sub-region (GMS). The Institute was the one and only laboratory for COVID-19 RT-PCR tests from January to July 2020 (done in the Virology Unit) until the National Public Health Laboratory (NPHL) at the National Institute for Public Health (NIPH) was able to start testing for COVID-19. During the year 2020, both laboratories were performing RT-PCR for COVID-19 accounting for 100% of the COVID-19 laboratory tests in Cambodia. In 2021, some additional laboratories will join this joint effort between NIPH and IPC to extend the lab capacity countrywide. The scientists and the senior management of the Institute were also deeply involved as technical experts to support the Royal Government of Cambodia (RGC) and the health development partners. One amazing achievement was that the Institute hosted one of the 22 WHO Global COVID-19 referral laboratories, receiving this designation in April 2020.
- Despite the COVID-19 health crisis, IPC has been able to pursue most of its collaborative research projects in its main fields of expertise on infectious diseases and spillover mechanism for emerging pathogens: antimicrobial resistance (AMR) including tuberculosis, malaria, immunology, entomology, rabies, HIV/viral hepatitis, arboviruses (dengue, chikungunya) and avian/seasonal flu. This scientific report details the achievements of the research teams and groups during this year.
- The high number ($n = 69$) and impact factor of the publications confirm the great scientific achievements from the previous years.
- The Institute was able to remain open to the public for health services (Medical Biology Laboratory (LBM), Vaccine Centers (VACC) including 3 rabies prevention centers) as well as the Laboratory for Environment and Food Safety (LEFS). Those three entities are also strongly contributing to several research projects together with the research units and external partners.
- In addition of the excellence in research and health services, the Institute continues to focus on high-quality: the LBM is accredited ISO 15189 (with a successful external audit in November 2020) and the Institute is preparing for the ISO 17025 accreditation of the LEFS and the metrology entities for the end of 2021.
- The organization of the bi-annual Scientific Advisory Board (SAB) was done in 2020 and was successfully conducted in February 2021 (virtual) with a new board of national and international experts.
- The senior Management also prioritized the continuous improvement of the local staff and capacity-building training activities such as research management, leadership and professional development skills and, but delayed some to 2021 due to the health crisis such as English courses that are now accessible to a large and diverse group of staff within IPC.
- An important modernization of the campus is in preparation, ranging from having better health coverage for the local staff to ensuring a more secured entrance for the visitors and staff by the end of 2021. The IT component of the Institute has been upgraded and the set-up of the mini-sequencing platform has been prepared for early 2021.
- The leadership team was consolidated to give better visibility to some of the units and groups. In August 2020, Dr. DUONG Veasna was appointed as Head of Virology and Dr. Erik KARLSSON as Deputy Head.
- As a better visibility of the Institute is important, a part-time consultant for communication was appointed to work at the Director's Office to harmonize and facilitate lay communications. The development of a new website was launched in 2020 and implemented in April 2021. The scientific seminars were suspended during most of 2020 but resumed in virtual format in early 2021.

- Strengthened regional collaborations including with institutional partners from Vietnam, China, Thailand, Myanmar, Laos, Singapore and Australia continued despite the health crisis and travel bans. Within the IPIN regional hub, a workshop to define innovative projects for AMR was prepared, and was conducted virtually in February 2021.
- Existing partnerships with the Cambodian hospitals (in Phnom Penh and in the provinces) and the universities (University of Health Science (UHS), the Royal University of Phnom Penh (RUPP), the Royal University of Agriculture (RUA), the Institute of Technology (ITC) were strengthened. A new Memorandum of Understanding (MoU) was signed with University of Puthisastra (UP).
- The Institute is providing mentorship and training for Cambodian and international students (about 50 students hosted, which unfortunately is half of the number hosted in 2019 due to the ongoing health crisis).
- The International Master's Degree in Infectious Diseases Science, co-organized by UHS and the University of Paris-Saclay (UHS) moved to its second year despite the challenge of the COVID-19 health crisis.

3. PRESENT, PERSPECTIVES AND CHALLENGES

3.1 The Institute in 2020

The *Institut Pasteur du Cambodge* remains a bridgehead of Pasteur-inspired research on infectious diseases in Cambodia. The Institute, a very active member of the *Institut Pasteur International Network* (IPIN), is a government-approved non-profit research institution. The Institute was created in 1953 and reopened in 1992 after the signature of an agreement with the Royal Government of Cambodia (RGC), which was renewed by an endorsement in 2013. The Institute is under the senior oversight of the Cambodian Ministry of Health (MoH), on behalf of which it carries out high excellence life sciences and health research, public health and health services as well as training and education activities. The Institute has reinforced this mission over the past 25 years in collaboration with national and international institutions. It contributes to the diagnosis, research, and prevention of the priority infectious diseases and emerging pathogens in Cambodia and the Greater Mekong Sub-region. It plays a major role in microbiological monitoring and research in cooperation with national and international partners, as well as several IPIN Institutes, first and foremost the *Institut Pasteur* in Paris. It hosts local researchers and international researchers, some being affiliated to *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 and other parasites, immunology, epidemiology and clinical research, and entomology), its facilities are among the most efficient in Cambodia and Greater Mekong sub-region (GMS) in the areas of pathogen diagnosis, study of transmission, analysis of biological and genetic biomarkers for severity and antimicrobial resistance of microorganisms. In 2020, the significant scientific advances were published through 69 scientific articles. Despite the COVID-19 health crisis, projects of excellence were pursued thanks to the insight and perseverance of highly dedicated teams. The scientists were able to secure several research contracts and to carry out their research; the main findings are being detailed in this scientific report.

The Institute was able to face the COVID-19 crisis thanks to key involvement from IPC's technical and administrative staff, critical support from the Cambodian MoH, and from many other partners including donors. It is noteworthy that research projects and service activities in 2020 made up the largest share of IPC resources by far, comprising 94% of the annual budget and 6% from the French Ministry for Higher Education, Research and Innovation (MESRI). One explanation for this important change in the operational budget repartition is that for the year 2020 the COVID-19 PCR testing effort (around 200,000 tests) on request from the MoH for the public response. It is anticipated that with the development of a strong laboratory network with molecular biology capacities in the country, this operational budget will be different by the end of 2021.

In December 2020, IPC had a staff of around 300 comprising 10 nationalities, including eight local senior scientists (*Personnel Scientifique de Recrutement Local*), two expatriates at the senior management level (one international technical expert from MEAE and one from IP), nine expatriate scientists (six from IP, one CIRAD and two from IRD), several international senior scientists and research engineers (fully paid through research projects) and five post-doctorates. Several full or part-time staff also support IPC as service contractors, and visiting scientists come for a few weeks to several months from abroad.

The Institute is usually intensely involved in university-level training, targeting students in biology, biomedical sciences, engineering, and master's and doctoral students from different universities in Cambodia, France and other countries. In 2020, the number decreased because of the ongoing health crisis from 96 in 2019 to 52 new students (38 Cambodians and 14 foreigners, including 5 PhD students). IPC strategy focuses on the promotion of careers for young Cambodian scientists by providing them with a high-excellence scientific environment.

3.2 The Institute's organization and governance

The revised organizational chart is presented at the end of this report (Annex 1).

The Institute is composed of:

- one administrative and financial department;
- health services: medical biology laboratory (LBM), laboratory for environment and food safety (LEFS), vaccine centers (VACC) including three anti-rabies treatment centers;
- five research units: malaria and molecular epidemiology (MME), epidemiology and public health (EPI-SP), immunology (IMMUNO), medical and veterinary entomology (ENTOMO), virology (VIRO);
- four research groups: bacterial AMR (within LBM), clinical research and on “One Health” (within EPI-SP) and Geo-Health.

The Institute activities and financial matters are reviewed by the Liaison Council chaired by HE the Minister of Health and co-chaired by the Director General of *Institut Pasteur* (Paris). The other attendees are: HE the Ambassador of France in Cambodia, representatives of several national authorities (Ministries of Economy and Finances (MEF), Ministry of Agriculture, Forestry and Fisheries (MAFF) and Ministry of Education, Youth and Sports (MEYS)) and international agencies (WHO, FAO, UNICEF, etc.) as well as the main international IPC partners.

The scientific activities are also reviewed every 2 years by the Scientific Advisory Board, its last session having been held on 6-7 December 2018), the SAB was finally conducted virtually early in February 2021. The scientific strategy is adapted based on the recommendations from the Liaison Council and the SAB.

In addition, at the end of January each year, the Chief Financial Officer (CFO) sends the consolidated financial statement (Year N-1) and the narrative with the planned operational budget (Year 1) to the *Institut Pasteur* (International Affairs Department). An external financial audit is also performed in April each year.

The Institute operating budget is organized by research contracts, health services, MESRI and grants from different sources. Table 1 describes the main trends over the nine years (2012 – 2020).

Operating Budget: Respective Share by Percentage of Three Main Components by Year, 2012-2020									
	2012 (%)	2013 (%)	2014 (%)	2015 (%)	2016 (%)	2017 (%)	2018 (%)	2019 (%)	2020 (%)
Research Contracts	40	48	57	58	60	57	57	55	39
Health services	30	27	21	25	29	32	32	35	55*
MESRI	20	17	14	12	11	11	11	10	6
Total Share of Budget	90	92	92	95	100	100	100	100	100

*18% from health services and 37% are exceptional incomes from COVID-19 funding (including donations)

Table 1. Budget from 2012 until 2020

3.3 The Governing Bodies' Main Recommendations in 2020

The challenges and future outlook are related to the three IPC missions: life sciences and health research activities, public health and health services, teaching and training activities. The objectives include the recommendations made by the Liaison Council and the Scientific Advisory Board.

3.3.1 Main comments from the 2020 Liaison Council meeting (27 May 2020)

Due to the COVID-19 pandemic, the Liaison Council was partially conducted through video-conference.

HE MAM Bunheng (President and Chair) welcomed the new Director (Dr. Laurence BARIL) and the new Chief Financial Officer (Mr. Christophe MOUSSET) and thanked IPC for the contribution in the efforts to combat COVID-19.

Prof Stewart COLE (Co-Chair) highlighted the importance of the scientific achievements (such as in the areas of dengue fever, rabies, malaria, antibiotic resistance, tuberculosis, avian flu, HIV/viral hepatitis, and mosquito control) achieved by the IPC's teams and the successful collaborations with the MoH and the MAFF.

All attendees congratulated IPC for being on the front line of the fight against COVID-19 with the Cambodian CDC and other partners, and for having been designated as a WHO Global Reference Laboratory for COVID-19. IPC's approach, combining surveillance, mapping, and research, is crucial to controlling, monitoring, and learning more about COVID-19 in the region. The effort for transfer of capacities and technical know-how within Cambodia and at regional level (with *Institut Pasteur du Laos* for RT-PCR COVID-19) was also acknowledged by the Council.

The 2020 Liaison Council hosted two Ambassadors: HE Mrs. Eva NGUYEN-BINH, Ambassador of France in Cambodia and HE Pablo KANG, Ambassador of Australia in Cambodia. Indeed, IPC would like to foster closer scientific ties with Australia. As an example, antimicrobial resistance (AMR) is one of the priority areas for cooperation and is the subject of a joint post-doctoral fellowship with the University of Melbourne's Peter Doherty Institute of Infection and Immunity. The post-doctoral fellow started in January 2021 in the AMR group of the LBM.

The support from France and European Cooperation to IPC was acknowledged by all board members through multiple channels including the French Agency for Development (AFD), the German Cooperation (GIZ), the Irish and EU cooperation (Medilabsecure project). This mobilization helped the Institute to respond to the COVID-19 crisis very early on.

3.3.2 Main recommendations of the Scientific Advisory Board meeting (1-4 February 2021)

The scientific advisory board (SAB) was delayed from December 2020 but was eventually conducted virtually over 1-4 February 2021.

The chair was Prof. Sharon LEWIN from the Peter Doherty Institute for Infection and Immunity at the University of Melbourne (Australia) and the co-chair & rapporteur Prof Anavaj SAKUNTABHAI (*Institut Pasteur* Office in Japan).

The other members were: Prof Linfa WANG from Duke - National of University Singapore; Prof Ivo MUELLER from Walter and Eliza Hall Institute (Australia) and *Institut Pasteur*; Prof. Ian SUTHERLAND, US Naval Medical Research Unit-2 (based in Cambodia); Prof Sylvain BRISSE (*Institut Pasteur*, France); HE Prof SAPHONN Vonthanak, UHS; Prof SORN San, MAFF and Prof TOUCH Visalsok, MEYS.

In the current section, only the overall comments are reported:

"The SAB was very impressed by the quality and impact of the IPC activities over the last 2 years. The focus of work is diverse but addresses key health challenges faced by Cambodia. There has been an impressive increase in funding in some laboratory groups, as a result of the COVID-19 pandemic but also through the award of a large number of competitive grants, including awards from the NIH, ANRS and other funders.

The quality of the presentations was excellent with most group leaders taking on board the recommendation from the last SAB to minimize the scientific detail and ensure there is a focus on strategic direction and to include a SWOT analysis. While recognizing that forward planning can be challenging given the funding model of IPC, a clear vision for the future is essential for all laboratories and the institute as a whole.

The SAB acknowledge and applaud the substantial contribution and recognition of IPC in establishing COVID-19 testing facility for Cambodia. It was clear that the virology laboratory in particular has been required to work very hard over this period and have done extraordinarily well. COVID-19 has also impacted every research group given the significant social restrictions, however, the institute as a whole appears to have navigated these challenges very well under the expert leadership of Dr. Baril.

COVID-19 presents many opportunities for IPC in both technology transfer and refining its vision of where they can make the greatest contribution to public health in Cambodia. Future needs and challenges such as genomic testing and vaccine surveillance will be important to plan for now. Long term opportunities for the discipline of public health and infectious diseases will also likely emerge and IPC should be well-positioned to greatly assist in these plans for the country”.

From the SAB report, the **nine SAB Institute recommendations** will be highlighted in the two next sections of the scientific report.

3.4 Institutional objectives

SAB Institute recommendation (N°1) - One of the main goals for the IPC Management and the Senior Scientists is to develop a 5 year strategic plan before the next SAB (tentatively planned December 2022). As this effort will need time and the support of an external consultant, this scientific report will start to address some of the SAB Institute recommendations as those currently listed in the SAB report including highlights in addition to top priorities and the overall direction proposed.

3.4.1 IPC continues to develop its partnerships

The Institute is a key national element of Cambodian research in life sciences and health research with a strong focus on endemic and emerging infectious diseases. As demonstrated with the COVID-19 crisis, IPC is strongly integrated in the Cambodian network both for public health response and for research.

What is true for COVID-19 also applies to all high expertise fields for other infectious diseases and emerging pathogens. This local network is successful thanks to a solid partnership with universities, hospitals, and other governmental and non-governmental institutions.

In addition, IPC continues to strengthen its regional and international partnerships acknowledging that the *Institut Pasteur International Network* (IPIN) is an important component of its international visibility. In November 2020, the IPIN Directors meeting (Yaoundé, Cameroon) confirmed two main priorities: rabies and AMR. The Institute is fully aligned with those two priorities. Rabies prevention and control both in human and animal population is a key priority since more than a decade under the leadership of Dr. Ly Sowath (Deputy Director) and AMR is a key priority with the creation of the bacterial AMR group in partnership with IRD (LMI-DRISA), *Institut Pasteur*, University of Melbourne and CIRAD. Both thematic areas involve several units and laboratories through a multi-disciplinary approach.

SAB Institute recommendation (N°3) - The SAB suggests to enhance collaboration and networking.

The fourth part the scientific report details all the key achievements and on-going collaborations per entity and per the crosscutting activities currently developed for the key research priorities: malaria, emerging infectious diseases, rabies, HIV/viral hepatitis, respiratory infections and AMR including tuberculosis.

Several of the SAB recommendations will need to be implemented before the next SAB, among them:

SAB Institute recommendation (N°4) - The SAB advised to invest in administrative support.

SAB Institute recommendation (N°5) - The SAB advised to recruit for grant management support.

SAB Institute recommendation (N°6) - The SAB advised to prioritize key international partners.

Several actions for better project management by entities will be implemented in place in 2021. Of note, a junior grant manager was appointed early in 2020 but she was not able to stay in Cambodia after a few months due to the COVID-19 crisis. This is an important position to be reconsidered for recruitment in 2021 with an already experienced staff. To better identify the key scientific partners, a detailed list of ongoing grants and partnerships is being developed by the Director's Office and will be available by mid-2021. In addition, several Memoranda of Understanding (MoU) and Memoranda of Agreement (MoA) were signed over the past 5 years and details are provided in the Table 2 below.

No	Name of Institution	Duration	Type (MoU/MoA)
1	Institute of Technology of Cambodia	2016 - 2021	MoA
2	University of Health Sciences (UHS)	October 2018 – Sept. 2023	MoU
3	University of California (USA)	March 2019 – Feb. 2022	MoU
4	Calmette Hospital	August 2019 - July 2024	MoA
5	University of Puthisastra	June 2020 - May 2025	MoU
6	University of Georgia Research Foundation & University of Kentucky Research Foundation (USA)	December 2020 – Nov.2023	MoU*
7	Mission Rabies (UK)	April 2021 - March 2026	MoU
8	Ministry of Environment**	in processing	MoU
	**Nagoya protocol		*Tripartite

Table 2. MoU and MoA signed over the past 5 year

Several other MoUs are still in discussion to strengthen regional and international collaborations.

In 2020, a tripartite collaborative agreement was signed between *Institut Pasteur*, Doherty Institute at the University of Melbourne (Australia) and IPC to strengthen research on AMR.

To give more visibility and to strengthen the current or ready to start future collaborations, IPC Management has undertaken several official visits. To note, a human resource representative visited IPC from *Institut Pasteur* for the first time to any of the Institutes of the IPIN. Also we have had visits from several Ambassadors, funders, and research institutions.

No	Name of Organization	Date	Country
1	Cambodia Oxford Medical Research Unit (COMRU) Dr. Paul Turner	7-Jan-20	UK
2	ANRS – Dr. Bruno Spire (Coordinator South of ANRS site)	13-14 Jan 2020	France
3	Human Resource (<i>Institut Pasteur</i>) Mrs. Rebeca Parreno-Castro	20-Jan-20	France
4	ECOMORE 2 Project Mrs. Sylvie Guillemaut (<i>Institut Pasteur</i>)	20-27 Feb 2020	France
5	LEFS – External Audit – Dr. Malika Gouali	20-26 Jan 2020	France
6	General Inspection - French MEAE Inspector : Mr. Jean Paul Seytre	22-Jan-20	France
7	Expertise France – Mrs. Véronica NOSEDA	09 Feb - 04 March 2020	France
8	CIRAD- Dr. Francois Roger (ASTRE)	17-18 Feb 2020	France
9	Sanofi Pasteur (Corporate) – Dr. Guy Houillon	5-Feb-20	France
10	ANRS – Dr. Claire Rekacewicz	10-Feb-20	France
11	Fondation Mérieux – Dr. François Xavier Babin	12-Feb-20	France
12	CIRAD – Prof. Philippe Girard (Regional Direction)	14-Feb-20	France
13	Embassy of the United States of America in Cambodia Ambassador HE W. Patrick Murphy	7-Apr-20	US
14	Embassy of the United Kingdom in Cambodia Ambassador HE Tina Redshaw	7-Apr-20	UK
15	Embassy of France in Cambodia Ambassador: HE Eva Nguyen Binh	13-Apr-20	France
16	Embassy of Malaysia in Cambodia Ambassador: HE Eldeen Husaini	28-Apr-20	Malaysia

No	Name of Organization	Date	Country
17	Embassy of Australia in Cambodia Ambassador: HE Pablo KANG	27-May-20	Australia
18	Representatives from Embassy of Hungary in Cambodia (after F3 event)	November 2020	Hungary
19	Embassy of the People's Republic of China	5-Feb-21	Chinese
20	Embassy of Japan in Cambodia and JICA	8-Feb-21	Japan

Table 3. Officials visit at the IPC

Finally, the two figures below represent the breakdown of the main research by countries and by funders at IPC in 2020.

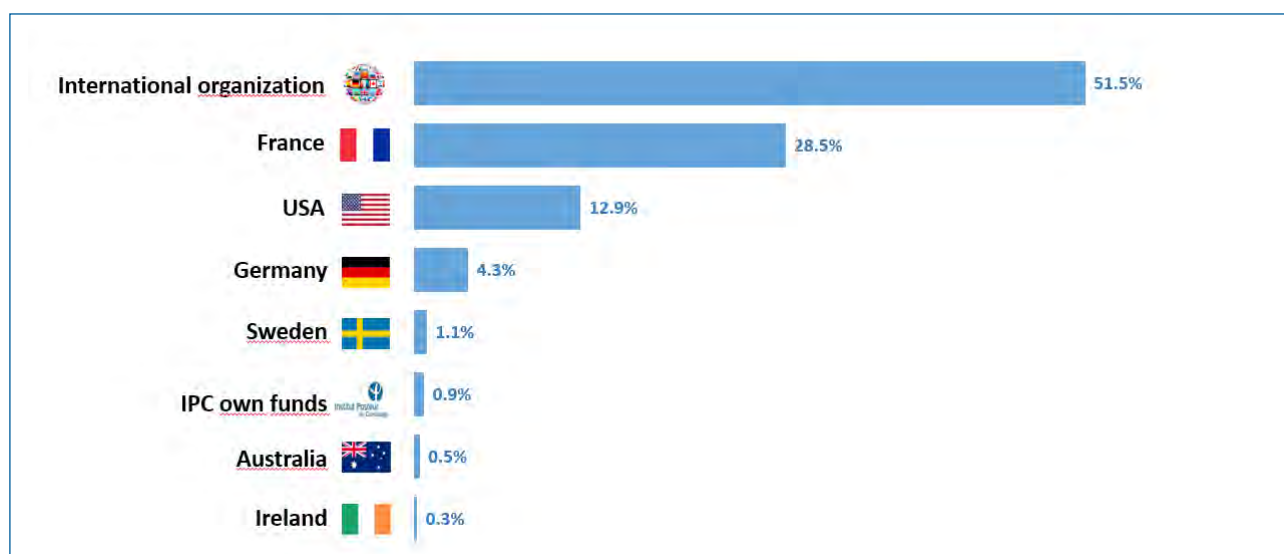


Figure 1. Breakdown of the main research by countries

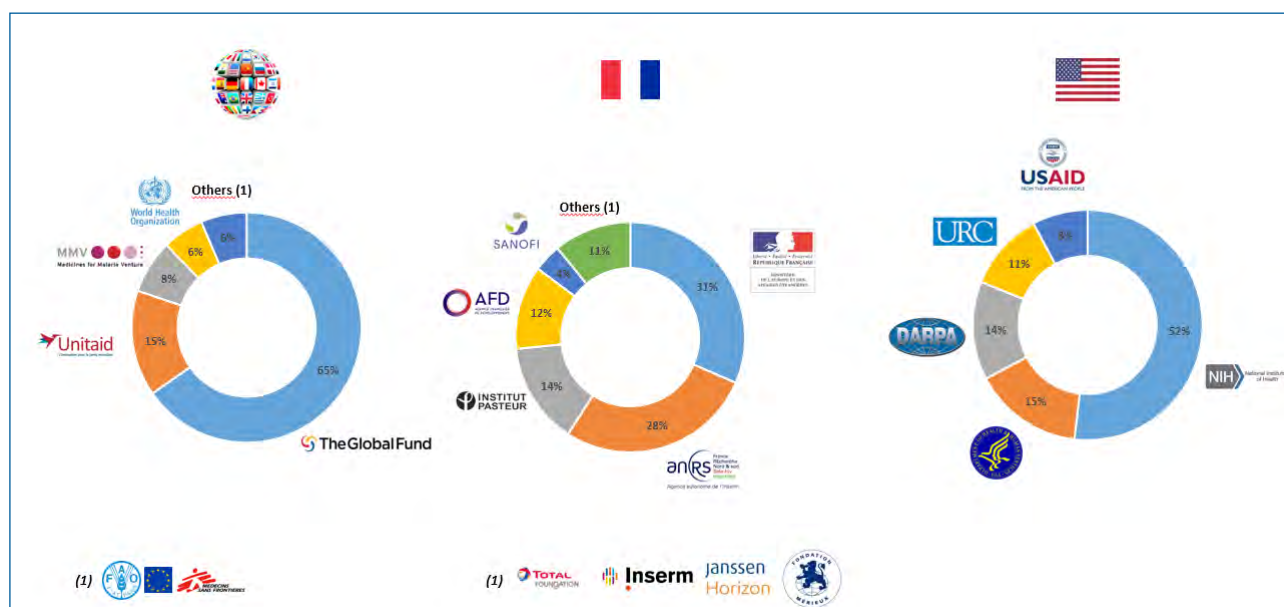


Figure 2. Breakdown of the main research by funders

3.4.2 IPC continues to develop scientific capabilities

SAB Institute recommendations (N°8 and 9) - The SAB suggests to articulate a clear mechanism for technology transfer to local organizations and diversity in leadership. This section and the above one together address in part these two recommendations.

IPC contributes by several means to the development of scientific capabilities. More than 90% of the staff, including senior researchers and managers, is Cambodian but scientists or trainees from ten nationalities were represented in 2020. IPC continues to host senior researchers from different institutions such as *Institut Pasteur* (Paris), CIRAD, for the “one-health” component and IRD (AMR and Geo-Health). IPC continues to give leadership opportunities internally to highly talented Cambodian scientists, engineers, technicians and managers.

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, PharmD., MD, DVM) enrolled from Cambodian and foreign universities. It is vital to make these training and mentoring activities more visible. All scientists based at IPC including colleagues from CIRAD and IRD are contributing to these educational efforts. In 2020, 52 students were hosted by IPC; this only half as many as in 2019 (n=96) mainly due to the COVID-19 crisis.

IPC contributed significantly to the successful launching of the International Master’s Degree in Infectious Disease Studies at UHS and Paris Saclay Universities (UPS) through teaching activities. With travel bans, the master’s-level courses were transformed to be fully on line in 2020-2021. The second year was launched. In addition, IPC created four grants for students (3 Cambodian women and 1 Indonesian woman) starting Master’s Year 1 to offer gender equity as the other 5 students admitted in the first year held fellowships. Now the plan is to for IPC to have two new fellowships per year (starting 2021-2022) and to continue to support the four students in Year 2. In addition to the financial contributions, IPC appointed two senior scientists as points of contact for the Master’s program to facilitate communication with UHS and UPS: Dr. PEAN Polidy (PSRL, Deputy Head of the Immunology Unit) and Dr. Jean POPOVICI (Senior Scientist, Malaria Molecular Epidemiology Unit).

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 resumed using the webinar mode in March 2021. Figure 3 below summarizes the numbers of students for 2021 up to April 2021.

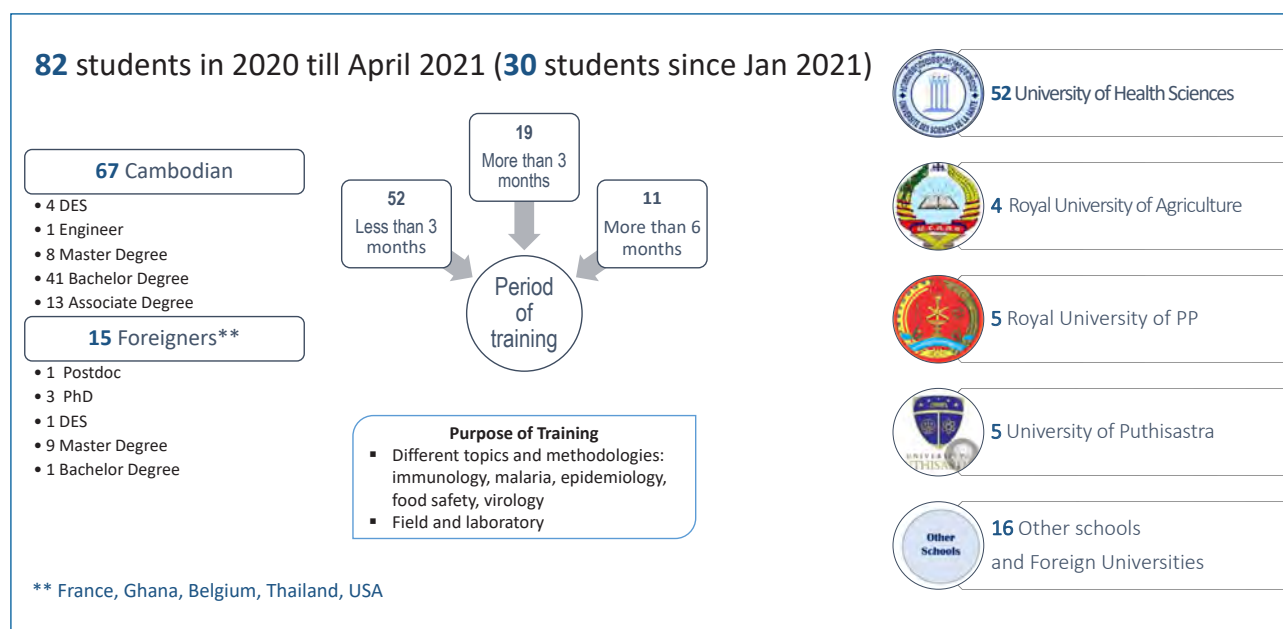


Figure 3. Numbers of students for 2020 up to April 2021

3.4.3 IPC continues to develop its infrastructure

IPC continues to improve laboratories and infrastructure for epidemiology, virology, malaria, entomology, immunology, LBM, LEFS and the vaccination centers (Phnom Penh, Battambang and Kampong Cham Rabies vaccination centers). In 2020, a focus was put on the new equipment for the virology (including BLS-3 facilities for the Emerging pathogens platform) and immunology units and a functional BLS-2 in the PRR Building (for the single cell analysis platform).

For 2021, a new insectarium for entomology and a new animal facility are foreseen. The PRR building was constructed in 2014 under the auspices of the French Alliance for Life Sciences and Health (AVIESAN) and hosts the malaria and entomology units. In addition, to better welcome visitors and ensure security of the campus, the entrance of IPC will be rebuilt (pending evolution of the construction of the Calmette Hospital's new building adjacent to IPC and the availability of external financial support). In addition the mini sequencing platform will be installed early 2021 in the PPR Building; acquisition of a MiSeq and recruitment of new staff members (a manager and 2 technicians). In total three platforms will be available to all scientists.

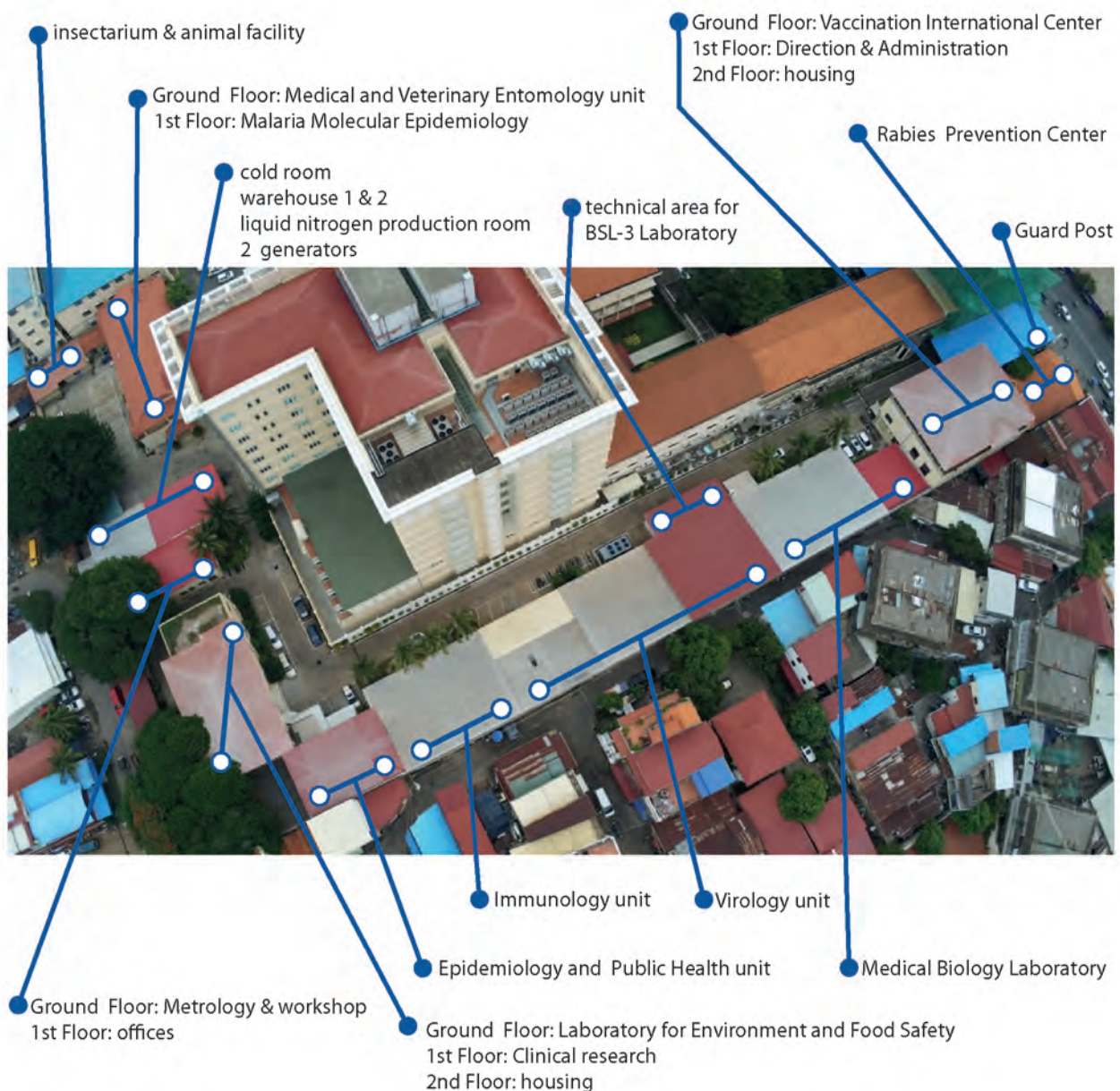


Figure 4. Current organization of the campus

3.4.4 IPC continues to develop its staff and to make careers attractive

An attractive institution is one with a common culture and goals that respects roles and responsibilities at all levels of the organization. This also includes particular attention to gender equity.

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 are encouraged. This is very important in domains where technologies are evolving rapidly such as life sciences and health research. Also, it is important to identify young talent to help those with it to later contribute to middle management and leadership of the institution.

A group training on research management was organized for the scientific staff in July 2020 (two virtual sessions) and a few senior scientists benefited from individual coaching to develop leadership and managerial skills for those highly talented local staff.

A managerial training was developed and implemented for the non-scientific and scientific middle and higher-level staff to ensure a unified and common vision and language in the institution.

A private health insurance (staff and family) scheme will be implemented in 2021 (target June) and a retirement scheme should be implemented in July 2021 in compliance with new government requirements.

In addition, a new training and career development plan will be prepared by the human resource team (with support from a consultant). This will be done simultaneously with an update of the IPC internal regulations, with the expectation of implementation in 2022. These initiatives were discussed with the new team staff delegates whose six members were elected in June 2020 for a two-year period.

Together, 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 to the mutual benefit of the staff and the Institution.

No	Course Name	From Date	To Date	Participants	Unit
1	Kapititude Training (biosafety and biosecurity)	1-Jan-20	1-Dec-20	21	All Units
2	Vaccinology course (<i>Institut Pasteur</i>)	10-Feb-20	6-Mar-20	1	Vacc
3	Theoretical SMQ	19-Feb-20	NA	6	LBM
4	Good Clinical Practice	March-20	September-20	10	Epi Sante
5	Introduction to Clinical Research	March-20	September-20	18	Epi Sante
6	Introduction to Best Clinical Laboratory Practice	March-20	January-21	10	Epi Sante
7	Biobanking	9-Mar-20	1-Dec-20	1	HSEQM
8	ICH-GCP (International Conference of Harmonization - Good Clinical Research Practice)	April-20	September-20	11	Epi Sante
9	Introduction of Collection and Reporting of Adverse Events	April-20	January-21	12	Epi Sante
10	ISO 15189	June-20	July-20	4	LBM & HSEQM
11	Introduction to Informed Consent	June-20	September-20	14	Epi Sante
12	Data Safety and Monitoring	June-20	September-20	12	Epi Sante
13	Introduction of Data Management in Clinical Research	June-20	January-21	11	Epi Sante
14	How to conduct GCP-Inspections_audits of Clinical Investigation Site	June-20	September-20	8	Epi Sante
15	Les enfants et la recherche Clinique	June-20	September-20	10	Epi Sante
16	Viral Hepatitis Course	June-20	July-20	6	Epi Sante

No	Course Name	From Date	To Date	Participants	Unit
17	Covid-19 test (Extraction + Amplification)	1-Jul-20	NA	4	LBM
18	Management and leadership skills	2-Jul-20	3-Jul-20	20	All Units
19	First AID Initiation	23-Jul-20	24-Jul-20	24	All Units
20	The Occupational Health and Safety	23-Jul-20	NA	4	LBM
21	Labor Law Compliance	6-Aug-20	7-Aug-20	2	ADM
22	Transformational Coaching for Leaders	30-Sep-20	30-Sep-20	1	ADM
23	Training of HPV, CTNG PCR Test	12-Oct-20	16-Oct-20	4	LBM
24	Personal Growth & Development	19-Oct-20	20-Oct-20	31	All Units
25	Human Resource Management System (Italent)	October-20	NA	All IPC Staff	LBM
26	ISO17025	5-Nov-20	16-Nov-20	19	LEFS and Other
27	Shipping and Transport of Regulated Biological Material	NA	NA	1	LEFS
28	Management and leadership in research	NA	NA	1	LEFS

Table 4. Main trainings done in 2020 and number of participants

3.4.5 IPC continues to develop its visibility and shares its scientific perspectives

SAB Institute recommendation (N°2) - The SAB advised IPC to invest in internal and external communications.

The IPC is a non-profit research institution and the operating budget has evolved over the years as described in Table 1 above. In 2020, fundraising initiatives were started and IPC benefitted from Cambodian donors' support for equipment. To give more external visibility to IPC, a communication team, a new website (activated in April 2021), improved social networking, and training for journalists were developed and implemented. The IPC teams contributed to several press interviews, books, and documentaries. In addition, the Director participated in many press conferences with the media on request of the MoH and at regional and international level workshops to explain the key role of IPC in supporting the response to emerging diseases.

The main challenge for IPC, however, is to maintain and develop its excellence in the sciences.

SAB Institute recommendation (N°7) - The SAB advised IPC to capitalize on major opportunities.

In response to this recommendation, in 2019 and 2020, efforts were made to secure innovation capacities through various initiatives.

First, the organizational chart (Annex 1) was adapted to give more visibility to some activities in addition to the five research units. This resulted in the following actions:

- Setting up the AMR bacterial group within the LBM with support from IRD LMI- DRISA and the arrival of a research engineer in 2019 (from IRD) and of a post-doc in bioinformatics as part of a tripartite agreement between *Institut Pasteur*, Doherty Institute in Melbourne and IPC;
- Increasing focus on more neglected pathogens such as leptospirosis and melioidosis by applying to the G4 call on AMR from *Institut Pasteur* to have a scientist based in Cambodia (response expected June 2021);
- Restructuring of the clinical research group within the EPI-SP unit with a new organization, a new training curriculum to facilitate continued successful implementation of new projects on tuberculosis, HIV/viral hepatitis and chikungunya (following the outbreak of mid-2020);
- Creation of the "Geo health group" led by an IRD researcher to include Geospatial Information System (GIS) and remote sensing capacity in health. The setup of a dashboard for the follow-up of the COVID-19 activities in collaboration with CDC-MoH proved the importance of this group. The group also has a key role in the environmental component of the One Health approach for viral, bacterial and parasitic diseases and for a regional footprint.
- Better defining of the animal health component through the One Health group led by a senior researcher from CIRAD. The long collaboration between IPC and CIRAD on the risk of spillover was essential in setting up

a project to understand the risk of emergence of new coronaviruses and to increase understanding of the role of dogs in rabies transmission in Cambodia.

- In 2020, IPC joined two large NIH-funded networks: PI-CREID on emerging infectious diseases coordinated by *Institut Pasteur* and another on *Plasmodium vivax* coordinated by the University of Maryland. In 2021, IPC will join a flu/respiratory NIH and Defense Advanced Research Projects Agency (DARPA) network.

Second, some of the key activities were better structured and new projects are anticipated to start in 2021 or 2022. It is important to maintain a strong multi-disciplinary approach within IPC but also to develop projects within the Asia-Pacific hub to account for the richness and complementarity of the ten Institutes in this region.

- For AMR, a workshop within the Asia-Pacific hub and the *Institut Pasteur* of the IPIN was prepared in 2020 and conducted in 2021 for and by the scientists from the ten Institutions based in the region (from Iran to New Caledonia). IPC and *Institut Pasteur* in Korea were the coordinating entities: seven cross-cutting innovative projects were identified and the search for funding will start in 2021.
- Three rabies prevention centers are now fully operational. However, this activity offered to the public remains challenging. The cost structure is unsustainable and has a negative balance as the vaccine price is very high compared to the public price for the three intra-dermal sessions; this is a topic for discussion with MoH in 2021. The database was cleaned and updated to facilitate new projects and new collaborations. For 2021, there is still a need to convince MoH, WHO and FAO to prioritize and take action to better control animal and human rabies in Cambodia.
- A multidisciplinary technical working group for rabies was initiated, meeting on a bi-monthly basis. A new scientist joined the virology unit in January 2021 to support the rabies activities and help to develop projects within the IPIN. She will work closely with the *Institut Pasteur* and other institutes based in Africa on oral dog vaccination to better control human rabies. Also, a massive open online course (MOOC) on rabies with *Institut Pasteur*, *Institut Pasteur de la Guinée* and IPC should start in 2021.
- Response to emergencies in an urgent and efficient mode: this is one of the strengths of IPC and the COVID-19 crisis has demonstrated the importance of maintaining capacities and capabilities at the utmost high standards in a country like Cambodia. The agility of IPC to support the public health response for COVID-19 under the coordination of the MoH in parallel with continuing research efforts (including the One Health approach) was acknowledged at national and international levels.

