Scientific Report 2021

Institut Pasteur du Cambodge

Pasteur Network

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NOTE

Please be informed that in sections 2, 3 & 5 the text is both in English and French language, French translation being in blue color.
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1. List of Acronyms

AAS	Atomic Absorption Spectrophotometer
ABR	Antibiotic Resistance
ACIP	Action Concertée Inter-Pasteurienne (Institut Pasteur)
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 (USA)
AFRICAM	Africa Cambodia: project integrated to PREZODE initiative
AFTC	Anonymous Free Testing Center
AIRD	Inter-établissements de recherche pour le développement International
AVSF	Agronomes et vétérinaires sans frontières
AIV	Avian Influenza Viruses
AMP	Antimicrobial Peptides
AMR	Antimicrobial Resistance
ANRS-MIE	Agence Nationale de Recherche sur le SIDA, les Hépatites et les maladies infectieuses et émergentes (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
ARCIMED	Antimicrobial Resistance Circulation along the Mekong and its Delta
AS	Arsenic
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	Burkholderia pseudomallei
BSL	Biosafety level
BIRDY	Bacterial Infections and Antibiotic-Resistant Diseases among Young Children in Low Income Countries
BMGF	Bill and Melinda Gates Foundation
BOD	Burden of Disease
CANARIES	Consortium of Animal Networks to Assess Risk of Emerging Infectious Diseases through Enhanced Surveillance
CARAMAL	Community Access to Rectal Artesunate for Malaria
C-CDC	Cambodian Center for Disease Control and Prevention
CD	Cadmium
CEG	Community Epidemiology Group
CIRAD	Centre de Coopération Internationale en Recherche Agronomique Pour le Développement, CIRAD (French Agricultural Research and International Cooperation Organization)
CEEIR	Centers for Excellence in Influenza Research and Response
CENAT	National Center for Tuberculosis and Leprosy Control (Cambodia)
CERFIG	Centre de Recherche et de Formation en Infectiologie de Guinée
CIN	Cervical Intraepithelial Neoplasia
CNM	National Center for Parasitology, Entomology, and Malaria Control (Cambodia)
CNRS	Centre National de la Recherche Scientifique
COFRAC	French Accreditation Committee (Comite Francais d'Accreditation)

COVID-19	Coronavirus Disease 2019
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
DTG	dolutegravir
DTRA	Defense Threat Reduction Agency (USA)
DVI	Dengue Vaccine Initiative
EC	Escherichia coli
EID	Emerging Infectious Disease
ENTOMO	Medical and Veterinary Entomology Unit of Institut Pasteur du Cambodge
EPH	Epidemiology and Public Health Unit of Institut Pasteur du Cambodge
ERIG	Equine Rabies Immunoglobulins
ESBL-E	ESBL-producing Enterobacteriaceae
FAO	Food and Agriculture Organization of the United Nations
FAVN	Fluorescent Antibody Virus Neutralization
FCI	Flow Cytometric Immunophenotyping
FE	Lead
FLDs	Fragmented and Loop Primer Ligated dsRNA Sequencing
FQ-Rs	Resistant (ce) to Fluoroquinolones
FRNT	Foci Reduction Neutralization Test
FSPI	Solidarity Fund for Innovative Projects- France
GCRF	Global Challenges Research Fund
GDAPH	Department within the Cambodian Ministry for Agriculture, Forestry and Fisheries
GF	Global Fund to Fight AIDS, TB and Malaria
GIS	Geospatial Information System
GISAID	
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
Hcase	High-level Cephalosporinase
HCV	Hepatitis C Virus
HDN	Human Baited Double Net Traps
HEPAR	Hepatitis E and ARENAvirus Project
HEV	Hepatitis E Virus
HFMD	Hand-Foot-and-Mouth Disease

HITS	High Impact Transformative Science
HIV	Human Immunodeficiency Virus
HS-RDT	High Sensitivity Rapid Diagnostic Test
HSeQM	Hygiene Security Quality Metrology
HLC	Human-land Catching Barrier Screens
HLA	Human Leukocyte Antigen
IAS	International Accreditation Service
IAV	Swine Influenza A Virus
ICEMR	International Center of Excellence in Malaria Research
ICP-OES	Inductively Coupled Plasma - Optical Emission Spectrometry
ILI	Influenza-like Illnesses
IMMUNO	Immunology Unit, IPC
INF	Interferon
INRAE	Institut national de la recherche pour l'agriculture, l'alimentation et l'environnement
INSERM	Institut National de la Santé et de la Recherche Médicale (France)
IP	Institut Pasteur
IPC	Institut Pasteur du Cambodge
IPL	Institut Pasteur du Laos
IRD	Institut de Recherche pour le Développement (Institute for Research and Development-France)
IRIS	Immune reconstitution inflammatory syndrome
ITC	Institute of Technology (Cambodia)
IVC	International Vaccination Center
IVI	International Vaccine Initiative
JEV	Japanese Encephalitis Virus
KBH	Kantha Bopha Hospital
KP	Klebsiella pneumoniae
LAM	Live Animal Market
LBM	Laboratoire de Biologie Médicale – Medical Biology Laboratory (Service open to public)
LEFS	Laboratory of Environment and Food Safety Laboratory (Service open to public)
LORV	Loei River Virus
LMICs	Low-middle Income Countries
LMI-DRISA	International Joint Laboratory "Drug Resistance in Southeast Asia" (IRD)
LSHTM	London School of Hygiene and Tropical Medicine
LTBI	Latent Tuberculosis Infection
MAFF	Ministry of Agriculture, Forestry, and Fisheries (Cambodia)
MALDI-TOF MS	Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry
MDR	Multidrug resistant(ce)
MEAE	Ministère de l'Europe et des Affaires Etrangères (French Ministry for European and Foreign Affairs)
MEF	Ministry of Economy and Finance (Cambodia)
MERS	Middle East Respiratory Syndrome
MESRI	Ministère de l'Enseignement Supérieur, de la Recherche, et de l'Innovation. (French Ministry for Higher Education, Research and Innovation)
MEYS	Ministry of Education, Youth and Sports (Cambodia)
MIE	Maladie Infectieurse Émergente (Emerging Infectious Disease)
MiVEGEC	Maladies Infectieuses et Vecteurs Écologie, Génétiques, Évolution, et Contrôle (IRD, France)
MinION	DNA sequencing using Oxford Nanopore Technologies
miRNA	Cell-free circulating microRNA biomarkers
MLST	Multilocus Sequence Typing
MOE	Ministry of Environment

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
MME	Malaria Molecular Epidemiology Unit (Institut Pasteur du Cambodge)
MMV	Medicines for Malaria Venture
MP-AES	Microwave Plasma Atomic Emission Spectroscopy
MSP	Main Spectra Projection
MTB	Mycobacterium Tuberculosis
MRSA	Methicillin-resistant Staphylococcus aureus
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
NCHADS	National Center for HIV/AIDS, Dermatology and Sexually Transmitted Diseases (Cambodia)
NDCP	National Dengue Control Program (Cambodia)
NGO	Non Governmental Organization
NIAID	The National Institute of Allergy and Infectious Diseases (US)
NIC	National Influenza Center
NIH	National Institutes of Health (USA)
NIP	National Immunization Program
NIPH	National Institute for Public Health (Cambodia)
NABM	Nomenclature of Medical Biology Acts (NABM)
NGS	Next Generation Sequencing
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 Organization for Animal Health
PCR	Polymerase Chain Reaction
PEP	Post Exposure Prophylaxis
PHC	Primary Health Care
PICREID	Pasteur Institute – Center for Research for Emerging Infectious Diseases
PhD	Doctor of Philosophy (degree)
PHD	Public Health Department
PIB-Net	Pasteur International Bioresources Network
PMI	President's Malaria Initiative (USA)
PN	Pasteur Network
POC	Point of Care
POCTs	Point of Care Test(s)
PPR	Programme Prioritaire de Recherche
PREDICT	Pandemic Preparedness for Global Health
PREEMPT	Preventing Emerging Pathogenic Threats
PrEP	Pre-exposure Prophylaxis
PREZODE	Preventing Zoonotic Disease Emergence
PRR	Platforme Régionale de Recherche
PSI	Population Services International

PSRL	Locally recruted scientific employees Personnel Scientifique de Recrutement Local
PTR	Programme Transversal de Recherche (funding mechanism - Institut Pasteur Paris)
PVDBP	P. vivax Duffy binding protein
QA/QC	Quality Assurance/Quality Control
QFT	Quantiferon
RABV	Rabies Virus
RAI3	Regional Initiative Artemisinin Resistance
RAS	Resistance-Associated Substitution
RBC	Red Blood Cell
RDT	Rapid Diagnostic Test
RGC	Royal Government of Cambodia
RNA	Ribonucleic Acid
RUA	Royal University of Agriculture (Cambodia)
RUPP	Royal University of Phnom Penh (Cambodia)
RPC	Rabies Prevention Center
RSV	Respiratory syncytial virus
SARI	Severe Acute Respiratory Illness
SAB	Scientific Advisory Board
SADS	Swine Acute Diarrhea Syndrome Coronavirus
SARS-CoV-2	Severe Acute Respiratory Illness, COVID-19- 2
SEA	Southeast Asia
SeOV	Seoul ortho hantavirus
SHCH	Sihanouk Hospital Center of Hope (Cambodia)
SIV	Swine Influenza Virus
SMRU	Shoklo Malaria Research Unit
SNP	Single Nucleotide Polymorphism
SOP	Standard Operating Procedures
STI	Sexually Transmitted Infections
SVR	Sustained Virological Response
TB	Tuberculosis
TBD	Tick-borne Disease
TGW	Transgender Women
TLR	Toll-like Receptors
TWG	Technical Working Group
UGA	University of Georgia (USA)
UHS	University of Health Sciences (Cambodia)
UMD	University of Maryland
UMR	Unité mixte de recherche (joint research units) France
USAID	United States Agency for International Development
UP	University of Puthisastra (Cambodia)
US CDC	US-Centers for Disease Control and Prevention
UTI	Urinary Tract Infections
VS	Vaccination Service
VEG	Veterinary Epidemiology Group
VACC	International Vaccination Center (service open to public)
VIRO	Virology Unit, Institut Pasteur du Cambodge
VL	Viral Load
VOC	Variants of Concern
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. Institut Pasteur du Cambodge in 2021

2.1. General presentation of the Institute

The Institut Pasteur du Cambodge (hereinafter abbreviated as IPC or the Institute), created in December 1953 is a non-profit research institution operating under the high patronage of the Cambodian Ministry of Health (MoH). The Institute also falls under the purview of Institut Pasteur in Paris, France as concerns its scientific and technical approaches and plans. Its statutes and operational systems are codified in the agreement signed between the Royal Government of Cambodia and the Institut Pasteur in Paris signed in 1992 and since modified through two amendments.