Third, the development of (mini) platforms is necessary to acquire the most recent state of the art technologies for research. In 2020, a MALDI-ToF was acquired and based at LBM. The staff are now trained to use it for antibacterial diagnosis but also for research. Three formal platforms were identified to share costly equipment within IPC and also with external collaborators, as explained below:

- A platform for emerging pathogens based in the virology unit which benefits from the support of a grant from German Cooperation (GIZ) including the support for a scientist to coordinate it. This platform is running the high containment facility (BLS-3, open in 2008). In 2021, an external technical audit will be conducted to ensure that biosafety and biosecurity requirements are meeting the current regulations. This facility is the only one in Cambodia and has shown its importance not only for COVID-19 but also with the alerts on avian flu, chikungunya and many other pathogens presenting themselves in 2020.
- A platform for sequencing (with the acquisition of a MiSeq from Illumina) will be set up in early 2021 thanks to the support from French Cooperation (through the Ecomore 2 Project via AFD) and European Cooperation (Medilabsecure Project, EU-DEVCO). It will be coordinated both by Virology and MME. This platform will need new competencies such as bioinformatics, biogenomics but also a structuring of the IT database component.
- A single cell analysis platform, unique in Cambodia, coordinated by the Immunology unit. This project has support from both the Wellcome Trust (UK) and GIZ (Germany).

SAB Institute Recommendation (N°5, continued) - The SAB recognized a gap in biostatistics.

There are some remaining gaps to fill to facilitate the work of IPC's scientists. As an example, despite the large size of the magnitude of the epidemiology and public health unit, there are still some needs for support in biostatistics. There are several ways to address the gap and they are not mutually exclusive: a strong partnership with external teams (the model adopted by the clinical research group), hiring consultants on a case-by-case basis, and increasing capabilities through training (for biostatistics adapted to laboratory data, the company IQLS will set up an on-line training for the junior scientist in 2021).

3.4.6 Publications

The last part of this section is dedicated to one of the major research indicators, that is, scientific publications.

In 2020, 69 scientific publications were realized compared to 49 in 2019 (an increase of almost one third). Figures 5 through 8 below summarize the position of IPC staff as authors and the impact factors of the journals. Between 2019 and 2020, the positions as authors were similar but the impact factors of the journals improved. More details on each of the publications are presented in the next section. All publications are available on the website (<https://www.pasteur-kh.org/publications>).

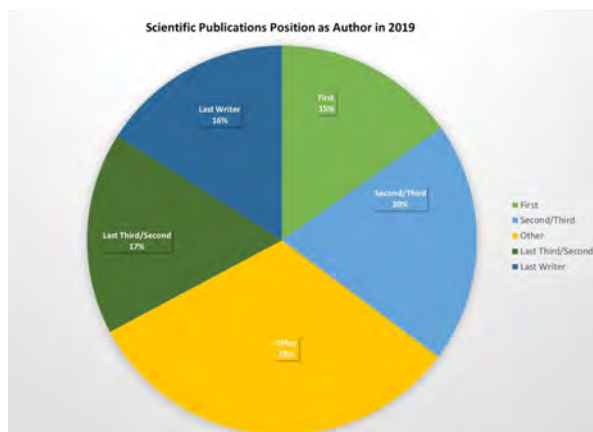


Figure 5

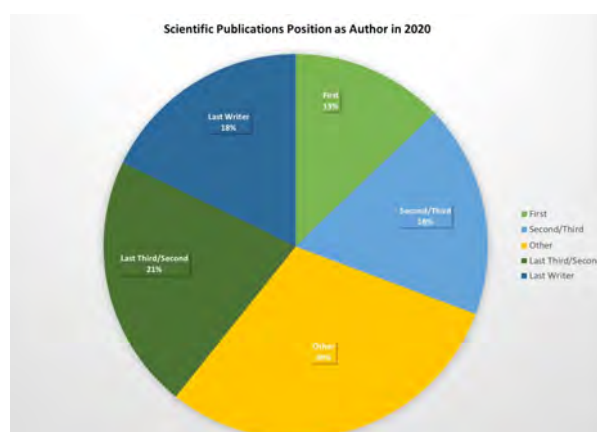


Figure 6

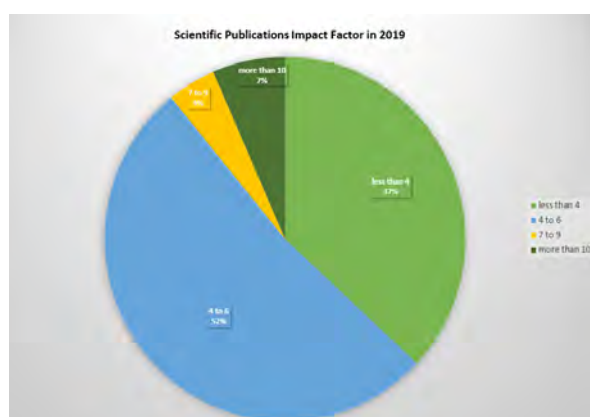


Figure 7

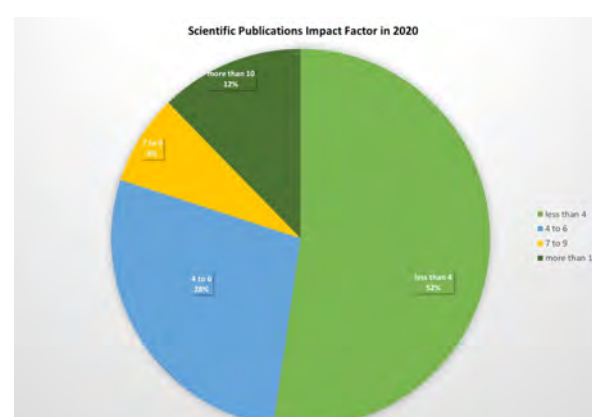


Figure 8

3.4.7 IPC strengths and challenges

In summary, the Institute has a lot of strengths and opportunities:

- It is fully recognized as part of the Cambodian research landscape with successful collaborations not only with the MoH but also with MAFF and soon with the MoE; it will continue to develop its collaborative network with other universities and research institutions in Cambodia and in the GMS;
- IPC now has greater international visibility through its research projects, publications, and networks but also though its capacity to contribute to global health efforts (eg. several referral laboratories)
- The structure of the platforms, the bio-bank (consolidated by the future MoU with the MoE), and the efforts towards the accreditation process will support opportunities for collaboration and diversification of funding agencies and of cross-cutting work within IPC;
- There is now a budget that facilitates the development of new capabilities by combining hosting foreign scientists and supporting the leadership and development skills of its scientific and non-scientific local staff;
- IPC has improved its lay communication and the number of scientific publications;
- IPC has focused on the well-being of its staff and gender equity and will soon implement a new health insurance system and the national retirement and social security scheme.

Despite these positive factors, there remain challenges:

- The foreign staff still has the leading roles and the leadership-building of the local staff needs to be reinforced;
- The COVID-19 crisis was an opportunity to have exceptional financial support to acquire equipment and to develop soft and hard skills; it is important to continue to seek new financial opportunities for the Institute;
- Support to researchers from grant preparation, implementation and recognition will need to be further developed and promoted;
- New equipment received requires new know-how and the integration of new competencies, but some gaps remain such as biostatistics and mathematical modeling skills;
- The Institute will need to modernize its campus (including the security component) and strengthen its administration to reinforce its current middle management component.

The next section shows the richness and the diversity of the research projects and health services.

4. 2020 ACTIVITIES AT INSTITUT PASTEUR DU CAMBODGE

4.1 Malaria Molecular Epidemiology Unit

4.1.1 Functional structure

Benoit Witkowski has headed 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 one head of Unit (B. Witkowski-IP permanent researcher), one deputy head (Nimol Khim-IPC permanent researcher), two contractual researchers (Amelie Vantaux & Jean Popovici), one postdoc (Camille Roesch), three PhD students (Melissa Mairet-Khedim, Anais Pepey, Kutub Ashraf) and seventeen technical & administrative staff. Since December 2016, the malaria unit at IPC is associated with Jean Christophe Barale's group at the Pasteur Institute in Paris to form the Pasteur International joint research unit (PIU) named Malaria Translational Research Unit (MTRU).

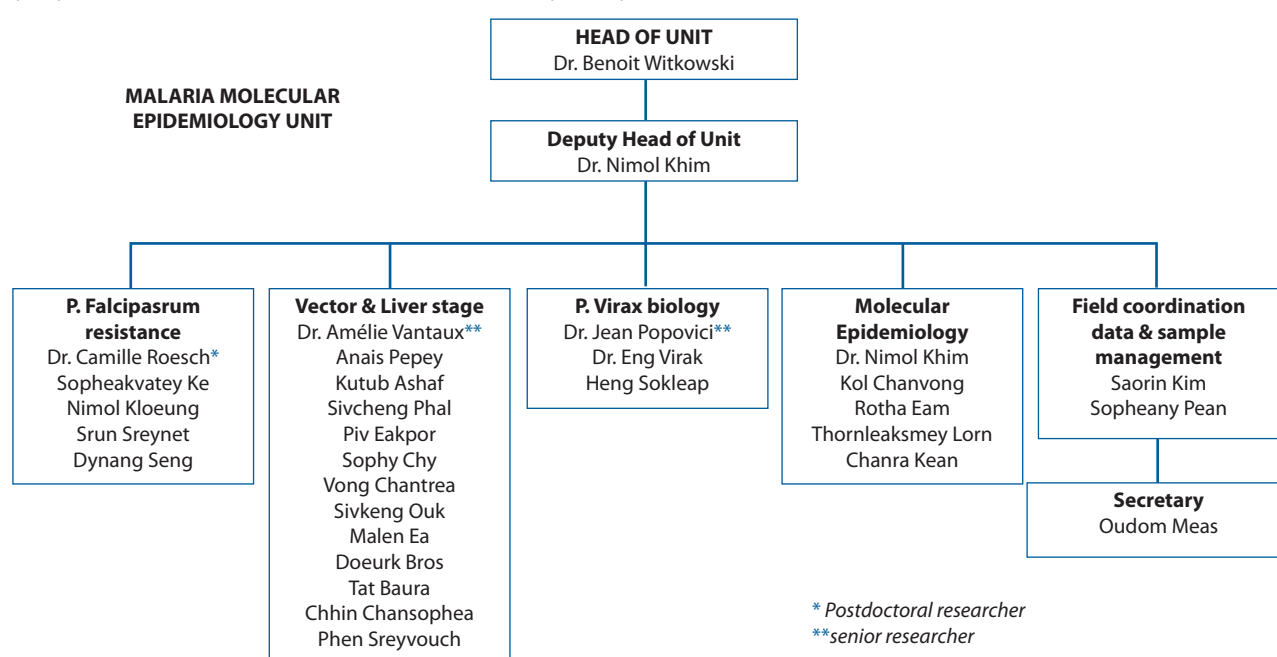


Figure 9. Malaria Molecular Epidemiology unit organogram

Malaria Molecular Epidemiology unit collaborator	
National	International
CNM Ministry of Health ITC URC UHS	Institut Pasteur Institut Pasteur International Network WHO MMV University of Georgia MORU UCSF Institut Cochin MSF University of Gottenburg INSERM CNRS TAD SANOFI NMPE Lao NMCP Myanmar NMCP WEHI CWRU

Table 5. Malaria Molecular Epidemiology unit collaborations

4.1.2 Research Programs – Major Achievements in 2020

a) Chemotherapy of Malaria Parasites

The drug resistance topic is a major and historical research focus of our unit. This initiative is mostly dedicated to clinical resistance to artemisinin-based combination therapy (ACT) drugs through our basic scientific activities. Our research aims to study antimalarial drug resistance at the regional level mostly but also through collaboration with several sites in South Asia and Africa. The research program also includes the evaluation of novel antimalarial drugs and the research on the cellular mechanisms of resistance. In regard to this theme we have built a solid network that includes the National Malaria Control Programs (NMCPs), the World Health Organization (WHO) and research institutions such as Institut Cochin, the Medicines for Malaria Venture (MMV), INSERM, *Institut Pasteur* and IPIN. This theme is also the research core of our PIU. These activities are coordinated by Camille Roesch and Nimol Khim. In terms of achievement we have demonstrated the circulation of parasite resistant to Artesunate amodiaquine (ASAQ) in Cambodia with new insights on testing methodology and molecular stigmata associated with resistance. Data were published in 2020 (Mairet Khedim 2020, Clinical Inf. Dis.). We also confirmed the circulation of ART-R parasites in Papua New Guinea. We monitored drug resistance in Cambodia, Laos, Vietnam, Bangladesh, Sudan and Yemen. From this, data were obtained related to the evolution of drug resistance in the parasite population in line with implemented treatments. These data are expected to be published in 2021 (Mairet-Khedim, unpublished 2021). We are also actively collaborating with several sites in Africa in the area of emerging drug resistance and drug efficacy. Notably, a large study based on ART-R emergence in Uganda, Democratic Republic of Congo and Nigeria were performed. The results are expected to be published in 2021.

In collaboration with INSERM and CPC we have participated in the clinical evaluation of DHA-PPQ in Cameroon. This study suggests the absence of resistance in Cameroon and the extremely high efficacy of this treatment as opposed to the situation in Asia. These data have been submitted for publication (Mairet-Khdeim et al 2021). As part of a long term collaboration with MMV, we are challenging new lead compounds against resistant parasites that circulate in Asia. An important set of data was generated and these findings submitted for publication (Nardella et al. 2021 submitted) or will be submitted in 2021 (Roesch, unpublished 2021). Finally, a collaborative project exists between IPC and IPL on lumefantrine resistance. This theme is and will remain a core activity for our unit. Funding has been obtained to make our future research plan sustainable.

Collaborations	WHO (P. Ringwald), IP (A. Scherf, F. Nardella), University of Gottenburg (P. Sunnerhagen), INSERM (A. Berry), PIU (JC Barale), CNM (R. Leang), IPMC (D. Mazier)
Funding	MMV, IP PTR, BMGF, UNITAID, SRC

Therapeutic Options against *P. vivax* Liver Stages.

This research area is one of the most recently developed in the unit. These activities are overseen by Amelie Vantaux. Because this research aims to fill the gap regarding treatment of *P. vivax* dormant stages, such activities are highly important from a public health perspective. The main characteristic of *Plasmodium vivax* is to develop in patient's liver particular cellular stages called hypnozoites. These hypnozoites remain silent for a variable duration until they exit their dormancy. When awoken the hypnozoites develop to hepatic schizonts 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 (PQ) and is currently in use worldwide. However, in certain patients presenting G6PD deficiency, primaquine treatment leads to severe hemolysis that may cause patient death. Unfortunately, Cambodia is one of the malaria hotspots where G6PD deficiency is the most widespread. For these patients primaquine usage is unsafe or presents an unfavorable risk/benefit balance. For these reasons the development of new drug active against these parasite stages without toxic effects, will be essential for vivax elimination. This program is supported by a long term collaboration with University of Georgia-USA (Steven Maher, Dennis Kyle) and MMV via an annual MMV grant and a NIH R01 grant. This collaboration started in 2017. In 2020, we evaluated 12,000 compounds against *P. vivax* hypnozoites. Some of these compounds show a clear inhibitory signal without signs of cytotoxicity at active concentrations. These results have allowed for extended investigations including mode of action and pharmacomodulation. These activities will be carried out in 2021 within the framework of a R01 NIH grant obtained in late 2020. In parallel, we have investigated the interaction existing between ACT drugs and PQ in the context of targeting hypnozoites. Additional research is ongoing to identify associated mechanisms. In conclusion, this recently included theme is now an important activity for our unit, making it one of the few centers worldwide with this capacity and activity.

Collaborations	University of Georgia, UGA (D. Kyle, S. Maher), Shoklo Malaria Research Unit, SMRU (F. Nosten), Northeastern University College of Science, NUCS (R. Manestch)
Funding	MMV RD150022, NIH R01 AI153290-02

b) Biology of *P. vivax* Invasion

The difficulties in controlling *P. vivax*, the limited drug classes available and the tolerability issues were major determinants in the decision to develop new vaccination approaches. Most of those are based on proteins involved in the invasion process of the parasite. The mechanisms mediating *P. vivax* invasion are not yet totally understood. In addition, evolutionary processes in the parasite population could lead to an altered efficacy of some vaccination targets. We have therefore developed a research program aiming to understand the host-pathogen mechanism associated with invasion mechanisms. These activities are overseen by Jean Popovici. We have demonstrated that pvdbp amplification significantly alters inhibitory action on the humabs on parasites invasion. We have also shown a clinical significance between presence of a neutralizing antibody and infection with amplified parasites. Ultimately these results showed that the vaccination approach could be jeopardized by parasites' evolution to reach immune resistance. These data were published in the journal Nature Communication (Popovici & Roesch et al 2020). Since PvDBP is possibly not the only mediator of invasion, we have extended our investigation to other proteins such as RBP2b. These activities are not yet complete yet but aim to determine whether or not this protein could represent a relevant vaccination target. The host variation is also an important aspect to understand. In this context and in collaboration with Toulouse University Hospital and INSERM, we have investigated the impact of Duffy antigen negativity in the invasion of *P. vivax*.

Collaborations	Case Western Reserve University, CWRU (C. King), Walter and Eliza Hall Institute, WEHI (Wa-iHong Tham), INSERM (A. Berry), IP (C. Chitnis)
Funding	IPC Internal Funding, NIH ICERM NIH-U19-IM

c) Malaria and Malaria Vectors Epidemiology

Our unit is part of the Asia-Pacific International Centre of Excellence in Malaria Research (Asia-Pacific ICERM) which aims at addressing the key challenges to malaria elimination in the Asia-Pacific Region. 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 moderate and high transmission in Papua New Guinea to low, highly focal transmission in Cambodia were carried out. We have established a cohort of participants in Keoseima (Monduliri, Eastern Cambodia). This cohort of 700 individuals was followed monthly during the first year and quarterly during the second year of study. Out of this cohort, important information on malaria prevalence and risk of infection were obtained. The first findings from these results were published in 2020 (Sandfort 2020). Other publications that will more precisely describe the malaria situation are under preparation. We've also determined an important feature for malaria control: the extremely common co-carriage of *P. falciparum*/*P. vivax* and the lack of sensitivity of vivax detection technique at health center (HC) level. All of these combined findings will be followed up with a detailed questionnaire, and the detailed data collected will be used for modelling purposes in partnership with IP (M. White). The vector is the main denominator of malaria transmission. No malaria elimination initiatives were or will be successful without a strong focus on the vector itself. 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 ICERM. Aims of this program are bringing a better understanding of vectors in Cambodia. This project that will address several medical entomology questions which can be grouped under vector epidemiology. Aspects to be investigated are the spatial distribution of the anopheles, their behavior, their phylogeny, tropism and ultimately their potency to carry human parasites. This project is conducted in Monduliri Province, which has high malaria prevalence and the expected outcomes will enable design of further vector control initiatives. In 2017-2018 we collected a total of 3,920 *Anopheles* mosquitoes during the rainy and dry seasons from four ecological settings (villages, forested areas near villages, rubber tree plantations and forest sites). Using odor-baited traps, we were able to show that *anopheles* mosquitoes were mainly zoophilic. Interestingly, 67% of the samples attracted by human baited traps were collected in forest sites. Overall, 20% of collected *Anopheles* were active during the day, with increased day biting during the dry season. 3,131 samples were identified morphologically as fourteen different species, and a subset was also identified molecularly allowing determination of twenty-nine *Anopheles* species indicating a high mosquito diversity which was also observed in the malaria vectors. Molecular screening for *Plasmodium* sp. presence indicated that 3.6% of collected *Anopheles* were positive, most for *P. vivax* followed by *P. falciparum*. These

results highlight some of the key mechanisms driving residual human malaria transmission in Cambodia, and illustrate the importance of diverse collection methods, sites and seasons to avoid bias and better characterize *Anopheles* mosquito ecology in Southeast Asia (Vantaux et al 2021).

A second part of the work on transmission focused on GPS-tracking of local human populations (360 participants) to better understand human movements in time and space as well as select thirty-seven mosquito collection sites based on human density and land cover types. Quarterly mosquito collection started in October 2019 and took place over one year. A total of 6,692 *anopheles* mosquitoes were collected and are currently being screened for malaria parasites. The characterization of the vegetation cover of Cambodia, or any classification of it was not currently available and the most recent dataset dated from 2010, we used Satellite SPOT 6/7 imagery from March 2018 with a 6.5 meter resolution to determine land use in our study area. Land cover mapping revealed a predominance of fields (39%) in the study area, followed by forest (35%) then plantations (24%). Wetland (1%) and urban environment (1%) represented only a small part of the study area. Villages were surrounded by mixed environments, and the study area included large areas of forest on the eastern part and a lot of field on the western side. Since data on land use from the same area exists from 1988, 1998 and 2008, the land use map produced from 2018 was used to compute different landscapes metrics indices to quantify evolution of deforestation, forest fragmentation and landscape diversity. As expected, the deforestation keeps expanding, as diversity indices increase and fragmentation indices decrease (Pepey et al. 2020). We are now analyzing human behaviors in time and space to better understand local transmission. This data, along with the mosquito collection and genetic analyses will allow a deeper understanding of malaria transmission patterns in Cambodia.

Collaborations	WEHI (I. Mueller), Burnett Institute (L. Robinson), CWRU (C. King), IP (Michael White), CNM (D. Lek)
Funding	NIH ICEMR initiative NIH-U19-IM

d) Methods and Interventions in Malaria Control

The malaria unit is committed to action aiming to control or eliminate malaria in Cambodia. It is now clear that forests represent the main areas for the risk of malaria infection. Therefore forest-goers are the population most at risk to contract malaria in Cambodia. As forests represent the key transmission area it is also where resistance to treatment develops and evolves. Consequently, elimination of malaria there is critical. In collaboration with IPC's public health unit, our unit is involved in two malaria elimination programs conducted in Monduliri, Stung Treng & Kratie Provinces. The intervention was implemented in Monduliri Province, while the two other provinces served as controls. The specific role of the malaria unit is the laboratory component of these initiatives. This includes molecular diagnosis, drug resistance and entomology. The prevalence of malaria in Monduliri site was tested by PCR over two cross sections in the intervention trial in 2020. In the four weeks preceding the intervention, approximately 18.2% (26/143) of participants had a *Plasmodium vivax* infection and 1.4% (2/143) had a *Plasmodium falciparum* infection. During cross sectional survey CSS1, 6.6% (13/196) of malaria infections (all *Plasmodium vivax*) and at the final CSS 4.5% (9/199) of malaria infections; 0.5% (1/199) were *Plasmodium falciparum* and 4.0% (8/199) *Plasmodium vivax*. In the control area, a total of 955 samples were analyzed from Kratie Province. PCR analysis was performed for on all 955 samples. Of these, 11.3% (108/955) samples from Kratie were positive for the presence of *Plasmodium spp*. Speciation was possible for 84.3% (91/108) of samples. The inability to confirm the parasite species on certain samples could be attributed to too low parasite density. Of the samples, 97.8% (89/91) were confirmed to present non-falciparum parasites (*P. vivax* & *P. malariae*) and 2.2% (2/91) presented positive results for *P. falciparum* presence. The overall prevalence of *P. falciparum* infections was 0.2% (2/955) and 9.3% (89/955) for non-falciparum species. Analysis of 1,528 samples from Stung Treng Province were analyzed. PCR analysis was performed for 1467/1528 samples. The presence of *Plasmodium spp* was found to be present in 10% (147/1467) of the samples, Speciation was possible for 81.6% (120/147) of the samples. As in Kratie Province, it was not possible to confirm the parasite species on certain samples due to too low parasite density. In addition, 93.3% (112/120) samples were confirmed to present non-falciparum parasites (*P. vivax* & *P. malariae*) and 6.7% (8/120) presented positive results for *P. falciparum* presence. The overall prevalence of *P. falciparum* infections was 0.5% (8/1467) and 7.6% (112/1467) for non-falciparum species.

Collaborations	P. Piola (Public Health Unit, IPC), Malaria Consortium, PFD, CNM
Funding	Global Fund RAI2, Initiative 5%

Evaluation of point of care G6PD test.

Southeast Asia is a main foci of human G6PD deletion. This genetic trait implies severe adverse events under certain drug administration, especially primaquine. As PQ is the only treatment registered that acts against dormant forms of *P. vivax*, its use is essential in the control of this parasite. Therefore, tests have been developed to safely administer PQ in the at-risk population. The existing methods used to measure G6PD activity and adapted to field condition were so far only qualitative (spot test, rapid diagnostic test). The qualitative aspect is an important limitation that restricts ability to generate valid results. To safely administer PQ to females, there was a need for quantitative field implementable assays. In this context we are therefore evaluating novel G6PD quantitative field tests and determining whether these devices can safely identify G6PD deficient individuals. We screened more than 1,400 individuals with G6PD point of care (POC) and the results compared with spectrophotometric gold standard methodology. The definitive conclusion will be available at completion of the project. This initiative, carried out in association with Impact Malaria, CNM, CDC, Population Services International (PSI) and under the President's Malaria Initiative (PMI)/USAID funding, will offer important insights to optimize case management of a *P. vivax* radical cure in Cambodia. The project will be completed in 2021.

Collaborations	PSI, CDC (J. Wang), PMI (M. Thigpen)
Funding	PMI/USAID 444ZCAMBODIA

4.1.3 Research Programs –Outlook for 2021

Research plan overview

Cambodia has officially set a malaria (all genera) elimination target for 2025. In addition to the malaria burden in Cambodia and its public health impact, eradication of malaria in Cambodia takes on a global importance to avoid further emergence and spread of antimalarial resistance. However, drug resistance is only one side of malaria challenge in Cambodia and several question need to be addressed to reach a more complete understanding of this disease. Thus, the MMEU research plan has a holistic approach to the malaria problem that is structured around 3 main research axes that aims to 1) better understand the dynamics of malaria in Cambodia, 2) identify therapeutic options and drug resistance (multi-centric), 3) propose novel approaches to malaria control.

a) Dynamics of Malaria

ICEMR program

Field investigation was completed in 2020, and the activities in 2021 will focus on generating lab data and subsequent data analysis. We will investigate, in collaboration with IP & WEHI, different biological metrics that may enable better understanding of epidemiology. A serological analysis will be performed to define the degree of malaria exposure in the population. We will determine whether asymptomatic may represent a reservoir for resistant parasites. All of these lab-based data will be linked and associated to epidemiological information. We will also investigate the parasite population structure through the amplicon sequencing approach. All this information should bring a new insight on malaria epidemiology and will enable a better tailoring of specific control measures. In addition, we will provide additional metrics in term of malaria prevalence in the frame of regional artemisinin resistance initiative (RAI3) intervention project (see below).

Collaborations	WEHI (I. Mueller), Burnett Institute (L. Robinson), CWRU (C. King), IP (Michael White), CNM (D. Lek)
Funding	NIH ICEMR initiative NIH-U19-IM

Vector-Human Geospatial Study

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, population connections etc. In addition to molecular detection and genotyping of *Plasmodium spp.* infections we will use novel analysis tools to quantify human exposure to mosquito bites by assaying blood samples against anopheline salivary gland proteins (aSGPs) as markers of exposure. A third aspect we will investigate in this project is the immune characteristics of the human population. A transfer of competence 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 aims will help develop or validate methods that could significantly improve the efficiency of malaria control, especially for medium and low transmission settings. Developing and implementing such methods will be particularly crucial for addressing the challenges inherent in eliminating malaria in the Asia-Pacific.

Collaborations	WEHI (I. Mueller), Burnett Institute (L. Robinson), IP (Michael White), CNM (D. Lek)
Funding	NIH ICEMR initiative NIH-U19-IM

b) Therapeutic & Drug resistance Epidemiology

The issue of drug resistance has decreased in Asia due to the reduction of cases, however we have demonstrated its emergence in the Pacific and in Africa. This question thus remains highly relevant. The drug resistance epidemiology in GMS will remain as emergence of new strains could jeopardize the malaria control measures while investigation in other sites (Africa, Pacific, and Asia) will increase as early emergence of drug resistance has been factually identified. Funding has been attained to perform these activities.

Collaborations	WHO, NMCP
Funding	Global Fund RAI3

Drug candidate

The antimalarial therapeutic approaches still present some important gaps that need to be filled. This is mainly focused on *P. vivax* anti-hypnozoite approaches and *P. falciparum* resistance. *P. vivax* is still widely present in Asia, Africa (eastern and Madagascar) and South America while ACT resistant *P. falciparum* has emerged in Africa. Therefore, our efforts to identify a molecule suitable to these situations will be maintained. In addition, an evaluation of Artemisia extract in term of efficacy and drug resistance selection will be investigated. The funding necessary to perform these activities is already in place.

Collaborations	UGA (D. Kyle, S. Maher), SMRU (F. Nosten), NUCS (R. Manestch)
Funding	MMV RD150022/ RD-13-0002, NIH R01 AI153290-02

Therapeutic Efficacy of Primaquine

To date amino-8-quinoline are the only drugs that provide effective anti-parasitic response against hypnozoite. Among those, primaquine is the only drug registered in Cambodia for that purpose. So far, there has not been rigorous assessment of the therapeutic efficacy neither data on globally recommended regimen in Cambodia. Therefore, we aim to launch a randomized PQ efficacy trial to determine whether the recommended regimen could support *P. vivax* control in Cambodia. Delayed by COVID-19, this trial will be launched in April 2021 and will include 300 patients. Alongside this clinical trial we are also aiming to better decipher parasite relapses chronology and mechanisms.

Collaborations	UMD (D. Serre)
Funding	NIH R01AI146590-01A1

c) Novel approaches to Malaria Control

SERO-T&T

While *P. falciparum* is on the cusp of elimination in Cambodia, *P. vivax* appears to be much more recalcitrant to the measures implemented. The experience gained from the ICEMR project indicates that a vast proportion of individuals living in endemic areas and having "at risk" activities are carriers of hypnozoites. These infections are mainly silent for several months but these individuals represent a reservoir for further transmission. Our objective is to launch an operational study in 2021 aiming to detect and treat these carriers. The method will be the sero-diagnosis coupled with PQ

therapeutics (serological test and treat). This early approach is aimed to evaluate the feasibility and thus to determine if a roll out of this methodology would make sense in Cambodia.

Collaborations	WEHI (I. Mueller), CNM (D. Lek)
Funding	Global Fund RAI3

4.1.4 Support to National Authorities

IPC's malaria unit is part, as are all IPC research units, of the Cambodian MoH's overall program. Specifically, our unit is a main collaborator and a main technical support of Cambodia's national malaria control program National Center for Parasitology, Entomology and Malaria Control (CNM). Our unit provides its support to drug efficacy studies that are conducted yearly in Cambodia. Additionally, we have provided molecular confirmation of the presence of *P. knowlesi* in Cambodia.

4.1.5 Teaching and Training

a) Students

PhD students:

Melissa Mairet-Khedim: *P. falciparum* resistance (defense in June 2020)

Camille Roesch: biology of *P. vivax* invasion (defense in May 2020)

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

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

b) Teaching

The Malaria Molecular Epidemiology Unit staff are significantly involved in the international Master's of Infectious Diseases at the University of Health Science (Phnom Penh) and the University of Paris Saclay. All confirmed researchers in the unit are involved in lectures, module development and master's program organization.

4.1.6 Publication List

- 1. Forest malaria in Cambodia: the occupational and spatial clustering of *Plasmodium vivax* and *Plasmodium falciparum* infection risk in a cross-sectional survey in Mondulkiri province, Cambodia.** Sandfort M, Vantaux A, Kim S, Obadia T, Pepey A, Gardais S, Khim N, Lek D, White M, Robinson LJ, Witkowski B, Mueller I. *Malar J.* 2020 Nov 19;19(1):413. doi: 10.1186/s12936-020-03482-4. PMID: 33213471
- 2. Keep the quality high: the benefits of lot testing for the quality control of malaria rapid diagnostic tests.** Incardona S, Bell D, Campillo A, Cunningham J, Arie F, Fandeur T, Luchavez J, Luna CA, Ménard D, Nhem S, Sornillo JB, Witkowski B, Katz Z, Dittrich S, Ding XC. *Malar J.* 2020 Jul 13;19(1):247. doi: 10.1186/s12936-020-03324-3. PMID: 32660630
- 3. Identification of compounds active against quiescent artemisinin-resistant *Plasmodium falciparum* parasites via the quiescent-stage survival assay (QSA).** Reyser T, Paloque L, Ouji M, Nguyen M, Ménard S, Witkowski B, Augereau JM, Benoit-Vical F. *J Antimicrob Chemother.* 2020 Oct 1;75(10):2826-2834. doi: 10.1093/jac/dkaa250. PMID: 32653910
- 4. Clinical and in vitro resistance of *Plasmodium falciparum* to artesunate-amodiaquine in Cambodia.** Mairet-Khedim M, Leang R, Marmai C, Khim N, Kim S, Ke S, Kaury C, Kloeung N, Eam R, Chy S, Izac B, Bouth DM, Bustos MD, Ringwald P, Arie F, Witkowski B. *Clin Infect Dis.* 2020 May 27;ciaa628. doi: 10.1093/cid/ciaa628. Online ahead of print. PMID: 32459308

5. **Plasmodium vivax Liver and Blood Stages Recruit the Druggable Host Membrane Channel Aquaporin-3.** Posfai D, Maher SP, Roesch C, Vantaux A, Sylvester K, Péneau J, Popovici J, Kyle DE, Witkowski B, Derbyshire ER. *Cell Chem Biol.* 2020 Jun 18;27(6):719-727.e5. doi: 10.1016/j.chembiol.2020.03.009. Epub 2020 Apr 23. PMID: 32330444
6. **Impact of the first-line treatment shift from dihydroartemisinin/piperaquine to artesunate/mefloquine on Plasmodium vivax drug susceptibility in Cambodia.** Roesch C, Mairet-Khedim M, Kim S, Lek D, Popovici J, Witkowski B. *J Antimicrob Chemother.* 2020 Jul 1;75(7):1766-1771. doi: 10.1093/jac/dkaa092. PMID: 32211790
7. **Amplification of Duffy binding protein-encoding gene allows Plasmodium vivax to evade host anti-DBP humoral immunity.** Popovici J, Roesch C, Carias LL, Khim N, Kim S, Vantaux A, Mueller I, Chitnis CE, King CL, Witkowski B. *Nat Commun.* 2020 Feb 19;11(1):953. doi: 10.1038/s41467-020-14574-9. PMID: 32075983
8. **DNA Methylation Bisubstrate Inhibitors Are Fast-Acting Drugs Active against Artemisinin-Resistant Plasmodium falciparum Parasites.** Nardella F, Halby L, Hammam E, Erdmann D, Cadet-Daniel V, Peronet R, Ménard D, Witkowski B, Mecheri S, Scherf A, Arimondo PB. *ACS Cent Sci.* 2020 Jan 22;6(1):16-21. doi: 10.1021/acscentsci.9b00874. Epub 2019 Nov 27. PMID: 31989022
9. **Performance of Ultrasensitive Rapid Diagnostic Tests for Detecting Asymptomatic Plasmodium falciparum.** Yeung S, McGregor D, James N, Kheang ST, Kim S, Khim N, Ly P, Sovannaroeth S, Witkowski B. *Am J Trop Med Hyg.* 2020 Feb;102(2):307-309. doi: 10.4269/ajtmh.19-0349. PMID: 31820711
10. **Significant efficacy of single low dose primaquine compared to stand alone artemisinin combination therapy in reducing gametocyte carriage in Cambodian patients with uncomplicated multidrug resistant Plasmodium falciparum malaria.** Vantaux A., Kim S., Piv E., Chy S., Berne L., Khim N., Lek D., Siv S., Muka-ka M., Talyor W. R. and Ménard D. **2020. Antimicrobial Agents and Chemotherapy.** 64:e02108-19.
11. **Studying land cover changes in a malaria endemic Cambodian district: considerations & constraints.** Pepey A., Souris M., Vantaux A., Morand S., Lek D., Mueller I., Witkowski B., Herbreteau V. **2020 Remote Sensing.** rs12182972.

4.2 Epidemiology & Public Health Unit

The Epidemiology and Public Health (EPH) unit, composed of approximately 45 members, performs operational research studies on major public health challenges in Cambodia. International guideline-changing clinical studies are done by the Clinical Research Group (CRG), while the Community Epidemiology Group (CEG) leads community-based studies. Our collaboration with CIRAD has allowed to develop a One Health Group. Almost all studies are in close collaboration with other units of IPC.

4.2.1 Functional structure

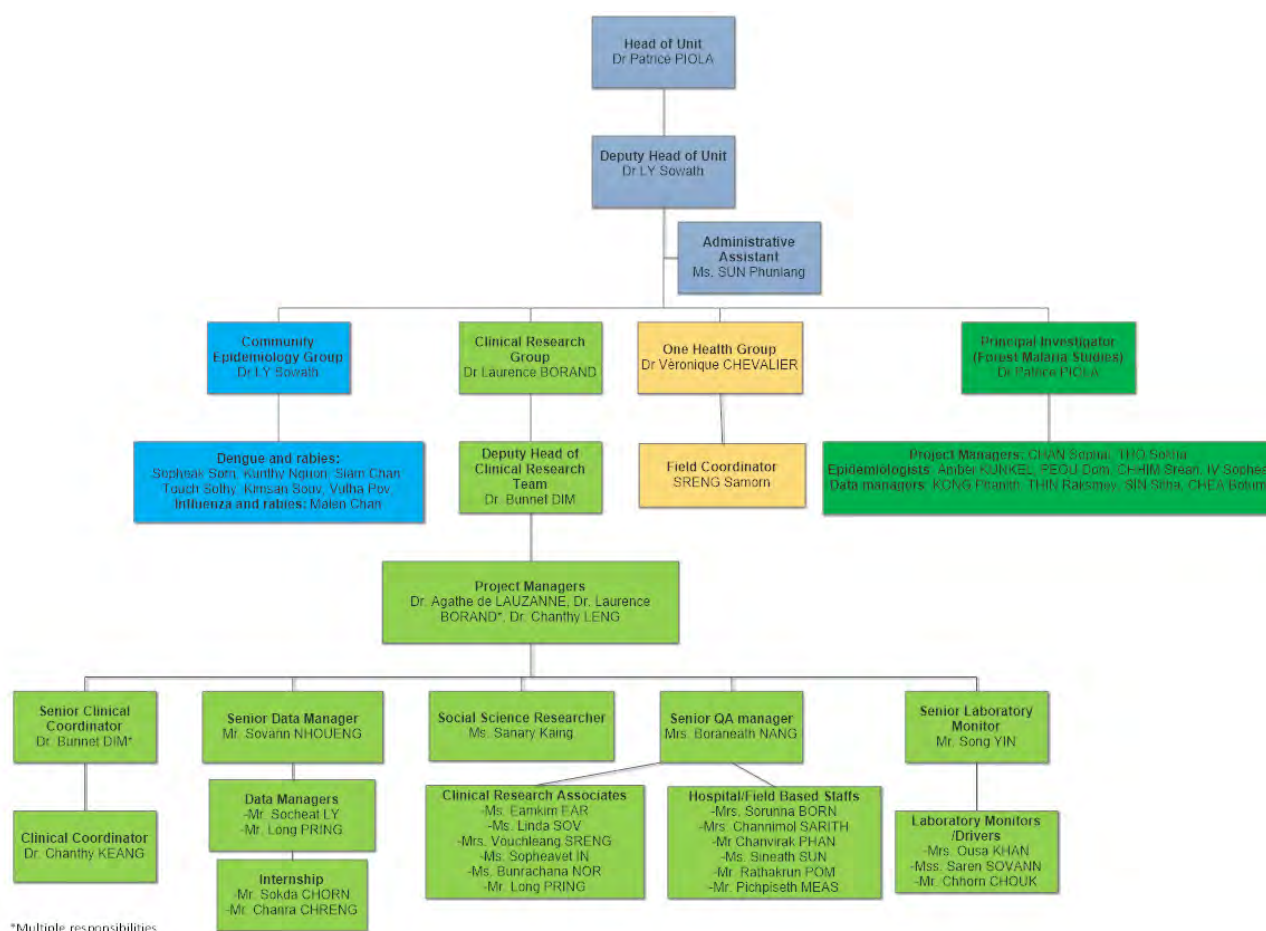


Figure 10. Epidemiology and Public Health unit organogram

The EPH research unit is headed by Dr. Patrice PIOLA and 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, led by Dr. Laurence Borand, has a long history of guideline-influencing trials to improve prevention, diagnosis and treatment of adults and children living with HIV, tuberculosis and hepatitis. This group also runs prospective studies on vaccine preventable diseases.
- The One Health Group, 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 One Health Group include rabies, Japanese encephalitis, and Nipah Virus, AMR and beta coronaviruses. Most CIRAD projects include a human component implemented by the OHG 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 the National Center for HIV/AIDS, Dermatology and Sexually Transmitted Diseases (NCHADS), the National Center for Tuberculosis and Leprosy Control (CENAT), the National Maternal Child Health

Center (NMCHC), the National Immunization Program (NIP), the National Veterinary Research Institute (NaVRI), the Council for the Development of Cambodia (CDC), and CNM to 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), Kampong 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 et les maladies infectieuses émergentes* (ANRS-MIE), the 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 Avénir, Gillings Public Health Fellowship, the World Health Organization, UNITAID, L'Initiative (Expertise France), and the *Agence Française de Développement* (AFD).

4.2.2 Research Programs - Major Achievements In 2020

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 a 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 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.

Collaborations	Team Leader: Sowath Ly. Immunology Unit (T. Cantaert), Virology Unit (V. Duong), Vaccination Unit (Y. Peng)
Funding	<i>Institut Pasteur du Cambodge</i> (2019-2021)

Immunogenicity Assessment of Subjects Receiving Rabies Post-Exposure Prophylaxis in Cambodia (RAB00056)

This work allows description of the humoral immune response (rabies virus neutralizing antibodies or RVNA) titers measured with Fluorescent Antibody Virus Neutralization test (FAVN) at different time points: at baseline before the first PEP dose, 14 days and 28 days after the first PEP injection in two study groups. One group of 120 participants will receive intramuscular (IM) protocol and another group of 120 receives Intradermal (ID) protocol with a follow-up of participants' health status at 6 months after the exposure. ID protocol is per the WHO recommended three sessions (Day 0, 3 and 7) of two-site injections with 0.1 mL vaccine per site. IM protocol is WHO recommended four sessions (Days 0, 3, 7 and 14) of one-site injection using 0.5 mL vaccine per site.

Collaborations	Team Leader: Sowath Ly. Immunology Unit (T. Cantaert), Virology Unit (V. Duong), Vaccination Unit (Y. Peng)
Funding	Sanofi Pasteur RAB00056 (2020-2022)

Immunity persistence after abridged intradermal rabies PEP: The RESIST-3 study

The aim of this study is to assess whether the ID regimen (3 sessions/1-week) confers long-term immunity equivalent to that conferred by TRC intradermal regimen (4 sessions/1-month), and to explore the characteristics of humoral and cell-mediated protection and response before and after boosting, by group. The plan is to recruit 90 patients for the study in Cambodia, of any age, gender or immune status identified for having received ID PEP through 4 sessions/1 month or 3 sessions/1 week at least two, five or 10 years earlier, with no rabies boosting since then. The work will determine and compare baseline (pre-booster) and post-booster RVNA titers in people who received PEP 2, 5 and 10 years earlier after a bite by a dog, using both RFFIT and FAVN.

Collaborations	Team leader: Sowath Ly. Immunology Unit, (T. Cantaert), Virology Unit (V. Duong), Vaccination Unit (Y. Peng), <i>Institut Pasteur</i> (P. Parize)
Funding	Action Concertée Inter-Pasteurienne (ACIP) n° 403-2020 (2021-2022)

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). Demographic parameters, bite incidence risk factors, human/dog relationships, bite management behavior are available: a publication has been submitted on this topic. Serological analyses of post-vaccination sera have been completed. A second paper on the latter topic is under preparation.

Collaborations	Team Leaders: Véronique Chevalier, (CIRAD), Sowath Ly (IPC), Uppsala University, National Institute of Hygiene and Epidemiology Vietnam
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.

Collaborations	Team Leaders: Véronique Chevalier (CIRAD), <i>Institut Pasteur</i> (L. Dacheux), <i>Institut Pasteur de Madagascar</i>
Funding	<i>Institut Pasteur</i>

Rabies Control 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, pet owner acceptability and feasibility. Demographic parameters, bite incidence risk factors, human/dog relationships, bite management behavior are available: a publication has been submitted. Serological analyses of post-vaccination sera have been achieved. A second paper is under preparation.

Collaborations	Team leaders: Véronique Chevalier, (CIRAD), Sowath Ly (IPC),
Funding	Région Occitanie

ZooCov

Naturally hosted in wild animals such as bats, beta coronaviruses (beta-CoVs) are responsible for severe and fatal human respiratory infections. Taking advantage of existing knowledge, on-going projects and a strong partnership, we propose a One Health–cross-sectoral and interdisciplinary approach associating medical sciences, ecology, epidemiology and social sciences (1) to improve the knowledge on “wet market”, wild animal meat trade, consumption practices and drivers in Cambodia (2) to address potential or existing risks at the animal-human interface that constitutes the wildlife meat market and consumption in Cambodia, and the link between drivers and this risk, (3) to develop a flexible and integrated early-detection system of viral spill-over events. To achieve these goals, we will implement dedicated sociological, epidemiological and virological surveys in two provinces of Cambodia, namely Monduliri and Stung Treng where wildlife trade and wild meat consumption have already been documented. Predictors of human exposure to beta coronaviruses –practices, handled and/or consumed species, location, season, will be identified using field data and *ad hoc* statistical models. We will then use the results of these surveys and participatory modeling tools to co-design with stakeholders an improved and adapted version of the existing wildlife health surveillance system for Cambodia, focusing on the detection of beta-CoVs

Collaborations	Team Leaders: Véronique Chevalier CIRAD, Institut de Recherche pour le Développement (IRD), Hong Kong University (HKU), Wildlife Conservation Society (WCS), World Wildlife Foundation (WWF)
Funding	ANR, Région Occitanie, Pasteur Asia Foundation

b. Dengue, Zika and Chikungunya

CHIK PREG Study: Mother-to-Child Chikungunya Infection at Jayavarman VII Hospital

Data on clinical characteristics and consequences of CHIKV infection on obstetrical outcomes are limited in resource-limited countries. The objective of this study is to characterize the clinical signs of CHIKV during pregnancy and obstetrical outcomes among confirmed CHIKV infected pregnant women in one large hospital in Cambodia in the context of CHIKV infection.

Collaborations	Team Leaders: Laurence Borand (Epidemiology Unit), Tineke Cantaert (Immunology Unit), Veasna Duong (Virology Unit), Immunology Unit, Virology Unit, Jayavarman VII Hospital
Funding	ANRS-MIE - IPC

c. HIV and/or Tuberculosis Infection

DATURA – ANRS 12424 Clinical Trial: Determination of Adequate Tuberculosis Regimen in Adults and Adolescents Hospitalized with HIV-associated Severe Immune Suppression ($CD4 \leq 100$ cells/ μ L)

Mortality in people entering late into HIV care with a tuberculosis (TB) co-infection is high. These patients present with malabsorption of TB drugs leading to lower efficacy of TB treatment. Corticosteroid are also known to reduce mortality and occurrence of immune reconstitution inflammatory syndrome (IRIS) in this population. The main objective of this multi-centric study is to estimate the impact of an intensified initial phase of TB treatment on mortality at 48 weeks among HIV-infected adults and adolescents hospitalized for TB with $CD4 \leq 100$ cells/ μ L in comparison with the standard TB regimen. This study is under preparation.

Collaborations	Team leaders: Laurence Borand (Clinical Research Group), Calmette hospital, Kossamak Hospital, Khmer- Soviet Friendship Hospital, NCHADS,
Funding	ANRS-MIE

LILAC-TB - ANRS 12394 Study: 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. Recruitment and follow-up of patients is ongoing.

Collaborations	Team leaders: Laurence Borand (Clinical Research Group), Pean Polidy (Immunology Unit), Immunology Unit, Sihanouk Hospital Center of Hope
Funding	ANRS-MIE

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 multi-center (seven countries) research project, aiming at improving the diagnosis of childhood tuberculosis through decentralization of TB diagnosis and systematic tuberculosis diagnostic in vulnerable children. Recruitment and follow-up of patients is ongoing.

Collaborations	Team Leader: Laurence Borand. (Clinical Research Group) CENAT, National Pediatric Hospital, Kampong Cham and Takeo Hospitals, Batheay and Ang Rokar Districts' health facilities
Funding	UNITAID <i>L'Initiative</i>

OPTICAM: Optimizing Latent TUBERCULOSIS Treatment INITIATION in Cambodia among People Living with HIV

The aim of the project is to improve Latent Tuberculosis Infection (LTBI) treatment uptake in people living with HIV (PLHIV) in Cambodia by assessing the impact of an intervention combining the use of shorter TPT regimen based on weekly isoniazid and rifapentine for 3 months (3HP) procured through a secured supply chain and a full healthcare worker capacity-building and PLHIV information package- based on previously identified barriers- as compared to the current practice of 6-month daily isoniazid based TPT regimen (6H), on the TPT coverage among PLHIV attending adult OI/ART clinics in Cambodia. Current status: Identification of barriers to TPT is completed. The intervention phase is under preparation.

Collaborations:	Team Leader: Laurence Borand (Clinical Research Group), NCHADS, CENAT, and the Clinton Health Access Initiative (CHAI).
Funding:	<i>L'Initiative</i>

d. Hepatitis

TA-PROHM - ANRS 12345: Tenofovir as Prevention of Hepatitis B Transmission for Mothers

Despite effective primary prophylaxis, the hepatitis B virus (HBV) remains an important 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 used to further decrease the risk of vertical transmission, especially in areas where WHO-recommended serovaccination is inaccessible. This project aims to prevent mother-to-child transmission (MTCT) by reducing the HBV viral load in mothers by offering antivirals, typically initiated starting week 24 of pregnancy. Patient recruitment and follow-up are completed.

Collaborations	Team Leaders: Laurence Borand (Clinical Research Group), Patrice Piola (Epidemiology Unit), Veasna Duong (Virology Unit), 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.
Funding	ANRS-MIE

4.2.2.2 Bacteriological Diseases And Antibiotic Resistance

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). The study was completed and two nested ancillary studies are ongoing. These are (1) Assessment of the serological status to diphtheria toxin in low and middle-income countries and (2) Characterization of the main relevant genes carried by *B. pertussis* strains collected in Cambodia as part of the PERILIC study.

Collaborations	Team leader: Laurence Borand (Clinical Research Group), Gauthier Delvallez (Medical Biology Laboratory) Rabies Vaccination Center at IPC, National Immunization Program, National Pediatric Hospital, several provincial hospitals, private clinics and health centers
Funding	<i>Fondation Total</i>

A Hospital Based Case-Control Study to Identify Risk Factors of Leptospirosis and to Improve Post-Disaster Management of Emerging Diseases

While extreme weather events such as floods are associated with leptospirosis outbreaks, little is known about the magnitude of leptospirosis incidence in Myanmar where floods are *a priori* increasingly frequent. Leptospirosis in Myanmar is suspected to be endemic, but remains underdiagnosed. We contributed to the design, protocol writing and technical support 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 are the National Health Laboratory of Myanmar, and the *Institut Pasteur de Nouvelle Calédonie*.

Collaborations	Team leader: Patrice Piola (Epidemiology Unit), NHL Myanmar (Htay Htay Tin, Khin Nyein Zan, May July), <i>Institut Pasteur de Nouvelle Calédonie</i> (Cyrille GOARANT), <i>Institut Pasteur</i> (Jane DEUVE)
Funding	ECOMORE 2 (AFD 2017-2020)

FSPI ARCAHE

This activity is led by the LBM and LMI DRISA. The objective of this project is twofold (1) to identify the sources of emergence and spread of resistant bacteria in Cambodia using a “One Health” approach, and (2) 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 study started at the end of October 2020. The epidemiology unit was involved in the Hospital Work Package (Calmette Hospital in Phnom Penh and Battambang Hospital) to recruit hospitalized patients with bacterial infections (both resistant and non-resistant) and the Animal Work Package (Battambang) to sample animals in households from patients with antibiotic resistance. The Principal Investigators are Cheng Sokleaph (IPC LBM) and Anne-Laure Bañuls (IRD); patient recruitment is ongoing.

Collaborations	Team leader: Véronique Chevalier (CIRAD), Patrice Piola (Epidemiology Unit). IRD (Anne-Laure BANULS, Mallory HIDE), Medical Biology Laboratory (CHENG Sokleaph, Gauthier DELVALLEZ), CIRAD (CHEVALIER Véronique), Calmette Hospital (BORY Sotharith), Battambang Hospital (CHIEK Sivhour)
Funding	<i>Fonds de Soutien pour les Projets Innovants</i> (FSPI)

4.2.2.3 Parasitological Diseases

Blocking Malaria Transmission in Vulnerable Forest Populations through Forest Malaria Workers: A Key for Malaria Elimination in Cambodia

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 there are 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 totaling 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 were trained to develop the necessary skills to work and control malaria inside forests. Following the presentation of Year 1 results in February 2020 to the scientific committee, we implemented a monthly Intermittent Preventive Treatment of Forest Goers (IPTfg) inside the study forests from June 2020 to January 2021. Of notice, this intervention has since then been adopted and implemented by the Ministry of Health in Cambodia and Laos as an aggressive malaria elimination measure. Malaria cases notified in neighboring health centers were monitored to estimate the effectiveness of the intervention. Main collaborators in this research are CNM and Partners for Development.

Collaborations	Team leader: Patrice Piola, Sophea IV (Epidemiology Unit). Partners for Development (PfD), National Center for Parasitology, Entomology and Malaria Control (CNM), World Health Organization (WHO), Malaria Molecular Epidemiology Unit
Funding	<i>L'Initiative Canal 2</i> : 17SANIN205

Comparison of Effectiveness of Forest-Based Malaria Control Interventions in Large Forests of Cambodia

The overarching objective of this study was to eliminate malaria infections inside forests (and consequently in surrounding villages) within a year (August 2019 to August 2020), through continuous in-forest active mass screening and treatment (MSATs with RDTs) and continuous passive detection. 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. The assumption is that forest goers are a mobile community accustomed to the harsh working condition inside forests and are the best placed to become malaria workers inside them, where transmission occurs in Cambodia. A tailored Android application was developed to support FMWs across all components of this project. The data uploaded from this MHealth application was used in near real-time to monitor, track (GPS) and evaluate all field activities and malaria indicators. Preliminary results in 2020 showed a very low sensitivity of RDTs among forest goers (7% for *P. falciparum* and 3% for *P. vivax*), contributing to the decision to implement the IPTfg mentioned in the above study. Main collaborators are Partners for Development, Malaria Consortium, ITC, and CNM.

Collaborations	Team leaders: Patrice Piola, Srean Chhim, Dom Peov (Epidemiology Unit). Malaria Consortium (MC), Partners for Development (PfD), National Center for Parasitology, Entomology and Malaria Control (CNM), Malaria Molecular Epidemiology Unit (IPC), Institute of Technology of Cambodia, World Health Organization (WHO), UNOPS
Funding	Resistance to Artemisinin Initiative 2 (Global Fund) - Operational Research QSE-M-UNOPS

Cambodian Forest People: Anthropological Study of an Often Marginalized Society Important to Malaria Elimination

Many activities take place in Cambodian tropical forests. Hence, it is likely an important place of human flows and movement, which can form a network of people's movements. This situation may cause a spread of forest disease. Based on this, we conducted a study in 2020 in Stung Treng, Kratie and Monduliri forests about the perception of malaria and its treatment by forest-dwelling or forest-working societies. Then, we focused more on the relationships, the uses and the significance of tropical forest for the population that spends time there. For example, being aware of the importance of forest products for the incomes of forest-goers can explain their presence in malaria areas. Through this entry point, the team is attempting to find an efficient approach to eliminate this disease.

Collaborations	Team leaders: Théphanie Sieng, Patrice Piola (Epidemiology Unit). Malaria Consortium (MC), Partners for Development (PfD), National Center for Parasitology, Entomology and Malaria Control, <i>Institut de Recherche pour le Développement</i> (Frédéric Bourdier)
Funding	<i>L'Initiative Canal 2</i> : 17SANIN205 Resistance to Artemisinin Initiative 2 – Operational Research QSE-M-UNOPS

4.2.3 Research Programs - Outlook for 2021

HEPEDIAC – ANRS 12420 Clinical Trial: Pilot Therapeutic Study of DAA treatment for Children and Adolescents with Active HCV Infection in Cambodia

Transmission from mother to child is the main route of acquisition of Hepatitis C (HCV) mono-infection and of HCV/HIV co-infection in children. Approximately 25% of HCV-infected children spontaneously clear the virus but the 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 sofosbuvir/daclatasvir combination for children at least 6 years old and adolescents with active HCV infection. This study is currently under preparation.

Collaborations	Team Leader: Laurence Borand (Clinical Research Group). NCHADS and OI/ART sites, Jayavarman VII Hospital, Kantha Bopha 1 and 2 Hospitals
Funding	ANRS-MIE

DENTHOM - Study of Dengue-Like Illness in Kampong Thom Province, Cambodia

We will evaluate the occurrence of dengue and dengue-like syndromes in Kampong Thom Province through surveillance of children and adult inpatients at three referral hospitals in Kampong Thom Province and at Jayavarman VII Pediatric Hospital in Siem Reap Province. Laboratory follow-up of suspected cases will be performed. The coupling of epidemiological and entomological data, associated with spatial and temporal analysis explaining the transmission hotspots will help define the link between mosquito surveillance and dengue incidence. We will also evaluate the seroprevalence of arboviral infection in Kampong Thom Province through a serosurveillance study.

Collaborations	Team Leader: Sowath Ly (Epidemiology Unit). Immunology Unit, (T. Cantaert), Virology Unit, (V. Duong), Entomology Unit (S. Boyer), National Dengue Control Program of the Ministry of Health, Provincial Health Department of Kampong Thom province and Jayavarman VII Pediatric Hospital in Siem Reap province
Funding	NIH-PICREID (2021-2024) (1U01AI151758 – 01)

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.

- L. Borand is member of the Cambodia Committee for TB Research (CCBR), contributed to the Join Program Review (in charge of the Research Section) and National Strategic Plan for the TB National Program in 2019/2020
- Dr. Dim Bunnet and L. Borand are part of the technical working group (TWG) on HIV/TB
- L. Borand participated to the Elimination Prevention of Mother to Child Transmission of HIV, Syphilis and Hepatitis B guideline preparatory workshops organized by the National Maternal and Child Health Center (NMCHC) and consultative workshop on the Implementation of National Strategic Plan and financing for the Viral Hepatitis Program
- P. Piola Two lecture sessions- Introduction to Outbreak Investigation and Response (IPC, 2019).
- Two epidemiology staff provided lectures on GPS and Quantum GIS mapping software for officers of the Cambodian Veterinary Applied Epidemiology (CAVET) program, Ministry of Agriculture
- S. Ly helped in the supervision of two officers of the Cambodian Veterinary Applied Epidemiology (CAVET) Program, Ministry of Agriculture and US-CDC, 2018-2019
- S. Ly's team provides support in data management for Covid-19 lab testing (IPC and CDC-MoH)

4.2.5 Teaching and Training

Teachings

- Masters of Infectious Disease (UHS, IPC and Paris Saclay University):
 - Patrice Piola: BioStatistics Module (2020, 2021)
 - Patrice Piola : Clinical Trials Module (2020)
 - Laurence Borand/ Dim Bunnet/ NHOEUNG Sovann/ Ly Socheat/ Nang Boraneath: Module Data Management and Good Clinical Practices (2020)
- Chest X-RAY Training to diagnose childhood tuberculosis from 16 – 20 Dec 2019 provided by Dr Dim Bunnet to health center and district hospital staff.
- Extensive and repeated trainings on pediatric TB, including TB workout and notably naso-pharyngeal aspirate collection and assay with Gene Xpert Ultra, chest X-ray interpretation, severe pneumonia standard of care performed by the Clinical Research Group.

Trainings

- PhD Student:
 - Sophea IV (Univ. Toulouse 2018 - 2020)
- Masters Students
 - Dim Bunnet (LSHTM clinical Trials – 2019-ongoing)
 - Nhoeung Sovann (UHS – Epidemiology – from 2021)
 - Fanny Velardo (CNAM-Pasteur, Public Health, 2019)

- Student Internships
 - Chorn Sokda (Bachelor Geography and Land Management -2019-ongoing)
 - Thuch Somaly (Bachelor Geography and Land Management -2019-2020)
 - Chheng Chanra (Bachelor Geography and Land Management -2020-ongoing)

4.2.6 Publications List

1. **Autoantibody Profiling in Plasma of Dengue Virus-Infected Individuals.** Vo, HTM, Duong, V, Ly, S, Li, QZ, Dussart, P, Cantaert, T. *Pathogens*. 2020 Dec 18;9(12). doi: 10.3390/pathogens9121060. PMID: 33352902
2. **Decreased Type I Interferon Production by Plasmacytoid Dendritic Cells Contributes to Severe Dengue.** Upasani, V, Scagnolari, C, Frasca, F, Smith, N, Bondet, V, Vanderlinden, A, Lay, S, Auerswald, H, Heng, S, Laurent, D, Ly, S, Duong, V, Antonelli, G, Dussart, P, Duffy, D, Cantaert, T. *Front Immunol*. 2020;11:605087. doi: 10.3389/fimmu.2020.605087. PMID: 33391269
3. **Hepatitis B e Antigen (HBeAg) Rapid Test and Alanine Aminotransferase Level-Based Algorithm to Identify Pregnant Women at Risk of HBV Mother-to-Child Transmission: The ANRS 12345 TA PROHM Study.** Segeral, O, Dim, B, Durier, C, Prak, S, Chhim, K, Vong, C, Pech, S, Tiv, S, Nem, B, Hout, K, Nouhin, J, Chhun, S, Borand, L. *Clin Infect Dis*. 2020 Dec 17;71(10):e587-e593. doi: 10.1093/cid/ciaa282. PMID: 32188982
4. **Efficacy and tolerability of artemisinin-based and quinine-based treatments for uncomplicated falciparum malaria in pregnancy: a systematic review and individual patient data meta-analysis.** Saito, M, Mansoor, R, Kennon, K, Anvikar, AR, Ashley, EA, Chandramohan, D, Cohee, LM, D'Alessandro, U, Genton, B, Gilder, ME, Juma, E, Kalilani-Phiri, L, Kuepfer, I, Laufer, MK, Lwin, KM, Meshnick, SR, Mosha, D, Mwapasa, V, Mwebaza, N, Nambozi, M, Ndiaye, JA, Nosten, F, Nyunt, M, Ogutu, B, Parikh, S, Paw, MK, Phyto, AP, Pimanpanarak, M, Piola, P, Rijken, MJ, Sriprawat, K, Tagbor, HK, Tarning, J, Tinto, H, Valea, I, Valecha, N, White, NJ, Wiladphaingern, J, Stepniewska, K, McGready, R, Guerin, PJ. *Lancet Infect Dis*. 2020 Aug;20(8):943-952. doi: 10.1016/S1473-3099(20)30064-5. PMID: 32530424
5. **Pregnancy outcomes and risk of placental malaria after artemisinin-based and quinine-based treatment for uncomplicated falciparum malaria in pregnancy: a WorldWide Antimalarial Resistance Network systematic review and individual patient data meta-analysis.** Saito, M, Mansoor, R, Kennon, K, Anvikar, AR, Ashley, EA, Chandramohan, D, Cohee, LM, D'Alessandro, U, Genton, B, Gilder, ME, Juma, E, Kalilani-Phiri, L, Kuepfer, I, Laufer, MK, Lwin, KM, Meshnick, SR, Mosha, D, Muehlenbachs, A, Mwapasa, V, Mwebaza, N, Nambozi, M, Ndiaye, JA, Nosten, F, Nyunt, M, Ogutu, B, Parikh, S, Paw, MK, Phyto, AP, Pimanpanarak, M, Piola, P, Rijken, MJ, Sriprawat, K, Tagbor, HK, Tarning, J, Tinto, H, Valea, I, Valecha, N, White, NJ, Wiladphaingern, J, Stepniewska, K, McGready, R, Guerin, PJ. *BMC Med*. 2020 Jun 2;18(1):138. doi: 10.1186/s12916-020-01592-z. PMID: 32482173
6. **Frequency, risk factors, and complications of induced abortion in ten districts of Madagascar: results from a cross-sectional household survey.** Ratovoson, R, Kunkel, A, Rakotovo, JP, Pourette, D, Mattern, C, Andriamiadana, J, Harimanana, A, Piola, P. *BMC Womens Health*. 2020 May 6;20(1):96. doi: 10.1186/s12905-020-00962-2. PMID: 32375746
7. **Tools to accelerate falciparum malaria elimination in Cambodia: a meeting report.** Lek, D, Callery, JJ, Nguon, C, Debackere, M, Sovannaroeth, S, Tripura, R, Wojnarski, M, Piola, P, Khean, ST, Manion, K, Nguon, S, Kunkel, A, Vernaev, L, Peto, TJ, Dantzer, E, Davoeung, C, Etienne, W, Dondorp, AM, Tuseo, L, von Seidlein, L, Guintran, JO. *Malar J*. 2020 Apr 15;19(1):151. doi: 10.1186/s12936-020-03197-6. PMID: 32293452
8. **Comparison of Japanese Encephalitis Force of Infection in Pigs, Poultry and Dogs in Cambodian Villages.** Ladreyt, H, Auerswald, H, Tum, S, Ken, S, Heng, L, In, S, Lay, S, Top, C, Ly, S, Duong, V, Dussart, P, Durand, B, Chevalier, V. *Pathogens*. 2020 Sep 1;9(9). doi: 10.3390/pathogens9090719. PMID: 32882890
9. **Klebsiella pneumoniae carriage in low-income countries: antimicrobial resistance, genomic diversity and risk factors.** Huynh, BT, Passet, V, Rakotondrasoa, A, Diallo, T, Kerleguer, A, Hennart, M, Lauzanne, A, Herindrainy, P, Seck, A, Bercion, R, Borand, L, Pardos de la Gandara, M, Delarocque-Astagneau, E, Guillemot, D, Vray, M, Garin, B, Collard, JM, Rodrigues, C, Brisse, S. *Gut Microbes*. 2020 Sep 2;11(5):1287-1299. doi: 10.1080/19490976.2020.1748257. PMID: 32404021

10. **“Health in” and “Health of” Social-Ecological Systems: A Practical Framework for the Management of Healthy and Resilient Agricultural and Natural Ecosystems.** De Garine-Wichatitsky, M, Binot, A, Ward, J, Caron, A, Perrotton, A, Ross, H, Tran Quoc, H, Valls-Fox, H, Gordon, IJ, Promburom, P, Ancog, R, Anthony Kock, R, Morand, S, Chevalier, V, Allen, W, Phimpraphai, W, Duboz, R, Echaubard, P. *Front Public Health*. 2020;8:616328. doi: 10.3389/fpubh.2020.616328. PMID: 33585387
11. **Nipah virus circulation at human-bat interfaces, Cambodia.** Cappelle, J, Hoem, T, Hul, V, Furey, N, Nguon, K, Prigent, S, Dupon, L, Ken, S, Neung, C, Hok, V, Pring, L, Lim, T, Bumrungsri, S, Duboz, R, Buchy, P, Ly, S, Duong, V, Tarantola, A, Binot, A, Dussart, P. *Bull World Health Organ*. 2020 Aug 1;98(8):539-547. doi: 10.2471/BLT.20.254227. PMID: 32773899
12. **Systematic or Test-Guided Treatment for Tuberculosis in HIV-Infected Adults.** Blanc, FX, Badje, AD, Bonnet, M, Gabillard, D, Messou, E, Muzoora, C, Samreth, S, Nguyen, BD, Borand, L, Domergue, A, Rapoud, D, Natukunda, N, Thai, S, Juchet, S, Eholie, SP, Lawn, SD, Domoua, SK, Anglaret, X, Laureillard, D, Team, SAT. *N Engl J Med*. 2020 Jun 18;382(25):2397-2410. doi: 10.1056/NEJMoa1910708. PMID: 32558469
13. **Serological Evidence for Japanese Encephalitis and West Nile Virus Infections in Domestic Birds in Cambodia.** Auerswald, H, Ruget, AS, Ladreyt, H, In, S, Mao, S, Sorn, S, Tum, S, Duong, V, Dussart, P, Cappelle, J, Chevalier, V. *Front Vet Sci*. 2020;7:15. doi: 10.3389/fvets.2020.00015. PMID: 32064271
14. **Dynamics and diversity of mosquito vectors of Japanese encephalitis virus in Kandal province, Cambodia.** Boyer S., Peng B., Pang S., Chevalier V., Duong V., Gorman C., Dussart P., Fontenille D., Cappelle J. 2020. *Journal of Asia-Pacific Entomology*, 23 (4) : p. 1048-1054.

Abstracts and Presentations

- **51st Union World Conference on Lung Health, 20 to 24 October 2020**, virtual conference. Barriers to Tuberculosis Preventive Therapy Initiation in People Living with HIV in Cambodia: patients and clinicians perspective. Marion Mora, Tan Eang Mao, Long Pring, Song Yin, Kunreath Seak, Socheat Ly, Sokda Chorn, Somaly Thuch, Bruno Spire, Laurence Borand.
- **51st Union World Conference on Lung Health, 20 to 24 October 2020**, virtual conference. Understanding Barriers To Latent Tuberculosis Treatment In People Living With HIV In Cambodia: Results From The KAP Survey And Facility Assessment From The OPTICAM Study. Jennifer Campbell, Kunrath Seak, Bunnet Dim, Sovann Nhoueng, Sokun Chay, Emi Okamoto, Sharvani Saraf, Penh Sun Ly, Tan Eang Mao, Laurence Borand.
- **1st Union World Conference on Lung Health, 20 to 24 October 2020**, virtual conference. Knowledge, Attitudes and Practices on Childhood TB among Health Care Workers in Sub-Saharan Africa and South-East Asia. B. Joshi , H. Font , M. Bonnet , L. Borand , R. Moh , G. Breton , C. Khosa , E.Wobudeya , O. Marcy , J. Orne-Gliemann, TB-Speed Study Group.

4.2.7 Perspectives of the Epidemiology and Public Health Unit

At three years:

- Extend research topics to Non Communicable Diseases (i.e. diabetes and tuberculosis; cancer and hepatitis)
- Extend regional collaborations
- Diversify funders and collaborations
- Hire a senior biostatistician for background validation of all statistical outputs and results.
- Develop a paperless study environment, including e-CRFs

At five years:

- Reinforce support to national health authorities through the relevant research findings
- Develop the capacity building for a national middle management within the unit
- Train national staffs
- Increase our attractiveness to national talented researchers (masters and PhDs)
- Welcome and attract Postdoctoral scientists

Perspectives of CIRAD

V. Chevalier will leave Cambodia in June 2021 and will be replaced by H. Guis, a veterinarian epidemiologist CIRAD with expertise in geomatics. H. Guis will keep developing the One Health research activities in collaboration with other IPC units and the CIRAD Grease network. V. Chevalier will keep coordinating the ZooCov project as well as the two rabies-related projects. She will be involved in building two main One Health initiatives in which IPC will be partner, i.e. :

- PREZODE in Action in Southeast Asia as a proposal to the IDRC. The objective of PREZODE in Action in Southeast Asia is to implement the PREZODE framework to reduce zoonotic risk emergence within 3 pilot countries in Africa and South East Asia. The specific objectives in each pilot countries will be to 1) to improve the understanding of the risk of zoonotic transmission between animals (both domestic and wild) and humans and co-develop solutions to tackle them with the people facing those risks, 2) to co-develop or strengthen existing early detection system to ensure rapid action to prevent emerging disease spread, 3) to ensure relevant stakeholder engagement including representation of minorities and gender equity from local to national level by co-developing prevention solutions and system together with them and to embed those solutions within national policies to ensure sustainability of the action, 4) To build a strong and permanent science-society-policy dialog enable to define relevant health management strategies.
- HORIZON-CL6-2021-BIODIV-01-11: What else is out there? Exploring the connection between biodiversity, ecosystems services, pandemics and epidemic risk

4.3 Immunology Unit

The Immunology Unit at IPC was founded in 2018 with a major emphasis to investigate host immune responses to pathogens of major public health importance in Cambodia. Our laboratory is focused on three major research axes, focusing on immune responses during flavivirus infections, improvement of vaccination strategies for rabies post-exposure prophylaxis (PEP) and identification of new immune-related biomarkers for infectious diseases. Moreover, we are hosting a transversal single cell analysis platform.

4.3.1 Functional structure

The Immunology Unit at IPC has nine team members based in Phnom Penh, under the leadership of a Head of Unit and organized as per the chart below.

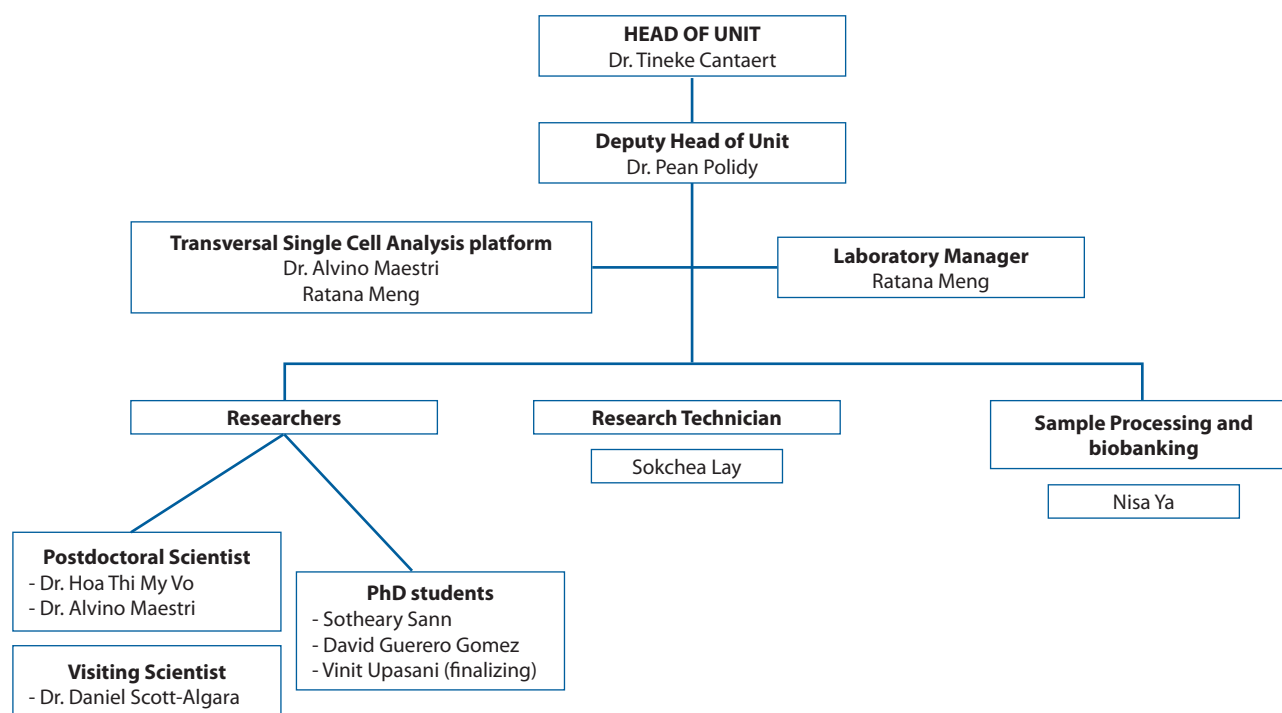


Figure 11. Immunology unit organogram

4.3.2 Research Programs – Major Achievements In 2020

Axis 1: Immune responses during Flavivirus infection

Dengue viruses (DENV) infect up to 390 million individuals yearly, of which 500,000 cases require hospitalization. Since 2012, dengue is the most common vector-borne viral disease among 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 in populated urbanized areas, contributing to the spread of DENV. DENV is a member of the family Flaviviridae, and consists 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).

Aim 1.1 Antibody-independent B cell functions

DENV replicates in primary immune cells such as dendritic cells and macrophages, which contribute to dissemination of the virus. Susceptibility of other immune cells such as B cells to direct infection by DENV and their subsequent response to infection is not well defined. In a cohort of 60 Cambodian children, we showed that B cells are susceptible to DENV infection. Moreover, we show that B cells can support viral replication of laboratory adapted and patient-derived DENV strains. B cells were permissive to DENV infection albeit low titers of infectious virions were released in cell supernatants. CD300a, a phosphatidylserine receptor, was identified as a potential attachment factor or receptor for entry of DENV into B cells. In spite of expressing Fcγ-receptors, antibody-mediated enhancement of DENV infection was not observed in B cells in an in-vitro model. Direct infection by DENV induced proliferation of B cells in dengue patients in vivo and plasmablast/plasma cell formation in vitro. To summarize, our results show that B cells are susceptible to direct infection by DENV via CD300a and the subsequent B cell responses could contribute to dengue pathogenesis (Upasani et al, *Frontiers Immunol* 2021).

Aim 1.2 Discovery of novel biomarkers for severity at hospital admittance

The clinical presentation of dengue virus (DENV) infection is variable. Severe complications mainly result from exacerbated immune responses. Type I interferons (IFN-I) are important in antiviral responses and form a crucial link between innate and adaptive immunity. Their contribution to host defense during DENV infection remains understudied, as direct quantification of IFN-I is challenging. We combined ultra-sensitive single-molecule array (Simoa) digital ELISA with IFN-I gene expression to elucidate the role of IFN-I in a well-characterized cohort of hospitalized Cambodian children undergoing acute DENV infection. Higher concentrations of type I IFN proteins were observed in blood of DENV patients, compared to healthy donors, and correlated with viral load. Stratifying patients for disease severity, we found a decreased expression of IFN-I in patients with a more severe clinical outcome, such as dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). This was seen in parallel to a correlation between low IFNα protein concentrations and decreased platelet counts. Type I IFNs concentrations were correlated to frequencies of plasmacytoid DCs, not DENV-infected myeloid DCs and correlated inversely with neutralizing anti-DENV antibody titers. Hence, type I IFN produced in the acute phase of infection is associated with less severe outcome of dengue disease. (Upasani et al, *Frontiers Immunol* 2020).

Aim 1.3 Autoantibody profiling in dengue-infected individuals

Autoimmune syndromes following dengue can be observed in long term follow up. Anti-DENV antibodies are cross-reactive with surface antigens on endothelial cells or platelets and could be involved in the pathogenesis of dengue. However, no studies have analyzed the autoantibody repertoire and its roles in dengue pathogenesis. Hence, we aimed to describe the autoantibody profile in dengue patients with different disease severities. We utilized a protein array with 128 putative autoantigens to screen for IgM and IgG reactivity in plasma obtained from healthy donors (n = 8), asymptomatic individuals infected with DENV (n = 11) and hospitalized dengue patients (n = 21). Even though the patient cohort is small, we show that 80 IgM and 6 IgG autoantibodies were elevated in DENV infected patients compared to age-matched healthy donors. Individuals undergoing a primary DENV infection showed higher amounts of IgG autoantibodies, not IgM autoantibodies, compared to individuals undergoing secondary infection. No differences were observed between asymptomatic and hospitalized dengue patients. Nineteen autoantibodies, which react against several coagulation and complement components, correlated with platelet counts in severe dengue patients. This current study provides a framework to explore a possible role of candidate autoantibodies in dengue immunopathogenesis. (Vo et al, *Pathogens* 2020).

Aim 1.4 Investigation of TLR2-mediated responses in dengue virus infection

Vascular permeability and plasma leakage are immune-pathologies of severe dengue virus (DENV) infection, but the mechanisms underlying the exacerbated inflammation during DENV pathogenesis are unclear. Here, we demonstrate that TLR2, together with its co-receptors CD14 and TLR6, is an innate sensor of DENV particles inducing inflammatory cytokine expression and impairing vascular integrity in vitro. Blocking TLR2 prior to DENV infection in vitro abrogates NF-κB activation while CD14 and TLR6 block has a moderate effect. Moreover, TLR2 block prior to DENV infection of peripheral blood mononuclear cells prevents activation of human vascular endothelium, suggesting a potential role of the TLR2-responses in vascular integrity. TLR2 expression on CD14 + + classical monocytes isolated in an acute phase from DENV-infected pediatric patients correlates with severe disease development. Altogether, these data

identify a role for TLR2 in DENV infection and provide insights into the complex interaction between the virus and innate receptors that may underlie disease pathogenesis. (Aguilar-Briseno et al, Nat Commun 2020).

Axis 2: Biomarkers of infectious diseases

Aim 2.1 ANRS N°12358: “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 microRNA-150 and microRNA-148a might be a biomarker that could discriminate HIV/TB coinfection from HIV or TB mono-infection and healthy individuals. Also, the expression levels of miR-148a and miR-223 could be a diagnostic biomarker of IRIS in TB/HIV infected patients and they may play a role in the pathogenesis of IRIS. In 2020, we have planned to analyze exosomal microRNA in the plasma. However, this experiment have been delayed due to the SARS-CoV2 pandemic.

Aim 2.2 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)”.

Additional tools are urgently needed not only to help diagnose TB but also to assess the response to TB treatment in empirically treated patients. In a previous study, we found that IL-1Ra plasma concentrations dropped dramatically after two months of TB treatment (Nouhin J, J Infect Dis 2017). The objective of this proof-of-concept study is to demonstrate that IL-1Ra concentrations significantly decrease earlier within two weeks following TB treatment initiation in adults with documented TB. In parallel, we are assessing two other biomarkers: IP-10 and sCD163. Patient enrollment will start in March 2020. The inclusions of participants and follow-up for all group categories in Ivory Coast and Cambodia are currently ongoing. In the Cambodia site, to date, 28 participants were enrolled: 22/20 TB+/HIV- and 6/30 TB+/HIV+. The measurement of biomarkers has been delayed because of the slow rate of enrollment of the participant in the study and the impact of SARS-CoV-2 pandemic.

Axis 3: Vaccine responses to rabies virus (RABV) vaccination

The WHO endorsed a new shortened protocol of PEP in the April 2018 guidelines (13). This “*Institut Pasteur du Cambodge* 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.

4.3.3 Research Programs – Outlook for 2021

Axis 1: Immune responses During Flavivirus Infection

Aim 1.1 Unravel B cell responses in asymptomatic DENV-infected individuals

In 2018, we conducted a field study in order to collect a new cohort of asymptomatic, acute infected individuals and a cohort of hospitalized patients in collaboration with the Virology Unit and Epidemiology and Public Health Unit, IP Cambodia. Biological specimens and associated data were obtained at day 0, day 3, day 5, day 10 and day 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 clear the virus. In previous work we revealed that these individuals show an increased activation of the adaptive immune compartment regulated by proper feed-back mechanisms, leading to elimination of viral infection without excessive immune activation (Simon-Lorieri et al, Scie Transl Med, 2017). Here, we demonstrated that B cell activation and plasma cell development pathways are differentially regulated between the 2 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/ Detailed investigation of the polyclonal anti-DENV serum response of asymptomatic individuals to uncover novel correlates of protection.

Collaborations	Virology Unit, (V. Duong) Structural Virology Unit, IP Paris (G. Barba-Spaeth and M. Flamand) Antibodies in Therapy and Pathology Unit, IP Paris (P. Bruhns) CB UTECHS, <i>Institut Pasteur</i> (M. Hasan)
Funding	PTR 2019-2021 (PTR 2019-212) Janssen Horizon grant 2019-2022

Aim 1.2 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. More in detail, whereas the abundance of afucosylated IgG1 is increased in hospitalized

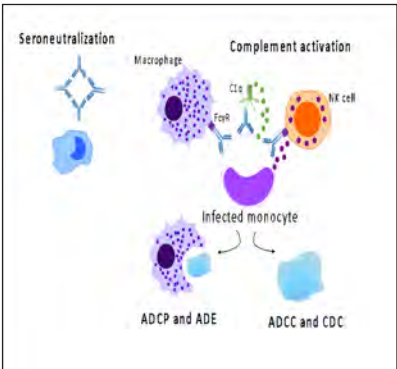


Figure 12. Cell based assays for antibody effector functions analysis.