The Institute is one of the 33 members of the Pasteur Network (PN) that brings together 25 countries on five continents. IPC's laboratories are at the full disposal of the Ministry of Health of Cambodia for any studies or research relevant to the prevention of illness and the protection of public health.

IPC's activities comprise four components: (1) biomedical research with a specialization in infectious diseases (2) support and capacity building to public health in Cambodia and the sub-region, (3) provision of health services (laboratory, immunization), and (4) training and education. The scientific foci of the IPC are infectious disease and public health challenges and issues among which illnesses related to arboviruses, respiratory viruses, rabies, malaria, and antimicrobial resistance of microorganisms, and zoonoses are priorities. These complex scientific themes, particularly those that involve pathogens with complex life cycles that can involve humans, mammals, and arthropods, could not be effectively addressed without complementarity between the Institute's units and its specialists (entomologists, doctors, veterinary scientists, immunologists, epidemiologists, mammalogists and others) or without its high-level technical platform, including a level-3 security laboratory and an animal research facility.

Créé en décembre 1953, l'IPC est un établissement de recherche scientifique à but non lucratif, déclaré d'utilité publique, placé sous le haut patronage du ministère de la Santé du Cambodge et sous la responsabilité de l'Institut Pasteur sur les plans scientifiques et techniques. Ses statuts et son mode de fonctionnement sont définis par la « Convention entre l'État du Cambodge et l'Institut Pasteur à Paris », signée en 1992 et modifiée depuis par deux avenants.

L'IPC fait partie du Pasteur Network qui rassemble 33 établissements dans 25 pays, sur 5 continents.

Les laboratoires de l'Institut Pasteur du Cambodge sont à la disposition du Ministère de la Santé du Royaume du Cambodge pour toutes les études et recherches relevant de la prévention des maladies et la défense de la santé publique, demandées par les services compétents.

Les activités de l'IPC s'articulent autour de 4 axes (i) la recherche dans le domaine biomédical et plus spécialement les maladies infectieuses, (ii) l'appui à la santé publique dans le pays, mais aussi dans la sous-région, (iii) la mise à disposition services dans le domaine de la santé (laboratoires, vaccination) et (iv) la formation.

Les thématiques scientifiques de l'IPC concernent différentes maladies infectieuses, les problèmes de santé publique au Cambodge et en ASE, parmi lesquelles, les maladies liées aux arbovirus, les virus respiratoires, la rage, le paludisme, la résistance aux antimicrobiens et les zoonoses. Ces thématiques notamment celles relatives à des agents pathogènes ayant un cycle complexe faisant intervenir l'homme, un mammifère et un arthropode, ne peuvent être abordées que grâce à la complémentarité de ses équipes pluridisciplinaires (entomologiste, médecin, vétérinaire, immunologiste, épidémiologiste, mammalogiste…) et à la qualité de son plateau technique : laboratoire de sécurité de niveau 3, animalerie…

2.2. The Institute's Governance and organization

2.2.1. Governance

Institut Pasteur du Cambodge is led by a Director and is monitored by a Liaison Council. The IPC Director is appointed by the Director General of the Institut Pasteur in Paris, France, in consultation with the MOH of the Kingdom of Cambodia. The Deputy Director of IPC is named by the Director in consultation with the MOH from within the pool of Cambodian scientists serving in the national public service and who have doctorates in biological or public health.

On an annual basis, the Institute's activities are reviewed by the Liaison Council presided by His Excellency the Minister of Health of Cambodia. The Council is composed of ten high-ranking members of the Cambodian Government or from its universities. The Director General of the Institut Pasteur in Paris, the Ambassador of France to Cambodia, and representatives of key international organizations in the health arena (WHO, UNICEF) round out the membership.

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

The scientific activities are also reviewed every two years by the Scientific Advisory Board, its last session having been held in early February 2021. The scientific strategy is then adapted based on the recommendations from both the Liaison Council and the Scientific Advisory Board.

L'Institut Pasteur du Cambodge est dirigé par un directeur et son fonctionnement est suivi par un Conseil de Liaison. Le Directeur de l'Institut Pasteur du Cambodge est nommé par le Directeur Général de l'Institut Pasteur, avec l'agrément du Ministère de la Santé du Royaume du Cambodge.

Le Directeur-adjoint de l'IPC est nommé par le Directeur de l'IPC en concertation avec le Ministre de la Santé au sein des scientifiques cambodgiens appartenant à la fonction publique, docteur ès science en biologie ou en santé publique

Les activités de l'Institut sont examinées chaque année par le Conseil de Liaison, présidé par SE le Ministre de la Santé. Ce Conseil est composé de 10 membres de haute rang notamment au sein de l'Etat, de l'Administration ou des Universités du Royaume du Cambodge ainsi que le Directeur Général de l'Institut Pasteur à Paris et l'Ambassadeur de France au Cambodge et les représentants d'organismes internationaux dans le domaine de la santé (OMS, UNICEF).

Sur le plan financier, chaque année, les états financiers consolidés semestriels et le budget prévisionnel de l'Année N+1 sont transmis à l'Institut Pasteur (International Division). Un audit financier de chaque clôture comptable est également réalisé par un cabinet d'audit indépendant.

Les activités scientifiques sont également revues tous les 2 ans par le Conseil Scientifique, sa dernière session s'est déroulé début février 2021. La stratégie scientifique est adapté en fonction des recommandations du Conseil de liaison et du Conseil scientifique.

2.2.2. Organization

The organogram is shown at the end of this report in Annex 1.
The Institute is composed of:

- A management unit comprising the Director, Deputy Director, and the Director of Administration and Finance;
- Administrative, financial and logistical services:
- Five research units, malaria and molecular epidemiology (MME), epidemiology and public health (EPH), immunology (IMMUNO),medical and veterinary entomology (ENTOMO), virology (VIRO);
- Health services including medical biology laboratory (LBM), a laboratory of environment and food safety (LEFS), vaccination service (VACC) including three anti-rabies treatment centers;
- Public health structures comprising a national reference center for influenza housed within the virology unit, the WHO regional H5 reference laboratory (global reference lab), a free-of-charge anonymous/confidential HIV testing site, and rabies centers at three different sites that provide pre and post exposure prophylaxis at a fee.
- Three technical platforms: 1) a biobank, 2) a sequencing platform, and 3) flow cytometry.

L'organigramme est présenté à la fin de ce rapport (Annexe 1).
L'Institut est composé de :

- une direction composée de 3 directeurs : directeur, directeur adjoint et directeur administratif et financier;
- des services administratifs, financiers et logistiques ;
- cinq unités de recherche : Epidémiologie moléculaire du paludisme, épidémiologie et santé publique (EPI-SP), immunologie (IMMUNO), entomologie médicale et vétérinaire (ENTOMO), virologie (VIRO);
- des entités de services dans le champ de la santé : un laboratoire de biologie médicale (LBM), un laboratoire environnement et sécurité alimentaire (LEFS), un service de vaccination englobant un centre de vaccinations internationale et un centre antirabique sur trois sites ;
- des structures de santé publiques : (i) centres de référence hébergés par l'Unité de virologie (centre national de référence pour la grippe, WHO Regional H5 Reference Laboratory, WHO Global Reference Lab), un centre de dépistage anonyme et gratuit du VIH, un centre antirabique sur 3 sites assurant la prophylaxie post-exposition à prix coutant ;
- 3 plateformes techniques : une biobanque, une plateforme de séquençage, une plateforme de cytométrie de flux.

2.3. Human Resources

As at the 31 December, 2021, the Institute had a team of 266:

- 248 with IPC contracts (of which 55 are MOH civil servants);
- Eight are Cambodian scientists (one research director and seven research fellows);
- 30 expatriates, including 11 on institutional contracts (one MEAE, seven IPP, two IRD, and one from the French Agricultural Research and International Cooperation Organization-(CIRAD);

and 15 on IPC contracts, including two PhD Students).
IPC has personnel from 11 different nationalities, with 88% being Cambodian nationals.

Au 31 décembre 2021, l'Institut comptait 266 collaborateurs. Parmi elles :

- 248 disposent d'un contrat IPC (dont 55 sont des fonctionnaires du Ministère de la santé)
- 8 sont des scientifiques statutaires cambodgiens (1 Directeur de recherche, 7 chargés de recherche) ;
- 30 sont des expatriés : 11 sont des expatriés en contrats institutionnels (1 MEAE, 7 IPP, 2 IRD, 1 CIRAD), 15 en contrats IPC (dont 2 étudiants en PhD).

Le personnel est issu de 11 nationalités différentes dont 88% de Cambodgien.

Diversity and Leadership

The Institute prioritizes gender balance and equity; 54% of staff members are women. The development of scientific leaders and other national professionals are an area of great importance to IPC. Of the 16 management positions (3 directors, 8 research or service unit heads and 5 service support managers (12 are men (75%) and 8 (50%) are Cambodian Nationals.

L'IPC donne une grande importance à l'égalité homme/femme : 54% du personnel sont des femmes.
Le développement du leadership des scientifiques et la promotion des cadres nationaux sont des axes prioritaires.
Parmi les 16 fonctions de management (3 directeurs, 8 responsables d'unités de recherche ou d'entités de services, 5 responsable de services d'appui), 12 sont des hommes (75%) et 8 (50%) des cambodgiens.

2.4. Finances

The majority of IPC's revenue comes from defined research contracts funded by donors, from the services offered by IPC including those preventing and treating rabies, and from a subsidy from the Ministère de l'Enseignement Supérieur, de la Recherche, et de l'Innovation (French Ministry for Higher Education, Research and Innovation- MESRI) via the Institut Pasteur in Paris. The Royal Government of Cambodia does not directly fund IPC, but provides a significant contribution in the form of a tax and customs exemption.
Details on the different revenue streams are shown in Tables 1 on next page. The share of income attributable to services was exceptionally high in 2020 and 2021 as a result of funds earned by providing COVID-19 tests. This revenue stream allowed important investment valued at 1.2 million Euros in 2020, excluding those previously in progress.