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 glycosylation profile could modulate antibody-effector functions and/or affinity maturation and the production of neutralizing antibodies conferring protection from severe disease (manuscript in preparation).

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 1). 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.

Collaborations	Virology Unit, (V. Duong) Rockefeller University, lab of Molecular Genetics and Immunology (J. Ravetch) Virus and Immunity Unit, <i>Institut Pasteur</i> (T. Bruel)
Funding	HHMI/Wellcome International Research Scholars Program (2017-2021) (208710/Z/17/Z) PTR 2019-2021 (PTR 2019-212)

Aim 1.3 Understand the role of different CD4+ T cell subsets in progression of DENV infection.

The contribution of CD4+ T cells in protection from disease or development of immunopathogenesis after dengue infection is less clear, however, recent data has pointed towards a protective role of CD4+ T cells during infection. By immunoprofiling of asymptomatic individuals, our previous work has shown an association between proliferation and activation of the CD4+ T cell compartment with asymptomatic outcome of infection, irrespective of previous dengue infection or viral load (Simon-Loriere, Duong et al, Scie Transl Med, 2017). However, detailed information is lacking on activation and function of different subsets of CD4+ T cells in asymptomatic and hospitalized dengue patients and their pattern of cross- reactivity on a single cell level during secondary infection is unknown. Hence, we aim to understand the contribution of homotypic and heterotypic DENV- specific T cells to disease development or protection after infection.

Regulatory T cells control unwanted immune responses like autoimmune reactions. Indeed, we have shown the impact of a non-functional Treg compartment on peripheral B cell tolerance in patients with primary immunodeficiencies. In the context of infectious diseases, Tregs can have both beneficial and harmful effects. Tregs subsets and functions are understudied during dengue infection. We hypothesize that defects in the regulatory T cell compartment contribute to immunopathology of dengue infection as observed in severe dengue. Hence, we aim to understand the function of regulatory T cells during the course of dengue infection in mild and severe dengue cases.

Collaborations	University of Hasselt, Belgium (M. Kleinewietfeld) Oxford University, London (M. Dominguez-Villar) Virology Unit, (V. Duong)
Funding	HHMI/Wellcome International Research Scholars Program (2017-2021) (208710/Z/17/Z)

Aim 1.4 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)

Collaborations	Laboratory of Malaria and Vector Research, NIAID, NIH (J. Manning) MiVEGEC Unit, IRD, UR224 (D. Misse) Entomology Unit, (S. Boyer)
Funding	Calmette-Yersin RIIP PhD program (2020-2022)

Aim 1.5 PI-CREID: Center of Research of Emerging Infectious Diseases

IP Cambodia is part of the NIH funded consortium PI-CREID. Here, the Immunology Unit is involved in aim 4, study of host adaptive immune responses to emerging infectious diseases in South-East Asia. In this framework, we have established a single cell analysis platform at IP Cambodia (see below). We will increase our insight into the adaptive immune response (Both B and CD4 T cell responses) at a single cell level and the sequence-function relationship of human antibodies generated during infectious diseases (DENV, RVFV, CCHFV) by combining sequencing at a single cell level with antibody repertoire analysis. We will study function and characterize structure at a single antibody level. Moreover, we aim to perform detailed immunoprofiling of flavivirus encephalitis. We will provide novel understanding of the role of cellular immunity in DENV disease. The proposed activity will allow the implementation of infrastructure and an analysis pipeline for outbreak preparedness in areas where viruses with potential pandemic threats circulate.

Collaborations	Functional Genetics of Infectious Diseases Unit (A. Sakuntabhai) Virology Unit, (V. Duong) Epidemiology and Public Health Unit, (S. Ly) Entomology Unit, (S. Boyer)
Funding	NIH PICREID (1U01AI151758 – 01)

Axis 2: Biomarkers of infectious diseases

Aim 2.1 Micro-RNA as potential biomarkers of infectious diseases

miRNAs have been identified in numerous diseases, particularly in cancer, that show as potential novel diagnostic and prognostic biomarkers with high specificity and sensitivity. The detectable miRNAs in body fluids and tissue with high stability provide an abundant source for miRNA-based biomarkers. The microRNAs detection in biofluid by flow cytometry technique, which has been validated and setup in our lab, allow us to screen up to 69 microRNAs in a small sample volume and can be used in different projects. Our project proposals in the pipeline for the year 2021 are (1.) a grant proposal PTR 2021 on microRNA in dengue (in collaboration with Dr. Carolina SCAGNOLARI, IP Rome), (2.) Biomarkers associated with tuberculosis severity in HIV and/or non-HIV infected patients (ANRS-MIE- *Appel d'offre Septembre* 2021), (3.) Biomarkers associated with the clinical outcome of HBV MTCT, (ANRS No 12345's ancillary study) (ANRS MIE-*Appel d'offre Septembre* 2021). (4.) Biomarkers for guiding and monitoring anti-microbial treatment (pilot study) (IPC funding, April 2021), (5.) Biomarker of Schistosomiasis infection, in collaboration with CNM/MOH (funder needs to be identified).

Axis 3: Vaccine responses to rabies virus (RABV) vaccination

We are establishing a biobank for the 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.

Patient inclusions are continuing from 2020.

Collaborations	Virology Unit, (V. Duong) Epidemiology and Public Health Unit, (S. Ly/L. Borand) Vaccination center, (Y. Peng)
Funding	Internal project (<i>Institut Pasteur du Cambodge</i>)

Axis 4: Investigation of adaptive immune responses in SARS-CoV-2 infected patients

The duration of immunity of SARS-CoV-2 will dictate the course of the COVID-19 pandemic and the post-pandemic dynamics. Hence, understanding factors influencing the kinetics and the quality of the memory immune response in different populations is crucial. Antigenic exposure influences duration and quality of immune responses. Viral shedding is variable among SARS-CoV-2-infected individuals. However, it is currently unknown if the duration of viral shedding is associated to the development or persistence of the humoral response to SARS-CoV-2. Due to the surveillance system in Cambodia, all individuals arriving at the airport are screened for SARS-CoV-2 infection. All laboratory confirmed cases are quarantined. Due to our collaboration with the Virology Unit, a WHO reference center for SARS-CoV-2 detection, we have been able to re-contact these individuals 6-9 months after laboratory confirmed infection. All cases have been followed up virologically extensively at IPC. We have observed that the length of the viral shedding is highly variable, ranging from 4 to 50 days. Most of the individuals remained asymptomatic or showed mild symptoms. Our aim is to perform a comprehensive analysis of the memory immune response to SARS-CoV-2 as a function of viral parameters in the COVID-19 Cambodian cohort. This includes the investigation of humoral responses to SARS-CoV-2 and seasonal CoV, determination of antibody effector functions and analysis of the functional S, M and N specific T cell response.

Single cell analysis platform

We have established a transversal single cell analysis platform. We have purchased and implemented a BD FACSAria Fusion cell sorter and a 10x genomics Chromium Controller. 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 internal collaborative projects with Malaria Molecular Epidemiology Unit and Virology Unit will be performed with the equipment. Moreover, the platform will support other research programs in Cambodia and in the region through workshops, training and enhanced collaborations. It will help visibility of IPC internationally and attract foreign scientists. We will train Internship students from both UHS and UP in state-of-the-art Immunology techniques.

Funding	Wellcome Trust Multi User Equipment (218310/Z/19/Z) GIZ (contract: 81255619C, project: 17.2006.9-002.00) NIH PICREID (1U01AI151758 – 01)
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4.3.4 Support to National Authorities

Tineke Cantaert, PhD and Polidy Pean, MD, PhD: Members of steering committee for the International Master's Degree in Infectiology and coordinators of the immunology module in master's year 1 (University of Health Sciences, Phnom Penh, Cambodia and Universite Paris Saclay, Paris, France).

4.3.5 Teaching and Training

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

- 10 hours/year each of teaching the immunology module, Master's of Medical Biology, University of Health Sciences
- Member of the steering committee International Master's of Infectious Diseases and coordinator of the immunology module.

PhD student:

- Sotheary SANN: 2019-2022: The student is enrolled at the University of Hasselt, Belgium. Visits to UHasselt are covered by a BOF/BILA grant of the Flemish Government
- David GUERERO GOMEZ: 2020-2022: the student is enrolled at the University of Montpellier, France.
- Internship/thesis students:
- Sara VAN DE KERKHOVE: University of Antwerp, Belgium: Master Thesis Biomedical Sciences: November 2019-June 2020
- Stanley DINKO, University of Health Sciences/University Paris Saclay: May 2020-September 2020

4.3.6 Publication List

Awards and Grants Approved in 2020

FELLOWSHIPS AND RESEARCH SUPPORT	
2020-2021	<i>Institut Pasteur</i> COVID-19 Taskforce Evaluation of anti-SARS-CoV-2 antibody effector functions in a Cambodian patient cohort PI: Tineke Cantaert
2020-2025	NIH/NIAID CREID network Inter-regional study of transmission, adaptation and pathogenesis of viruses with pandemic potential in Southeast Asia and West/Central Africa PI: Anavaj Sakuntabhai, country PI: Tineke Cantaert
2020-2025	Wellcome Trust Multiuser Equipment Grant Advancing flow cytometry for the on-site study of tropical infectious diseases PI: Tineke Cantaert

Publication list 2020

1. **Decreased type I Interferon production by plasmacytoid dendritic cells contributes to severe dengue** Upasani V, Scagnolari C, Frasca F, Smith N, Bondet V, Vanderlinden A, Lay S, Auerswald H, Heng S, Laurent D, Ly S, Duong V, Antonelli G, Dussart P, Duffy D, Cantaert T. *Frontiers Immunol*, 2020 Dec 17;11:605087. doi: 10.3389/fimmu.2020.605087. PMID: 33391269
2. **Autoantibody profiling in plasma of dengue virus-infected individuals.** Vo HTM, Duong V, Ly S, Li QZ, Dussart P, Cantaert T. *Pathogens*, 2020 Dec 18;9(12):1060. doi: 10.3390/pathogens9121060. PMID: 33352902
3. **Comparison of dengue case classification schemes and evaluation of biological changes in different dengue clinical patterns in a longitudinal follow-up of hospitalized children in Cambodia.** Dussart P, Duong V, Bleakley K, Fortas C, Lorn Try P, Kim S, Choeung R, In S, Andries AC, Cantaert T, Flamand M, Buchy P, Sakuntabhai A. *Plos Neglected Tropical Diseases*, 2020 Sep 14;14(9):e0008603. doi: 10.1371/journal.pntd.0008603. PMID: 32925941
4. **Aedes mosquito salivary components and their effect on the immune response to arboviruses.** Guerrero D, Cantaert T, Missé D. *Frontiers in Cellular and Infection Microbiology*, 2020 Aug 7;10:407. doi: 10.3389/fcimb.2020.00407. PMID: 32850501
5. **TLR2 on blood monocytes senses dengue virus infection and its expression correlates with disease pathogenesis.** Aguilar-Briseno JA, Upasani V, Ellen BMT, Moser J, Pauzuolis M, Ruiz-Silva M, Heng S, Laurent D, Choeung R, Dussart P, Cantaert T, Smit JM, Rodenhuis-Zybert IA. *Nature Communications*, 2020 Jun 23;11(1):3177. doi: 10.1038/s41467-020-16849-7. PMID: 32576819

4.4 Virology Unit

4.4.1 Functional Structure

IPC's virology unit's activities are directed toward biomedical research, the surveillance and monitoring of infectious diseases and support to public health emergencies (Figure 13). These activities can be divided into five main components, 1) arboviruses (e.g. dengue, Zika, chikungunya and Japanese encephalitis), 2) respiratory syndromes (seasonal, avian influenza, COVID-19 and other respiratory viruses), 3) zoonotic and emerging pathogens (e.g.: coronaviruses, hantavirus, Nipah virus and other emerging viruses), 4) HIV and viral hepatitis, and 5) other viruses (rabies, enteroviruses, etc.) The cross-cutting activities comprise of cell culture, virus isolation, sequencing, Biosafety level-3 (BSL-3) laboratory, animal facility, quality, security and hygiene and administrative and stock management. The unit comprises thirty-eight staff including seven PhDs, one PhD candidate, four master's degree holders and two medical doctors.

Within each of these topics, the Virology Unit has developed numerous research programs. Most of these programs are conducted in collaboration with the IPC's epidemiology and public health unit, entomology unit and immunology unit. These programs focus on infectious diseases of concern to the Cambodian population.

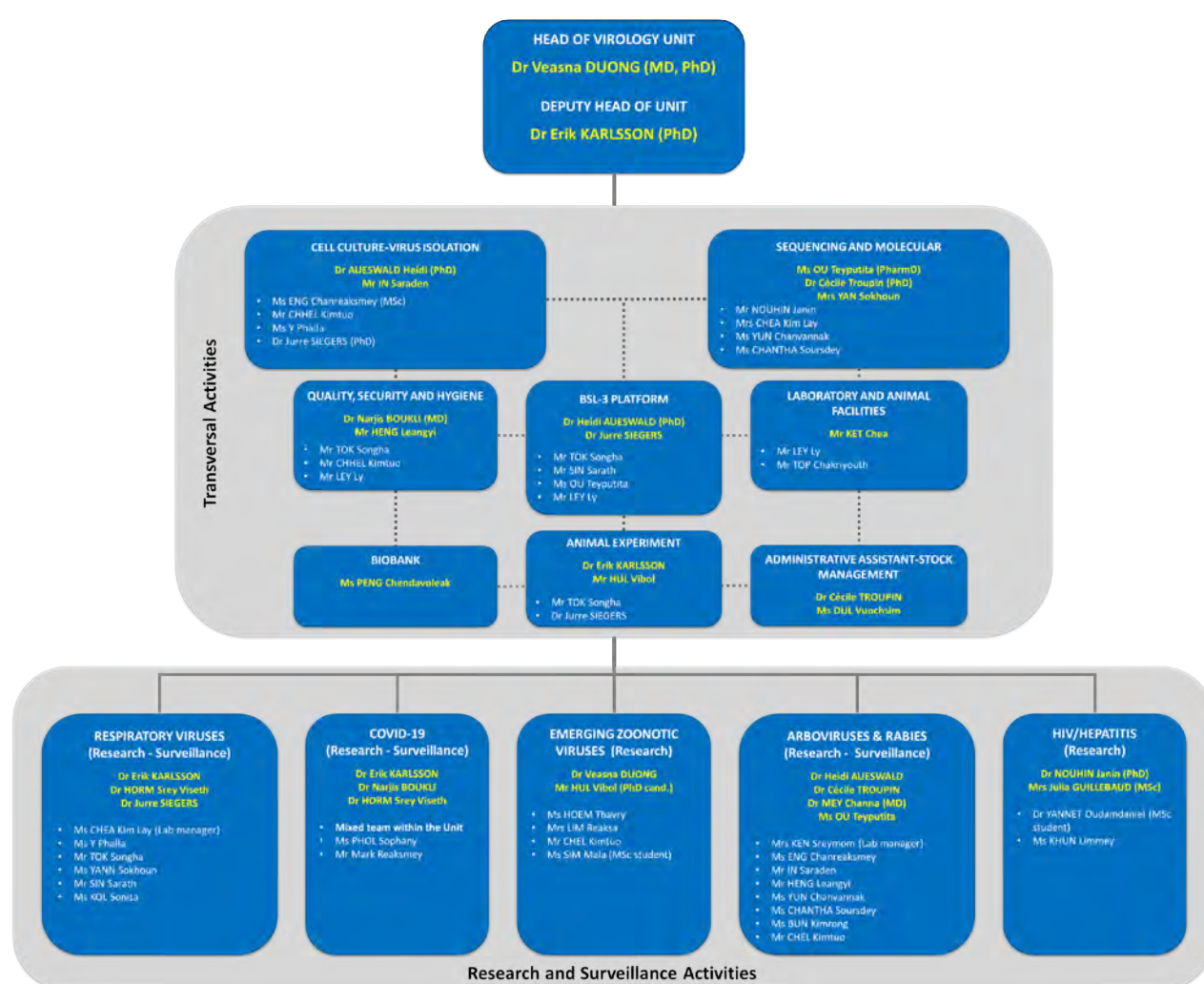


Figure 13. Virology unit organogram

4.4.2 Research Programs - Major Achievements in 2020

Arboviral Diseases

IPC undertook research titled “Description of The Intra-Subject Time-Course of Viraemia during the Acute Phase of Chikungunya Infection and The risk of Chikungunya Infection and Immune Response Characteristics within Household Members in Cambodia.”

Chikungunya is a viral disease transmitted to humans by infected mosquitoes. It is caused by the chikungunya virus (CHIKV). There is currently no vaccine or specific drug against the virus. The current treatment is focused only on relieving disease symptoms. The unpredictability of chikungunya outbreaks combined with its frequently short duration pose considerable difficulties in initiating a trial before the risk of infection in the community has fallen to low levels. Hence, there is a lack comprehensive understanding of the duration of CHIKV viremia in patients and the risk of transmission in household settings. This information will help improve understanding of the clinical feasibility of potential new treatments.

Research Objective

- To characterize the kinetic viremia during the acute phase of chikungunya virus infection.
- To determine the risk of CHIKV infection in household members of an index case.
- To study the immune innate and adaptive immune response in CHIKV patients.

The study is being carried out in Kampong Thom Province between December 2020 and December 2021. Patients will be recruited at Kampong Thom Provincial Referral Hospital, two other district referral hospitals in Baray and Staung Districts, Kampong Thom Province, Javarman VII Hospital in Siem Reap and Kantha Bopha Hospital in Phnom Penh and among household members of CHIKV confirmed cases. Blood samples will be collected for virological and immunological tests at IPC.

Collaborations	<i>Institut Pasteur du Cambodge:</i> Virology Unit (V. Duong); Epidemiology-Public Health Unit (S. Ly); Immunology Unit (T. Cantaert) Evotec (Hugh Watson)
Funding	Evotec International GmbH/EVT-CHIK-03C/04C

a. DenThom: Study of Dengue-like Illness in Kampong Thom Province, Cambodia

Community scale studies have been conducted in Cambodia before, yielding valuable novel insights into asymptomatic dengue occurrence and transmission patterns, with important implications for vector control and for our understanding of dengue transmission. In addition, we have started characterizing the acute immune response in these asymptomatic carriers, which has implications for vaccine design. However, many research questions remain unanswered. Among these are 1) questions around spatial and timely circulation of various DENV genotypes and serotypes, 2) evolution of the adaptive immune responses over time and correlations to the initial innate responses and disease severity, 3) the discovery of novel biomarkers of severity and the optimization of novel diagnostic assays and d) the influence of dynamics of ecological variables (land use, meteorological) on the changing mosquito populations and dengue transmission.

The primary objective is to study the prevalence and incidence of dengue and dengue-like illness in Cambodia by implementing a novel study in Kampong Thom, a province in central Cambodia that is a major transport hub, and where information on dengue transmission and circulation is lacking.

The full description of this project is detailed in an immunology unit report. The virology unit will collaborate with the epi-public health unit in the implementation of this project and regarding the diagnosis of arboviruses using serological and molecular tools in humans and mosquitoes.

Collaborations	<i>Institut Pasteur du Cambodge:</i> Virology Unit (V. Duong); Epidemiology-Public Health Unit (S. Ly); Immunology Unit (T. Cantaert); Entomology Unit (S. Boyer) <i>Institut Pasteur</i> (A. Sakuntabhai)
Funding	NIH collaborative agreement (1-IPC-NIH-U01-AS-2020)

b. ZIKAlliance: A Global Alliance for Zika Virus Control and Prevention

The virology unit at IPC participated in WP6 of this H2020-SC1-2016-2017 consortium focusing on vector competence experiment of ZIKV strains from Africa, the Americas and Asia with Cambodian *Aedes aegypti* mosquitoes.

The results of the experiment showed that the dissemination rate of the three ZIKV strains were similar in *A. aegypti* from Phnom Penh. However, a higher transmission rate was observed in African strains (Dak84) compared to Malaysian (MAS66) and Martinique (MRS_OPY) strains. The MAS66 had the lowest transmission rate among the three strains (Figure 14).

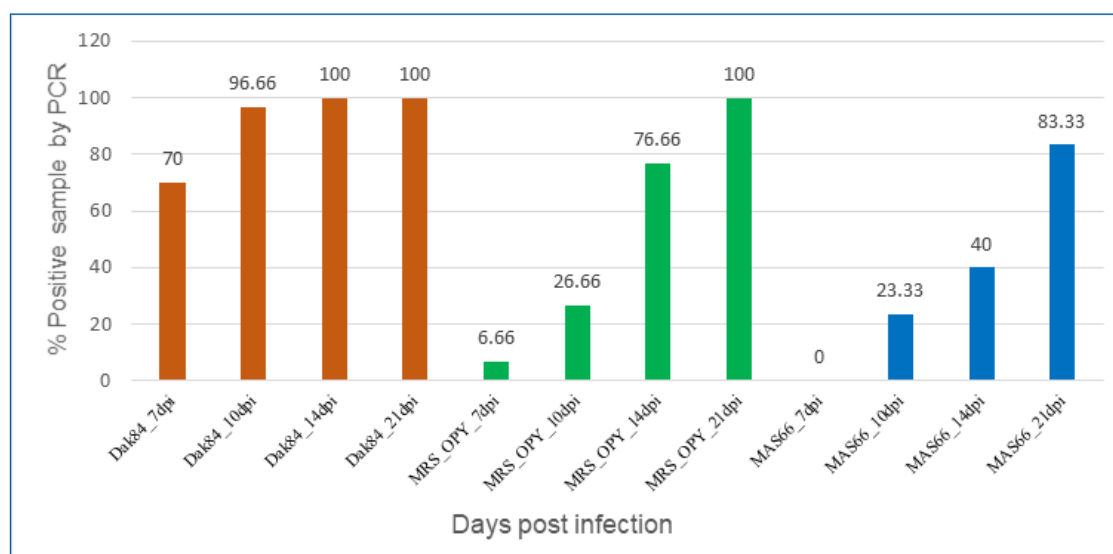


Figure 14. Percentage of positive saliva of *A. aegypti* from Phnom Penh at Day 7, 10, 14 and 21 Post-infectious Blood Meal

Collaborations	<i>Institut Pasteur du Cambodge</i> : Virology Unit (V. Duong); Entomology Unit (S. Boyer); <i>Institut Pasteur</i> (Anna-Bella Failloux).
Funding	HORIZON-2020 (European Union)

c. PREEMPT – Understanding Arbovirus Emergence and Changing the Approach to Intervention.

Arboviruses 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 mosquitoes from various field sites to map mosquito densities and dynamics. The collected mosquitoes were partly shipped to IP Paris for NGS for the identification of the mosquito species and the virome of the single mosquitoes. 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 mosquitoes 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 sent to IP Paris for further investigation and sequencing.

Collaborations	<i>Institut Pasteur du Cambodge</i> : Virology Unit (P. Dussart and H. Auerswald), Entomology Unit (S. Boyer), <i>Institut Pasteur</i> (C. Saleh, C. Koh)
Funding	DARPA

d. Respiratory Viruses

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2); Novel Coronavirus Disease 2019 (Covid-19)

Following the detection of a cluster of cases of pneumonia of unknown etiology in Wuhan, China in December, 2019, IPC began to immediately organize to screen individuals in the event that any cases were detected in Cambodia. Through IPC's previous involvement in the PREDICT program, IPC was quickly able to mobilize and was prepared to test suspected COVID-19 samples in early January 2020. In addition, IPC quickly obtained and established protocols for the real-time molecular diagnosis of SARS-CoV-2 through WHO provided protocols. As such, using our BLS-3-level capacity in conjunction with multiple diagnostic tests, IPC ensured diagnostic capacity in Cambodia very early and was approved as a first line laboratory for diagnosis of COVID-19 (SARS-CoV-2) on January 21st, 2020 (Figure 15). On January 27th, IPC confirmed the first COVID-19 case (a traveler from Wuhan) in Cambodia. As of the end of December, 2020, IPC has received and tested over 180,000 human samples covering approximately 120,000 individuals with 370 confirmed cases of COVID-19. In April 2020, the work done at IPC in response to the global COVID outbreak was recognized by naming the virology unit as a WHO COVID-19 Global Reference Center. In addition, IPC has been working closely with the Cambodian Centers for Disease Control and Prevention (CCDC), which is the coordinating entity designated for notification of suspected cases, and the COVID-19 sampling system as a whole. In addition, the epidemiology and public health unit at IPC is using its extensive experience to facilitate data management, reporting, and contact tracing.

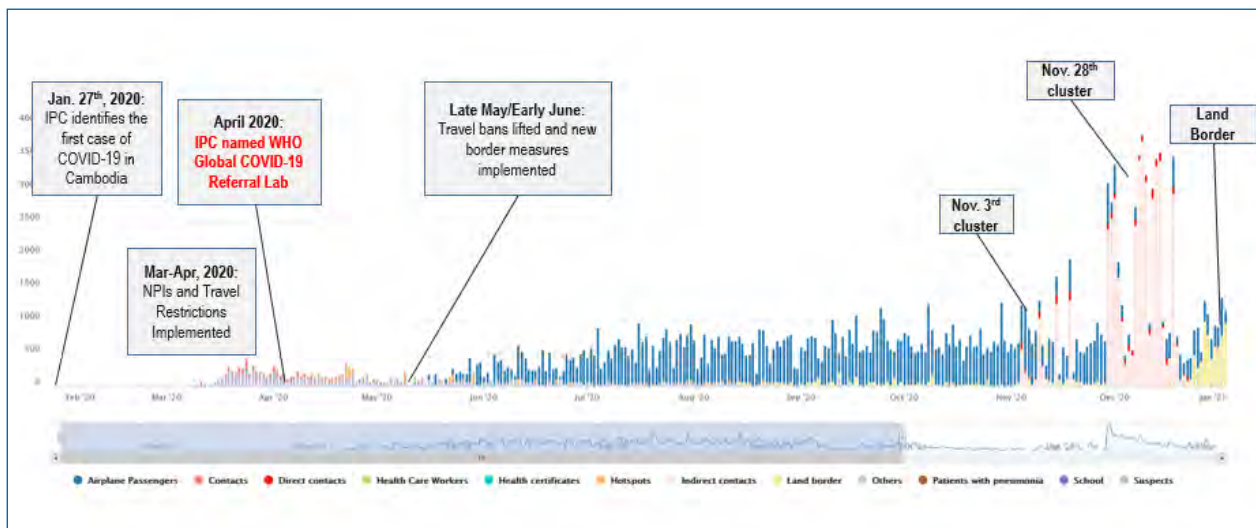


Figure 15. Number of Samples Tested for SARS-CoV-2 at Virology Unit, IPC Since January 20, 2020

Viral Isolation and Titration

Having a BSL-3 level facility and experience in isolating numerous types of viruses, IPC was quickly able to establish viral isolation and titrating (both TCID₅₀ and PFU) for COVID-19 in Cambodia. Briefly, SARS-CoV-2 isolates were successfully generated by inoculation of Vero cells with filtrated swap sample from infected individuals. The identity of all isolates was confirmed by qRT-PCR. From passage two on, the virus isolates showed a cytopathic effect (CPE) on Vero E6 cells which allowed the set-up of titration methods for the determination of infectious virus (TCID₅₀ and plaque assay). Additionally, the culture in a human lung cell line (A549) and a human laryngeal cell line (Hep-2) was established. SARS-CoV-2 showed no CPE in these cell lines and grew to lower titers (1-2 log₁₀) than in Vero cells. Generally, passaging increased the titer of detectable infectious virus that could be reached 3dpi (day of 50-70% CPE in Vero cells when infected with MOI 0.01) from 10² pfu/mL at passage 2 to 10⁷ pfu/mL at passage 6. At the current time, 23 viral isolates are available from patients identified in Cambodia. This strain biobank is vital for our continued validation and technical improvement work but also for establishing serological assays for sero-epidemiological surveys and contact tracing efforts. Further isolation attempts are made on all possibly viable samples as available.

Molecular Diagnostics (qRT-PCR)

At the beginning of the global outbreak/pandemic, IPC quickly obtained and established protocols for the real-time molecular diagnosis of SARS-CoV-2 through WHO-provided protocols. As such, IPC was highly involved in the initial validation and use of *Charité* Virology and Hong Kong University assays for screening for possible COVID-19 cases. Comparison of sensitivity/specificity of both tests against each other with variable reagents including viral isolates generated in-house. Throughout 2020, IPC continued to maintain and improve diagnostic protocols for COVID-19, including validation of numerous research/diagnostic protocols and commercial kits. Overall, IPC validated and has protocols available for five different conventional qRT-PCR tests and five commercial assays. Some of these technol-

ogies were also transferred for use by the National Institute of Public Health for their use, as part of capacity-building and for testing of samples in their laboratories.

Testing of Novel Diagnostics, Extraction Machines, Alternative Sample Collection Methods and Tests

In addition to standard and commercial assays, IPC has been actively involved in validating of testing novel technologies and protocols for alternative procedures and reagents for testing SARS-CoV-2 samples. In 2020, IPC tested and/or validated three point-of-care technologies (GeneXpert, GeneReach Central, GeneReach POKKIT Mini) and four extraction machines (Taco Mini, Indical Indigo, Thermofisher DUO Prime, and Kingfisher Flex systems) for use in COVID-19 diagnostics. The comparison of these machines and reagents led to recommendations for test usage and selection in several regions of the world and a manuscript describing some of the comparisons is in preparation and is expected to be submitted in mid-2020 for publication.

In addition to methods of processing samples, other sample types and alternate means of detection are very important to both testing and speed of outbreak responses. In 2020, IPC tested one saliva-based kit and two Rapid Antigen Tests using both laboratory standard samples and clinical material for usage in SARS-CoV-2 diagnosis with disappointing results. Further analysis and other tests will be further investigated in 2021.

Testing Inactivation Techniques for SARS-CoV-2 Samples

Within the laboratory, non-propagative work with samples containing the virus requires, at a minimum, Biosafety Level-2 (BSL-2) techniques and facilities. Therefore, handling of SARS-CoV-2 samples was (and remains) of major concern, especially in areas and conditions where biosafety and biosecurity for specimen handling is difficult to maintain, such as in rural laboratories or basic field testing sites. Inactivation through physical or chemical means can reduce the risk of handling live virus and increase testing ability worldwide. We assessed several chemical and physical inactivation techniques employed against SARS-CoV-2 isolates from Cambodian COVID-19 patients and demonstrated that all chemical (AVL, inactivating sample buffer and formaldehyde) and heat treatment (56°C and 98°C) methods tested completely inactivated viral loads of up to $5 \log_{10}$. This work was accepted for publication in 2020.

Testing Alternative Extraction Techniques for SARS-CoV-2 Samples

Testing is the limiting factor for understanding and controlling the COVID-19 pandemic. The global shortage of material and reagents led to several attempts streamlining the direct detection of SARS-CoV-2 mainly by skipping the RNA extraction step. One of the first investigations described an extraction-free workflow with a heat-inactivation step at 98°C followed by cooling at 4°C for 2min. We used this procedure and additionally tested longer incubation periods at 4°C as we considered practical circumstances that would make a longer storage necessary. We observed no influence of the timespan of storage itself on the outcome of the RT-PCR. This indicates that there is insignificant RNA degradation occurring in the heat-inactivated samples. However, the use of heat-inactivated, crude samples without RNA extraction for direct SARS-CoV-2 detection led to a significant decline in sensitivity as 77% of samples turned into false-negative results. We observed an average increase of at least 2 Ct values for virus culture samples, and 8-9 Ct values for patient samples due to extraction-free procedure. Our results also showed a drastic increase of false-negative results applying the Berlin RT-PCR protocol. However, based on our results and other reports especially with weak positive patient specimen IPC decided not to recommend the elimination of the RNA extraction step from the molecular detection workflow for SARS-CoV-2 as this leads to a dramatic loss of sensitivity. Nevertheless, the shortage of material and reagents is an enormous problem that should be addressed, especially under resource-limited conditions. Further investigations are needed.

SARS-CoV-2 Sequencing

From the initial outbreak until the present day, sequencing has been an integral part of the global understanding and response to COVID-19. In conjunction with our partners in-country (NIPH, NIAID/NIH, NAMRU-2), sequencing of the first positive case of COVID-19 in Cambodia (<https://www.biorxiv.org/content/10.1101/2020.03.02.968818v1>) was quickly established and submitted to GISAID (www.gisaid.org). Sequencing of further isolates was also conducted and sequences of select batches of cases and clusters were performed throughout 2020 to monitor for lineages, variants, and any other mutations. We also worked closely with collaborators at IP Paris to establish amplification and sequencing techniques to utilize in primary, clinical samples to allow for greater sequencing and these results will be vital for viral epidemiology and further studies. These efforts have allowed us to integrate the ARTIC primer protocol with our Nanopore MinION sequencer to quickly obtain full genomes of SARS-CoV-2 positive samples. These techniques were invaluable in helping to determine the genetic lineage, putative origin, and case linkages in the November 3rd and November 28th "events" (Figure 16). A manuscript detailing the virological and epidemiological characteristics of these strains sequenced in 2020 is in preparation and expected to be submitted for publication in mid-2020.

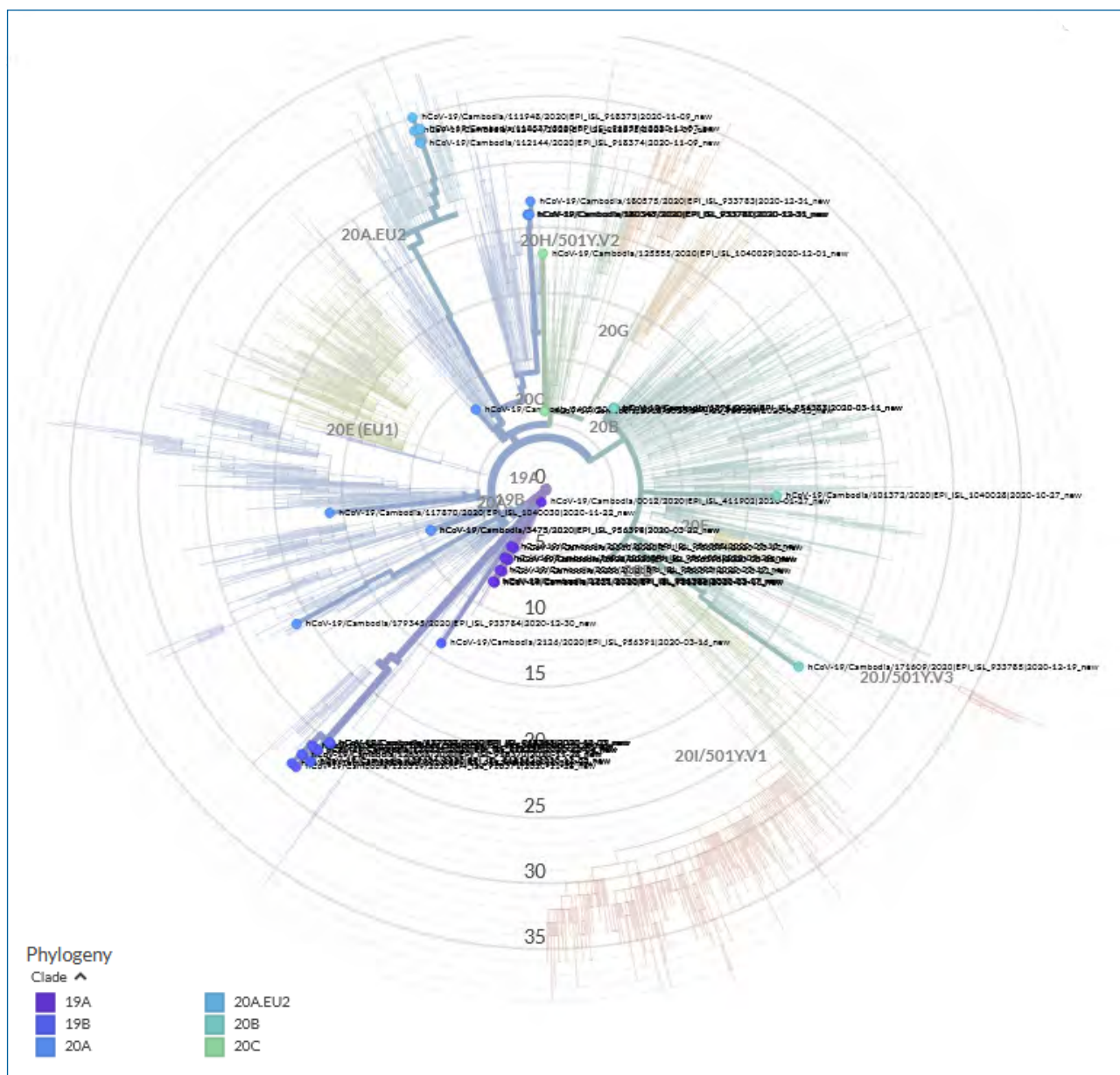


Figure 16. Phylogenetic Tree of SARS-CoV-2 Strains Detected in Cambodia in 2020

Establishment and Testing of SARS-COV-2 Serological Assays

To understand the real spread of the virus including asymptomatic, apparent and mild presentation, epidemiological investigations based on serological testing is inevitable. Serological assays are also necessary to monitor vaccine trials and vaccination programs. Using our biobank of viral isolates and serum samples from outbreak investigations, we have also been able to establish in-house serological methods (IFA, ELISA, PRNT, FRNT, MIA) and a biobank of serum from COVID-19 confirmed cases which are vital to our repertoire of serological understanding of SARS-CoV-2 infections

The establishment of these serological methods will also be used for further testing of human samples from Cambodia (individuals involved in wild animal trade, live animal markets, and long-term follow-up of confirmed cases). Additionally, these tests are vital for the evaluation of commercial assays to provide recommendations for broad serological testing in the developing Covid-19 laboratory network in Cambodia.

PRNT Establishment

Based on previous arbovirus neutralization assays, we developed various formats of neutralization assays (PRNT, FRNT) using in-house isolates to use as a “gold standard” to evaluate other in-house and commercial assays as well as to utilize in serological case investigation.

IFA Establishment

The immunofluorescence assay (IFA) was implemented with SARS-CoV-2 infected Vero cells. Briefly, cells were infected at MOI 0.01 and 2 dpi harvested by centrifugation, washed with 1xPBS and mixed with the same amount of uninfected cells before plated on IFA slides. Due to the fixation of the cells with acetone they are not infectious anymore and the further procedure was done outside of BSL3. Serial dilutions (starting with 1:20) dilutions of heat-inactivated human sera were applied on the fixed cells. After washing, anti-human IgG and IgM fluorescein isothiocyanate (FITC) conjugated antibodies were used for visualization of complexes of viral antigen and human antibody by ultraviolet (UV) light microscopy. This method can be used to measure IgM and/or IgG antibodies. However, due to the time and person-consumption nature of this procedure, it was discontinued, as ELISA and neutralization tests (NT) are sensitive and high-throughput methods and therefore preferable.

ELISA Establishment

The in-house ELISA was set-up by using virus culture supernatant. The virus is precipitated and concentrated from the supernatant of virus culture on Vero cells by polyethylene glycol and inactivated by UV light for fifteen minutes. The inactivated virus is used as antigen (concentration of 100 ug/well) for the coating on Polysorb microtiterplates with 1M bicarbonate buffer, pH 9.6 overnight at 4°C. Afterwards, plates are washed with 1xPBST and blocked with 3% milk in PBST (blocking buffer) for 1h at room temperature. As the ELISA is used for screening purposes, a 1:40 sample (serum or plasma) is prepared in blocking buffer and incubated for 2 hours at room temperature on the coated plates. After further washing steps with PBST follows 1h incubation with anti-human IgG HRP-conjugated antibody, and further washing with PBS. Visualization is done by adding ABTS peroxidase substrate for 20 minutes. The optical density at 405 nm is measured with a microplate photometer. The ELISA was set-up by using the NIBSC reference plasma panels 20/118 and 20/130. Based on these a threshold of OD₄₀₅ ≥ 1 is considered positive.

MIA testing

As an alternative to standard immunoassays, a multiplex microsphere-based immunoassay (MIA) was set-up at *Institut Pasteur*. Commercially available, recombinant SARS-CoV-2 antigens (S1 and S2 proteins) were coupled to microspheres following an approach previously used for other emerging pathogens. Additionally, SARS-CoV-1 (N and S1 proteins), MERS (S and S1 proteins), hCoV 229E (N protein), hCoV HKU1 (S1 protein) and hCoV NL63 (N protein) were also used to investigate serological cross-reactivity. These assays are currently being investigated and work is projected to be completed in mid-2021. The MIA will be adapted further with the support from Jessica Vanhomwegen's group to test prospective and retrospective animal samples of interest, especially from various bat species.

Serological study on human samples

ELISA and NT (plaque reduction neutralization test, PRNT) were used to analyze the Covid-19 surveillance samples received at IPC. Until now 326 plasma and serum samples (plus the NIBSC reference plasma samples) were comparatively analyzed. The in-house IgG ELISA was thus found to have a sensitivity of 87% and a specificity of 69% when compared to the PRNT. We also used these samples to evaluate the Roche Elecsys immunoassay (qualitative detection of total anti-SARS-CoV-2 antibodies; using N protein) revealing a sensitivity of 79% and a specificity of 69% when compared to the PRNT. A subset of these samples (n=34) was also used for the evaluation of the Genscript SARS-CoV-2 neutralization antibody detection kit, the Medomics IgM/IgG rapid test, and the SD Biosensor IgM/IgG rapid test.

Collaborations	<i>Institut Pasteur du Cambodge</i> : Virology Unit (E. Karlsson, V. Duong, H. Auerswald, et al) WHO, MoH/CCDC, USCDC, <i>insitut Pasteur</i> , HKU, Duke-NUS, NIH/NIAID
Funding	WHO, GIZ (contract: 81255619C, project: 17.2006.9-002.00), ANRS, ECOMORE, Private Partners

Avian Influenza in Cambodia: Molecular Characterization of HPAI A (H5N1) Virus, Virus Evolution, Drug Resistance, Animal-Human-Environment Interface Survey for HPAI in Live Bird Markets (LBMs)

Despite the huge shift in focus and burden to the COVID-19 pandemic we were able to retain the majority of avian influenza work in Cambodia, albeit it being slightly delayed from original 2020 plans.