Les recettes de l'IPC proviennent principalement des contrats de recherches sur des projets définis octroyés par des bailleurs, des revenus générés par les différentes entités de service et le centre antirabique, et de la subvention du MESRI via l'Institut Pasteur à Paris. Le Royaume du Cambodge ne subventionne pas directement l'Institut mais apporte une contribution indirecte très importante à travers notamment l'exemption de taxes et d'impôts accordée à l'IPC.
La part des différents types de recettes selon leur provenance est précisé au tableau ci-contre (tableau 1).
La part des recettes émanant des services est exceptionnellement élevée en 2020 et 2021 compte tenu des fonds obtenus à travers la réalisation des tests COVID-19. Ces revenus exceptionnels ont permis des investissements particulièrement importants qui ont atteint plus de 1,2 M€ en 2021 (hors immobilisations en cours).
Les investissements réalisés ont été particulièrement importants et ont atteint plus de 1,2 M€ en 2021 (hors immobilisations en cours).
Les deux figures ci-dessous représentent la distribution des financements par pays et par bailleurs de fonds en 2021.

Table 1. Revenues by Source (2012-2021)

	2012	2013	2014	2015	2016	2017	2018	2019	20120	2021
Research contracts	46%	52%	60%	58%	59%	56%	56%	54%	40%	22%
Health services	29%	25%	21%	23%	25%	29%	30%	34%	51%	71%
MESRI grant	20%	17%	14%	12%	11%	11%	11%	10%	6%	3%
Other revenues	5%	5%	6%	7%	5%	5%	2%	2%	2%	5%
	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%

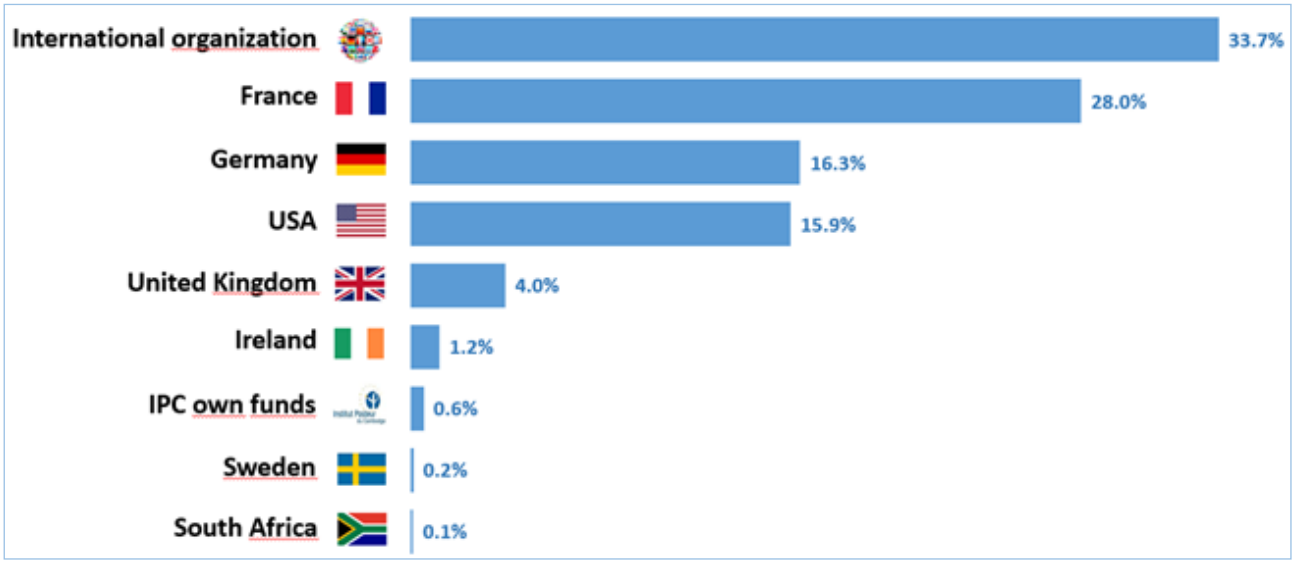


Figure 1. Breakdown of the research funds received by donor country

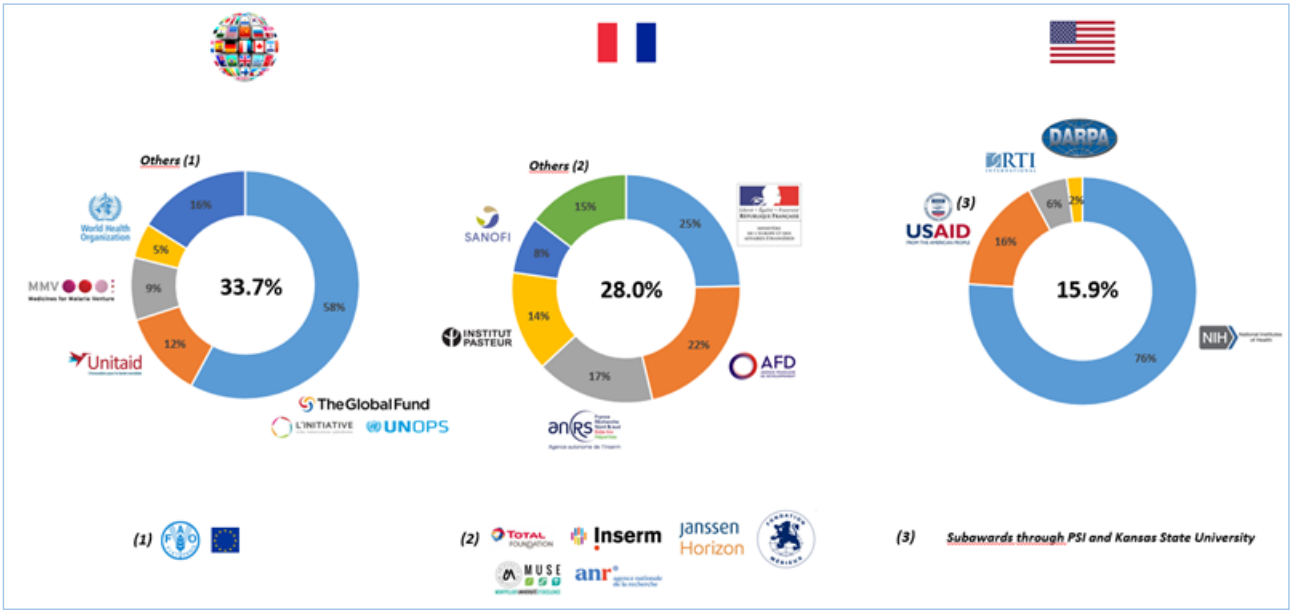


Figure 2. Revenues by Source (2012-2021)

2.5. Publications 2021

IPC's research and public health activities are detailed in later sections . A summary of these are presented in figures 3 and 4. below. Research activities done in 2021 comprise 49 articles published by scientists affiliated to IPC, appearing in peer-reviewed international journals with impact factors (IF) more than 0. Among them are 25 as first or last author and 30 with an IF greater than or equal to 4..

Les activités de recherche et de santé publique sont détaillées par entités dans la suite du rapport.
Pour l'ensemble de l'Institut une synthèse de la valorisation de ces activités à travers une analyse des publications est présentée ci-dessous aux figure 3 et 4. Les activités de recherche ont été valorisées en 2021 par 49 articles publiés par des scientifiques affiliés à l'IPC dans des revues internationales référencées à comité de lecture ayant un impact facteur supérieur à 0, dont 25 en tant que premier ou dernier auteur et 30 avec un IF supérieur ou égal à 4.

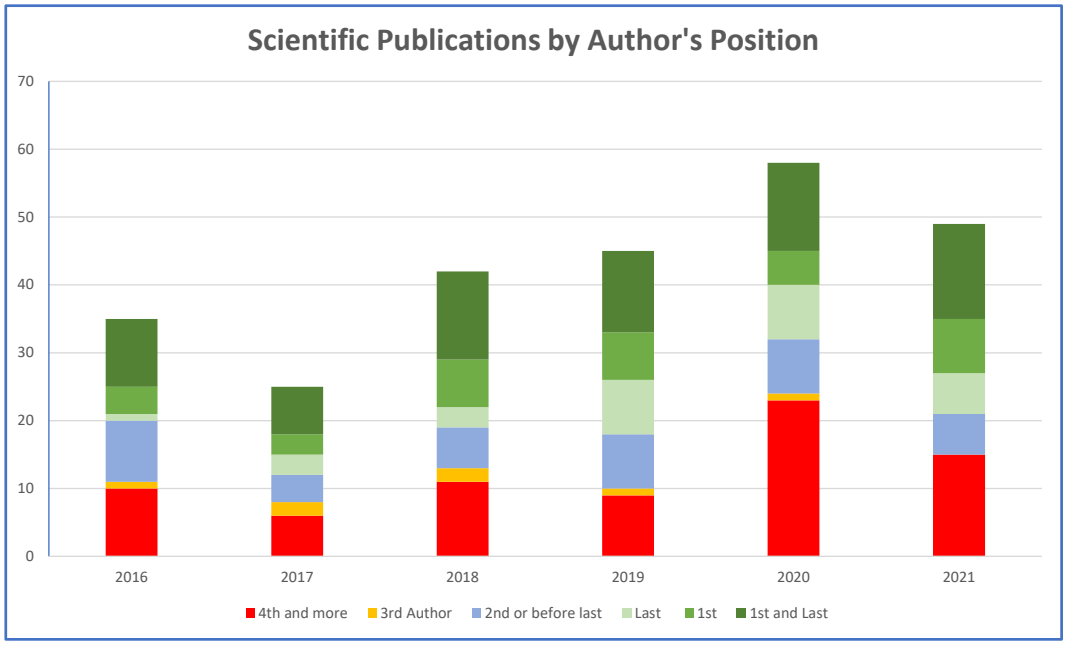


Figure 3. Scientific Publications by Author's Position (2016-2021)