As the Cambodian National Influenza Centre (NIC) and WHO H5 Reference Laboratory (H5RL), IPC supports the Cambodian Ministry of Health and Ministry of Agriculture, Forestry and Fisheries in the confirmation of influenza infections in humans and animals. In 2020, 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 characterization. In past years, IPC laboratory testing confirmed the detection of outbreak samples, 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, and 2017 to 2020. 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-2021.

Avian Influenza Surveillance in Cambodia Border Regions

In 2017, in collaboration with the FAO and NAHPRI, we sought to establish avian influenza virus (AIV) surveillance in Cambodian border regions to obtain a greater understanding of the dynamics of cross-border movements of avian influenza viruses into Cambodia and to obtain molecular profiles of the circulating influenza viruses in Cambodia. 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. During the 2020 surveillance, we again collected 5,120 poultry samples from 2,560 birds (paired tracheal and cloacal swabs).

Analyses is complete on both 2017-2018 and 2018-2019 periods and final analysis and sequencing of the 2020 sessions is underway and several manuscripts are in preparation (see below). One manuscript detailing the 2017-2018 efforts has been published. This collaboration continues for the 2021-2022 season.

Sequencing of Market Environmental Samples to Investigate the Diversity of Influenza Viruses Circulating in Cambodian Poultry

Since 2011, we 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 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 2019 were sequenced and phylogenetic, antigenic, and molecular risk assessment are underway in collaboration with WHOCC in Melbourne, Hong Kong University, and other partners as described 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 (Figure 17).

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 in final stages and a paper detailing these findings is expected mid-2021.

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 during the month of 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. 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 reassort in the region. A paper detailing these initial findings was published in 2019.

Throughout the 2020 season, A/H7Nx viruses continued to be detected in waves. These samples are currently being sequenced and a detailed report of A/H7Nx circulation and evolution within Cambodian LBMs is expected in mid to late 2021.

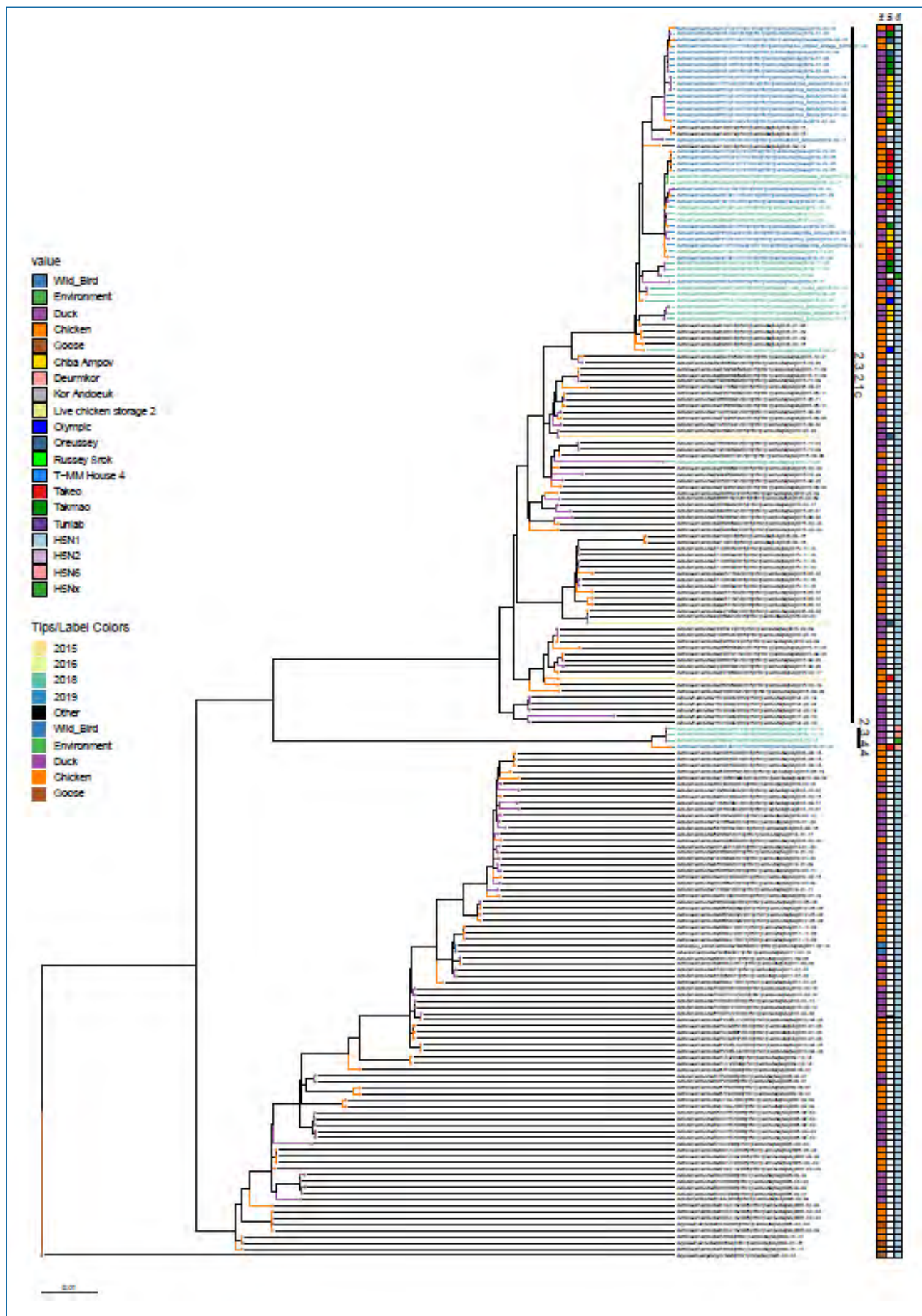


Figure 17. Phylogenetic Tree of Cambodian A/H5N1 Viruses Identified Between 2004 and 2014

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 similar to Bangladesh, China and Vietnam and live bird market (LBM) workers are exposed to these viruses. All A/H9 viruses identified so 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 between 2014 and 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 roles.

Longitudinal Serological Surveillance for AIV infection in poultry workers

Following a sero-survey in 2015, and similar to what was 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 epidemiology unit at IPC in conjunction with NAHPRI in four provinces. In 2020, the same four sessions were planned for collection in the same locations; however, due to restrictions related to the COVID-19 pandemic, only two sessions implemented during the year. Four further sessions are planned for 2021-2022.

In terms of serological analyses, hemagglutination inhibition assays (HAIs) were performed for the 2015, 2017-2018 and 2018-2019 LBM worker samples against all four human seasonal strains for the given time periods, as well as against appropriate A/H5N1, A/H5N6, A/H7Nx, and A/H9N2 strains. Serology on 2020 samples is underway. Correlation between seroconversion and poultry handling risks as well as other sociodemographic data are being analyzed and a publication is expected to be published jointly with the A/H9N2 phylogenetics and antigenic characteristics (as described above) in mid-2021.

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/PSN 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. These studies were slated to continue in 2020; however, field missions and participation was significantly impacted by COVID-19. It is hoped these studies will be restarted in 2021.

Poultry Market Supply Chain Study in Phnom Penh, And Upstream Evaluation of Avian Influenza Viruses' Circulation

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 festivals 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 started in January 2019 with seven middlemen near 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 the linkage between Cambodia and Vietnam. Data from the 2018-2019 tracking is still being analyzed and assessed for quality and attempts are being made to confirm points of interest and novel hotspots (work was delayed in 2020 due to COVID-19), including those identified in the 2017-2018 study. A manuscript is in preparation for these studies and is expected in mid to late 2021.

e. Swine Influenza Viruses

With one of the world's fastest-growing economies, demand for protein, especially pork, is increasing in Cambodia. While swine production in Cambodia was traditionally characterized by backyard, small-scale farming efforts, larger confinement farms have increased in recent years. Further, urbanization and expansion have caused many previously isolated farms to be surrounded by urban sprawl and thus surrounded by houses and small roadside businesses³⁰. Imported pigs (via both legal and illegal routes) from Vietnam and other surrounding countries are becoming more common. Overall, expansion of the swine industry in Cambodia coupled with negligible biosecurity and mixed farming of pigs and poultry create a major risk for human, swine, and avian influenza strains to mix and transmit.

To better understand swine IAV diversity, epidemiology, prevalence, and disease dynamics in Cambodia, virological surveillance in swine from backyard farms in Cambodia was completed in 2011–2013. Those studies found that 1.5% of sampled pigs were positive for triple reassortant H3N2 viruses similar to human H3N2 viruses previously isolated in Southeast Asia. Because of the risk of reassortment between influenza viruses circulating in pigs and poultry as well as targeting periods associated with increased trade and consumption of poultry and a concomitant increase in circulation of avian influenza virus in the region, active surveillance was undertaken in July 2017–May 2018 in border provinces during and after four major national festival periods in Cambodia: Lunar New Year (Jan–Feb), Khmer New Year (Apr), Pchum Ben/Qing Ming (Hungry Ghost festival; Sep–Oct), and Bon Om Touk (Water Festival; Nov). Nasal swabs from swine were collected from at least 100 random swine per province per session (5 sites/province x 20 swabs/site), preferably from swine 5–12 weeks of age to capture viruses from naïve pigs, for a total of 1,048 swine samples. IAV was detected in 6.1% (64/1,048) of swine samples. Prevalence was greater in samples of swine from slaughterhouses (8.4%) versus farms (4.2%), suggesting likely amplification of virus through the production chain. At the third visit, IAV prevalence was higher in slaughterhouses (19.5%) than farm samples (3.2%). Several differences were also noted in prevalence among provinces. A/H1 and A/H3N2 swine lineages were detected as well as A/H5 and H9N2 in Cambodian swine is concerning for zoonotic potential. In 2020, swine samples were sent to collaborators at Duke-NUS for attempts at full genome sequencing to understand the viruses detected in these animals and has been completed (Figure 18).

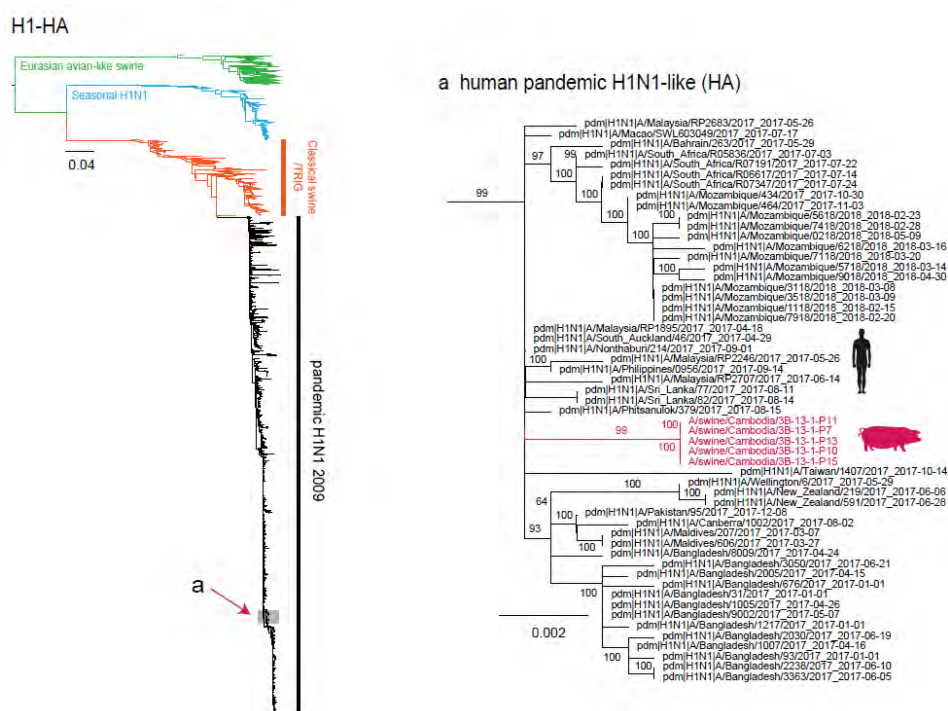


Figure 18. Phylogenetic Tree of HA Gene of H1N1 Detected in Swine in Cambodia

To understand risk of transmission between humans and swine, we also completed preliminary human serological studies in swine workers in Cambodia through a collaboration between the *Institut Pasteur du Cambodge* and the NIPH) of the MoH. HAI analyses were completed in 2020 and approximately 30% (n=53) of 174 sampled individuals seroconverted or had high pre-existing titers to at least one seasonal IAV strain within four sampling efforts between 2017 and 2018. The majority were positive for H3N2, which was the commonly circulating strain during the 2017 influenza season in Cambodia. Eight swine workers seroconverted against H9N2 during the sampling period, irrespective of seasonal titers. The majority of these H9N2 serologically positive workers (n=6/8) were from farms that also raised poultry. A detailed analysis of these results has been initiated in collaboration with the Peter Doherty Institute for Infection and Immunity and WHOCC in Melbourne, Australia and a paper detailing swine surveillance, virology, and human sero-prevalence is expected in mid-2021.

Further swine work is funded through the NIAID funded Centers for Excellence in Influenza Research and Response for 2021-2028 and is under further consideration for funding from other sources.

Collaborations	<i>Institut Pasteur du Cambodge</i> : Virology Unit (E. Karlsson), Epidemiology Unit (S. Ly, M. Chan) MoH/CCDC, NAHPRI/GDAH, FAO, WHO CC Melbourne/Peter Doherty Institute, Duke-NUS, WHO, RVC, AAHL/CSIRO, London School of Tropical Medicine and Hygiene, UHS
Funding	US-DHHS, FAO

f. 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. 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 collected 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 classify viruses of interest, especially RSV, coronaviruses, and paramyxoviruses. These samples, plus those identified in 2020, were shipped to collaborators at Duke-NUS in Singapore for sequencing in late 2020 for next generation sequencing and phylogenetic analysis. Analysis of the etiology database was completed in 2020 and we are actively collaborating with epidemiologists and biostatisticians for the best way to analyze and present the data. A manuscript describing the 2014-2019 data between SARI and healthy children as well as reasons for increased mortality from influenza between 2016 and 2017 compared to between 2014 and 2015 is in preparation and is projected to be submitted mid-2021.

Collaborations	<i>Institut Pasteur du Cambodge</i> : Virology Unit (E. Karlsson) MoH/C-CDC, WHO CC Melbourne/Peter Doherty Institute, Duke-NUS, WHO, US-CDC, NIPH; Kantha Bopha Hospital; NAMRU-2
Funding	USDHHS, USAID, FAO, WHO

g. 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 phylogenetics/

molecular analysis, antigenic testing and mammalian studies in conjunction with Dr. Stacey Schultz-Cherry at St Jude's Children's Hospital in Memphis, Tennessee 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 in mid-2019 and the full risk assessment was completed in 2020 and a manuscript is expected by mid-2021.

Collaborations	<i>Institut Pasteur du Cambodge</i> : Virology Unit (E. Karlsson), NAHPRI/GDAH, MAFF, FAO, Free the Bears, Phnom Tamao, WCS, St. Jude Children's Hospital
Funding	LACANET, USDHHS

h. Surveillance of Avian Influenza and Identification of Hotspots of Spillover between Poultry and Wild Birds

The recent emergence of H7N9 in China, emergence and global spread of H5Nx clade 2.3.4.4. and continual H5N1 outbreaks in domestic poultry highlight the need to understand the prevalence as well as genetic and phenotypic diversity of avian influenza virus circulating in wild bird reservoirs in Southeast Asia. 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 sought 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 commenced and sites were selected for this study. Final funding approval is pending from DTRA in mid-2021; however, preliminary sampling commenced in mid-2020.

Collaborations	<i>Institut Pasteur du Cambodge</i> : Virology Unit (E. Karlsson) NAHPRI/GDAH, MAFF, MoE, FAO, WCS
Funding	Defense Threat Reduction Agency (DTRA), FAO

ZOONOSES

During PREDICT-2, "Surveillance for emerging zoonotic disease threats and behavioral risk characterization in high-risk communities in Asia and Africa", a study spanning from 2015 to 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. Following the emergence of COVID-19, we obtained a 6-month extension from January to June 2020 to screen samples in our biobank collected under PREDICT programs from 2010 to 2018 to search for putative SARS-CoV-2-like beta coronaviruses (betaCov).

A total of 430 archived samples from six bat families and two carnivorous mammal families, including 162 oral swabs and 268 rectal swabs, were retrospectively tested with a pan-coronavirus (pan-CoV) hemi-nested RT-PCR9. Sixteen out of 430 (3.72%) samples tested positive for CoV by pan-CoV hemi-nested PCR. Eleven were classified as alpha coronaviruses and five as betaCoV. Two of the five betaCoV samples further tested positive using a RT-qPCR targeting the RdRp gene of sarbecoviruses. Both samples came from Shamel's horseshoe bats (*Rhinolophus shameli*) sampled in December 2010 in the Steung Treng Province in northeastern Cambodia. The two sequences are closely related to SARS-CoV-2, exhibiting 92.6% nucleotide identity across the genome and identical genomic organization. Phylogenetic analysis using full genome sequences shows that RshSTT182 and RshSTT200 represent a new sub-lineage of SARS-CoV-2 related viruses, despite the geographic distance of isolation (Figure 19).

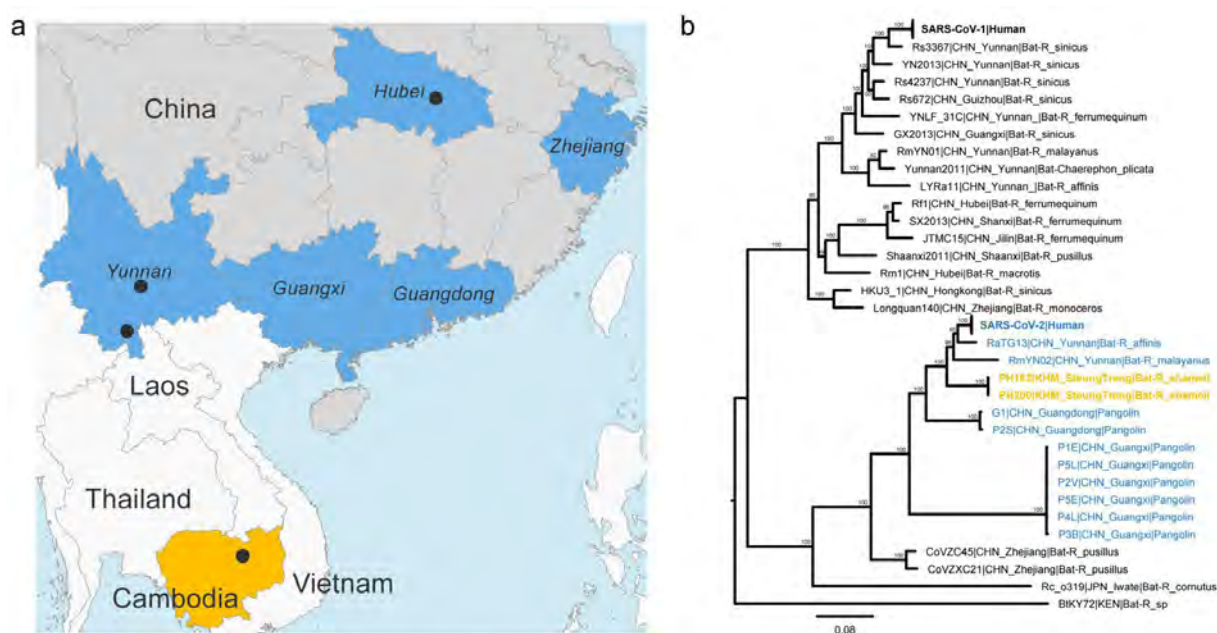


Figure 19. Phylogenetic analysis of SARS-CoV-2 and representative sarbecoviruses and geographical context. a. Maximum likelihood phylogeny of the subgenus Sarbecovirus (genus Beta coronavirus; $n = 37$); b. map of parts of China and Southeast Asia. Regions where viruses of the SARS-CoV-2 lineage were sampled are colored as in the tree. A dot indicates a sampling site when known.

Collaborations	Institut Pasteur du Cambodge: Virology Unit (V. Duong); Epidemiology-Public Health Unit (S. Ly) Ministry of Agriculture, Forestry and Fisheries: National Animal Health and Production Research Institute; Forestry Administration Ministry of Health: Cambodian CDC Wildlife Conservation Society (WCS), University of California Davis (UC Davis)
Funding	USAID (AID-OAA-A-14-00102)

HEPAR: Rodents as Reservoirs for Hepatitis E Virus (HEV), Arenavirus and Other Rodent-Borne Viruses and Risk Assessment of Infection in Humans in Cambodia

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 peri-domestic *Rattus* species and zoonotic infection in human has been recently reported.

The study objective was to investigate HEV, Arenavirus and other rodent-borne virus infections in rodents and estimate the sero-prevalence of those rodent-borne virus infections in human in both urban and rural environments.

A cross-sectional prospective study in selected open markets of the capital Phnom Penh and rural villages in Kampong Cham, Preah Sihanouk and Monduliri Provinces during dry and rainy seasons is being carried out. Rodent trapping sessions and blood sampling and data collection on human exposed to rodents were conducted during rainy and dry seasons over a one-year period (June 2020-May 2021).

Among 661 rodents trapped, 336 were collected between June and August 2020 (rainy season) and 325 between January and February 2021 (dry season). After humane culling, the following samples were collected for all rodents: oral and rectal swabs, serum, bladder/urine, kidney, spleen, liver, lung, heart, brain. After homogenization and RNA extraction, liver samples were tested for the presence of HEV-C by real-time RT-PCR. A total of nineteen positive samples (2.9%) were detected, and ten have already been sequenced and confirmed as HEV-C.

Similarly, individual pools of liver, kidney, spleen, and lung were tested for the presence of Arenavirus by real-time RT-PCR. Among the 336 pools tested, twenty-eight tested positive (8.3%) and sequencing of PCR products identified them as Cardamones virus.

Individuals working in markets in Phnom Penh, and communities living in villages or sites where rodents trapping were implemented, were interviewed concurrently with trapping sessions. Blood was drawn for each for sero-prevalence study. A total of 887 participants were recruited. Serological analysis are pending to detect antibodies against human HEV, HEV-C, and Arenaviruses using either commercial or in-house ELISA.

Due to COVID-19 restrictions, rodent trapping sessions and interviews and sampling sessions in human in Preah Siha-nouk Province could not be conducted during the dry season 2021.

Collaborations	<i>Institut Pasteur du Cambodge</i> : Virology Unit (V. Duong); Epidemiology-Public Health Unit (S. Ly) Ministry of Agriculture, Forestry and Fisheries: National Animal Health and Production Research Institute; Forestry Administration Ministry of Health: Cambodian CDC
Funding	Internal project (<i>Institut Pasteur du Cambodge</i>)

ZooCoV: Toward an Integrated Surveillance of Potential Zoonotic Beta Coronaviruses in the Wild Animal Value Chains in Cambodia

Beta-CoVs were responsible for three major respiratory infection outbreaks worldwide over the last two decades, including the current COVID-19 one, involving viral spill-over at the human/animal interface. Data on knowledge on animal reservoir, as well as on cultural, sociological, and ecological factors driving Beta-CoVs spread to humans and emergence are limited. In Cambodia, wet markets represent a major supply of animal products, and wild meat consumption is prevalent. These practices raise major safety issues: the potential transmission of pathogens from wildlife to the human population of Cambodia.

The study objectives are to 1) provide new knowledge on wild meat trade chains in Cambodia, 2) document the diversity of Beta-CoVs circulating through these chains, 3) describe and understand the practices and perceptions of the bush meat trade and consumption at the human/wildlife interface, and 4) develop a flexible and integrated early-detection system of viral spill-over events.

The virology unit is responsible for WP3 “risk quantification at the human/wildlife interface”.

Activities are focused in two pilot provinces- Mondulkiri and Stung Treng- where various study sites were selected to conduct a wildlife and bush meat trade survey in the human population. Preliminary results are include that over the four field missions conducted during rainy (August and October 2020) and dry (February and March 2021) seasons, 901 individuals potentially exposed to wildlife and/or wild meat were included, 590 (65.5%) in Mondulkiri, and 311 (34.5%) in Stung Treng. Serum was collected for each participant.

By 13 April 2021, 560 sera have been tested for detection of antibodies against SARS-CoV-2 using in-house ELISA and FRNT tests. No sample was detected with anti-SARS-CoV-2 antibodies. Luminex® technology in multiplex will be used as well to characterize immune response of participants regarding their antibodies profile to various Coronaviruses SARS-CoV-1, SARS-CoV2, MERS-CoV, hCoV-OC43, hCoV-NL63, hCoV-HKU1, and hCoV-229E (in collaboration with the TRANSVIHMI, IRD).

Concurrent to human sampling, wildlife sampling was implemented in both provinces. By 10 May 2021, various samples from different wildlife species (n=84) were collected and are being screened for Coronavirus by RT-PCR. Additional field missions to collect rodents and bats samples are planned in the upcoming months.

Collaborations	<i>Institut Pasteur du Cambodge</i> : Virology Unit (V. Duong); Epidemiology-Public Health Unit (S. Ly) CIRAD (V. Chevalier) IRD (Martin Peeters) Wildlife Conservation Society Flora and Fauna International Ministry of Agriculture, Forestry and Fisheries: National Animal Health and Production Research Institute; Forestry Administration Ministry of Health: Cambodian CDC
Funding	ANR, Région Occitanie, Pasteur Asia Fondation -16297

RhinoKhov: Detection of SARS-CoV related virus in *Rhinolophus* bats in Cambodia

Bats of the genus *Rhinolophus* are known to be the natural reservoirs of SARS-CoV 1 and 2. Our preliminary data shows that a seasonal peak in coronavirus circulation exists in these species during the massive presence of juveniles, between August and October in Cambodia. A cross-sectional study during this period would therefore make it possible to study the diversity of coronaviruses currently circulating in *Rhinolophus* in particular SARS-CoV related viruses.

To detect the presence of viruses close to SARS-CoV in populations of *Rhinolophus* in Cambodia, two field missions were conducted between August and October 2020 in Steung Treng where *karst massifs* shelter caves hosting populations of four species of horseshoe bats. Biological samples (oral and rectal swabs) were collected from captured bats in caves and tested for CoV.

Among 478 samples tested by family level CoV nested RT-PCR and specific RT-PCR targeting the RdRp gene of sarbecoviruses, fifteen were positive for CoV belonging to the alpha coronavirus genus by nest RT-PCR and one was positive for SARS-CoV-2-related virus by real time RT-PCR. Full genome sequencing for the sample positive for SARS-CoV-2 related virus is ongoing.

Collaborations	<i>Institut Pasteur du Cambodge</i> : Virology Unit (V. Duong) CIRAD (J. Cappelle) Ministry of Agriculture, Forestry and Fisheries: National Animal Health and Production Research Institute; Forestry Administration
Funding	MUSE-16297

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 these 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 of the virology unit in collaboration with the University of Aix-Marseille started aiming to develop 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.

The rodent samples and human sera collected in the HEPAR Project will be used for the detection and characterization of mammarenaviruses in rodents and for the detection of antibodies against this virus in humans.

Collaborations	<i>Institut Pasteur du Cambodge</i> : Virology Unit (V. Duong and V. Hul) Aix-Marseille Université (X. de Lamballerie and B. Coutard) Campus France
Funding	Internal project (<i>Institut Pasteur du Cambodge</i>)

Continuation 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 IPC with sponsorship from the Defense Threat Reduction Agency, Cooperative Threat Reduction, Biological Threat Reduction Program (BTRP) 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 multi-sectoral, multi-level approach to integrating programs, policies, legislation, and research to achieve better "One Health" outcomes.

CANARIES continued in 2020, albeit virtually, with the establishment of a steering committee, an official charter, a website (<http://www.canarieshmhp.org>) and other social media, and regular weekly meetings with funders. The con-

sortium as a whole is actively writing manuscripts, grants, and other collaborative efforts; a second consortium meeting is being planned for 2021.

Collaborations	<i>Institut Pasteur du Cambodge</i> : Virology Unit (E. Karlsson) Multiple Collaborations Globally
Funding	Defense Threat Reduction Agency (DTRA), UKGCRF

HIV AND VIRAL HEPATITIS

Clinical Utility of Resistance-Associated Substitutions Characterization in Patients Infected with HCV Genotype 6 Failing Direct-acting Antiviral Treatment in Cambodia

Direct-acting antivirals (DAAs)-based treatment targeting multiple regions of Hepatitis C Virus (HCV) have proven to have remarkable efficacy 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 caused by emergence of DAA resistant-associated substitutions (RASs) in HCV genomes. 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, better knowledge of the RASs' profile presenting in HCV genotype 6 strains and their clinical impacts is urgently needed.

The main objective of the present 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 approaches, 3) to investigate whether the emergence of post-treatment RASs is associated with the selection of pre-existing substitutions among minor viral variants. 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.

Collaborations	<i>Institut Pasteur du Cambodge</i> : Virology Unit (J. Nouhin) Médecins Sans Frontières (JP. Dousset and M. Le Paih) <i>Institut Pasteur</i> (E. Simon-Lorière)
Funding	Internal project (<i>Institut Pasteur du Cambodge</i>)

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 rapid diagnostic tests (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 not receiving 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 May 10, 2021, 1,193 women were included in the study and had an HBV VL performed. HbsAg ELISA was performed for 842 babies at delivery timing on DBS with a positivity rate of 9.4% (79/842).

This study is being done in partnership with the EPH (L. Borand), ANRS (O. Segéral) and Calmette Hospital (C. Chhun).

Collaborations	<i>Institut Pasteur du Cambodge</i> : Virology Unit (J. Nouhin and Julia Guillebaud) Clinical Research Group (L. Borand) ANRS (O. Segéral) Calmette Hospital (C. Chhun)
Funding	ANRS 12345

4.4.3 Research Programs –Outlook for 2021

ARBOVIRAL DISEASES

CHIKV: Genetic and Phenotypic Characterization of CHIKV from 2020 Outbreak in Cambodia

Chikungunya virus (CHIKV) was first detected in Cambodia in 1961 and reemerged in 2011. After a decade, an outbreak of CHIKV infection occurred in 2020 with a large outbreak and rapid spread across the country. Preliminary sequencing of the E gene showed that the current outbreak strains belonged to an East/Central/South African genotype without E1 residue change A226V. Three additional mutation sites E1 of CHIKV were observed in patients.

Our hypothesis is that the new mutations observed in E gene and potentially other mutations throughout the genome might play an important role in the current outbreak. We plan to conduct comparative viral fitness assays of the CHIKV isolates in different mosquito cell lines and human cell lines. Additionally, we will conduct structural analysis of the mutations observed in E1 and E2 genes. These mutations might reflect antigenic drift in comparison to other CHIKV isolates.

An *in vivo* experiment will be conducted to test whether the new mutations observed in CHIKV isolated in 2020 provide any phenotypic advantage in the main mosquito vectors. We will conduct vector competence studies using CHIKV isolates (Asian genotype) from 2011 and 2020 and other Asian and African strains in both *A. aegypti* and *A. albopictus*, to estimate the infection, dissemination and transmission rates.

Collaborations	<i>Institut Pasteur du Cambodge</i> : Virology Unit (V. Duong) ; Entomology Unit (S. Boyer)
Funding	Internal project (<i>Institut Pasteur du Cambodge</i>)

SEASONAL AND AVIAN INFLUENZA VIRUSES

Animal-Human-Environment Interface Studies for AIV in Live Bird Markets (LBMs) (2021-2022)

The expected outputs of this study are 1) to improve understanding of the movement of AIVs around and into Cambodia mainly through border regions, 2) further establish baseline data and continue tracking sero-prevalence and infection rates of LBM workers in high risk markets and at the Cambodia/Vietnam border region, 3) implementation of Nanopore sequencing of circulating AIVs using the MinION including generation of SoPs and potential raw data for further bioinformatics training, and 4) conduct risk assessment of the impact of COVID-19 pandemic on AIV and changes in poultry farming and production as a function of economic/sustenance needs.

Collaborations	<i>Institut Pasteur du Cambodge</i> : Virology Unit (E. Karlsson), Epidemiology Unit (S. Ly, M. Chan) MoH/C-CDC, NAHPRI/GDAH, FAO, WHO CC Melbourne/Peter Doherty Institute, Duke-NUS, WHO, RVC, AAHL/CSIRO
Funding	FAO (LAO/RAP/2020/09)

Animal-Human-Environment Interface Studies for Swine Influenza Viruses (2021-2028)

This study is expected to produce four key outputs: 1) improved understanding of the movement of swine around and into Cambodia particularly through border regions, 2) undertake and describe a value chain assessment, gender roles, economic impacts of COVID-10 and ASF, 3) further establish baseline data and continue tracking seroprevalence and infection rates among swine workers in Cambodia, and 4) implementation of Nanopore sequencing of circulating SIVs using the MinION including generation of SoPs and potential raw data for further bioinformatics training.

Collaborations	<i>Institut Pasteur du Cambodge</i> : Virology Unit (E. Karlsson), Epidemiology Unit (S. Ly, M. Chan) MoH/C-CDC, NAHPRI/GDAH, FAO, WHO CC Melbourne/Peter Doherty Institute, Duke-NUS, WHO, RVC, AAHL/CSIRO, City University Hong Kong, CIRAD, AVSF
Funding	NIH/NIAID (CEIRR 2021-2028); IDRC (Pending)

Surveillance of Avian Influenza and Identification of Hotspots of Wild Bird/Poultry Interface (2021-2023)

Final funding approval is pending from DTRA in mid-2021 with sampling to commence in Q3 of 2021.

Collaborations	<i>Institut Pasteur du Cambodge</i> : Virology Unit (E. Karlsson) NAHPRI/GDAH, MAFF, MoE, FAO, WCS
Funding	Defense Threat Reduction Agency (DTRA)

ZOONOSES

Continuation of CANARIES (2021-2025)

The consortium as a whole is actively writing manuscripts, grants, and other collaborative efforts; a second consortium meeting is being planned for 2021.

Collaborations	<i>Institut Pasteur du Cambodge</i> : Virology Unit (E. Karlsson) Multiple Collaborations Globally
Funding	Defense Threat Reduction Agency (DTRA)

Hantavirus Detection and Characterization in Rodents and Human Seroprevalence Study in Cambodia

Hantaviruses are widespread across the world. In Asia, there is much evidence of the circulation of these viruses in the eastern part of the continent (especially in Far-East Russia, China, and Republic of Korea) but the reporting of Hantavirus in Southeast Asia is more recent and scarce. In Cambodia, in 1998 we reported for the first time the presence of SEOV and Thailand Hantavirus (THAIV). SEOV was detected in brown rats (*Rattus norvegicus*) sampled in Phnom Penh and THAIV in black rats (*Rattus rattus*) collected in rice fields in neighboring villages. Very few data of serological evidence of infection in humans are available in Cambodia.

The project aims to 1) document the presence and diversity of Hantaviruses among rodents in urban and rural areas of Cambodia, and 2) estimating the seroprevalence of Hantavirus infection in human and identifying factors associated with Hantavirus infection and/or exposure.

The present study will be conducted in two steps: 1) development and standardization of serological tools for diagnosis of hantavirus and molecular assay for characterization of hantavirus strain in collaboration with different research units at IP in Paris (Evolutionary Genomics of RNA Viruses (EGRV), and Environment and Infectious Risks (EIR) including the French National Reference Center for Hantaviruses (NRCH) and the Laboratory for Urgent Response to Biological Threats), and 2) application of tools developed in the first step of the study to characterize hantavirus strains in rodents samples collected in HEPAR project (see above) and to evaluate seroprevalence of hantavirus in humans. In addition, we will identify factors associated with Hantavirus infection and/or exposure.

The expected outcomes are 1) the availability of a full range of diagnostic tools including multiplex Luminex assay, THAIV/SEOV specific ELISA assay, FRNT for confirmation, real time RT-PCR, NGS pipeline for full genome sequencing, 2) implementation at IPC and training of Cambodian investigators in those new diagnostic tools developed at EIR unit, France, 3) description of hantavirus diversity in Cambodia, prevalence of hantaviruses infection in rodents in different settings and seasons and collection of virus strains for further fundamental research questions including pathogenesis, and 4) first comprehensive measure of seroprevalence of hantaviruses infection in human and list of human behavior and practices that might put people at higher risk of transmission.

Collaborations	<i>Institut Pasteur du Cambodge</i> : Virology Unit (V. Duong, V. Hul and J. Guillebaud) <i>Institut Pasteur</i> : French National Reference Center for Hantaviruses (J.M. Reynes); Urgent Response to Biological Threats laboratory (J.C. Manuguera; J. Vanhomwegen and V. Caro)
Funding	NIH-PICREID (1U01AI151758 – 01)

4.4.4 Other Pending Funding for Research Programs

Detection of Emerging Viruses at Bat-Human Interface in Asia (DEBHIA)

- Name of the awarding body: *Institut Pasteur*, Paris
- Name(s) of grant holder(s): Veasna Duong
- Role in the project: Coordinator
- Objective: The objective of the DEBHIA project is to evaluate the circulation and the potential risks of emergence in Asia of four families of bat-borne viruses of major public health interest (Bunyaviridae, Coronaviridae, Paramyxoviridae and Rhabdoviridae), using both molecular and innovative multiplex immuno-assay (MIA) assays dedicated to these viruses.
- Funding Status: Pending

Karst Ecosystem Protection to Prevent Pandemics

- Awarding body: International Climate Initiative (IKI)
- Grant holder(s): Frank Momberg (Fauna & Flora International)
- Role in the project: Cambodia Coordinator
- Objective: The project rationale is that by 1) identifying potential hotspots for biodiversity and zoonotic disease transmission 2) developing natural protective barriers between humans and wildlife in these hotspots through extending and improving protected area management 3) by mitigating the risk of zoonosis where interaction does occur, and by 4) sharing learning and good practices regionally and globally, the project will dramatically reduce the risk of zoonotic disease transmission while significantly advancing conservation of the exceptional and globally-threatened karst biodiversity.
- Funding Status: Pending

Real-time Surveillance and Pandemic Prediction using GPS-Nano Biosensor Technologies in an Interdisciplinary Framework (Prediction2Response)

- Awarding body: Norway Research Council
- Grant holder(s): Courtney Waugh (Nord University) & Annelin Seppola (Nord University)
- Role in the project: Cambodia Coordinator
- Objective: The overall project objective is to develop a concrete and comprehensive plan for pandemic prediction, prevention, and response that incorporates an interdisciplinary approach (GPS tracking and implantable Nano biosensors for real time surveillance of viruses and miRNAs) so that science is translated into policy and action through governance and adequate communication.
- Funding Status: Pending

Biodiversity Conservation to Mitigate the Risks of Emerging Infectious Diseases (BCOMING)

- Awarding Body: European Union (HORIZON-CL6-2021-BIODIV-01-11)
- Grant holder(s): Julien Cappelle
- Role in the project: Cambodia Coordinator (WP2)
- Objective: The project aim is to co-construct innovations with all stakeholders of biodiversity hotspots to reduce the risk of infectious disease emergence through biodiversity conservation, restoration and surveillance.
- Funding Status: Pending

Understanding of SARS-CoV-2 Related Virus Distribution at the Human-Animal Interface in Southeast Asia

- Awarding Body: Defense Threat Reduction Agency (DTRA)
- Grant holder(s): Supaporn Wacharapluesadee, Veasna Duong and Linfa Wang
- Role in the project: Cambodia Coordinator
- Objective: The project will establish the bat research network in the Southeast Asia region (Cambodia and Thailand), aiming to better understand the origin of SARS-CoV-2 and diversity of SARS-CoV-2 related coronaviruses for improved preparedness of the next pandemic.
- Funding Status: Pending

NIH-K43: Surveillance of Pre-treatment and Acquired HIV-1 Integrase strand Transfer Inhibitor Resistance in Cambodia

It was believed that the introduction of dolutegravir (DTG) in low- and middle- income countries would reduce the incidence of HIV drug resistance, since the high durability of DTG has been demonstrated in clinical trials. However, few cases of DTG resistance mutations emerged. Because of diversity in patient populations, clinical practices and health infrastructures, the outcomes of DTG-based regimen in a “real-world” setting can be different from the clinical trials. Following the updated guideline of the World Health Organization, Cambodia switched its antiretroviral first-line regimen to a DTG-based regimen. To date, data of pre-treatment and acquired HIV drug resistance in patients receiving DTG-based regimen is not available. Therefore, it is important for Cambodia to have a surveillance system that would allow the rapid identification of viruses that are less likely to respond to DTG-based therapy.

The main objectives of the present study are to establish a national surveillance survey in Cambodia to closely monitor the rate of pre-treatment and acquired HIV drug resistance among adult and antiretroviral (ARV)-naïve patients receiving DTG-based regimen as first-line therapy.

A total of 450 ARV-naïve adult (≥ 15 years) patients initiating DTG-based first line regimen will be included from fifty out of sixty-seven adult ARV therapy sites in Cambodia over a period of one year. At inclusion, HIV RNA viral load and HIV drug resistance testing in HIV reverse transcriptase, protease and integrase genes will be assessed for all patients. The acquired HIV drug resistance in these genes will be assessed for patients with virological failure at months 6, 12, and 24. This surveillance survey would serve as an alert system for the national program to make informed and responsible decisions in response to the emergence of HIV drug resistance in Cambodia.

Collaborations	<i>Institut Pasteur du Cambodge: Virology Unit (J. Nouhin, and N. Boukli) and Clinical Research Group (Laurence Borand)</i> Stanford University (Robert William Shafer)
Funding	to be resubmitted to NIH K43 in 2021

EDOLICA project: Era of Dolutegravir-Based First-Line Regimen in Cambodia: a National Prospective Cohort to Evaluate HIV-1 Acquired Drug Resistance and Prevalence of Transmitted Drug Resistance

HIV RNA plasma viral load (HIV VL) is available in Cambodia for the monitoring of treatment. Nevertheless, there is still limited access to genotypic drug resistance testing, as it is not recommended. So far, data of acquired drug resistance (ADR) in patients failing DTG-based regimen is unavailable in the country. Moreover, in terms of HIV transmitted drug resistance (TDR) or pre-treatment drug resistance (PDR), data is scarce; only two studies were conducted since the first case of HIV was detected in the early 1990s and the last surveillance was carried out 13 years ago. At the same time, an increasing trend of TDR has been reported in neighboring countries. In this regard, it is of particular interest to closely monitor PDR and ADR in Cambodia.