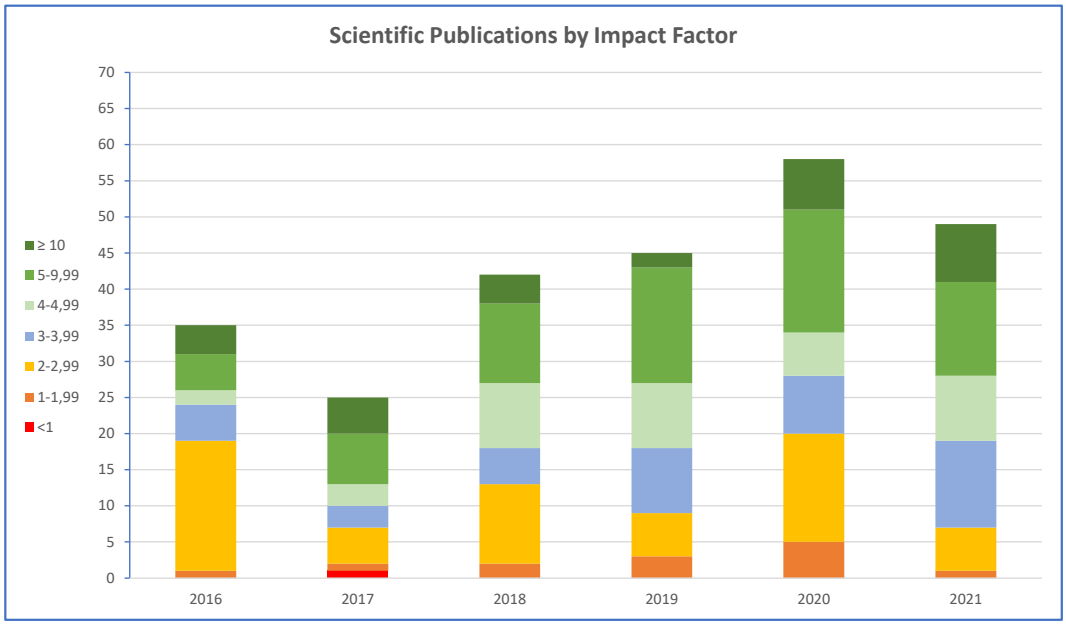


Figure 4. Scientific Publications by Impact Factor of the Journal (2016-2021)

2.6. Training and Internships 2021

IPC plays a major role in the training of university students. Its scientists participate in teaching offered by local universities, including the University of Health Science (UHS) in Phnom Penh, and welcomes many students for internships and practical experiences.

L'IPC est très impliqué dans la formation des étudiants de niveau universitaire. Les scientifiques de l'IPC participent à différents enseignements délivrés par les Universités de Phnom Penh (Université of Heath Sciences,…) et accueillent de nombreux étudiants en stage.

Student Internships

During 2021, 55 students interned at IPC. This is lower than the 80 welcomed in 2020 due to constraints posed by the continuing COVID-19 epidemic. Among the 2021 interns, 48 were Cambodian nationals, while the others were either French, Indonesian, Thai or Columbian. Their university affiliations are as follows: University of Health Science (33), University Puthisastra (8), Royal University of Phnom Penh (3) while the remainder (11) were from various foreign universities in France, Belgium, and Germany. Two of the interns were medical students, six were PhD students, seven were in master's level studies, 29 were bachelor-level students, and 11 were working toward associates degrees.

Ainsi en 2021, 55 étudiants ont été accueillis en stage. Ce nombre est inférieur à celui de 2020 (80 étudiants) du fait de la situation liée au COVI-19.
Parmi ces étudiants, 48 étudiants sont cambodgiens, les 7 autres sont français, indonésiens, thaï ou colombien. Ils sont rattachés aux universités suivantes : University of Health Sciences (33), Université de Puthisastra (8), Royal University of Phnom Penh (3) et 11 à d'autres Universités étrangères (Françaises, allemandes, belges).
Leur accueil se fait dans le cadre d'étude de médecine (2), de PhD (6), de master (7), Bachelor (29), Associate Degree (11).

International Master's in Infectious Disease

As in the past, during 2021, IPC provided important and substantive support the the International Master's Program in Infectious Disease (infectiologie in French) jointly offered by UHS and l'Université Paris Saclay (UPS) by contributing lectures and curriculum content, and by providing IPC scholarships (there are two in year 1 of the program and another 2 in second year). Due to restrictions on movements and travel, the master's level courses were offered entirely online for the 2020-2021 years. In-person instruction restarted in 2022 and IPC made lodging available within the Institute compound for modest rates.
For the 2021-2022 academic year, seven new students (including 2 Cambodians) were admitted to the third cohort of first year students, 13 are now in master's year 2 (9 in infectious disease and 4 in other subject areas). As of 2019, 25 students benefited from this master's level training, with 22 in master's year 2.

Table 2. Students in the International Master's Course during three university years.

	M1	M2
2019-2020	P1 =9 students (4 cambodians)	
2020-2021	P2 := 9 students (4 cambodians)	P1 : 9 students (4 cambodians
2021-2022	P3 = 7 students (2 cambodgien)s	P2= 9 students (4 cambodian) + 4 students

Other than providing financial support to the UHS, IPC appointed two senior scientists as focal points for the master's program to facilitate communication between UHS and UPS. Namely these are Dr. Polidy Pean (PSRL), Head of Research and Deputy Chief of the Immunology Unit and Dr. Jean Popovici, Head of Research in the Malaria Molecular Epidemiology Unit .

En 2021, l'IPC a continué à contribuer de manière significative au soutien du Master International en Infectiologie co-organisé par l'UHS et l'Université Paris Saclay (UPS) par la participation aux enseignements et la délivrance de bourses IPC (2 en master 1 et 2 master 2). Avec les restrictions de déplacements, les cours de niveau master ont été transformés pour être donnés entièrement en ligne en 2020-2021. Les enseignements en présentiel ont repris en 2022 et l'IPC a mis à disposition des étudiants un hébergement dans les locaux de l'Institut à un coût très modéré. Pour l'année universitaire 2021-2022, 7 étudiants ont été admis en master (dont 2 cambodgiens) au sein de la 3ème promotion master 1, et 13 en master 2 (9 issus du master 1 d'infectiologie et 4 d'autres masters). Ainsi, depuis 2019, 25 étudiants ont pu bénéficier de cette formation en master et 22 en master 2.
En plus des contributions financières, l'IPC a nommé deux scientifiques seniors comme points de contact pour le Programme de Master pour faciliter la communication avec l'UHS et l'UPS : Dr PEAN Polidy (PSRL, Chargé de recherche, Adjoint au Chef de l'Unité d'Immunologie) et Dr Jean POPOVICI (Chargé de recherche, Unité d'Epidémiologie Moléculaire du Paludisme).

Scientific Seminars

IPC held scientific seminars every other week in 2021, despite the various restrictions on meetings and travel. Sixteen were held while respecting social distancing, or using videoconference, as appropriate.

Des séminaires scientifiques sont organisés toutes les deux semaines à l'IPC En 2021, malgré les restrictions des déplacements, 16 séminaires scientifiques ont pu avoir lieu à l'IPC respectant les mesures de distanciation sociale et accessible par visioconférence.

2.7. Highlights in 2021

Human Resources

- Dr. Laurence Baril left her position as IPC Director on 31 August 2021. Professor André Spiegel succeeded Dr. Baril starting in September 2021.
- Dr Claude Flamand took over the leadership of the epidemiology and public health unit in November 2021 replacing Dr. Patrice Piola.
- IPC entered into a contract with a private insurance company to provide health coverage for IPC staff and their families. Previously, IPC had provided coverage directly. The new system became effective September 1, 2021.
- To strengthen pandemic surveillance and response, the Cambodian MOH formally appointed 36 IPC collaborators to the national civil service, starting on October 1, 2021.

- Le Dr Laurence BARIL a quitté la Direction de l'IPC le 31 août 2021. Le Pr André SPIEGEL lui a succédé à compter du mois de septembre 2022.
- Le Dr Claude FLAMAND a pris la responsabilité de l'Unité d'Epidémiologie et de Santé Publique en

novembre 2021 succédant au Dr Patrice PIOLA.

- La signature d'un contrat d'assurance santé avec une société d'assurance privée couvrant les dépenses de santé du personnel de l'IPC et de leur famille. Auparavant la couverture santé était directement prise en charge par l'Institut Pasteur du Cambodge. La nouvelle couverture santé a été mise en place à partir du 1er septembre 2021.
- Dans le cadre du renforcement de la lutte contre la pandémie, le Ministère de la Santé du Cambodge a titularisé comme fonctionnaire 36 collaborateurs de l'Institut Pasteur du Cambodge à partir du 1er octobre 2021.

SARS-CoV 2 Surveillance

It is important to note and appreciate the excellent collaboration and coordination that IPC has with the MOH. For example, consolidation and synthesis of RT-PCR results and analysis of positive results were addressed by the MOH on a daily basis. Genotypic monitoring of variants was communicated via a weekly bulletin. When a new variant was detected, the information was immediately transmitted.

Il est important de souligner l'excellente coordination avec les structures du Ministère de la santé. Ainsi la synthèse des résultats des RT-PCR et les bulletins d'analyse des résultats positifs étaient adressés quotidiennement au MoH. Les résultats du suivi génotypique des variants étaient communiqués sur la base d'un rapport hebdomadaire. Quand un nouveau variant était détecté, l'information était transmise immédiatement.

PCR Testing

It should be recalled that the first confirmed case of COVID-19 in Cambodia was detected on 27 January 2020 in a passenger arriving from Wuhan. In April 2020, IPC's response to the global COVID epidemic was recognized by the WHO as a "COVID-19 Global Referral Center."

By the end of December 2020, IPC had tested over 180,000 human samples covering approximately 120,000 individuals, resulting in 370 positive confirmed cases. By December 2021, the Institute had conducted over 890,000 RT-PCR tests and identified (or confirmed when originally identified by the National Public Health Laboratory (NPHL) at the National Institute for Public Health (NIPH) or a regional laboratory). Tens of thousands of positive cases were identified as part of this ongoing surveillance and response.

Pour rappel, le 27 janvier 2020, l'IPC avait confirmé le premier cas de COVID-19 (un voyageur de Wuhan) au Cambodge. En avril 2020, le travail effectué à l'IPC en réponse à l'épidémie mondiale de COVID a été reconnu par l'OMS en attribuant à l'unité de virologie la position de « WHO COVID-19 global référence ».

Fin décembre 2020, IPC avait reçu et testé plus de 180 000 échantillons humains couvrant environ 120 000 personnes avec 370 cas confirmés de COVID-19. Au 31 décembre 2021, l'IPC avait testé plus de 890 000 RT-PCR et identifié (ou confirmé lorsqu'il était identifié par le NPHL au NIPH ou l'un des laboratoires régionaux) des dizaines de milliers de cas positifs dans le cadre de la surveillance et de la réponse.

Viral Surveillance of SARS-COV 2 Variants

While sequencing remains the gold standard for detection of SARS-CoV-2 variants, with the global propagation of different variants of concern, IPC tested, validates and used commercial kits for molecular detection of COVID-19 variants of concern, permitting rapid, sensitive, and specific detection in suspected cases.

In 2020, sequencing had limited utility for certain isolates in imported cases, but became essential for epidemic response to determine the likely origin of community "events" between November 3 and 28. This sequencing

was essential to ensure that clusters originating from imported cases were not linked to undetected community transmission. The 2020 sequencing covered 63 cases (5.9% of those reported at that time).
Following what is now referred to as the "20 February event of 2021", sequencing and identification of variants of concern became even more critical. In order to identify the introduction and spread of variants of interest, Cambodia adopted a genomic surveillance strategy where the samples were selected relative to the availability of those tested the previous day and attempts were made to extend tests and analysis across provinces and sample types to the extent possible. Prior to week 15 of 2021, all variant determinations were done through genomic sequencing. From week 15 onward, Cambodia began using the genetic mutation kit for S SARS-CoV-2. Between January 2021 and December 31, 2021, IPC sequenced 1,958 samples (1.7% of the total number of reported cases at that time) and submitted this information to GISAID, the global science initiative and primary source established in 2008 that provides open access to genomic data of influenza viruses and the coronavirus responsible for the COVID-19 pandemic. By the end of December 2021, the Institute had tested over 14,880 variant of interest samples by RT-PCR to surveille variants of interest circulation within Cambodia and arriving through its borders.

Bien que le séquençage reste le « gold standard » pour la détection des variants du SARS-CoV-2, avec la propagation mondiale des différentes variants d'intérêt (VoC) du COVID-19, l'IPC a testé, validé puis utilisé des kits commerciaux pour la détection moléculaire des lignées VoC du SARS-CoV-2 qui permettaient une détection rapide, sensible et spécifique de différents VoC dans les cas suspects.
En 2020, le séquençage était d'une utilisation limitée pour certains isolats de cas importés et est devenu essentiel pour la réponse à l'épidémie afin d'aider à déterminer l'origine approximative des « événements » communautaires limités des 3 et 28 novembre. Ce séquençage était essentiel pour garantir que ces clusters provenaient de l'importation et ne représentaient pas une transmission communautaire non détectée. Le séquençage total en 2020 a couvert 63 cas (5,9 % des cas déclarés à cette époque).
En 2021, à la suite de l'événement du 20 février, le séquençage et la détermination de la VoC sont devenus encore plus critiques. Pour détecter l'introduction et la propagation des variants d'intérêt, le Cambodge a adopté une stratégie de surveillance génomique où les échantillons ont été choisis en fonction de la disponibilité de ce qui a été testé la veille et des tentatives ont été faites pour étendre les tests et l'analyse à travers la province et le type d'échantillon dans la mesure du possible. Avant la semaine 15 de 2021, toutes les déterminations de variantes étaient effectuées par séquençage génomique. Après la semaine 15, le Cambodge a commencé à utiliser le kit de mutation du gène S SARS-CoV-2. Entre janvier 2021 et fin décembre 2021, l'IPC a pu séquencer 1 958 échantillons (1,70 % séquencé au total du total des cas signalés à ce moment-là) et les soumettre au GISAID. Fin décembre 2021, l'IPC avait testé plus de 14 880 échantillons de VoC par RT-PCR pour surveiller les variantes préoccupantes (VoC) circulant au Cambodge et y entrant.

Reduction in Laboratory and Vaccination Service Activities

IPC's various service entities and laboratories were strongly impacted by the COVID-19 epidemic. Specifically, curfews, lock-downs, and travel restrictions all reduced demand for services or made it difficult to impossible to obtain them. Beginning in April 2021, IPC's Medical Biology Laboratory began offering PCR tests for international travelers who required them. This high-demand service eventually made up 61.5% of laboratory activities in 2021, with about 17,000 tests being done. The Laboratory of Environment and Food Safety Laboratory (LEFS), the International Vaccination Center and the rabies prevention centers saw a decrease in their activities respectively, 27% (decline in activities, measured by the number of analyses done), 30% (decline in vaccinations carried out) and 23% (decline of persons treated).

Les activités des différentes laboratoires et entités de services ont été fortement affectées par les différents événements relatifs à la pandémie de COVID-19 (épidémie, mesures de confinement et de restriction de déplacement, …). A compter d'avril 2021, le LBM a mis en place la PCR COVID-19 au profit des voyageurs internationaux pour lesquels un résultat était demandé par les compagnies aériennes ou les autorités des pays de transit ou de destination finale. Cette activité a pris une place importante représentant 61,5% des activités du laboratoire en 2021 (17 000 test réalisés environ). Le LEFS, le Centre de vaccination internationale et le centre antirabique ont vu leurs activités baisser respectivement de 27% (nombre d'analyses effectuées), 30% (nombre de vaccinations effectuées) et 23% (nombre de personnes prises en charge).

Surveillance and Investigation of Avian Flu Virus

In 2021, IPC was involved in a study of two households in Battambang having experienced poultry deaths in January 2021, and of wild bird deaths in Prey Veng Province in May 2021. Samples related to these events were sent to the virology unit at IPC for sequencing following tests at the National Animal Health and Production Research Institute (NAHPRI) that found the samples to be positive for subtype A/HS. In February 2021, a One Health survey followed up a positive case of bird fluA(H9N2) found in a three-year old resident of Prasat Bakong District in Siem Reap Province. This work was a collaboration between NIPH, NAHPRI,CDC, IPC, WHO, CDC-USA).

En 2021, IPC a été impliqué dans l'enquête sur deux foyers associés à un événement de mortalité de volailles dans la province de Battambang en janvier 2021 et à une mortalité d'oiseaux sauvages à Prey Veng en mai 2021. Les échantillons associés aux deux événements d'éclosion ont été envoyés à l'unité de virologie de l'IPC pour séquençage après les tests au NAHPRI et ont été confirmés positifs pour le sous-type A/H5.
En février 2021, réalisation d'une enquête One Health suite à la détection d'un cas d'infection par le virus de la grippe aviaire A(H9N2) chez un enfant de 3 ans vivant dans le district de Prasat Bakong, à Siem Reap. (Collaboration NIPH, NAHPRI,CDC, IPC, OMS, CDC-USA).

Quality and Accreditation

The Medical Biology Laboratory (LBM) was successful in having its ISO 15189 accreditation confirmed by the French Accreditation Committee (COFRAC) for the third time with a scope of accreditation covering over 63% of analyses undertaken.
The metrology laboratory within the HSeQM service was accredited by an international accreditation service (IAS) for the measurement and calibration according to ISO/IEC Standard 17025:2017. To our knowledge, **this is the first accredited laboratory of this type within the Pasteur Network.**
LEFS initiated the accreditation process for norm ISO17025 with IAS and with the Department of accreditation (DA) of the Ministry of Industry, Science, Technology & Innovation. It is anticipated the accreditation will be granted in 2022.

Le LBM a confirmé pour la troisième fois son accréditation (Norme ISO 15189) par le COFRAC avec un champ d'accréditation portant sur plus de 63% des analyses réalisées.
La Laboratoire de Métrologie au sein du Service HSeQM a obtenu une accréditation par International Accréditation Service (IAS) pour la mesure et le calibrage selon la norme ISO/IEC Standard 17025:2017, General. C'est à notre connaissance le premier laboratoire de ce type accrédité dans le Pasteur Network.
Le LEFS a entamé en 2021 une démarche d'accréditation selon la norme ISO 17025 auprès de l'IAS et du Department of Accreditation (DA) du Ministry of Industry, Science, Technology & Innovation visant une accréditation au cours de l'année 2022.

Establishing of Sequencing Platform

In April 2021, IPC put in place a sequencing platform with the support of several stakeholders, notably l'Agence française de développement (AFD), MediLabSecure, The Embassy of the United Kingdom, the Wellcome Trust and WHO. This platform is intended to strengthen operational research and the public health response at IPC.

En avril 2021, l'Institut Pasteur du Cambodge (IPC) a mis en place une plateforme de séquençage avec le soutien de plusieurs parties prenantes, notamment : l'Agence française de développement (AFD), MediLabSecure, l'ambassade du Royaume-Uni, Wellcome Trust et l'Organisation mondiale de la santé. Cette plateforme est destinée à renforcer la recherche opérationnelle et la réponse de santé publique à l'IPC.

Improving security and service quality for patients: Infrastructure works.

Using support from the British Embassy in Cambodia, several important improvements were made to the physical infrastructure of the IPC entrance/reception. These works have multiple objectives:

- Creation of a zone dedicated to travelers requesting RT-PCR tests;
- Strengthening of security of the campus by limiting access to areas that are off limits to the public;
- Streamlining the reception process for all visitors.

Des travaux importants ont été menés à l'entrée de l'IPC grâce à l'appui de l'Ambassade de Grande Bretagne. Ces travaux avaient un double objectif :

- Créer une zone dédiée aux voyageurs venant réaliser un prélèvement en vue d'un test RT-PCT qui était exigé pour les déplacements (compagnies aériennes, pays de destination, …).
- Renforcer la sécurité du campus en restreignant l'accès aux zones n'accueillant pas du public.
- Améliorer la qualité de l'accueil à l'entrée de l'IPC (patients, clients, visiteurs…).

3. Challenges Facing the Institut Pasteur du Cambodge

3.1. The Governing Bodies' Main Recommendations in 2021

Liaison Council (27 May 2021)

During the period in question, Cambodia was deeply affected by the COVID-19 pandemic, as was the rest of the world. The Liaison Council Members recognized the quality and level of engagement of the IPC, which worked shoulder-to-shoulder with the MoH in the COVID-19 response.

Au cours de cette période marquée, au Cambodge comme dans le monde entier par la pandémie de COVID19, les membres du Conseil de liaison ont souligné la qualité du travail et de l'engagement de l'IPC au côté du Ministère de la santé au cours de la riposte contre l'épidémie de Covid19.