The primary objective is to assess the emergence of acquired drug resistance in HIV reverse transcriptase (RT) and integrase (IN) at 12 months (M12), 24 months (M24), and 36 months (M36) of ART in patients who experience virological failure of their DTG-based first-line regimen in Cambodia.

In the current context of clinical care of PLHIV in Cambodia where recommended ARV regimens are moving to a new era of a potent ART, it seems of particular interest to evaluate the emergence of acquired drug resistance in case of viral failure. Furthermore, there is a big gap of PDR information in Cambodia during the last decade. We believe that HIV PDR in RT and/or IN may influence the outcome of DTG-based first-line regimen in Cambodia. In addition, we hypothesize that PDR mutations which present as a low-frequency population may be selected during the treatment of DTG-based regimen and may become a major variant during VF. Determining whether the present of low-frequency variants is associated with VF has implications for monitoring of ART, and for improving our understanding of virus evolution under DTG selective pressure. Should our study demonstrate that PDR is associated with dolutegravir-based treatment failure, this would provide us a useful predictive biomarker for virological outcome of the ART. Our second hypothesis is that mutation outside IN coding region may emerge and be associated with failure to DTG-based regimen, in particular in absence of mutations in IN gene.

Collaborations	<i>Institut Pasteur du Cambodge</i> : Virology Unit (J. Nouhin, and N. Boukli) and Clinical Research Group (Laurence Borand) National Centre for HIV/AIDS, Dermatology and STD Control (Dr. Ly Penh Srun) University of Health Science (Prof. Saphonn Vonthanak) Virology Unit, <i>Hôpital Bichat-Claude Bernard</i> , Paris, France (Professor Diane Descamps)
Funding	Pending-Submitted to ANRS on March 2021

Phenotyping Assay for HCV Drug Resistance: To Be Submitted for IPC Internal Grant

Despite the efficacy of direct-acting antivirals (DAA), HCV remains a major concern for public health in the absence of a preventive vaccine and emergence of DAA resistance mutations. In a previous study, we investigated the cause of DAA (NS5A and NS5B inhibitors) treatment failure in Cambodian patients infected with HCV genotype 6, using next generation sequencing (NGS). We observed substitutions occurring at resistance-associated positions in NS5A (28K, 30G, 31I, 54H, and 62E/L) and NS5B (203H, 237A/N and 289A) coding regions either before or after DAA treatment in patients failing DAA treatment. Nevertheless, these substitutions have never been experimentally demonstrated to be associated with DAA resistance.

In this study, we aim to 1) develop a phenotyping assay to study DAA resistance of HCV genotype 6 which has been less studied unlike genotypes predominant in western countries and 2) to implement this phenotypic assay to evaluate clinical significance of the substitutions found in our previous study.

Sub genomic replicon-based virus culture using HCV cDNA amplified from patient plasma will be developed. We will then test antiviral activity of NS5A or NS5B inhibitors against sub genomic replicons constructed from patient plasma containing substitutions observed in our previous study.

The expected outcomes are an ability to acquire a phenotypic assay available to investigate HCV DAA resistance in Cambodia. In addition, we will be able to determine the clinical significance of newly emerged substitutions in NS5A and NS5B regions.

Collaborations	<i>Institut Pasteur du Cambodge</i> : Virology Unit (J. Nouhin, H. Auerswald and V. Duong) <i>Institut Pasteur Shanghai</i> (J. Zong)
Funding	Pending-Submitted as internal project (<i>Institut Pasteur du Cambodge</i>)

4.4.5 Support to National Authorities

National Dengue Surveillance in Cambodia

As part of a collaboration with the WHO and NDCP and within the framework of a national program 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 the clinical laboratory from IPC. These surveillance sites in Cambodian provinces are located within high risk areas of dengue hemorrhagic fever (high population density, presence of the vector, history of dengue in the region). Results from the monitoring of hemorrhagic syndromes are reported weekly to the various monitoring program 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. We continued to detect mainly DENV-2 in 2017-2018, while DENV-1 was still present in the country at a lower level. The recrudescence of dengue in 2018 was much higher compared to previous years, and 2019 was marked by a huge dengue outbreak with more than 68,000 hospitalized cases and forty-eight deaths reported countrywide. For comparison, in 2018, 24,684 cases and twenty-three 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 big dengue outbreak occurred in 2007 and 2012 and was caused by DENV-3 and DENV-2 serotypes respectively (Figure 20).

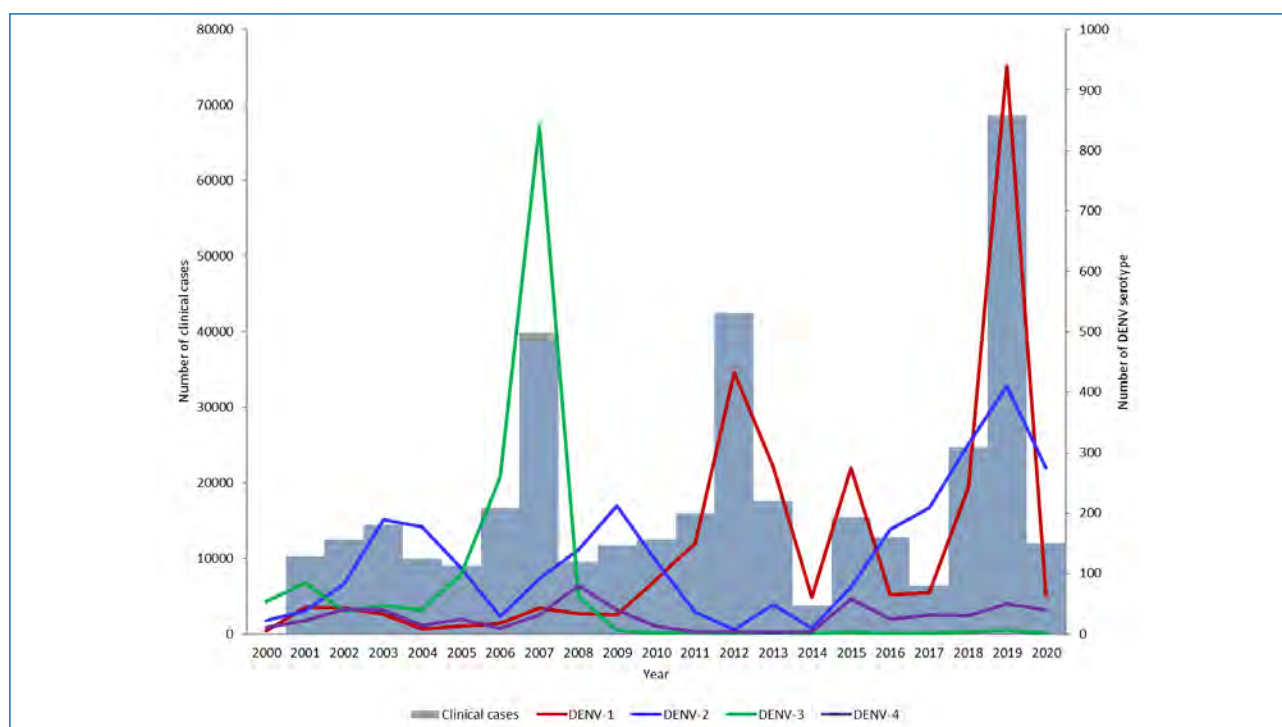


Figure 20. Dengue Virus Serotypes Circulation in Cambodia and Number of Dengue Clinical Cases Reported.

Cambodian National Influenza Centre

Seasonal Human Influenza Viruses Surveillance (Influenza-like Illness and Severe Acute Respiratory Illness)

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

The Influenza-like illness (ILI) surveillance established in 2006, in collaboration with the MoH and WHO, allows for the collection of influenza strains and data on seasonality. Currently, seven hospitals contribute to ILI surveillance: Kam-pot, Battambang, Kampong Cham, Mondulhiri, 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 analyzed by the National Institute of Public Health 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 Naval Army Medical Research Unit (NAMRU-2), and the Armed Forces Research Institute of the Medical Sciences (AFRIMS). In collaboration with Kantha Bopha Hospital, IPC has conducted 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. A manuscript detailing seasonal influenza circulation between 2016 and 2019 is being completed and is expected to be submitted in mid-2021 due to some delays caused by work on the COVID-19 pandemic.

Cambodia has two distinct seasons, the dry season which generally runs from November to April, and the rainy season which starts in May-June and ends in October-November. In Cambodia, influenza cases usually increase during the March to June period, and peaks between July and September, corresponding to influenza circulation in the temperate regions of the southern hemisphere, although low level year-round circulation of influenza occurs.

The current global COVID-19 pandemic has significantly altered both the surveillance and landscape of respiratory disease worldwide. Indeed, introduction of control measures in early 2020 to reduce the transmission and disease burden of Severe Acute Respiratory Virus coronavirus 2 (SARS-CoV-2) infection has shown a remarkable reduction in the rates of infection of many respiratory diseases. Since April/May 2020, influenza activity remained at historically low levels globally, despite continued, or even increased, testing for influenza in some countries. In the northern hemisphere, influenza activity remained below inter-seasonal levels following the 2019-2020 season, though sporadic influenza detections were reported in some countries. Interestingly, the majority of influenza detections have been in Southeast Asia and other tropical regions such as in Western Africa in 2020.

Due to a robust and swift response, SARS-CoV-2 was well managed and controlled in Cambodia throughout 2020 and the country had no widespread community transmission until March 2021. While influenza virus detection typically increases in Cambodia from April-June, few influenza viruses were detected in this period in 2020. This reduction coincided with the implementation of several COVID-19 non-pharmaceutical intervention (NPI) measures. Once international border restrictions were eased on 11 June 2020, Cambodia experienced an outbreak of influenza A(H3N2) that circulated in several provinces from July through to November 2020 (Figure 21). Indeed, influenza detections in sentinel surveillance systems began to increase in Cambodia resulting in a country-wide outbreak including clustered detections in closed/semi-closed systems (prisons/pagodas) and also spreading in the general community.

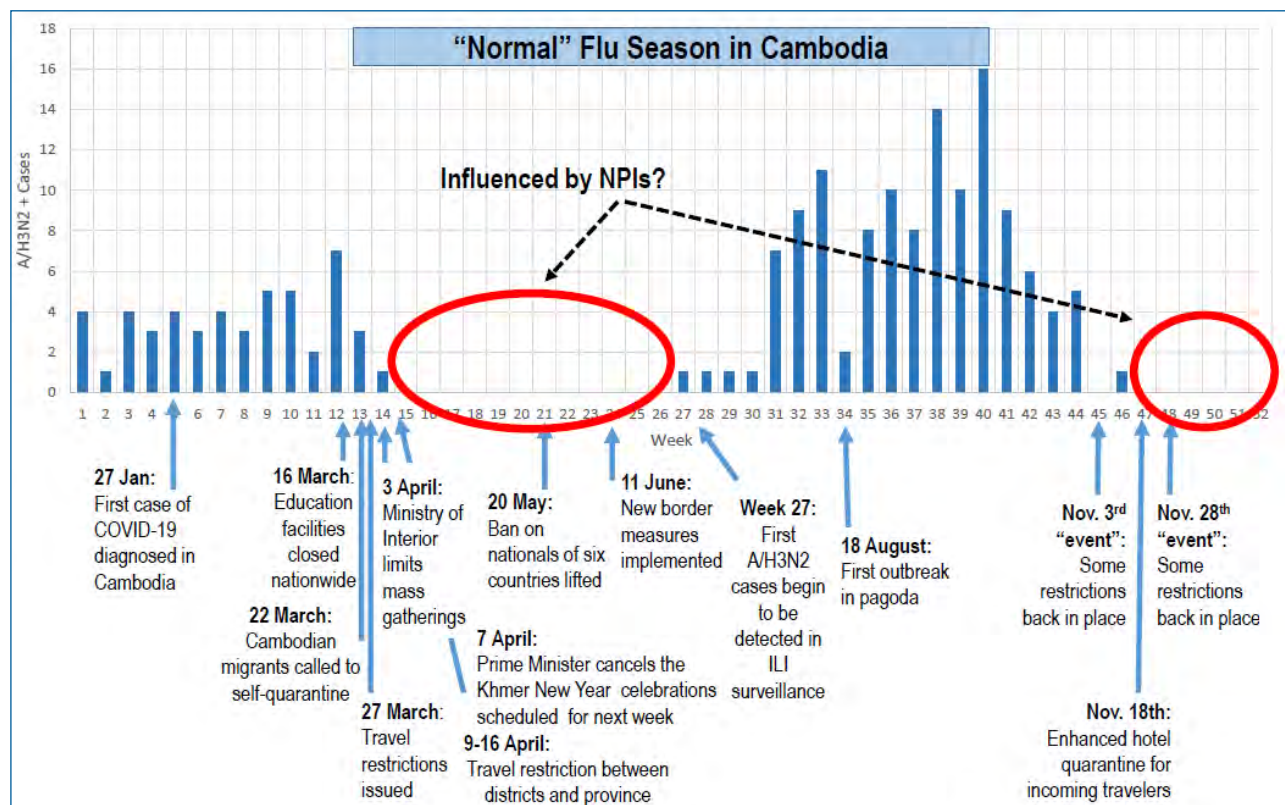


Figure 21. Positive H3N2 influenza samples from ILI surveillance in Cambodia, 2020.

Genetic and antigenic characteristics of influenza viruses detected during the outbreak were assessed to determine the degree of vaccine-match with the currently recommended A(H3N2) influenza vaccine and the likelihood of a more global spread in the future. All A(H3N2) viruses collected after July 2020 clustered within a major clade designated as 3C2a1b+131K. This emergent 2020 lineage contained all Cambodian A(H3N2) viruses collected since July 2020 and viruses collected from Bangladesh and India during August and December 2020, respectively. In contrast, viruses collected earlier in 2020 from Cambodia were dispersed among the other major H3N2 lineages, which also contained the 2020-2021 northern hemisphere and the 2021 southern hemisphere recommended influenza vaccine A(H3N2) vaccine virus component which was based on a A/Hong Kong/2671/2019-like viruses. They were similar to viruses collected during early 2020 in Australia and Indonesia and neighboring regions and appeared to be derived independently from viruses circulating in the northern hemisphere during 2019 suggesting an importation into the country.

Antigenic analysis showed that the late-2020 Cambodian viruses reacted best with A/Tasmania/503/2020-like viruses and viruses collected from Timor-Leste in July-August 2020. Again, these were distinct from early 2020 Cambodian viruses. Together this data indicates that the late 2020 Cambodian viruses were antigenically distinct from the previous vaccine viruses and may escape prior-immunity generated by infection or vaccination with these vaccine-like A(H3N2) viruses and a vaccine update may be required. Indeed, the prototypical Cambodian A(H3N2) strain from this period, A/Cambodia/e0826360/2020, was selected as the recommended composition for use in the 2021-2022 northern hemisphere influenza vaccine in February 2021. This virus was identified and first isolated at IPC and represents a first for Cambodia to be included in the vaccine.

One paper detailing the initial A(H3N2) outbreaks in mid to late 2020 has been published and a second which details the genetic and antigenic findings from the entire outbreak is in preparation and expected to be submitted in mid-2021.

Diagnostics for rabies infections

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

From February to June 2019, IPC 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 to rabies infection. During this period, we have 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 tested positive for rabies remains high at 68.8% (n=86). Similarly, due to COVID-19, we received only 164 specimen 2020 but the positive rate was 70% (Figure 22). The average percentage of positive dog heads observed from 2002 to 2020 is currently 51.5%.

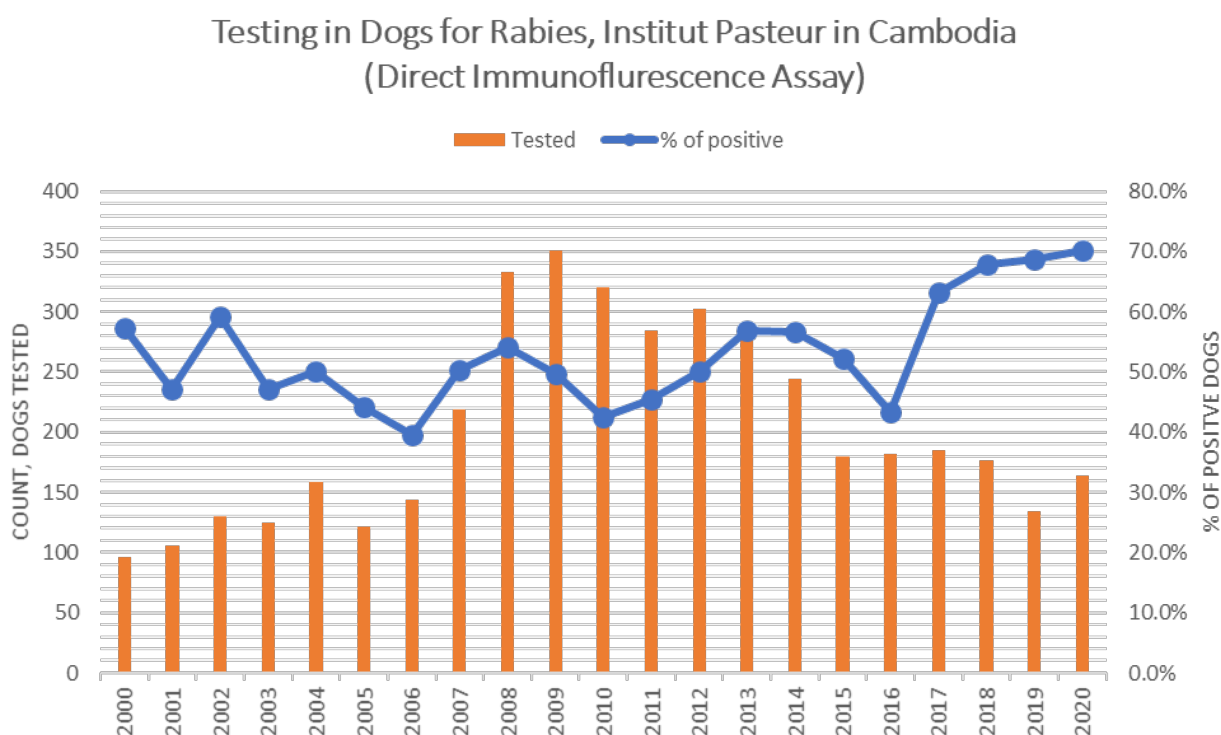


Figure 22. Testing in Dogs for Rabies, Institut Pasteur du Cambodge by Direct Immunofluorescence Assay

4.4.6 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 students and staff from institution and government partners in the fields of surveillance and research, conducted at the national, regional and international levels.

In 2020, the virology unit contributed to COVID-19 related training since the beginning of the crisis including the swab sampling/safe transportation training and ensured training/technological transfer for diagnosis to the NIPH and other regional laboratories. This key component started in early March 2020.

The unit hosts two master's students of Master of Infectious Disease at University of Health Sciences (UHS), two students from Royal University of Agriculture for their Doctor of Veterinary Medicine degree. One virology staff registered in a PhD program in the field of virology at Aix Marseille University spent four months in Xavier de Lamballerie lab in 2019 and he is now in his second year.

Scientists at the virology unit are actively involved in teaching the master's programs at UHS including Master of Infectious Diseases and Master of Medical Biology.

4.4.7 Publication List

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4.5 Medical and Veterinary Entomology Unit

The Medical and Veterinary Entomology Unit has recorded significant progress in a very short period: personnel increased from 9 to 12 persons between 2019 and 2020, field missions have increased 52% between 2019 to 2020 and the number of publications increased from 6 in 2019 to 11 in 2020.

4.5.1 Functional Structure

The Medical Entomology Unit was officially created on 1 October 2018 (N/Réf: N°413/IPC/DIR/2018) at the same time that Sebastien Boyer was recruited by *Institut Pasteur* in Paris for a permanent position. As of March 2021, the unit has twelve members, an increase in five over the previous year.

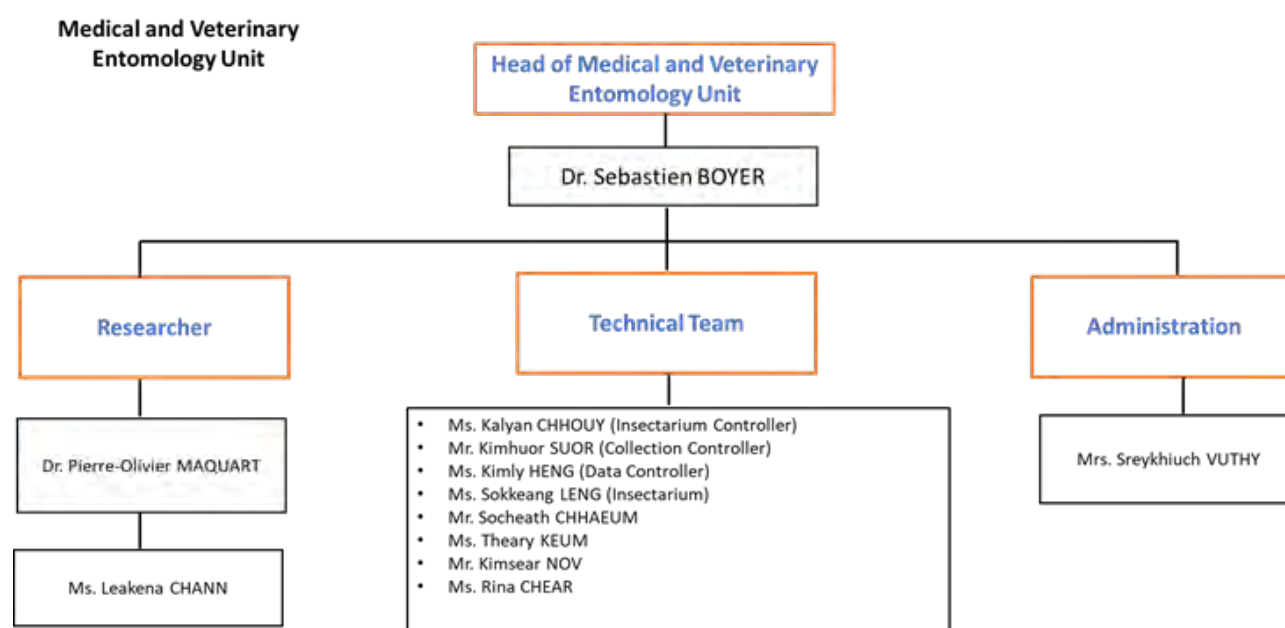


Figure 23. Medical and Veterinary Entomology unit organogram

4.5.2 Research Programs – Major Achievements in 2020

Field missions in 2020

During 2020 the Medical and Veterinary Unit undertook 32 field missions in Cambodia and 24 sampling missions in Phnom Penh City for a total of 496 mission days in the field.

ECOMORE 2 Project: Economic Development, Ecosystem Modifications, and Emerging Infectious Diseases Risk Evaluation

The ECOMORE 2 Project is funded by the AFD (*Agence Française de Développement*) while Panic project is financed by the European Union. The objective of the project was to determine if a successful integrated vector management (IVM) system 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 different interventions: 1) elimination of breeding sites with the help of students and their teachers, 2) scientific education and sensitization of school children, 3) the use of a *bacterio* insecticide (*Bacillus thuringiensis israelensis*) used as a larvicide in water jars and in large water reservoirs, and 4) use of an auto-dissemination insecticide (Pyriproxyfen) for the spreading of insect growth regulator larvicide and spores of *Beauveria bassiana* for slowly killing adult mosquitoes. The main expected study outcome would be the number of dengue-like fever cases in the villages around schools.

Field missions ended in February 2020 after a period of three years. The first article related to the diversity of mosquitoes in schools was published this year. We found that a high diversity of mosquitoes was present in the schools (61 species) of which more than 70% were potential vector species.

Within this project, we organized a dissemination meeting at Kampong Cham Province. During this meeting, Ms. Sony Yean presented the main activities and results to the 24 primary schools directors in which we worked, as well as to representatives of the Ministry of Health (PHD and CNM representatives), Ministry of Youth, Education and Sport, and Ministry of Agriculture, Forestry and Fisheries.

The project finished last year. It is planned that three articles will be written with these data.

Collaborations	Epidemiology and Virology Units, IRD (GeoHealth Group), <i>Institut Pasteur du Laos</i> <i>Institut Pasteur</i> , Ministry of Health, Ministry of Youth, Education and Sport Ministry of Agriculture, Fisheries and Forestry
Funding	ECOMORE 2, <i>Agence Française pour le Développement</i> (AFD) CZZ 2146 01

DARPA-PREEMPT Project: Preventing Emerging Pathogenic Threats

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

In 2020, five sites were sampled during both the dry and rainy seasons.

The two sites listed below were sampled in 2019:

- Prek Toal Wildlife Sanctuary, located in Battambang Province is recognized as an important sanctuary for migratory birds in Cambodia;
- Virachey National Park located in Ratanakiri Province, described as a primary forest hosting several primates (macaques, gibbons) and large mammals species (bears, elephant, muntjac deer).

Three new sites of interest for biodiversity were sampled this year: Changkran Roy Community Forest, Phnom Aural and Phnom Tnout Forests. Based in Siem Reap Province, The Changkran Roy Community Forest is a protected semi-evergreen forest in the area of Phnom Kulen. Based in Kampong Speu Province and in the Cardamom Mountains, the Phnom Aural Wildlife Sanctuary is a protected area with an important diversity of vegetation, and the presence of several bird and mammal species. In Preah Vihear Forest, Phnom Tnout is a 64,000 hectare protected community forest with more than 200 bird species, wild animals such as pigs, monkey, deer, banteng, sambar, silver langur, dhole and gibbons.

After years one and two, in total, more than 25,000 mosquitoes were collected representing an important biodiversity with at least 131 mosquito species. Two articles were written and submitted on this topic. Finally, the mosquitoes collected during year one were sent to *Institut Pasteur* in Paris to determine the presence of virus in the different species. New viruses were found in these mosquitoes and collaborators are currently describing them. In addition, one specific new virus was discovered on 4 different mosquito species, and six different viruses were found on one specific mosquito species. This particular mosquito species represents about 60% of all collected mosquitoes in Cambodia, highlighting the importance of this discovery.

Collaborations	Virology Unit , <i>Institut Pasteur</i> , Ministry of Environment
Funding	DARPA - HR001118S0017

FSPI Project: Surveillance and Prevention of Emerging Viruses in Cambodia and the Region

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

In addition, in Cambodia, there are neither university courses related to medical entomology, nor applied practical virology work available. To address critical public health needs in the country, the *Institut Pasteur du Cambodge* pro-

poses through this project, to train entomologists in the field by a training in medical entomology, and virologists for the national reference center for surveillance of arboviruses in Cambodia.

In 2020, twenty missions were undertaken for FSPI projects covering five different areas in five different provinces (Pailin, Preah Vihear, Kampong Saom, Battambang, Kampong Thom). These missions were the third to sixth missions done in the different areas. Unfortunately, missions during the dry season (March, April and May) could not be done due to the Covid-19 situation. These missions will be done in 2021. In 2020, the collaborators at *Institut Pasteur* in Paris described new viruses within mosquito species, and designed primers.

Data analysis will be completed at the end of the missions during the second 2021 semester.

Collaborations	<i>Institut Pasteur</i> , Ministry of Health, Wildlife Conservation Society IRD (GeoHealth Group)
Funding	FSPI 2019-17

Mosquito Species and Dynamics in Phnom Penh: Surveillance and Prevention of Emerging Viruses in Cambodia and the Region

The diversity, distribution and seasonality of mosquito species in Phnom Penh was yet unknown. A study on the dynamics of dengue vectors in Phnom Penh was undertaken in 2019 and 2020. The relative abundance of the different species will be analyzed according to different meteorological parameters, and the different types of urban environments surrounding pagodas. The sampling was done around pagodas since in the rapidly urbanizing Cambodian capital, pagodas will be left undeveloped and unchanged in the near future, allowing replication of this study over several decades and providing important time-series data. The objective will be to evaluate the dynamics, and the risk associated with the potential presence of mosquito vector species.

The field missions began in 2019. The sampling was done in forty different sampling points in Phnom Penh Pagodas between March 2019 and March 2020. During this period, 9,054 adult mosquitoes were collected, including 5,080 *Aedes aegypti* and 2,771 *Aedes albopictus*. These two dengue vector mosquito species represent 87% of all the mosquitoes sampled during the year. Their distribution in Phnom Penh shows that in high human density areas *Ae. Aegypti* is more common, whereas in more treed areas *Ae. Albopictus* is more likely to be the species found. In fact, one article was published on the presence of *Ae. Albopictus* in Phnom Penh that was never before described. Another article on the prediction of these arbovirus vector species in Southeast Asia, based in part from these data, is in progress. Two other articles will be written using these data.

This work is accomplished in collaboration with the Ministry of Cults and Religion, and the Ministry of Tourism.

Collaborations	Ministry of Cults and Religion, Ministry of Tourism
Funding	Internal project (<i>Institut Pasteur du Cambodge</i>)

Identification of mosquitoes

Since 2020, we have made efforts to develop a method for mosquito identification with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). The current methods for mosquito identification include both morphological and molecular methods. Identification by morphology is skill-dependent and is time-consuming while the identification by PCR is expensive. The 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 mosquitoes from Cambodia and to create a useful tool for the Ministry of Health and also for our current partners in Southeast Asia.

In 2020, we begun to implement a database by creating the reference spectra of 12 mosquito species with MALDI-TOF.

Based on the samples from the different field missions, and in order to further develop the Medical and Veterinary Entomology Unit including new methods for determining mosquito species, the molecular entomology must be strengthened. Moreover, we want to validate the use of this tool for determining different mosquito populations within one species. We used molecular entomology and MALDI-TOF MS to study the *Culex vishnui* mosquito species complex. This species complex is difficult to identify based only on morphological tools. In parallel to determine the number of species and the different populations within this species, we want to validate the use of MALDI-TOF MS as a method to study it.

Communication and Workshops in 2020

In 2020, conferences and communications were limited due to the Covid-19 crisis. In January 2020, we organized the third meeting of the working group with stakeholders at Phnom Penh for the Ecomore 2 Project. In November 2020, we presented our work at the *Alliance Française* in Siem Reap where an Ecomore 2 exposition was organized. We did a large audience presentation about the objectives and the methods of Ecomore 2.

Finally, related to FSPI project, we made a video on the project in the Khmer language with English subtitles that is available at the following YouTube address: <https://www.youtube.com/watch?v=-iwN--Lwsgs>

NIH PICREID Project: Pasteur Institute – Center for Research for Emerging Infectious Diseases (PICREID)

In August 2020, the Pasteur Institute – Center Research for Emerging Infectious Diseases (PICREID) project started its five-year implementation period. The PICREID project has the objective to establish a One Health approach in order to improve the capacity to respond rapidly and effectively to emerging infectious diseases outbreaks in Southeast Asia. The surveillance enhancement component of the PICREID project is based on RNA virus detection, understanding of endemic RNA virus transmission, determination of factors influencing RNA adaptations to new host and adaptive responses of emerging infectious diseases.

The mosquito component aims to study the dynamics of the main DENV vector species in Kampong Thom Province (*Aedes albopictus* and *Aedes aegypti*), to describe the mosquito behaviors, to characterize their ecological niches to further analyze and model the spatial distribution and the land-use effects on the dynamics. The other objective will be to model the risk of dengue by linking the number of dengue vectors and the number of dengue cases in humans. Finally, we also coupled the pathogen discovery objective with an entomological-based genomic surveillance, in collaboration with *Institut Pasteur* in Paris.

Collaborations	Immunology, Epidemiology and Virology Units, <i>Institut Pasteur</i> , Ministry of Health, IRD (Geo-Health Group)
Funding	NIH-U01AI151758-01

4.5.3 Research Programs – Outlook for 2021

DARPA-PREEMPT project

The DARPA-PREEMPT missions will finish in January 2021. We expect that in 2021 the identification of all collected mosquitoes and the determination of the presence of some viruses will be completed. We also expect to discover new viruses in remote areas. Therefore, after all individual mosquito identification, we will send samples to IP Paris for virus screening. The data analysis will be done in 2021 or 2022.

FSPI project

The FSPI project will end in March 2021 and ten more missions will be done in 2021. After that, trainings in virology and entomology should be completed. During 2021, we expect the morphological identification of all individual mosquitoes, the determination of all the virus families present in our mosquitoes, and a prevalence study of virus along an ecological gradient to model the risk.

The data analysis of virus families and mosquito diversity will be achieved in 2021. The study of virus prevalence, planned for 2021, will be analyzed in 2022.

INVALBO / KIM-RIVE Project

The characterization of *Ae. Albopictus* invasivity in Phnom Penh will be completed in 2021. The project objective is to determine the invasion modality of *Ae. Albopictus*. We will characterize the genomic diversity, genetic structuring of populations, dispersal and flow of genes along the anthropization gradients and the genetic inference from recent demographic history and evolution of to determine the modalities of the invasion of the urban environment of Phnom Penh and Europe.

Based on the samples obtained via FSPI projects (in forest) and in Phnom Penh, *Ae. Albopictus* will be analyzed by Dr. Michael Fontaine, IRD, Montpellier.

Resistance to insecticides

The resistance to temephos (a larvicide), permethrin and deltamethrin (adulticide) used in Cambodia were tested on the main dengue vector species. We demonstrated that *Aedes aegypti* species (4 populations) were resistant to the three currently used insecticides in this country. The results were shared with the Ministry of Health (CNM: National Center for Parasitology, Entomology and Malaria Control). These results were also presented at six national and four international conferences.

The bioassays that were commenced at the end of 2020 continued and should be completed in 2021 with the test of new larvicides and adulticides that will all the proposal of new alternatives to the Ministry of Health. We will test fifteen adulticides and three larvicides.

Wat'Health Project (FSPI project)

This project is expected to begin the middle of 2021 and run until the end of 2022. This project aims to determine the link between flooding and mosquito populations in Cambodia. We will define the relationship between floods and mosquito population, and evaluate the indirect sanitary risk in the studied area. We will also describe the resistance to insecticides.

Vector Competence

The study of vector competence of *Aedes aegypti* and *Aedes albopictus* for several viruses (DENV, CHIKV, ZIKAV) 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).

Factors Influencing Arbovirus Emergence in Anthropized Environments

In parallel of the PICREID-NIH project initiated in 2020, one post-doctoral student applied to work on this topic within in our unit starting in 2021.

Thus, within this project, we want to test these scientific hypotheses and improve knowledge on arboviruses distributed in mosquito species in order to anticipate the emergence of new vector-borne diseases, in the context of a changing environment. Three specific objectives are specified. The objectives of this project are to understand the temporal and spatial distribution of arboviruses in specific mosquito species, and to characterize discovered viruses related to the land use and inhabited areas.

Briefly, the main objectives are to identify and characterize new arbovirus(es), with the necessary successive steps: 1) screening mosquitoes collected in different environments monthly for arboviruses, 2) isolation of viruses and determination of known or unknown viruses, 3) taking cultures of all the viruses including new viruses inside PSL-P3 laboratory, 4) characterization of the host range with the use of different cell lines, and 5) development of detection method (RT-PCR) and testing of retrospective mosquito samples.

Veterinary Entomology

As for the Medical Entomology that was absent within the research landscape in Cambodia, our IPC unit wants to develop veterinary entomology. Based on a joint request of the Ministry of Agriculture (General Directorate of Animal Health and Production) and the Royal University of Agriculture (RUA), we will try to propose a developmental and research project during this year, based on babesiosis and trypanosomiasis as requested by the two national partners. It could be the first step on further studies on ticks responsible for animal and human pathogens' transmission.

4.5.4 Support to National authorities

The Medical and Veterinary Entomology Unit is working with 5 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 2020, with Ecomore 2, we organized a restitution meeting at Kampong Cham. During this meeting, Sony Yean presented the main activities and results to all the 24 directors of primary schools in which we worked, to representatives for Ministry of health (PHD and CNM representatives), Ministry of Youth, Education and Sport, and Ministry of Agriculture, Fisheries and Food.

Finally, technicians and responsible from the Ministry of Health, specifically the CNM, participated to the taxonomy training organized at IPC.

4.5.5 Teaching and Training

Mentorship

In 2020, Ms. Yim Chanmuneneath, a Cambodian Master's Degree year 2 student, did her internship in our Medical and Veterinary Entomology Unit. She came from the Royal University of Phnom Penh, and was in the Master of Science in Biodiversity for Conservation. Her work was on the factors influencing mosquito species dynamics and distribution in five provinces of Cambodia.

In 2020, Mr. Khin Chandara, also a second year Cambodian Master's student, did his internship in our unit as well. He came from the Royal University of Phnom Penh, and was in the Master of Science in Biodiversity for Conservation. His work was on the factors influencing the spatial distribution pattern and temporal dynamics of mosquito species in Phnom Penh, Cambodia.

In 2020, Ms. Dusadeepong Rutaiwan, a student from Thailand, completed the first year of her Master's program in Phnom Penh as part of the International Master of Biology of Infectious Diseases, organized by the University of Health Sciences and University Paris-Saclay. She worked on the database acquisition of mosquito species with MALDI-TOF.

Teaching

Finally, Sebastien Boyer was responsible of the module "Vector Borne Diseases and Vector Transmission" in the International Joint Master's year two of Infectious Disease Study: Biology of Infectious Diseases. The module represents 2.5 ECTS and 20 hours.

The module was created in 2020 and it took significant time to prepare and complete. The teaching was a module of 8 lectures of 1.5 hours. The exams and corrections were also done by our team.

Trainings

In 2020, the Thailand International Cooperation Agency (TICA) within the framework of the *Agence Française de Développement* (AFD) Project Ecomore 2 organized one training on mosquito systematics in Cambodia at IPC in January 2020. Technicians and scientists from CNM and the Malaria Unit (IPC) also participated in the training directed by Professor Theerapapth from Thailand.

In January 2020, we organized a training on mosquito taxonomy at IPC in conjunction with the FSPI project. Nil Rahola, from IRD in France, was responsible for this advanced two-week training.

In 2020, the staff also participated in a training in management (Sebastien Boyer) and in personal growth and development (one technician: Chhuoy Kalyan). All the technicians followed the Kaptitude training online course on the security and good laboratory practices.

Calmette & Yersin Grant

In 2020, Dr. Pierre-Olivier Maquart obtained a post-doctoral grant. The grant guarantees this position in the unit from January 2021 until December 2022.

Collaborations	Université Catholique de Louvain, UCL (D. Da Re, S. Vanwambeke)
Funding	Calmette & Yersin Post doc Grant, <i>Institut Pasteur</i> (2021-2022)

4.5.6 Publications List

1. **High diversity of mosquito vectors in Cambodian primary schools and consequences for arbovirus transmission.** Boyer S, Marcombe S, Yean S, Fontenille D. PLoS One, 2020, 15 (6), e0233669.
2. **Dynamics and diversity of mosquito vectors of Japanese encephalitis virus in Kandal province, Cambodia.** Boyer S, Peng B, Pang S, Chevalier V, Duong V, Gorman C, Dussart P, Fontenille D, Cappelle J. Journal of Asia-Pacific Entomology, 2020, 23:1048-1054
3. **A genomic amplification affecting a carboxylesterase gene cluster confers organophosphate resistance in the mosquito *Aedes aegypti* from genomic characterization to high-throughput field detection.** Cattel J, Haberkorn C, Laporte F, Gaude T, Cumert T, Renaud J, Sutherland IW, Hertz JC, Bonneville JM, Arnaud V, Noûs C, Fustec B, Boyer S, Marcombe S, David J-P. Evolutionary Applications, 2020:
4. **Novel genome sequences of cell-fusing agent virus reveal lack of phylogenetic codivergence with *Aedes aegypti* mosquitoes in South East Asia.** Baidaliuk A, Lequime S, Moltini-Conclois I, Dabo S, Dickson LB, Duong V, Dussart P, Boyer S, Shi C, Matthijnsens J, Guglielmini J, Powell JR, Gloria-Soria A, Simon-Lorière E, Lambrechts L. Virus Evolution, 2020, 6:veaa018
5. **Current Vector Research Challenges in the Greater Mekong Subregion for Dengue, Malaria, and Other Vector-Borne Diseases: A report from a multi-sectoral workshop March 2019.** Christofferson RC, Parker DM, Overgaard HJ, Hii J, Devine G, Wilcox BA, Nam VS, Abubakar S, Boyer S, Boonnak K, Whitehead SS, Rekol H, Rithea L, Sochantha T, Wellemes TE, Valenzuela JG, Manning JE. PLoS NTD, 2020, 14:e0008302
6. **Correlation of the Premise Condition Index (PCI) with adult and immature *Aedes* mosquitoes in Kampong Cham, Cambodia.** Hustedt J, Doum D, Keo V, Sokha L, Sam B, Vibol C, Boyer S, Liverani M, Alexander N, Bradley J, Prasetyo DB, Rachmat A, Lopes S, Rithea L, Hii J. American Journal of Tropical Medicine and Hygiene, 2020, tpm190453.
7. **First record of *Cheironotus parryi* Grey, 1848 (Coleoptera: Eucirinae) in Cambodia.** Maquart PO, Sin S, Chhorn S, Phak S, Boyer S, Phauk S. Cambodian journal of Natural History, 2020 2:31-33.
8. **Discrimination of *Uranotaenia* species (Diptera: Culicidae) from Madagascar based on morphology and wing morphometric traits.** Tantely ML, Randrianambinintsoa FJ, Rakotonirina AH, Woog F, Boyer S, Girard R. Zootaxa, 4801:171-178.
9. **Two new phlebotomine sandflies (Diptera: Psychodidae) from the forest edge in Madagascar: the anthropophilic *Phlebotomus artemievi* sp. nov. and *Sergentomyia maroantsetraensis* sp. nov.** Randrianambinintsoa FNJ, Depaquit J, Martinet JP, Golden CD, Boyer S, Robert V, Tantely LM. Parasitology Research, 2020.
10. **Strategic approach, advances and challenges in the development and application of the SIT for area-wide control of *Aedes albopictus* mosquitoes in Reunion Island.** Gouagna LC, Damiens D, Oliva CF, Boyer S, Le Goff G, Brengues C, Dehecq JS, Raude J, Simard F, Fontenille D. Insects, 2020, 11:770

4.6 Laboratory for Environment and Food Safety

The Laboratory for Environment and Food Safety (LEFS) was created in 1995 and its activities are mainly microbiological and chemical analysis in food and water. More specifically, its activities include:

- Identification and quantification of public health issues related to food and water consumption by the presence of pathogens (*Clostridium perfringens*, Coagulase positive Staphylococci, *Salmonella*...) and parasites;
- Promotion of hygiene in restaurants and food industries (training, consulting, auditing); and
- Assessing the ecological risk of heavy metals (As, Cd, Fe, Pb, etc.).