Scientific advisory board (1-4 February 2021)

The previous scientific advisory board (SAB) meeting was held virtually due to COVID-19 related reasons. The main recommendations of the council were described in the 2020 report. These include the following:

- Establish a five-year strategic plan to better define the role of IPC in the Cambodian public health arena.
- Improve both internal and external communication.
- Review and revise the organization's structure to improve collaboration and strengthen thematic work. Antibiotic resistance must be given more emphasis and visibility due to its importance.
- Improve capacity and support in biostatistics and support to research and financial management of research grants.
- Increase leadership diversity. The leadership of various units could be more diverse both as regards gender and country of origin. Programs need to be established to ensure expanded diversity and promote the growth of national scientists.

Le dernier Conseil scientifique a eu lieu par visioconférence compte tenu de l'épidémie de COVID-19. Les principales recommandations de membres du Conseil ont été rappelées lors du précédent rapport.

- Etablir un plan stratégique sur 5 ans permettant de définir le rôle de l'IPC dans l'environnement/la stratégie de santé publique cambodgienne.
- Améliorer la communication interne et externe.
- Revoir l'organisation actuelle pour améliorer la collaboration et le travail par thématique. La thématique antibiorésistance doit apparaitre plus visible compte tenu de son importance.
- Renforcer les compétence / l'appui en bio statistique et le soutien dans la recherche et la gestion des financements de la recherche (grant office).
- Augmenter la diversité dans le leadership. La direction des entités pourrait être plus diversifiée, en ce qui concerne le sexe ou le pays d'origine. Certains programmes doivent être mis en place pour assurer l'expansion de la diversité et assurer un leadership croissant des scientifiques nationaux.

3.2. Institutional issues, objectives and outlook

Objectives are in line with recommendations made at the last two liaison councils, some of which could not be implemented sooner due to the COVID-19 pandemic.

Certains de objectifs suivent les recommandations du ou des derniers Conseil de liaison qui n'ont pu être mis en œuvre du fait de la situation liée à la pandémie de COVID-19.

3.2.1. Maintain the IPC in its National and Regional Scientific and Medical Context

IPC maintains regular, frequent interactions and communications with the Ministry of Health and with Institut Pasteur in Paris. The Institute is integral to public health research efforts in Cambodia, and over 90% of its personnel is Cambodian. The IPC should preserve its efforts to mobilize and retain support from its different partners, both Cambodian and otherwise so that it can achieve its mission with maximum impact.

L'IPC maintient de nombreuses interactions avec le Ministère de la Santé et l'Institut Pasteur à Paris. L'Institut est au cœur du dispositif de recherche et de santé publique en santé au Cambodge. Plus de 90% du personnel est cambodgien. L'IPC a vocation à être mobilisé et soutenu par ces différents partenaires, cambodgiens et étrangers afin qu'il continue à remplir ses missions avec le maximum d'efficacité.

3.2.2. Develop its Partnerships with Scientific Institutions

Develop partnerships with national institutions

Partnerships with Cambodian Ministries (agriculture, education, environment, and others beyond the MoH) and its affiliated institutions, including but not limited to research centers, hospitals, universities, and the private sector are important to achieving IPC's goals and objectives. Reinforcing and expanding these partnerships and collaborations would be to the advantage of IPC.

In light of the similarity and complementary scopes of IPC and UHS's work, even closer collaboration could be envisaged in the fields of epidemiology and clinical research particularly as regards the work done by teams associated on ANRS site in Cambodia.

Les partenariats avec les institutions du Ministère de la santé et d'autres ministères (agriculture, éducation, environnement) telles que les Universités, les centres de recherche, les hôpitaux, le secteur privé sont clés pour que l'IPC puissent remplir ces missions.

Il convient de renforcer ces collaborations. Compte tenu de la proximité des missions et des champs d'activités avec l'UHS, des collaborations encore plus étroites peuvent être envisagées dans le domaine de l'épidémiologie et la recherche clinique notamment en s'appuyant sur le site ANRS.

Maintain and Strengthen the hosting of scientists from outside institutions, most notably from CIRAD and IRD

Maintaining collaboration with these two French research institutions by welcoming their scientists within IPC appears key to building a critical mass of scientists and the development of key scientific areas such as antibiotic resistance and veterinary sciences focusing on zoonoses.

Le maintien des collaborations avec ces deux institutions françaises de recherche par l'accueil de scientifiques hébergés au sein de l'IPC apparait clé pour renforcer des masses critiques de scientifiques et développer des thématiques de recherche (antibiorésistance, aspect vétérinaire des zoonoses,).

3.2.3. Guiding Scientific Strategy

Organize the 8th meeting of the SAB

The last scientific advisory board (SAB) meeting was held virtually in February 2021. To respect the intended 18-24 month schedule and taking into account changes having happened at PIC, it would be advantageous to organize the next SAB in 2023.

Le dernier CS a eu lieu par visioconférence en janvier 2021. Pour respecter la périodicité de 18 à 24 mois, tenir compte des différentes évolutions de l'IPC, il conviendra d'organiser le prochain Conseil scientifique en 2023.

Develop a 5-year strategic plan

The last two SAB meetings recommended the development of a 5-year strategic plan. This plan should be developed in concert with all IPC scientists as well as with key partners. The plan should also be coordinated with the MoH and the Institut Pasteur in Paris.

The said strategic plan should bring all personnel to a common vision for the Institute and to a clear understanding of major priorities and general orientation of goals and processes. The polan should define IPC's role in the Cambodian public health arena, and should demonstrate how IPC can build local scientific capacity and skills transfer to local health authorities and actors. This would permit IPC to concentrate on highly specialized public health issues as well as on research.

Il s'agit d'une recommandation importante des deux dernières réunions du Conseil scientifique.
Ce plan doit être établi en concertation avec l'ensemble des scientifiques de l'IPC ainsi qu'avec les partenaires les plus importants. Ce plan devra associer à la fois le Ministère de la Santé et l'Institut Pasteur à Paris.
Le plan stratégique doit amener tout le personnel à une vision commune de l'institut et à une compréhension claire des principales priorités et de l'orientation générale. Le plan stratégique devra définir le rôle de l'IPC dans l'environnement/la stratégie de santé publique cambodgienne. Il serait important de démontrer comment l'IPC pourrait contribuer au renforcement des capacités locales et au transfert de technologie aux autorités sanitaires locales. Cela permettrait à l'IPC de se concentrer sur des aspects hautement spécialisés de la santé publique ainsi que sur la recherche.

Lead a Strategic Review of Research on Malaria

The summer 2022 departure of Dr. Benoit Witkowski, the unit leader and of Dr. Patrice Piola who also had substantial involvement in the area of malarial epidemiology together with the large reduction in Plasmodium falciparum malaria incidence suggest a need for a redefinition of this unit's strategy for the upcoming five year period.

Le départ à l'été 2022 du responsable de l'entité (Dr Benoit WITKOWSKI), le départ du Dr Patrice PIOLA très impliqué dans le domaine de l'épidémiologie du paludisme, la réduction drastique de l'incidence du paludisme à Plasmodium falciparum doivent amener à redéfinir la stratégie de cette unité pour les 5 ans à venir.

Develop a Research Program on Antibiotic Resistance

The scientific council recommended further strengthening of research on and attention to antibiotic resistance at both the unit and multiunit levels and the creation of a multidisciplinary group. Taking into account ongoing projects (ARCHAE, RAMSES and different stakeholders and actors including LMB, LEFS), any planning and implementation should be done in close consultation with the International Joint Laboratory "Drug Resistance in Southeast Asia" (LMI-DRISA, IRD).

Le Conseil scientifique a recommandé une structuration encore plus forte de la recherche sur l'antibiorésistance (unité, programme transversal, …) et la création d'un groupe transversal, …). Compte tenu des différents projets en cours ARCHAE, RAMSES, des différents intervenants (LMB, Unité d'épidémiologie, LEFS), cette réflexion doit être menée en articulation avec le LIM IDRISA.

Develop and Expand Capacity in Bioinformatics

A working group was created in April regrouping different skills existing in this area at IPC.
Discussions were held demonstrating the need to recruit a senior scientist specialized in this area. The strategy would be to work towards having bioinformatics specialists in the different research areas within the Institute.

Un groupe de travail a été créée en avril regroupant les différentes compétences dans ce domaine à l'IPC. Des discussions ont eu lieu démontrant la nécessité de recruter un scientifique senior dans ce domaine. La stratégie serait de disposer à terme de différents bioinformaticiens dans les différents domaines de recherche de l'Institut.

3.2.4. Reduce the Administrative Tasks required of Scientists

This lightening of the administrative burden is necessary to avail more time to scientists to conduct research. Serious consideration should be given to the creation of a grant office that would assist scientists in the development and follow-up of projects and proposals. IPC has sought advice from the Institut Pasteur de Dakar, whose size approximates IPC's, and who have implemented this action and created such a structure. This objective is in line with recommendations 4 and 5 from the last SAB.
It is also foreseen that a new procurement system (automated) will be put in place that will integrate purchasing, billing and accounting. This would make the procurement process simple, decentralized, faster and more efficient.

Cette simplification est nécessaire afin de faire gagner du temps « recherche aux scientifiques. L'accès en temps réel des budgets de programme et la réflexion sur la création d'un « Grant Office » permettant d'aider les scientifiques dans le montage et le suivi des projets doit être étudié. Des contacts ont été pris avec l'Institut Pasteur de Dakar – institut de la taille de l'IPC – qui s'est doté d'une telle structure. Cet objectif suit les recommandation 4 et 5 du dernier Conseil scientifique.
Il est également prévu de mettre en place un nouveau processus de gestion des commandes intégré et automatisé de la demande d'achat à l'approbation de la facture et la comptabilisation. L'objectif est de rendre le processus achats simple, décentralisé, rapide et efficace.

3.2.5. Continue the Development of Quality Standards

IPC currently has two accredited units (LBM and the metrology laboratory) and a third that is in the process of accreditation (LEFS). consideration should be given to accreditation or certification of other research units, most notably the virology unit.

L'IPC dispose de deux entités accréditées (le LBM et le Laboratoire de métrologie) et d'un laboratoire dans le processus d'accréditation (LEFS). Il conviendra de réfléchir à l'accréditation ou à la certification des unités de recherche notamment l'Unité de virologie.