The laboratory provides services for the following analyses by using international protocol standards:

- Food and water microbiology, microbiological quality of surface samples;
- Physical-chemistry, quality of water samples;
- Identification of *Legionella pneumophila* in tap water, cooling towers, pools and spa water.

4.6.1 Functional Structure

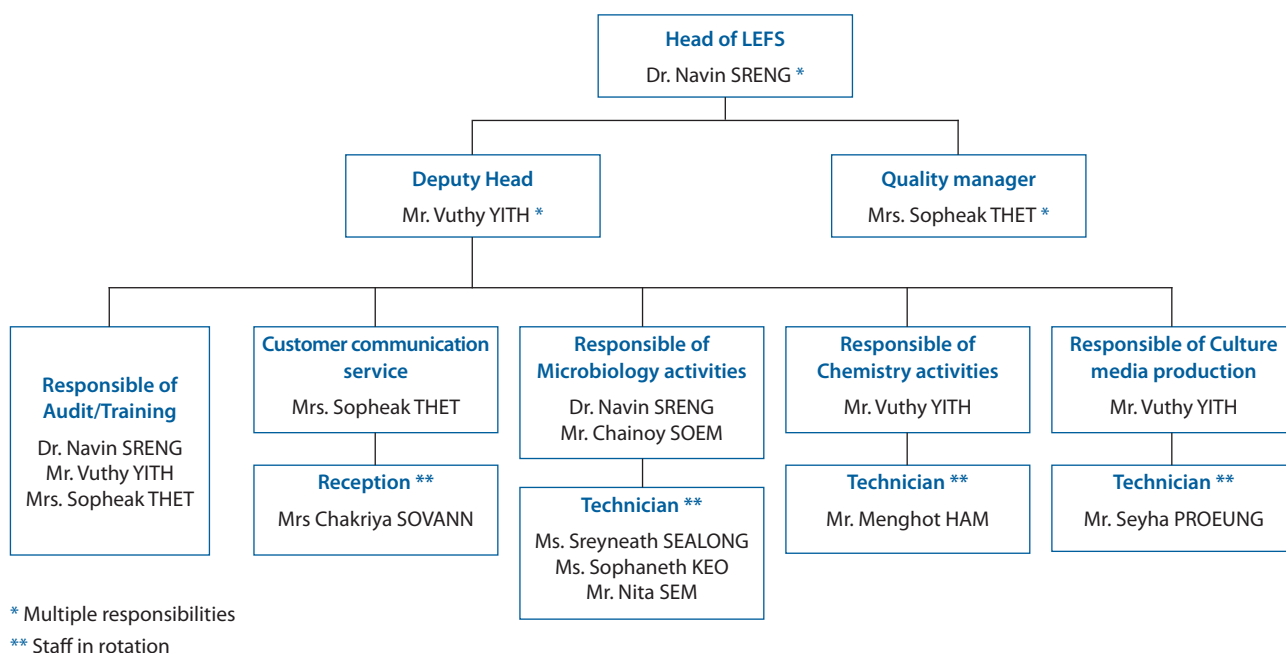


Figure 24. Laboratory for Environment and Food Safety organogram

Changes occurred to the team composition in 2020; two new technicians and one technical manager for food and water microbiology were recruited. These two technicians replaced staff members who resigned.

4.6.2 Daily Activities 2020

During 2020, the laboratory tested 7,838 samples comprising 2,521 samples of food, 3,367 water samples for microbiology testing and 1,950 water samples for chemical testing. Due to Covid-19 pandemic, we postponed all the training requests.

Compared to 2019, the number of total samples tested decreased by nine percent. The year 2020 has been a difficult one due to the Covid-19 pandemic. Entry restrictions have drastically reduced the number of tourists to Cambodia, and several hotels and restaurants have suspended operations or closed permanently. This resulted in a decrease in the number of samples at LEFS. With the number of international flights also declining, one of our largest customers suspended these requests for pesticide testing as they usually send to New Zealand for testing. The analytical activities over the last five years is shown in the table and figure below:

Analytical activity	Samples/Year				
	2016	2017	2018	2019	2020
Food microbiology testing	1,390	1,674	3,817	2,984	2,521
Water microbiology testing	1,810	2,256	2,985	3,477	3,367
Water chemical testing	1,738	1,681	1,930	2,082	1,950
Pesticide sample preparation	-	-	98	123	-
Total	4,938	5,611	8,830	8,666	7,838

Table 6. Evolution of LEFS Activities from 2016 to 2020

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

- Fifty-eight percent of food samples (1,456/2,521) were reported conformity to standards although six percent (89/1,456) were unsatisfactory;
- The unsatisfactory results were due to *Salmonella* contamination (19%), and high levels of hygiene indicators such as enterobacteriaceae (10%), total coliforms (21%) and *E.coli* (15%). More than 50% of *salmonella* positive food samples were meat products and fresh vegetables. The other products included salads, cooked food, and raw ready-to-eat foods.
- Thirteen percent of unsatisfactory food samples were co-contaminated with at least two different germs.
- Sixty-four percent of water samples for microbiology testing (2,147/3,367) for which test results were completed, 20% (440/2,147) were unfit for human consumption because of a fecal contamination.
- Twenty percent of ice cube samples (89/440) served in the restaurants and bars were found to be contaminated by fecal bacteria as coliforms, *E.coli* and intestinal enterococci.
- Fifty-eight percent of water samples undergoing chemical testing with conformity statement (1,131/1,950), 9% (98/1,131) were found to be unsatisfactory.

Quality management system

Documents	Total	In Application	In Reviewing Process
Procedures	27	5	22
Standard Operating Procedures	111	72	39
Forms	194	169	25

Table 7. Quality Management Documents in LEFS

LEFS has set itself the objective of obtaining ISO 17025 accreditation towards the end of 2021. For this, the drafting of various quality documents has been initiated, some of which have been validated and implemented in 2020 while others still have to be validated in 2021.

4.6.3 Research Programs - Major Achievements in 2020

FSPI ARCAHE: Antibiotic Resistance at the Human/Animal/Environment interface in a “One Health» Approach in Cambodia

Antibiotic resistance (ABR) is an increasing public health concern and threatens decades of infectious disease control efforts. The emergence and spread of ABR are mostly attributed to the overuse of antibiotics, whether among humans, animals, or in the environment. The development of ABR control and monitoring strategies has become a priority in low-middle income countries (LMICs), particularly in South East Asia where very high levels of ABR prevalence are reported. Cambodia is also confronting serious ABR. We observe the emergence of bacteria resistant to multiple antibiotics having a direct impact on human health. We do not know why and where ABR emerges and how it circulates between humans, animals and the environment in the country. This information is essential for the establishment of effective control. In partnership with other local public health actors in the country, IPC, the Institute of Research for

Development (IRD), CIRAD and two major hospitals in Cambodia, Calmette (Phnom Penh) and Battambang (Province of North-West), have gathered around this project to improve control of ABR using a “One Health” approach.

The **main objective** of the project is to explore the circulation of antibiotic resistant bacteria between humans, animals and the environment in Cambodia.

The **secondary objectives** are to:

1. To estimate the prevalence of antibiotic resistance in a main Phnom Penh hospital, Calmette Hospital, and in one provincial hospital, Battambang Provincial Referral Hospital, due to the elevated number of community infections.
2. To study the circulation of resistant bacteria in the environment (animals, soil, food, water) of patients with resistant bacteria from Battambang Hospital.
3. To contribute to the development of research capacities and scientific skills on ABR and the “One Health” concept.

LEFS’s responsibility in the project is part of secondary objective 2 (SO2) by working on food and environmental (soil and water) bacteria collected from the environment surrounding selected patients.

The project started in October 2020 and faced certain difficulties at its inception, which is why the first environmental samples were only collected in February 2021. Therefore, no results for the SO2 is currently available.

Collaborations	<i>Institut Pasteur du Cambodge</i> : LBM (S. Cheng and G. Delvallez), LEFS (N. Sreng), Epidemiology and Public Health (P. Piola) Institute of Research for Development (IRD) (A.-L. Bañuls) CIRAD (V. Chevalier)
Funding	Fonds de solidarité pour les projets innovants, French Ministry for Europe and Foreign Affairs (n° FSPI 2020-14)

Reducing Foodborne Pathogen Contamination of Vegetables in Cambodia: Innovative Research, Targeted Interventions, and Impactful, Cambodian-Led Engagement

Globally, diarrheal diseases are the greatest contributors to the burden of disease (BOD) in children. Cambodia has one of the highest child mortality rates in Southeast Asia, with diarrheal diseases causing 6% of childhood mortalities. Although unsafe water and poor sanitation have long been considered the prominent cause of diarrheal disease, recent estimates show that foodborne diseases heavily contribute to this disease burden (WHO, 2015; Havelaar et al., 2015). In Cambodia, the etiological agents responsible for most cases of diarrhea are often unknown, and comprehensive data regarding etiological agents for foodborne diseases are scarce. Nevertheless, the incidence of acute diarrhea in Cambodia is considered quite high across all socio-economic groups, ranging from 11% among the wealthiest and 18% among the least wealthy quintiles of the population, indicating that determinants of diarrheal disease extend beyond issues related to poverty (i.e. unsafe food).

Food safety efforts in Cambodia have focused largely on chemical contamination. Effective outreach and engagement programs are needed to increase awareness of the role of microbial pathogens in diarrheal diseases. Otherwise, it may be difficult to build incentives for programs or interventions addressing challenges not recognized by consumers and beyond. Most food consumed by Cambodians is purchased from informal markets, which are complex, fluid, and non-uniform with only loose regulation and weak sanitation. Thus, reducing foodborne disease in Cambodia will require significant focus on food obtained from these settings.

The **main objective** for this activity is to measurably reduce the prevalence of foodborne bacterial pathogen contamination of vegetables produced and sold in Cambodia. Ultimately, this will lead to reduced human exposure to foodborne bacterial pathogens via the consumption of vegetables, and reduced risk of foodborne disease. Our approach aims to bridge identified food safety gaps (described above) through the project specific goals described below:

The **secondary objectives** and sub-activities are described below:

1. Identification of Critical Control Points;
 - a. Identify two prominent bacterial pathogens associated with vegetable-borne disease(s).
 - b. Conduct a longitudinal study to map and characterize microbial pathogen contamination points, persistence, and transmission in vegetable-chains.
 - c. Use previously collected data to create a shared research agenda among all partners in terms of critical control points to be targeted for high-impact interventions.
2. Creation of Targeted Interventions ;
 - a. Identify and/or design interventions to reduce microbial contamination
 - b. Assess food safety awareness and willingness to adopt potential interventions for specific control points
 - c. Establish and strengthen food safety networks and public-private partnerships to promote adoptable interventions, identify early adopters, and help in positioning interventions.
3. Delivery of Data-Driven Engagement;
 - a. Create and deliver engagement programs to foster greater adoption of food safety interventions by farmers, collectors, vendors, and market management groups;
 - b. Deliver engagement programs that improve food safety awareness among consumers;
 - c. Measure impacts and efficacy of all engagement programs and refine outreach to produce greater reductions in foodborne disease risks associated with vegetable consumption

The project started in October 2020 and has a planned 3.5 year duration. IPC is currently in phase 1 of the project. LEFS is mainly involved in part 1 by collecting clinical data from children under the age of 15 with diarrheal diseases during the last 24 months in Phnom Penh, Battambang and Siem Reap and analyzing collected samples.

Collaborations	Kansas State University (J. Vipham), Purdue University (P. Ebner) <i>Institut Pasteur du Cambodge</i> (N. Sreng), Institute of Technology of Cambodia (C. Peng) Royal University of Agriculture (R. Chrun), World Vegetable Center (S. Ramasamy)
Funding	Feed the Future Innovation Lab for Food Safety, U.S. Agency for International Development (USAID) (n° A21-0346-S002)

4.6.4 Research Programs - Outlook for 2021

FSPI WAT-HEALTH

This project is a cooperation between IPC units (LBM, LEFS, Entomology, Epidemiology, GeoHealth-IRD), ITC, IRD, IPNC, RUA and CNRS. The main objective is to define the exposure level and to qualify the vulnerability of rural populations to health hazards related to floods in order to anticipate appropriate responses to protection issues. To do this, interdisciplinary approaches are used to combine different themes such hydrological dynamics, land use, waterborne bacteria and vector ecology, public health, agricultural practices and health risk models. *Leptospira* and *Bukhoderia pseudomallei* are the main waterborne bacteria detected in samples (water, soil, and vegetables) collected monthly over 18 months. Molecular approaches such qPCR and NGS will be performed to identify bacterial species and inter-species genetic diversity.

Collaborations	<i>Institut Pasteur du Cambodge</i> : LBM (S. Cheng and G. Delvallez), LEFS (N. Sreng), Entomology Unit (S.Boyer), Institute of Research for Development (IRD) (A.-L. Bañuls, M. Hidé, V. Herbreteau, S. Mas-suel and JP Venot)
Funding	<i>Fonds de solidarité pour les projets innovants</i> , French Ministry for Europe and Foreign Affairs (n° Pending)

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 as well as some private sector laboratories.

In 2020, as part of a national monitoring program, the Ministry of Health sent IPC 773 samples through sampling campaigns of industrial foods imported from different countries.

4.6.6 Teaching and Training

The laboratory supervised 8 trainees coming from different universities in Cambodia and in France for internships lasting between one and six months. The details of these are described below.

University	Number of students	Scholar Year	Period (month)	Date
University of Health Sciences (UHS)	5	Year 3	1.5	06/01/2020 - 25/02/2020 10/09/2020 - 17/10/2020 19/10/2020 - 28/11/2020
Royal University of Agriculture	2	Year 3	1.5	01/02/2020 - 15/03/2020
University of Toulouse, France	1	Master 2	6	09/03/2020 - 28/08/2020

Table 8. Internship Students at LEFS in 2020

4.6.7 Outlook for Upcoming 3 - 5 Years

The outlook of the LEFS for the upcoming three to five years comprise the following:

- Maintaining and improving the quality of service;
- Increasing laboratory visibility/recognition from the public;
- Extension of accredited parameters for food and water microbiological testing;
- Set up international standard methods for chemical analysis in water by using AAS/ICP-OES/MP-AES;
- Set up pesticide, heavy metal and antibiotic residue analysis on site;
- Extension of lab to Kampong Cham Province;
- Develop increasing cooperation with internal and external partners for research projects.

4.7 Medical Biology Laboratory

4.7.1 Functional Structure

IPC's Medical Biology Laboratory (LBM) provides a platform for comprehensive medical and biological analyses to both Cambodian and international clients. The lab aims supporting health professionals by offering a variety of *in vitro* diagnostic tests covering all medical areas. Currently, a panel of approximately 150 tests is offered to public and private hospitals, non-governmental organizations, and walk-in patients. In December 2018, the Medical Biology Laboratory of the *Institut Pasteur du Cambodge* became the first laboratory in Cambodia awarded ISO 15189 accreditation. In 2020, the laboratory renewed its accreditation for the second time following an external audit in November by COFRAC. Of 132,217 analyses performed in 2020, 101,705 out of are part of the scope of accreditation, which represents 77% of the laboratory activity.

LBM also hosts a bacteriology research laboratory with a dedicated team of researchers. Research projects largely focus on the diagnosis, surveillance, and molecular epidemiology of several pathogenic bacteria of particular public health importance and has an emphasis on antibiotic resistance.

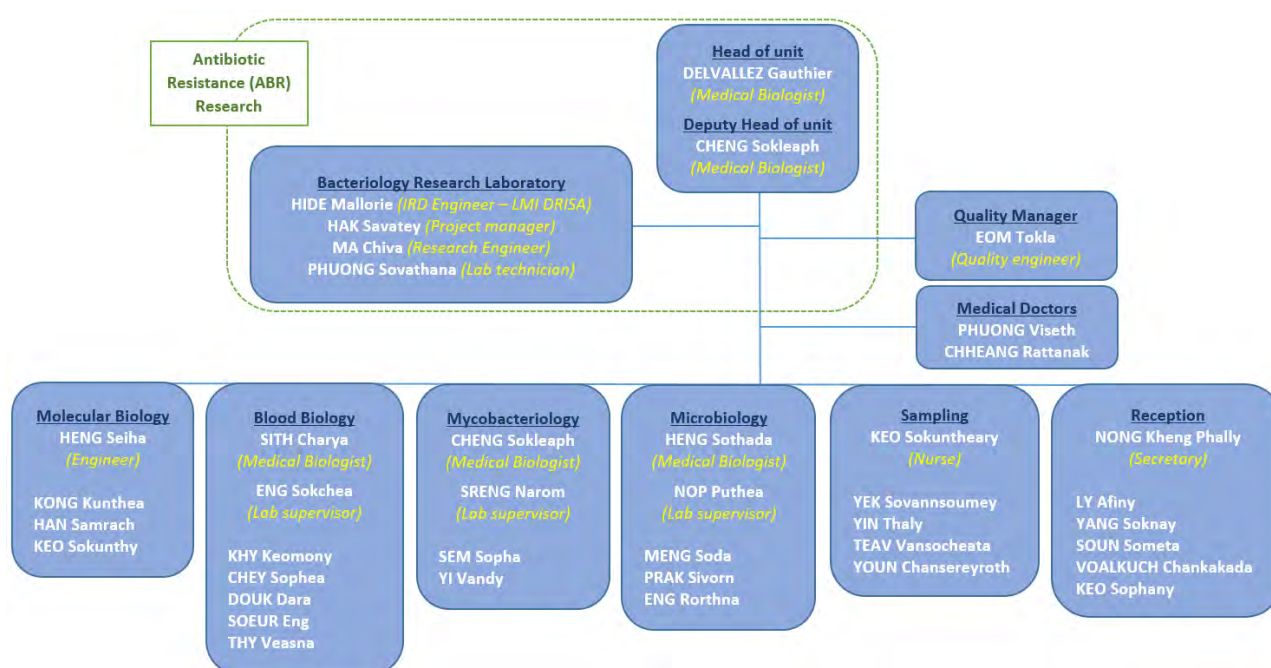


Figure 25. Medical Biology Laboratory organogram (31/12/2020)

Thirty-nine employees work in the LBM, including four employees fully dedicated to bacteriology research. The laboratory is composed of four medical biologists (including the head of unit and the deputy head), one quality manager, two engineers (one molecular biology engineer and 1 research engineer), 3 Lab supervisors, 13 technicians for routine activities, 1 project manager, 1 technician for research, 6 secretaries, 5 nurses and 2 medical doctors. 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”) (Figure 25).

4.7.2 Daily Activities

In 2020, LBM's activity decreased by 3.1% compared to 2019; the lab performed 132,217 analyses (Figure 26). The blood biology laboratory represents the lion's share of our activity (almost 90%) with analysis in hematology, biochemistry and immuno-serology. The other laboratories have a lower activity levels but offer a more specialized panel of analyses. Activity in 2020 was affected by the various events related to COVID-19 with an important decrease of patients between March and May after the first identification of COVID-19 in Cambodia and the implementation of travel restrictions and non-pharmaceutical interventions (NPIs). A second important decrease was observed at the end of 2020 following the two COVID-19 clusters identified in November (Figure 27).

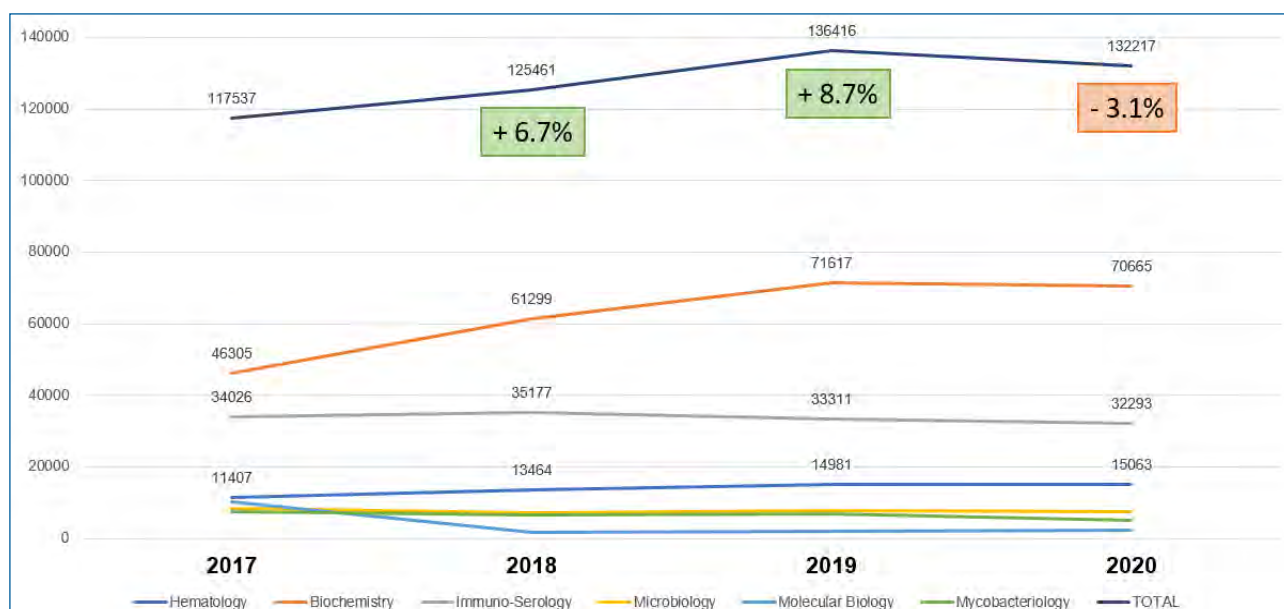


Figure 26. Number of analyses per year

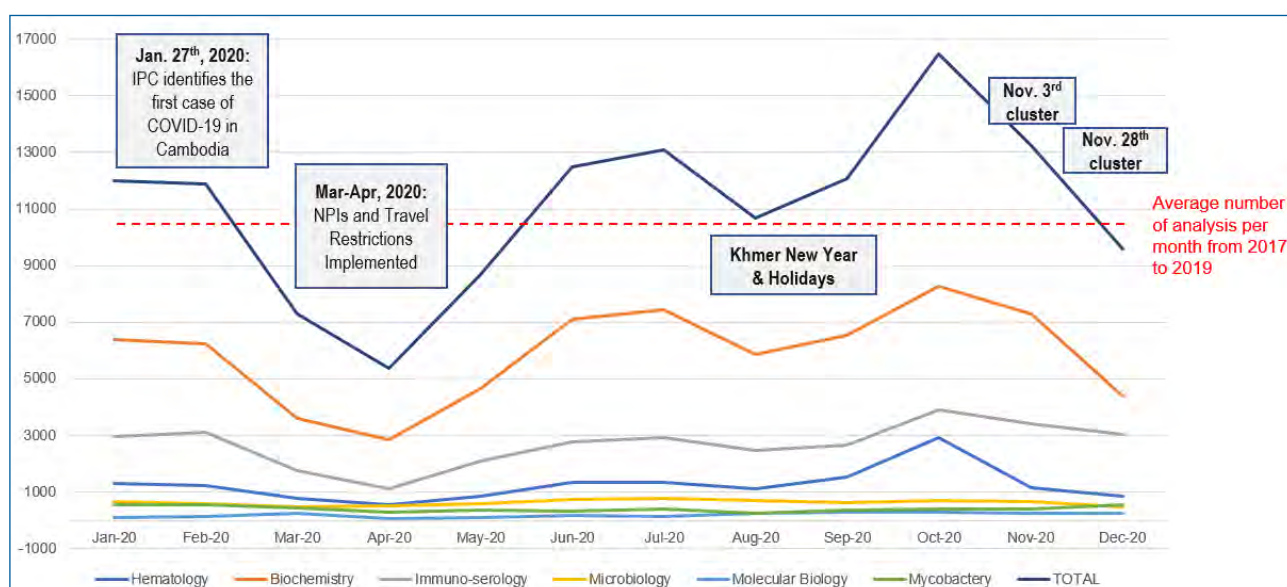


Figure 27. Number of analyses per month in 2020

The LBM was equipped with a MALDI-TOF MS in early 2020 and with a Cobas PCR Machine (ROCHE) in June. This allowed us to improve our work in microbiology and to be able to offer several new analyses such as COVID-19 PCR, High-risk Human Papillomavirus (HPV) PCR and *Chlamydia trachomatis* / *Neisseria gonorrhoeae* PCR. Other new analyses have been added to our catalogue in 2020 such as Flu rapid test (Flu A + B), StreptoTest for rapid detection of *Streptococcus A* and COVID-19 serology.

4.7.2.1 Tuberculosis

Mycobacteriology laboratory activities represented 3.8% of LBM activities in 2020. The total number of analyses was 5,185 in 2020, which represents a drop of 25.5% compared to the number of analyses in 2019 (6964). This decrease is due to the interruption of international travels in 2020, including a decrease in the activities of the International Office for Migration (IOM) NGO which is one of our major clients.

In 2020, 834 Xpert MTB/RIF Ultra tests were performed, of which 32.9% were MTB positive and 1.1% of positive samples were resistant to Rifampicin. Concerning resistance to second-line anti-TB drugs, the evolution of the resistance rate is presented in Figure 4.

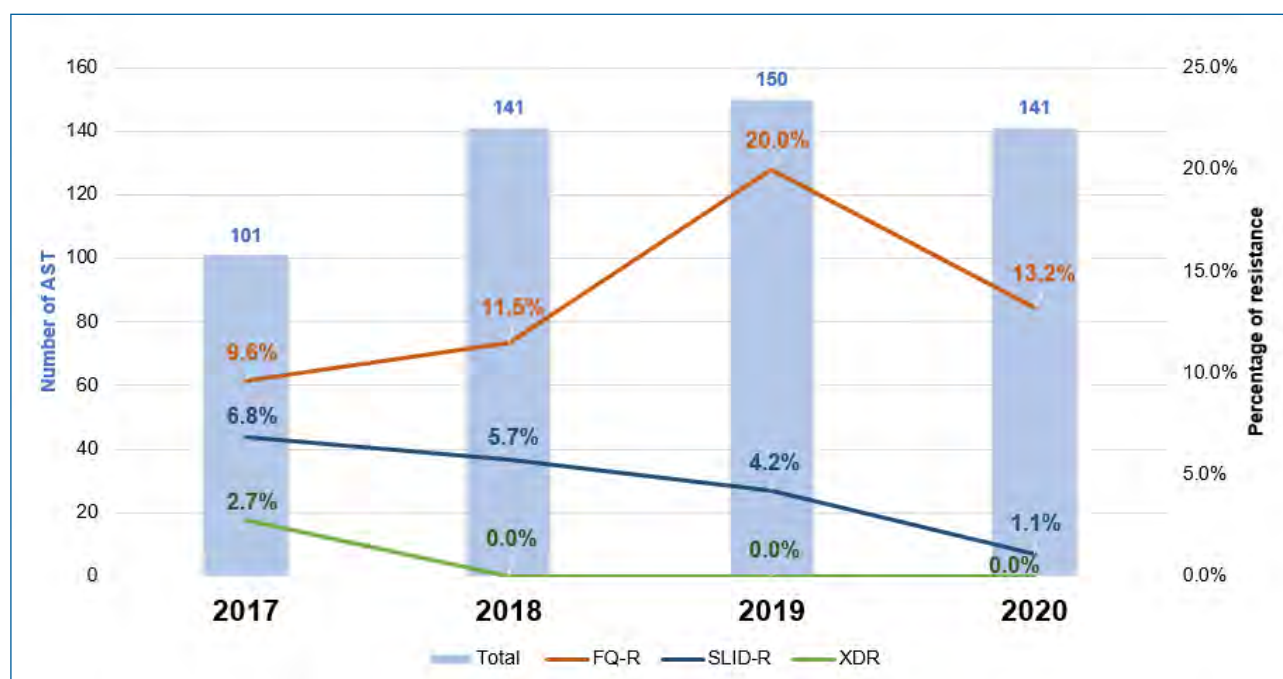


Figure 28. Evolution of resistance to second-line anti-TB drugs

4.7.2.2 Bacteriology

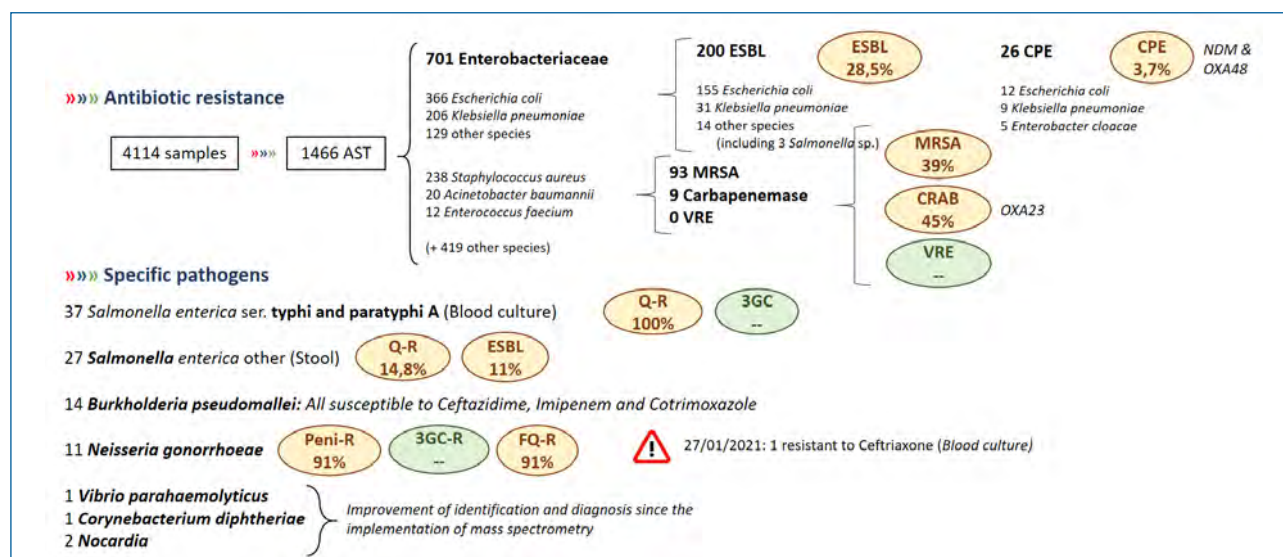


Figure 29. Bacteriological data in 2020

The main data of interest in bacteriology are presented in Figure 5. Concerning antibiotic resistance, the microbiology laboratory has performed 4,114 bacterial cultures in 2020 and 1,466 antibiotic susceptibility tests. The proportion of ESBL, CPE, MRSA and CRAB were 28.5%, 3.7%, 39% and 45%, respectively. Several specific pathogens were isolated including *Salmonella typhi* and paratyphi A, *Salmonella enterica* sp., *Burkholderia pseudomallei*, *Neisseria gonorrhoeae*, *Vibrio parahaemolyticus*, *Corynebacterium diphtheriae* and *Nocardia* sp.

Anonymous Free Testing Center (AFTC)

During 2020, 388 patients consulted the AFTC and benefited from free HIV serology. The HIV-positive population represents 17.3 % of the total number of AFTC consultants. We observed a high increase of HIV positive cases since 2018, which correlates with a significant increase of male sex workers in our patients' population (Figure 30).

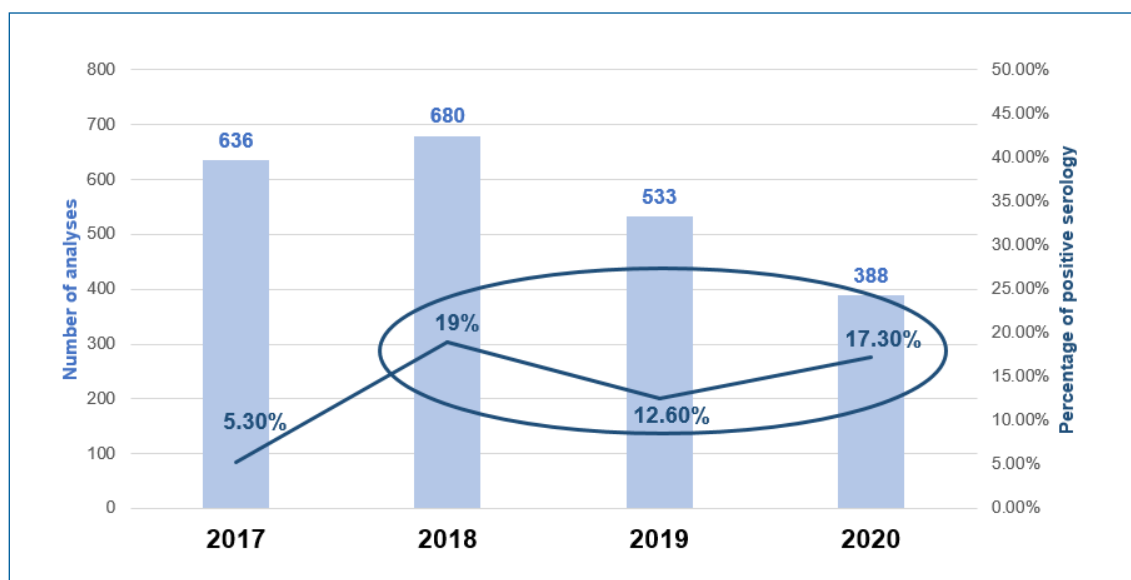


Figure 30. HIV serology at AFTC

HIV Nominative Serology

In 2020, the rate of seropositivity of nominative patients stayed stable with a rate at 3.7%. Prevalence may be over-estimated compared to the general population as many patients come to IPC to confirm HIV positive status from other laboratories. It is clear that the COVID-19 epidemic had a large negative impact on the number of HIV serology requests (Figure 31).

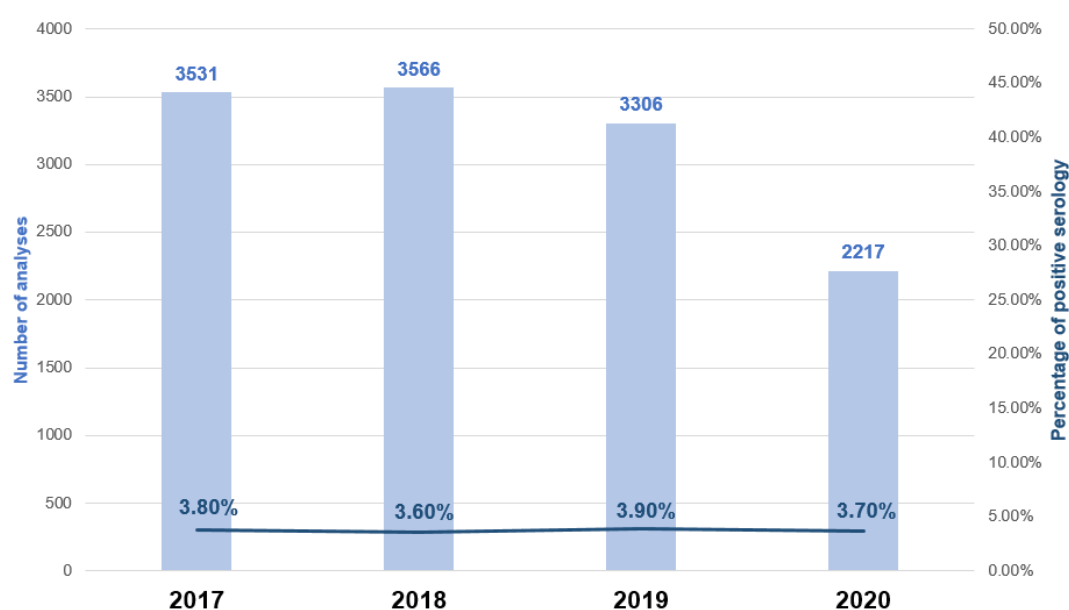


Figure 31. HIV serology at LBM

4.7.3 Research Programs – Major Achievements in 2020

International Joint Laboratory «Drug Resistance in Southeast Asia» (LMI DRISA)

Through the collaboration with the *Institut de Recherche pour le Développement* (IRD), since August 2018, we have hosted an engineer, Dr. Mallorie Hide, specialized in molecular biology. Her term at LBM is 4 years. She works on molecular epidemiology and drug resistance of *Mycobacterium tuberculosis*, *enterobacteriaceae* and *Burkholderia pseudomallei*. Dr. Hide has set up an analytic platform (“Laboratoire de recherche en Bactériologie” – LRB) dedicated to research in bacteriology thanks to the financial support provided by IPC and LMI DRISA.

4.7.3.1 Genetic diversity of M.tb in TB/HIV coinfecting patients in Cambodia with a focus on susceptibility to anti-TB drugs

The genetic diversity of *Mycobacterium tuberculosis* and susceptibility to anti-tuberculosis drugs in TB/HIV co-infected patients in Cambodia has been explored on a sample of 523 TB/HIV patient isolates. These samples came from five Cambodian hospitals and were studied using antibiotic susceptibility testing (AST), mycobacterial interspersed repetitive units - variable number tandem repeats (MIRU-VNTR) typing, Spoligotyping and single nucleotide polymorphism (SNP).

According to AST results, around 22% of the isolates were resistant to at least one antibiotic and among them, around 7% were multidrug resistant (MDR). MIRU-VNTR and spoligotyping revealed that isolates mainly pertained to the EAI (66, 19%) and Beijing (22, 06%) families and resistant isolates preferentially pertained to the Beijing family (both resistance to Streptomycin and PolyDrugResistance are statistically associated with the Beijing family). Unfortunately, SNPs cannot be directly related to resistance status and most of them are specific of either Beijing or EAI families, Manuscript is currently in preparation for publication.

Collaborations	Team Leader: M. Hide (IRD). MIVEGEC Unit, IRD, LMI DRISA (A.-L. Bañuls, S. Godreuil)
Funding	LMI DRISA, CAMELIA (ANRS 12278)

4.7.3.2 Epidemiology of urinary tract infections (UTI) and antibiotic resistance in pediatric Hospitals in Cambodia: emergence of Carbapenemase Producing *Enterobacteriaceae*

One of the studies initiated in 2020 concerns the antibiotic resistance in urinary tract infections, which are one of the most common bacterial infections in children. The aim of this study was to explore antibioresistance of the two most prevalent bacteria causing UTI, *Escherichia coli* and *Klebsiella pneumoniae*. For this purpose, a prospective study was conducted in June and July 2020 in two pediatric hospitals (Kantha Bopha Hospital, Phnom Penh) and Jayavarman VII Hospital, Siem Reap): 131 UTI samples were fully characterized using antibiotic susceptibility testing (AST), MALDI-TOF identification and PCR detection.

Our results reveal an important proportion of resistant isolates; around 78% of the isolates were ESBLPE, 36% were AmpC positive isolates and 23% were CPE. In the present study, we detected for the first time CPE in *E. coli* and *K. pneumoniae* isolated from pediatric UTIs. The emerging of CPE (with both *bla*_{NDM} and *bla*_{OXA48} involved) in Cambodia suggests an alarming spread of both ESBL-PE and CPE in the country. Identification of ESBL encoding genes (*bla*_{CTXM}, *bla*_{TEM}, *bla*_{SHV}, *bla*_{OXA1}) is in progress and bacterial typing and manuscript preparation are both forecasted in 2021.

Collaborations	Team Leaders: G. Delvallez (LBM) / M. Hide (IRD). MIVEGEC Unit, IRD, LMI DRISA (A.-L. Bañuls)
Funding	LMI DRISA

4.7.3.3 Colistin resistance in multidrug-resistant *Enterobacteriaceae* in Cambodia

The second study started in 2020 concerns Colistin resistance in multidrug-resistant *Enterobacteriaceae* in Cambodia. Colistin is considered a last-line antimicrobial for the treatment of infections caused by multidrug-resistant *Enterobacteriaceae* but the emergence of colistin resistant strains worldwide compromised its use. Colistin belongs to the antimicrobial class designated polymyxins which originate from the organism *Paenibacillus polymyxa*. In Cambodia, Colistin is one of the most commonly used antibiotics in pig and poultry farms but the circulation of colistin resistant strains in the human population is still poorly understood. In this context, we aim on exploring colistin resistance

among both carbapenemase- and extended-spectrum Beta-Lactamase (ESBL) -producing *Escherichia coli* (EC) and *Klebsiella pneumoniae* (KP) isolated from humans and cryoconserved at LBM.

Firstly, the minimum inhibitory concentration (MIC) of Colistin was estimated using broth microdilution for 269 resistant isolates of both EC and KP from LBM routine activity and identification of molecular determinants for Colistin-resistant bacteria (*mcr-like* genes, chromosomal mutations) is currently in progress. Concerning AST in the ESBL samples, around 7% of EC and 38% of KP had an MIC superior to 2µg/mL for colistin, whereas in the CPE, colistin-resistance represents less than 1% for EC and 11% for KP. In total, 20 isolates showed a colistin-resistant phenotype, mainly associated with the ESBL phenotype and the KP species. We have tried to identify the molecular determinant responsible for colistin-resistance among these isolates. The presence of plasmid encoded resistance genes was examined by PCR for the ten *mcr* variants and nucleotide mutations associated to the TCS are currently investigated by sequencing six different genes (*pmrA*, *pmrB*, *phoP*, *phoQ*, *mgrB*, *crrB*). Our preliminary results allowed identifying mutations for nine among ten EC resistant strains with the presence of *mcr-1*, *mcr-3* and *mcr-8* variants and several potential *mgrB* deletion/insertion. Concerning *mcr-8*, it has been first detected in China in 2018 in both poultry and fecal carriage and to our knowledge, this is the first description of *mcr-8* and TCS modifications in EC/KP from patients in Cambodia. In conclusion, our preliminary results revealed the existence of colistin resistance among multidrug-resistant bacteria in Cambodia. This resistance is associated to several *mcr* variants or chromosomal mutations suggesting multiple emergence phenomena in Cambodia. Molecular identification has to be finalized and EC/KP genotypes should be determined before publication.