3.2.6. Build Staff Capacity at IPC and Make it a More Attractive Place to Work

Strengthen the General Capacity of IPC Scientists

On this front, IPC would like to develop skills in biostatistics, in the writing of scientific articles and of proposals for funding. This may require some external training that would be funded by IPC.

Il s'agira de développer des compétences dans les domaines des bio-statistiques, de la rédaction d'articles et de projets. Les formations qui ne pourront être assurées en interne devront être financées par l'IPC.

Strengthening Management Skills

Continue ongoing efforts to build professionalism and skills in management, including supervision and mentorship, structure orientation at all levels.

Continuer les efforts entrepris dans le domaine du management et de l'encadrement à tous les niveaux, du cadre de contact au chef d'entité.

Develop its staff and make careers attractive

IPC would like to establish more direct linkages between senior management, the human resources department, and unit leaders through an annual meeting to identify and plan for the mentorship and follow up of young talent and employees with high potential.

This process will identify young talent as they develop their theses, and build pathways for individual staff members, both for scientific and management growth, guiding them towards rewarding careers that also fill national and international gaps. This year two students will start their theses in entomology, allowing IPC to introduce and test this approach, and to refine it over the next two years.

Un processus est à mettre en place à l'IPC en lien étroit entre Direction, Service des Ressources Humaines et Chefs d'Unité pour structurer cette démarche (réunion annuelle pour identifier et suivre les jeunes talents et hauts potentiels, construction et suivi d'un parcours de développement).

Il s'agit d'identifier les jeunes talents dès le niveau de la thèse et de leur construire un parcours scientifiques, managériales permettant d'envisager (deux étudiants vont commencer cette année leur thèse en entomologie, un est en cours de thèse en immunologie, deux scientifiques candidats à l'intégration dans les cadres scientifiques en 2022 et 2023).

Improve Workplace Safety and Security

The consultative committee on safety and security needs to be relaunched and occupational medicine needs to be better structured.

Le Comité consultatif d'hygiène et sécurité doit être relancé et la médecine du travail (aptitude à différents postes, vaccination) doivent encore être mieux structurée.

Improve IPC Infrastructure

The growth and evolution of activities over the last few years led to an increase in the workforce and the development of infrastructure that will soon reach their limits. It is time to seriously consider the need for specialized space in the next five to ten years. Strategically, what is possible at the current campus? A study should be undertaken by an accredited architectural firm to inform IPC's strategic plan regarding optimal development and modernization of the Institute's infrastructure.

Le développement des activités ces dernières années ont entraîné une augmentation des effectifs et un développement des infrastructures qui atteignent leurs limites. Il convient de mener une réflexion sur ce que devrait être le campus à un horizon de 5 à 10 ans. Cette réflexion doit s'appuyer sur le plan stratégique et sur une étude menée par un cabinet d'architecte permettant de mener une réflexion globale et cohérente sur le développement et la modernisation des infrastructures du site.

3.2.7. Fine-tune IPC's economic model

IPC is a not-for-profit organization. Its economic model rests on the successful pursuit of research and institutional grants, and earning of revenue through the offering of services such as vaccinations and laboratory analysis. The Medical Biology Laboratory activities need to be expanded to generate additional revenue. This is possible given the technical excellence and reputation it possesses. The laboratory activity measured "in B" according to the French nomenclature of biological acts which makes it possible to quantify the activity remains at a level that is too modest given the excellence of its technical platform and its potential to allow even more attractive prices.

IPC's laboratory is facing increasing competition from non-accredited laboratories and also suffers from accessibility constraints due to lack of parking for patients. The establishment of an off site sample-taking center warrants prompt attention, as does an overall review of LBM's general positioning in terms of price, value, and access.

L'IPC est un organisme à but non lucratif. Le modèle économique de l'IPC repose sur un financement provenant des contrats et subventions de recherche et de l'excédent de trésorerie dégagé par les entités de service (LBM, LEFS CIV).

Les activités du LBM doivent être développées. L'activité de laboratoire mesurée en B reste à un niveau trop modeste compte tenu de l'excellence de son plateau technique pour permettre des tarifs encore plus attractifs.

Le laboratoire rencontre le problème d'une concurrence importance de laboratoires non accrédités et une difficulté d'accessibilité (difficulté pour les patients de garer leur véhicule à proximité). L'ouverture d'un centre de prélèvement délocalisé est à étudier rapidement ainsi qu'une réflexion plus générale sur le positionnement du LBM en terme de prix

4. 2021 Activities at Institut Pasteur du Cambodge

4.1 Malaria Molecular Epidemiology

4.1.1. Functional Structure

The malaria molecular epidemiology unit (MMEU) has been led by Benoit Witkowski 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 (September 2017) of one head of Unit (B. Witkowski-IP permanent researcher), one deputy head (Nimol Khim-IPC permanent researcher), one IP researcher (Jean Popovici) two contractual researchers (Amelie Vantaux & Camille Roesch), two PhD student (Anais Pepey, Kutub Ashraf) and seventeen technical & administrative staff. Since 2016 the malaria unit at IPC has been associated with the group at Institut Pasteur in Paris led by Jean Christophe Baraleto from the Pasteur International Joint Research Unit (PIU) called the malaria translational research unit (MTRU).

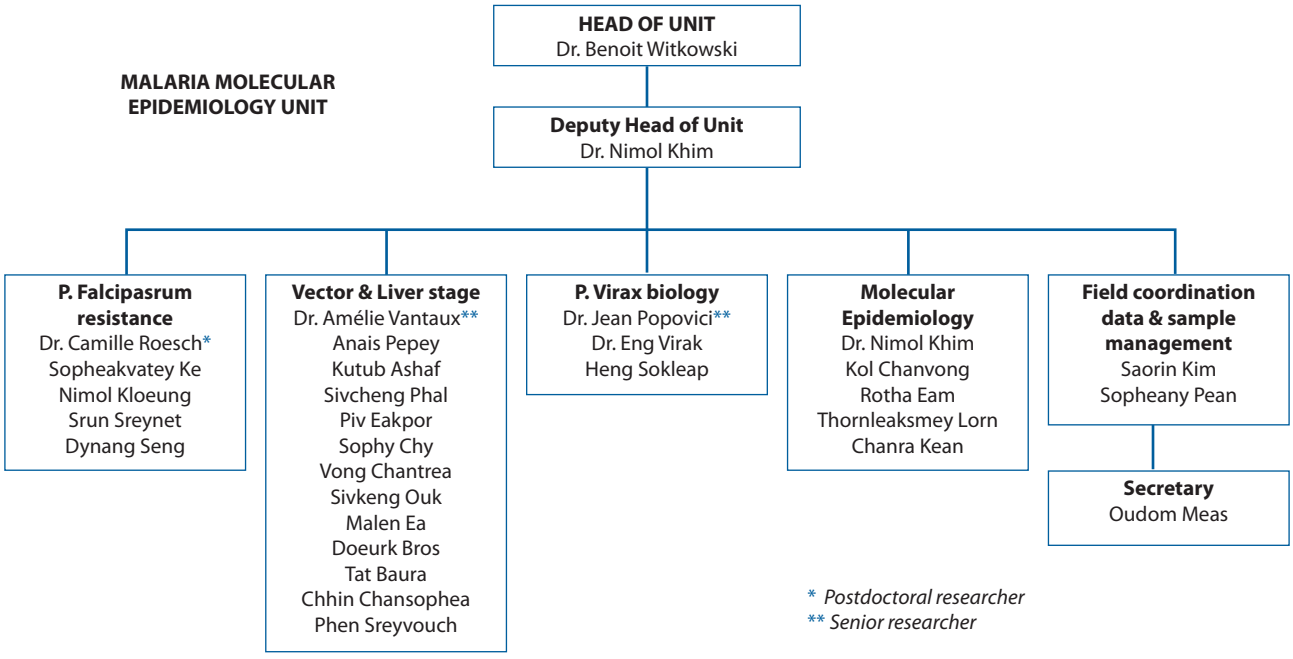


Figure 5. Malaria Molecular Epidemiology Unit Organogram

4.1.2. Research Programs – Major Achievements in 2021

Our unit's research activities are designed to provide insights useful for malaria programming. These projects are mainly focused on Cambodia's key public health challenges but have implications beyond the country. Our activities are divided in three main axes: the chemotherapy component in malaria control, the gap in *P. vivax* control and the design of innovative interventions through epidemiological knowledge.

Axis 1: Chemotherapy of Malaria Parasites

***P. falciparum* drug resistance surveillance.** The surveillance of drug resistance in the Greater Mekong Subregion (GMS) is a major activity. This program is supported by the GF with a two year project entitled RAI3-resistance. The main 2021 achievement was the description of parasite resistance profiles collected as part of therapeutic efficacy studies in Cambodia, Vietnam, Laos, Pakistan and Sudan. Several hundreds of samples were characterized in order to provide an accurate epidemiological picture of the resistance. The conclusion was that parasite resistance to the artemisinin based combination therapies (ACT) in use locally is still restricted to Mekong countries. A major persistence of multi resistant parasites was observed in Vietnam despite the change in national treatment guidelines. As mentioned no expansion of resistance was detected in Pakistan, corroborated by the optimal efficacy of the treatments used. In Sudan we observed the transmission of parasites that could be resistant to artemisinin derivatives with a relatively high prevalence. These parasites are unrelated to the haplotype detected in Asia which suggests the phenomenon of local emergence. Additional investigations are planned to determine whether these parasites represent a public health concern. A secondary objective was to determine the population structure of the parasites circulating in GMS. Preliminary results showed a remarkable drop in the parasite's diversity. In addition to its implications for surveillance, this research program builds on previous research activity on drug resistance in Cambodia. This aspect facilitates a better understanding of the mechanisms of counter-selection within the parasite population and led to a very important conclusion on the benefits of treatment policy set by Cambodia authorities in 2017. Data generated through this project are still being analyzed, the main results have been shared between the World Health Organization (WHO) and local health authorities and several proposed publications will be submitted in 2022.