Collaborations	Team Leader: M. Hide (IRD). MIVEGEC Unit, IRD, LMI DRISA (A.-L. Bañuls, S. Godreuil)
Funding	LMI DRISA

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

Last year, we also started working on the molecular epidemiology of *Burkholderia pseudomallei* (BP). BP is an environmental gram-negative bacterium causing a fatal human disease, known as melioidosis. Melioidosis is a disease of public health importance in Southeast Asia and Northern Australia. BP is transmitted to humans by contact with contaminated soil and surface water (percutaneous inoculation, inhalation, or ingestion) and most of cases occur during rainy and humid months. Melioidosis is endemic in Cambodia and the majority of cases are children (>60%) with head and neck infections whereas adults mostly present pneumonia and/or sepsis with known risk factors including diabetes mellitus, renal diseases, chronic lung disease, thalassemia, excessive alcohol consumption, male sex and occupational exposure. In Cambodia, culture-confirmed cases have been reported in 23 out of the 25 provinces but little is known about BP diversity in Cambodia. In this context, we aim to explore the genetic diversity of BP. For this purpose, 127 cryoconserved strains isolated from different hospitals in Phnom-Penh between 2016 and 2020 and previously identified as BP by API2One at LBM were studied using MaldiToF and Multilocus Sequence Typing (MLST). Identification with Maldi-ToF and blast on both the Bruker and Microbnet database allowed us to identify 104 isolates as BP species and 14 BP strains have been typed with MLST. 7 genes (*ace-gltB-gmhD-lepA-lipA-narK-ndh*) have been sequenced for these 14 strains and alignment with reference sequences confirmed that they clustered with BP. This small sample showed a high genetic diversity and when compared with sequence types available on Pubmlst for Cambodia, six new Sequence types were identified for Cambodia (manuscript in preparation).

Given these preliminary results and in order to better understand the epidemiology of melioidosis in Cambodia, we will continue with these investigations by initiating two new studies in 2021. The first one concerns an exhaustive sample of 122 isolates from pediatric melioidosis collected between June to November 2020 in Kantha Bopha Hospitals which will be explored using both a genetic approach and spatial and temporal analysis. The second study is an EcoHealth project entitled "Water and Health Risks in Cambodia – Wat-Health" (see 4.7.4.3).

Collaborations	Team Leader: M. Hide (IRD). MIVEGEC Unit, IRD, LMI DRISA (A.-L. Bañuls, S. Godreuil)
Funding	LMI DRISA; internal project (<i>Institut Pasteur du Cambodge</i>)

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

In this study, we aim to understand the emergence, spread and evolution of antibiotic resistance in *Mycobacterium tuberculosis* (M.tb) in Cambodia. To archive this objective, 404 isolates from MDR-TB presumptive patients between 2012 and 2017 were included in the study and analyzed by spoligotyping, 24-MIRU-VNTR, sequencing of drug resistance genes and drug susceptibility testing for first (FLD) and second line (SLD) drugs. Of 404 included isolates, 278 (68.8%) were resistant to at least one FLD and 126 (31.2%) were susceptible to all four FLDs tested. The result are divided into three parts. First, this work describes the genetic diversity of clinical M.tb isolates under study and their link with FLD resistance patterns. The two predominant families, Beijing and EAI, represent almost 90% of the sample. The analysis showed a significant association between the Beijing family and phenotypic drug resistance, in particular with MDR and quadruple resistance. The Beijing family also significantly associated with clustering, suggestive of the existence of recent transmissions. Secondly, while focusing on resistance to SLD, the data suggest that the proportion of multidrug resistance (XDR) and pre-XDR isolates remains low but is on the rise compared to previous reports. A high proportion of pre-XDR (75%) and XDR (100%) isolates also carried mutations associated with resistance to pyrazinamide (PZA). The Beijing family was predominant among these highly resistant isolates. One Beijing isolate, named XDR+ in this study, was resistant to all anti-TB drugs tested (FLD and SLD). A cluster comprising 2 pre-XDR and XDR isolates was observed, suggesting recent transmission of these strains. Finally, the genetic and phylogenetic analysis demonstrated a diversity of drug resistant patterns from mono-resistance to XDR and an important diversity of mutation patterns in each M.tb family and each cluster. These results suggest various evolutionary trajectories towards resistance to FLD and SLD. The Beijing family was also associated with MDR and XDR and low fitness cost mutations in resistant genes. The existence of compensatory mutations in some resistant isolates highlights that the M.tb population is evolving towards increasingly resistant strains with a high transmission potential. The data from this project produced three publications; one was accepted for publication in the Journal of Infection and Resistance in 2020 and two are in preparation for submission.

Collaborations	Team Leader: S. Cheng (LBM). MIVEGEC Unit, IRD, LMI DRISA (A.-L. Bañuls), National Center for Tuberculosis and Leprosy Control (S. H. Pheng)
Funding	LMI DRISA, French Embassy in Cambodia, Ministry of Education Youth and Sport, NIHE (PHC Lotus Project)

4.7.3.6 Antibiotic resistance at the human/animal/environment interface in a “One-Health” approach in Cambodia: ARCAHE

Emergence and spread of antibioresistance (ABR) are mostly attributed to the overuse of antibiotics, whether to humans, animals, or in the environment. The main objective of the project is to explore the circulation of antibiotic resistant bacteria at the interface between humans, animals and the environment in Cambodia. To achieve the objectives, the study has been subdivided in 3 main work packages (WP). WP1- Hospital will describe and compare the landscape of antibioresistance in patients from two hospitals in Cambodia: Battambang Provincial Referral Hospital (North-West Province) and Calmette Hospital (Phnom Penh). WP2- Patient environment aims to compare the resistant bacteria isolated from the patients of the Battambang Hospital and the resistant bacteria isolated from their environment (animals, food, soil, water and rats). Using next-generation sequencing approaches, WP3-Genetic identity card aims to study the genetic characterization of resistant bacteria isolated from patients of the two hospitals (WP1) and from patients' environment (WP2) and to get genetic information on the bacterial population diversity and antibiotic resistant genes circulating in the patient's environment. A final global data analysis will allow IPC to assess drug resistant bacteria genetic diversity and to identify potential cross-contaminations between humans, animals and their environment, to define any flow of resistance genes between environment (animals, water, soil, food) and humans. The project will also contribute to the development of research capacities and scientific skills on ABR and “One Health” concept. In 2020, we finished planning and implementation of the project with a pilot study started in October 2020.

Collaborations	Team Leader: S. Cheng (LBM). MIVEGEC Unit, IRD (A.-L. Bañuls, M. Hide, V. Herbreteau) Epidemiology and Public Health Unit (P. Piola), Calmette Hospital (S. Bory), Battambang provincial Referral Hospital (S. Chiek), CIRAD (V. Chevalier), Laboratory of Environment and Food Safety (N. Sreng), General Directorate of Animal Health and Production (GDAPH), MAFF (S. San)
Funding	Fonds de solidarité pour les projets innovants (n° FSPI 2020-14) French Embassy in Cambodia, French Ministry for Europe and Foreign Affairs

4.7.3.6 Aviesan SUD on antimicrobial resistance

The objective of this activity is to establish a research network in partnership with low and middle income countries (LMIC) scientists to strengthen local research expertise and develop research projects related to antimicrobial resistance (AMR). Countries involved are Cambodia, Madagascar, Côte D'Ivoire, Burkina Faso and France. Following the visit of the Aviesan south delegation to each country member in late 2019, a first workshop was conducted at INSERM in Paris in February 2020 from which two complementary multicenter and interdisciplinary projects (CircUs and RAM-SES) were developed and submitted for funding from PPR *Antibiorésistance*.

Collaborations	Team Leader: S. Cheng (LBM).
Funding	Aviesan SUD (pending)

4.7.4 Research Programs – Outlook for 2021

4.7.4.1 New AntiMicrobial Peptides (AMP)

The general objective of the study is to evaluate the antibacterial activity of a new set of eight synthetic AMPs against clinical isolates of relevant bacteria identified by global antimicrobial resistance surveillance system (GLASS) and mycobacteria. The antibacterial activity of AMP against relevant bacteria involved in human infections in Cambodia (*Mycobacterium tuberculosis*, *Acinetobacter* spp., *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus*) with different resistance statuses (ESBL, CPE, MRSA, Colistin and Fluoroquinolones resistance...) will be evaluated (experiments started in March 2021).

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)
- To measure the susceptibility of relevant resistant bacteria identified by GLASS against the eight new AMPs by comparing with sensitive bacterial strains

Collaborations	Team Leader: M. Hide, MIVEGEC Unit, IRD, LMI DRISA (A.-L. Bañuls)
Funding	LMI DRISA

4.7.4.2 Pediatric melioidosis in Cambodia

In order to figure out the epidemiology of melioidosis in Cambodia, we will study an exhaustive sample of 122 isolates from pediatric melioidosis collected from June to November 2020 in Kantha Bopha Hospitals using both a genetic approach and spatial and temporal analysis. *Burkholderia* species identification will be performed using Maldi-Tof and molecular typing and the genome of *B. pseudomallei* isolates will be sequenced by NGS.

Collaborations	Team Leader: M. Hide, MIVEGEC Unit, IRD, LMI DRISA (A.-L. Bañuls, S. Godreuil), GeoHealth Group-IRD (V. Herbreteau), Kantha Bopha Children's Hospitals (D. Laurent) Doherty Institute (T. Stinear)
Funding	LMI DRISA

4.7.4.3 FSPI Wat-Health 2021-2022

This Wat-Health project comprises interdisciplinary approaches around multiple interrelated themes, namely hydrological dynamics, land use, waterborne bacteria and vector ecology, public health, agricultural practices and health risk modeling. The main objective is to define the exposure and to qualify the vulnerability of rural populations to health hazards related to floods, in order to anticipate appropriate responses to protection issues. The "waterborne bacteria" work package will explore 1) human exposure, 2) genetic diversity and 3) spatial & temporal distribution of waterborne bacteria in Cambodia. Relevant waterborne bacteria, especially *Leptospira* and *Burkholderia pseudomallei*,

will be sampled every month over eighteen months in twenty-five environmental sites (water, soil, vegetables) in the Koh Thum region. Molecular approaches (qPCR, NGS) will allow identifying bacterial species and intraspecies genetic diversity and human exposure will be assessed through questionnaires and serological surveys.

Collaborations	Team Leader: M. Hide, MIVEGEC Unit, IRD, LMI DRISA (A.-L. Bañuls, S. Godreuil), IRD (French PI: S. Massuel), Entomology Unit (S. Boyer), Epidemiology Unit, LEFS (N. Sreng), GeoHealth Group-IRD (V. Herbreteau, ITC (Cambodian PI), <i>Institut Pasteur de Nouvelle Calédonie</i> (C. Goarant), RUA (JP Venot, IRD)
Funding	Fonds de solidarité pour les projets innovants (600k€), French Ministry for Europe and Foreign Affairs

4.7.4.4 *B. pertussis* genotyping from nasopharyngeal samples collected in Cambodia (PERILIC WP1)

The objective of this ancillary study 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.

Collaborations	Team Leader: M. Hide (IRD). <i>Institut Pasteur</i> (G. Noel), Clinical Research Group (L. Borand)
Funding	<i>Institut Pasteur</i>

4.7.4.5 A multicenter serosurvey on diphtheria immunity in children in low and middle-income countries (PERILIC WP2)

The objective of this ancillary study is to assess the level of anti-diphtheria toxin IgG in bio-banked serum samples obtained from children aged 3 to 15 having been primo-vaccinated (3 injections in the first year of life). Samples from children aged 3-4, 5-6, 7-8 years will first be tested and, based on preliminary results, additional samples collected from older children may be analysed. This will allow the calculation of the period leading the vaccine-induced immunity waning. This period will be determined by the time interval between the last vaccination and the youngest age of the children's group showing significant decrease in proportion of fully protected individuals.

Collaborations	Team Leader: G. Delvallez, (LBM). <i>Institut Pasteur</i> (G.Noel), Clinical Research Group (L. Borand)
Funding	<i>Institut Pasteur</i>

4.7.4.6 Prevalence of HPV among men who have sex with men (MSM) and transgender women (TGW) in Phnom Penh, Cambodia

Despite numerous publications outlining the increasing prevalence of rectal HPV in both western and Asian countries, screening for HPV is rarely performed in Cambodia when indicated largely due to cost and availability of testing. The primary objective of this study is to estimate the prevalence of Human Papilloma Virus among MSM and TGW in Phnom Penh, but also to describe sexual behavioural risks and the prevalence of HIV and syphilis in the participating population. Hence, this prevalence study is crucial for identifying the burden of HPV in both MSM and TGW populations in Phnom Penh, to assess the need for health services, and to inform policy making in Phnom Penh regarding HPV vaccination.

Collaborations	Team Leader: G. Delvallez (LBM), FHI360 (S. Wignall)
Funding	ROCHE Thailand (480 free PCR HPV Test), USAID, UNAIDS

4.7.4.7 Establishing microbial AMR genomics in Phnom Penh through genomic snapshot surveys of Multi-drug-Resistant Gram-Negative bacteremia

Prevention and control of AMR in Cambodia requires timely, effective, and high-resolution investigations which can be provided by genomic technologies. Microbial genomics has the potential to revolutionize the diagnosis, surveillance and control of AMR. This is why, in this research project, we aim to combine rapid advances in genomics with public health epidemiology and best practice bioinformatics to set up a new paradigm for AMR control and response in Phnom Penh. As one of the first stepping stones to achieving such an ambitious goal, here we plan to organize a series of snapshot surveys of a number of key multidrug resistant (MDR) Gram-negative pathogens causing bloodstream infections, in collaboration with partnering hospitals. We believe this would create a synergy between isolate supplying partnering hospitals and IPC which would define the genomic characteristics and population structure of pathogen specific outbreaks in return. Over the course of outbreaks of interest, transmission networks could be modeled for each species using integrated clinical and genomic epidemiological data.

Collaborations	Team Leader: K. Vandelannoote (LBM) <i>Institut Pasteur</i> (P. Glaser), Doherty Institute (T. Stinear)
Funding	<i>Institut Pasteur du Cambodge, Institut Pasteur, Doherty Institute</i>

4.7.4.8 [Sharing Research on AMR Network \(SHARENET\)](#)

AVIESAN has recently initiated the development of a collaborative research network on AMR with LMICs, called AVIESAN AMR SUD. The network involves scientists from Cambodia, Madagascar, Ivory Coast and Burkina Faso, together with scientists from French institutions. This SHARENET aims to expand the network to integrate experts from Laos, Mauritius, Senegal and Burkina Faso, together with scientists from Belgium, Italy and the Netherlands in order to reinforce the geographical distribution of the network (South-East Asia, Indian Ocean and Africa), strengthen its coherence, and enrich the collaboration. This extended network will bring together European scientists with diverse and complementary expertise in research on the burden, transmission, and risk factors of AMR, and human, animal and environment health scientist from LMICs implementing national actions plans for the fight against AMR.

Collaborations	Team Leader: S. Cheng (LBM) AVIESAN AMR SUD network
Funding	JPIAMR Network Plus (pending)

4.7.4.9 [Applied epidemiological insights from a tuberculosis outbreak in a captive bear population](#)

This project will investigate an outbreak of presumed human-origin tuberculosis in bears at a rescue center in Cambodia. This outbreak occurred over more than ten years, with 31 bear cases detected in a number of different locations within the rescue center. In 2011 a staff member at the rescue center developed tuberculosis, underwent treatment and made a full recovery. Preliminary genotyping already conducted has shown that the bear cases were caused by two genetically distinct infections of *M. tuberculosis* and the genotype of the human infection is identical to one of these infections, strongly suggesting involvement of human case in the transmission chain. This project will enrich and build on this preliminary molecular epidemiological work by utilizing the higher resolution and discriminatory power of WGS. Combining this information with epidemiological data will generate hypotheses regarding the index cases for each infection, as well as the timing and direction of transmission between cases. This is very important information for the prevention of future outbreaks, as well as having broader public health and conservation impacts.

Collaborations	Team Leader: S. Cheng (LBM) Free the Bears (K. Officer), Murdoch University (B. Jackson)
Funding	Murdoch University

4.7.5 Support to National Authorities

- With NCHADS: follow-up of HIV seropositivity
- Member of CENAT's TWG – Laboratory: Development of technical procedures guideline
- Member of CENAT's TWG on multi-drug resistant tuberculosis
- Technical working group on AMR with MOH
- Technical support on Quality with MOH
- COVID-19 sampling and PCR testing for Diplomatic and Official Visa after quarantine
- COVID-19 serology for contact tracing

4.7.6 Teaching and Training

Continuous training for staff

- Integrated Management System (IMS) training
- Management and Leadership Skills
- Cobas 4800 Roche / PCR COVID-19, Papillomavirus, CT/NG
- Online training ISO 15189
- Occupational Health and Safety
- E-learning 17025v2017

Internships

One of IPC's main missions is to contribute to teaching and training activities. The Medical Biology Laboratory has been proactive in the training of laboratory technicians and pharmacist from partner institutions in the fields of medical biology including hematology, immuno-serology, biochemistry, microbiology and molecular biology. In 2020, LBM received sixteen students for internship:

- Pharmacists (UHS): 5
- Laboratory Technicians (UHS & UP): 9
- Technicians from Calmette Hospital (Semen analysis): 2
- UHS Master Infectiology: 1

Thesis supervision

Ms Sokleaph Cheng completed her PhD in Health Biology, University of Montpellier (defense date: December 30th, 2020).

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

Master's courses at UHS

- Resistance mechanisms to anti-infectious agents: Antibiotics and Antibioresistance (G. DELVALLEZ)

4.7.7 Publication List 2020

1. **Evaluation of Loopamp Assay for the Diagnosis of Pulmonary Tuberculosis in Cambodia.** Cheng S, Pheng SH, Heng S, Marks GB, Bañuls A-L, Mao TE, Kerléguer A. BioMed Research International. 2020; 2020:6828043. doi: 10.1155/2020/6828043. PMID: 32596349.
2. ***Klebsiella pneumoniae* carriage in low-income countries: antimicrobial resistance, genomic diversity and risk factors.** Huynh BT, Passet V, Rakotondrasoa A, Diallo T, Kerléguer A, Hennart M, Lauzanne A, Herindrainy P, Seck A, Bercion R, Borand L, Pardos de la Gandara M, Delarocque-Astagneau E, Guillemot D, Vray M, Garin B, Collard JM, Rodrigues C, Brisse S. Gut microbes. 2020; 11(5):1287-99. doi: 10.1080/19490976.2020.1748257. PMID: 32404021.
3. **Human Outbreak of Trichinellosis Caused by *Trichinella papuae* Nematodes, Central Kampong Thom Province, Cambodia.** Caron Y, Bory S, Pluot M, Nheb M, Chan S, Prum SH, Lim SBH, Sim M, Sengdoeurn Y, Sovann L, Khieu V, Vallée I, Yera H. Emerging Infectious Disease journal. 2020; 26(8):1759. doi: 10.3201/eid2608.191497. PMID: 32687022.
4. **Resistance to Second-Line Anti-TB Drugs in Cambodia: A Phenotypic and Genetic Study.** Cheng S, Hide M, Pheng SH, Kerléguer A, Delvallez G, Sam S, Mao TE, Nguyen TVA, Bañuls AL. Accepted for publication in the Journal of Infection and Drug Resistance (Sub ID: 289907).

4.8 Vaccination Center

The Vaccination Center at the *Institut Pasteur du Cambodge* (IPC) is composed of one Vaccination International Center (VIC) in Phnom Penh and three Rabies Prevention Centers (RPC), one each in Phnom Penh, Battambang, and Kampong Cham Provinces.

4.8.1 Functional Structure

The Vaccination Center (VC) has 29 team members based in Phnom Penh and two provinces: 9 medical doctors (Head of Vaccination Center and 3 medical supervisors), 10 nurses, 2 administration staff, 1 data manager, and 1 person in charge of hygiene.

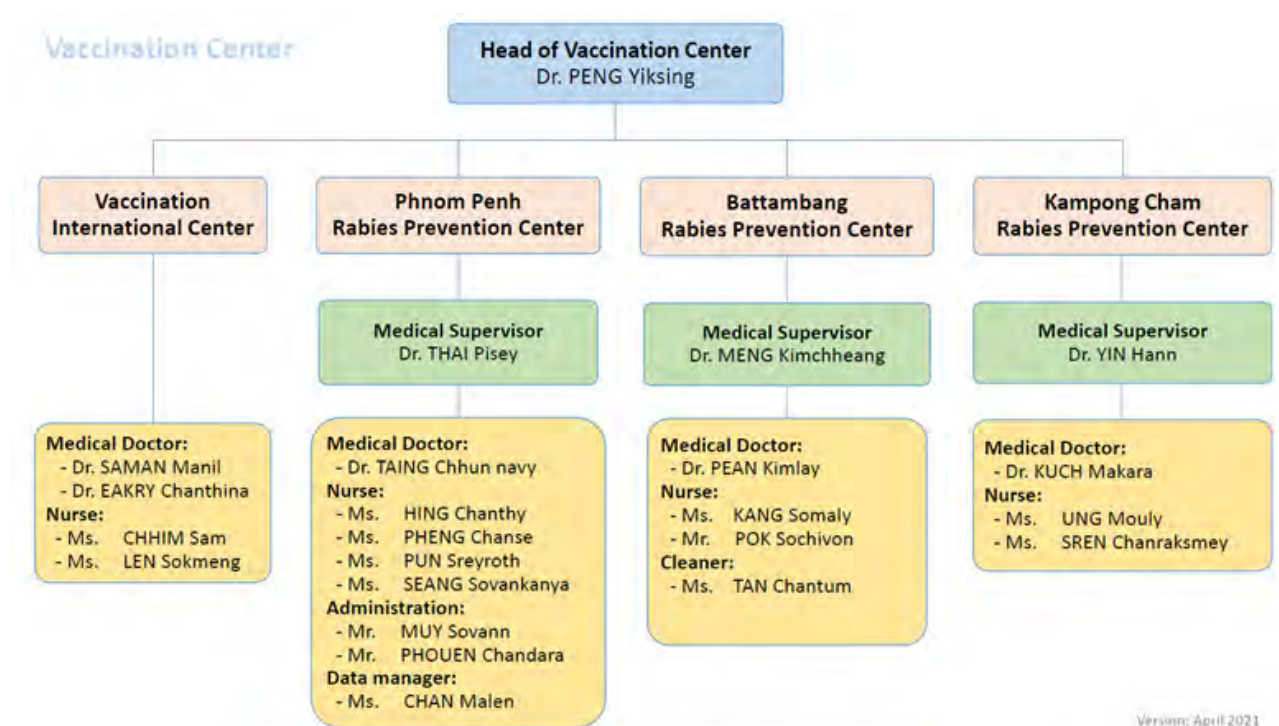


Figure 32. Vaccination International Center organogram

4.8.2 Rabies Prevention Centers

The three centers employ 18 full-time staff under the leadership of the head of the Vaccination Center. These centers provide post-exposure prophylaxis (PEP) against rabies including the administration of Equine Rabies Immunoglobulins (ERIG) at an affordable price to the public, as the treatment is subsidized by the *Institut Pasteur du Cambodge*.

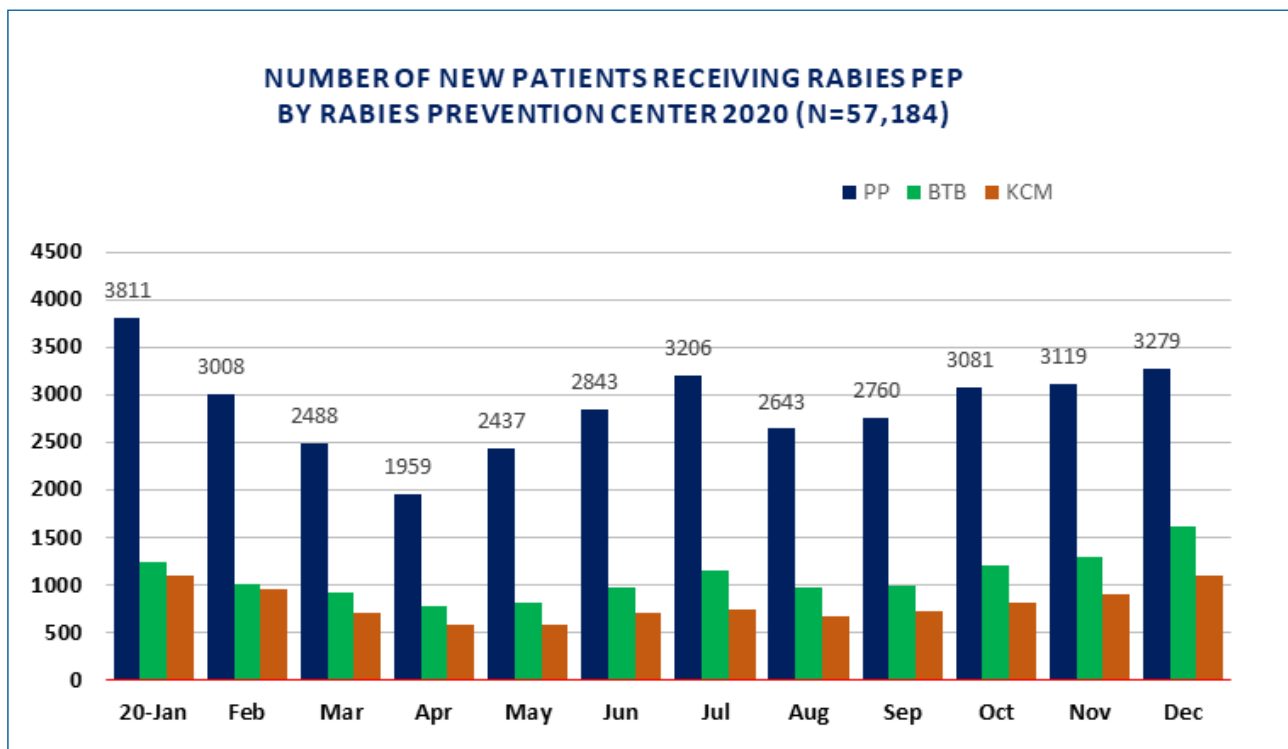
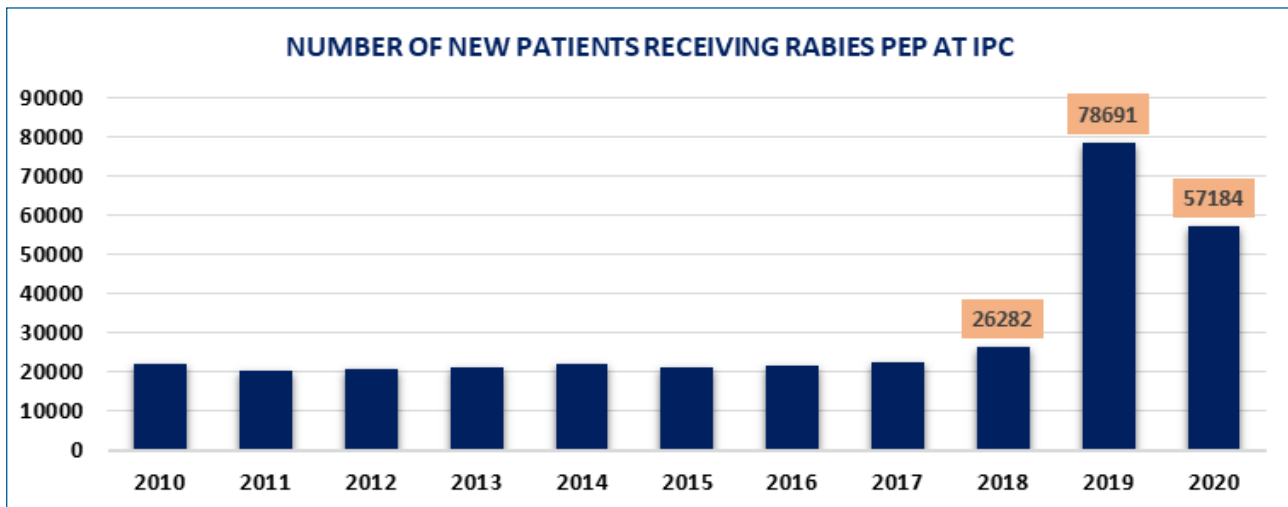
Since July 2018, IPC has offered a full rabies PEP intradermal protocol following the 2018 WHO recommended protocol, which consists of 3 sessions of 2-site ID injection using 0.1 mL vaccine per site (IPC protocol) to the public for \$15.

Diagnostic tests on brain samples of biting animals are done by the virology unit and timely results are provided free of charge to the patients even if samples are shipped from our two provincial PEP centers.

Rabies Prevention Centers Activities in 2020:

- Provided rabies post-exposure prophylaxis to 57,184 patients.
 - 34,634 patients received rabies PEP at the Rabies Prevention Center in Phnom Penh
 - 12,957 patients received rabies PEP at the Rabies Prevention Center in Battambang
 - 9,593 patients received rabies PEP at the Rabies Prevention Center in Kampong Cham

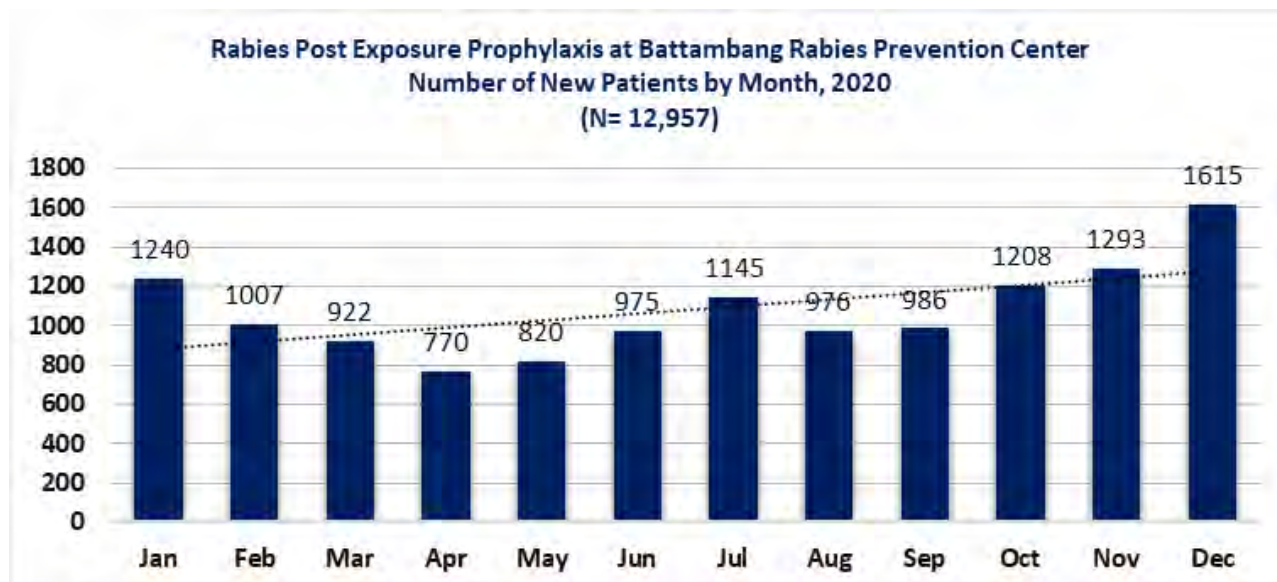
- A total of 178 animal heads were tested by immunofluorescence for rabies virus at the virology unit
 - 116 samples (65, 5%) were positive for rabies (115 dogs, 1 cat).
 - This information is regularly communicated to MoH and MoH CDC, FAO, and WHO.



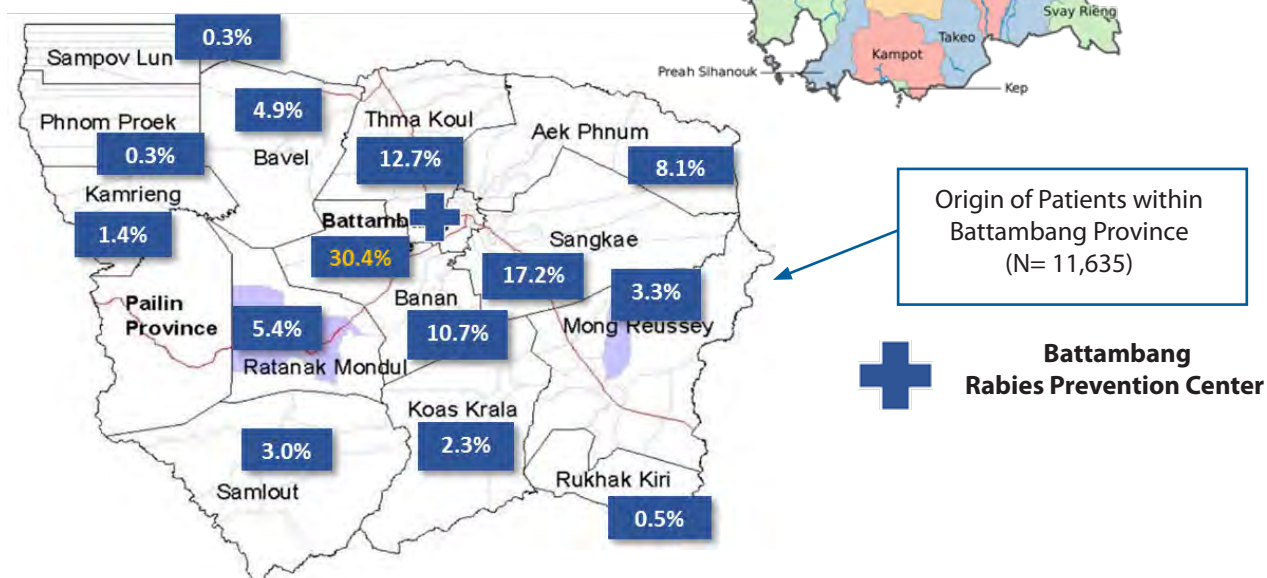
4.8.2.1 Battambang Rabies Prevention Center

The center located within the provincial hospital was opened in July 2018, following a memorandum of understanding signed on 25th December 2017 between the Battambang Provincial Health Department and the Director of *Institut Pasteur du Cambodge*. In this collaboration, the PHD contributes the building, water, and electricity supplies. The official inauguration was held on 28th September 2018.

This Center is expected to cover Battambang and 5 other neighbouring provinces.



Provinces	%
1. Battambang	89.8%
2. Banteay Meanchey	5.2%
3. Pursat	2.8%
4. Pailin	1.2%
Other	1%

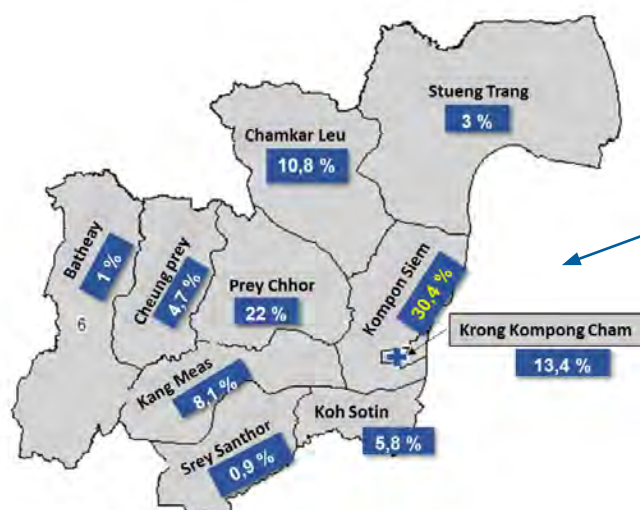


4.8.2.2 Kampong Cham Rabies Prevention Center

This PHD-IPC Rabies Prevention Center was opened on 7th March 2019 in Kampong Cham Province as part of the response to a sudden surge of patients seeking rabies prevention following wounding caused by dogs or cats. The center is located within Kampong Cham Provincial Referral Hospital using a temporary building provided by the hospital. The coverage may be extended to six other provinces of Northeast Cambodia.

A permanent facility is expected to be established in the future as part of the ongoing collaboration between IPC and the Kampong Cham PHD.

Provinces	%
1. Kampong Cham	73.4%
2. Tboung Khmom	21.5%
3. Kampong Thom	1.7%
4. Prey Veng	1.7%
Other	1.7%



Origin of Patients within
Kampong Cham Province
(N= 7,044)



**Kampong Cham
Rabies Prevention Center**

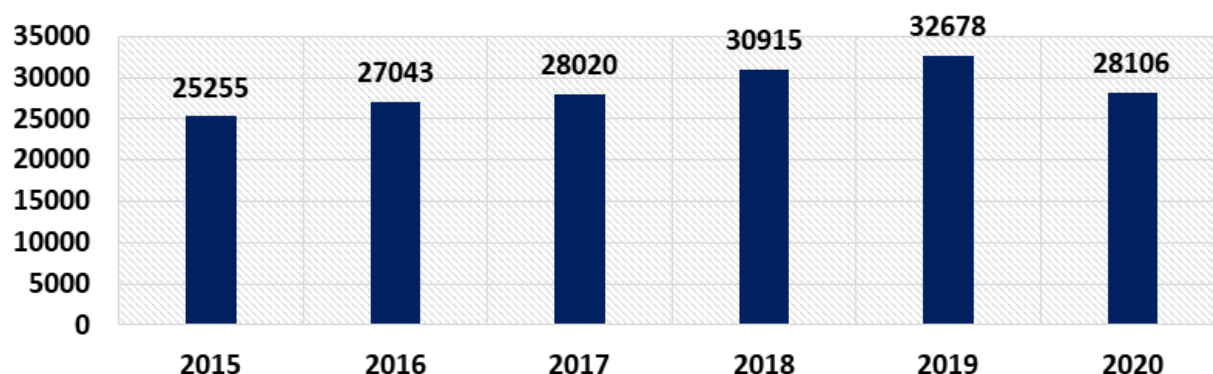
4.8.3 Vaccination International Center

The Vaccination International Center at the *Institut Pasteur du Cambodge* has a medical team of 4 full-time staff under the responsibility of the head of the Vaccination Center.

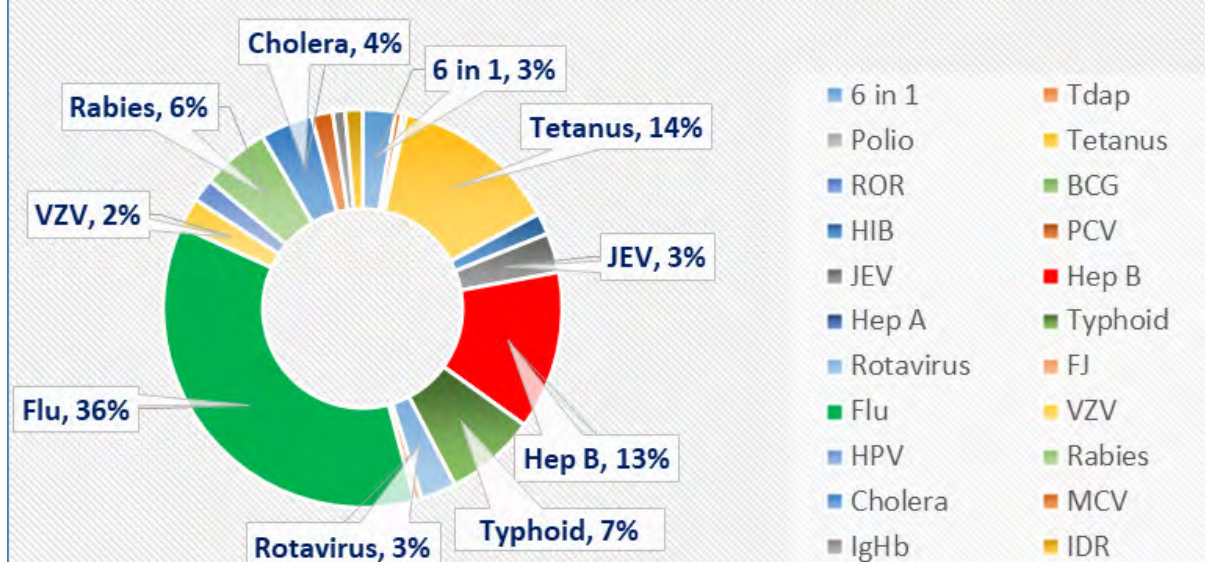
A wide range of vaccines (including those included in the national immunization program) and immunoglobulins is available at the Vaccination International Center. We maintain an international standard with qualified products, proper cold chain management, high-quality control, and professionalism.

In 2020, a total of 28,106 injections (including the immunoglobulins) were done as part of 16,126 vaccine protocols.

Vaccination International Center activity Number of injections, 2015-2020



Vaccination International Center Activity in 2020



4.8.4 Vaccination Center Vision for Next 2-5 Years

- **Contribution to the fight against rabies in Cambodia**

As an ASEAN member state, Cambodia has committed to eliminate rabies by 2030. In order to achieve this milestone 2 main action plans to fight rabies have been set up by IPC in collaboration with the Ministry of Health:

- Increasing accessibility to rabies PEP by improving the visibility of our centers.
- Raising awareness of rabies by implementing communication activities.

- **Develop a long term plan for the sustainability of the rabies prevention centers**

- Work with the different government institutions in Cambodia to develop sustainability plans.

- **Support and promote additional research**

- Enhance staff career development in research studies, focusing on young talent within the Cambodian staff.
- Continue to promote the vaccination center's research activities in close collaboration with other units of IPC.

5. CONCLUSION

The *Institut Pasteur du Cambodge* (IPC) continues to focus its 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, an environment and food safety laboratory). In 2020 through the response to the COVID-19 crisis, IPC has confirmed that it is a responsive and flexible institution that can rapidly react to any communicable disease health crisis. It actively contributes to capacity building and educational training of 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*. IPC will continue to develop high excellence research, provide direct health services, and offer teaching and training in life sciences and health research of critical importance to Cambodia and beyond. In order to fulfil its mission, IPC strives to improve the quality of life and well-being of the Cambodian population.

6. ANNEX

May 21st, 2021