Research Project Name	RAI3 Molecular Markers
Support	Global Fund through WHO
Project duration	2021-2023
External collaborator	NMCPS, WHO, CNM (Dr. Rithea Leang)

***P. falciparum* resistance emergence.** Although the drug resistance of *P. falciparum* to artemisinin derivatives (ART) is well described, in Africa, more work is needed to disseminate this knowledge. The existing knowledge base, tools and capacities that now exist position our unit to be part of drug resistance focused projects at the international level. Thus, we have joined the CARAMAL project managed by the Swiss Tropical and Public Health Institute (STPH). The rationale of this project was to measure the efficiency of single rectal dose of artesunate (RAS) administration by community workers in order to mitigate severe malaria impact. This initiative was set in three countries: Democratic Republic of Congo (DRC), Uganda and Nigeria. Our specific role was to determine whether this approach would leverage the selection of artemisinin resistant (ART-R) parasites. The study design included first determining K13 mutant emergence through the comparison of pre- and post-RAS periods. No resistance or putative resistance emergence was detected in DRC or Nigeria. Conversely, we determined a major increase in resistant parasites prevalence in Uganda (0% to 6.5%). Stratified analyses showed this proportion was even higher 28 days after RAS administration (21%). Genetic editing enabled us to show that the mutation detected conferred a resistance phenotype to the parasites. Through whole genome sequencing approaches we determined these mutations emerged locally. The detailed data related to this project are available at this stage in a preprint format (Awor & Khim et al. 2021 SSRN). The overall

conclusion was that RAS could promote, as do all monotherapies, the selection of ART-R in Africa. However, the RAS- by itself- is not the main factor responsible for the drug pressure on the parasite population and the issue of inadequate use of injectable artesunate in the adult population should now be addressed in these settings.

Name	CARAMAL-RESISTANCE
Support	UNITAID
Project duration	2019-2021
External collaborator	STPH (Dr. Manuel HETZEL), (Dr.Phyllis AWOR), Institut Cochin (Pr. Frederic ARIEY), IRD (Dr. Jérome CLAIN, Dr. Romain COPPEE)

***P. falciparum* resistance: alternative therapeutics.** In addition to improving drug resistance characterization, our unit is involved in a complementary objective focused on the determination of compounds that would be effective against resistant parasites. This activity has been supported by the Medicines for Malaria Venture (MMV) since 2013. The aim is to measure how major leading candidates in the new antimalarial pipeline would behave vs. clinical isolates from Cambodia presenting a broad range of resistance profiles. The main result is the identification of several new candidates presenting a notable antimalarial activity independently to the multi resistance profiles that circulate in Cambodia. These results are available in a recent publication (De Vries et al. Nature Com. 2022) and a complementary manuscript is in development (Roesch et al.). In addition to our contributions on drug discovery we've developed a secondary objective focusing on alternative therapeutics in Africa.

The use of *artemisia annua* and *afra* phytotherapies against malaria is an important recent trend in Sub-Saharan Africa that requires investigation on a high-priority basis. The justification for using said practices are yet unverified and could seriously jeopardize malaria control through the possible selection of resistant parasites. Therefore, we have initiated a research component aimed at generating evidence regarding artemisia based phytotherapies. We first evaluated the in vitro activity of *artemisia* against *P. falciparum* clinical isolates. We've determined that *artemisia annua*, the widespread used plant, presented important in vitro activity that was dramatically reduced by the resistance of the parasites to ART. We also determined the activity of artemisia afra was important and not modulated by the resistance but presented a low specificity and potentially significant toxicity. In addition we've evaluated the capacity of artemisia annua to select resistant parasites. Preliminary results suggest that the use of these phytotherapies may present issues regarding the emergence of resistant parasites in Africa. A manuscript detailing these results is being prepared and will be submitted for review and publication this year (Roesch et al.)

Name	ACT-R (MMV); Artemisia (AVIESAN)
Support	MMV/ AVIESAN
Project duration	2021-2022 (MMV); 2020-2021 (AVISEAN)
External collaborator	IP (Dr. Jean-Christophe BARALE), IPMC (Dr. Dominique MAZIER)

Therapeutic options against *P. vivax* liver stages. *P. vivax* is characterized by the formation of sleeping stages called hypnozoites responsible for the chronic nature of this infection. Because of this feature, *P. vivax* will be extremely difficult to eradicate. Although the amino-8-quinolines (primaquine (PQ) mainly) are standard therapeutics for the radical cure of *P. vivax* infection, these drugs present several issues making

them suboptimal for large scale population use. In certain patients presenting glucose-6-phosphate deshydrogenase (G6PD) deficiency, PQ treatment led to severe hemolysis that may be responsible for patient death. Unfortunately, Cambodia is one of the malaria hotspots where G6PD deficiency is the most widespread. For these patients PQ usage is unsafe or presents an unfavorable risk/benefit balance thus accelerating the need to discover a new class of drug able to target hypnozoites but that would be safer. We have developed an activity capable of medium throughput screening of drug targeting this stage making our unit one of the few laboratories able to perform this research at a global level. In terms. To date, 8,000 molecules have been screened using our platform in 2021. This activity is supported by MMV. In parallel we have developed a research program (National Institute of Health-(NIH) funded) with our collaborator from Uganda to explore the most promising hits family, better understanding their mechanism of action and evaluating their potency to become future effective and safe treatments.

Name	LS project (MMV) and (NIH)
Support	MMV/NIH
Project duration	2019-2024 (NIH); 2021-2022 (MMV)
External collaborator	UGA (Pr. Dennis KYLE, Dr. Steven MAHER, Dr. Anthony RUBERTO, Roman MANETSCH)

Filling the gaps on *P. vivax* elimination. *P. vivax* is now the main species encountered in GMS. In Cambodia it represents 90% of registered symptomatic cases. This parasite will be inherently more difficult to eradicate because of its specific biology that causes chronic infections but also because of the limitations in the methods developed so far. The overall aim of this axis is to fill this gap in order to help identify the most relevant future elimination strategies.

Deciphering malaria epidemiology in Cambodia. Our unit is part of the Asia-Pacific International Center of Excellence in Malaria Research (Asia-Pacific International Center for Excellence in Malaria Research (ICEMR) Program Director I. Muller/ L. Robinson) which aims at addressing the key challenges to malaria elimination in the Asia-Pacific. A coordinated set of in-depth studies into the epidemiology, entomology and biology of residual malaria transmission in three sites spanning the entire Asia-Pacific transmission gradient from moderately and high transmission in Papua New Guinea to low, highly focal transmission in Cambodia are carried out. In order to provide a comprehensive picture of malaria epidemiology we have established a cohort of 650 individuals in Mondulkiri Province. The follow-up was completed in late 2020 and the results analyzed in 2021 based using polymerase chain reaction (PCR). These data enabled us to identify several aspects of the malaria dynamic.

The first point is the quasi-elimination of *P. falciparum* consistent with the dramatic drop of documented cases. This suggests that the theory of a reservoir represented by asymptomatic carriers does not align with our observations. Conversely, we observed a persistence of *P. vivax* with an unchanged prevalence in asymptomatic infections despite the number of symptomatic cases reducing by a factor of 5 in the last two years. Importantly, the indicators from our cohort showed the populations experienced symptomatic infections as well but those were mainly undetected because of low parasitic densities. Altogether these data suggest *P. vivax* persists in a reservoir untargeted by current control measures. To better quantify this reservoir, a serology based measurement of the population exposure to *P. vivax* is currently ongoing. To better understand the different vector species ecology and their respective roles within malaria transmission, we

established 37 collection sites and four collection methods across a land use gradient in a district in Mondulkiri Province, Northeastern Cambodia having both high malaria prevalence and deforestation rates in 2019 and 2020. We collected mosquitoes from 24 to 72 hours per site, using human-baited double net (HDN) traps, human-land catching (HLC), barrier screens and Centers for Disease Control (CDC) light traps. We detected no significant difference between HLC and HDN relative abundance and prevalence, but the abundance and the diversity were greater in HDN traps. We collected 5,389 female *Anopheles* with HDN traps and determined the environmental variables associated with their prevalence or their abundance. We recorded that during the first round, *Plasmodium* prevalence was highest in fields (October-November, rainy season: 5.35 ± 0.05%) and during the two other rounds in the forest (January-February, dry season: 5.13 ± 0.22%; July-August, rainy season: 3.05 ± 0.04%). Prevalence was positively associated with forest cover in a 2 km radius and negatively associated with fragmentation in a 2 km radius. In addition, there were more Anopheles in fields and forest, compared to tree plantations and villages. Overall, deforestation, which is associated with increased temperatures and the expansion of fields, might increase vectors abundance and diversity, or to the contrary, drive malaria elimination as forest areas diminish and fragmentation increases. From a public health perspective, further attention and effort is required in fields and agricultural areas in addition to continued work in forests.

Since a SEROTAT study was implemented in Kaev Seima District we added a round of mosquito vector collection using human-baited odor traps in the field and forest sites to evaluate malaria prevalence in vectors before and after the intervention as well as to have a longitudinal overview of the evolution of malaria prevalence in the area. The 4,045 anopheles samples collected in 2021 are currently being processed.

We investigated the fine-scale mobility of forest-goers to understand how and when their daily movement patterns may affect malaria transmission by using questionnaires and global positioning system (GPS) data loggers for measuring mobility and by using parasites detection via RT PCR and anti-*Anopheles* saliva antibodies quantification via ELISA. Participants risk exposure varied according to their village forest coverage, all within a few kilometers of each other; participants from outside the forest were significantly more exposed than participants from the forest fringes. The GPS data did not highlight risk factors linked to the time spent in a specific environment, particularly the time spent in the forest was not associated with a higher probability of detecting malaria. The levels of antibody response among participants were significantly higher in the rainy season and for participants using mosquito protection in the deep forest. We conclude that the exposure to *Anopheles* infectious bites is similar across study participants, as they all belong to the risk population sharing the same mobility habits and socio-economic characteristics. *Plasmodium vivax* relapses and participants' immunity may interfere with the observed malaria prevalence, explaining the lack of evidence of risk heterogeneity. Finally, after training of one of our PhD students in Melbourne, we transferred the Deep Amplicon Sequencing (DAS) method to our lab in Phnom Penh in 2021. We used samples from the two ICEMR epidemiological studies (GPS follow-up and cohort studies) and the vector collections carried out in our field site in Mondulkiri Province. Amplification was not successful in about half of the samples, for both *P. falciparum* and *P. vivax*. Because most of the samples were from *Anopheles* vectors (low volumes) and from asymptomatic cases (low parasite density), we can expect that the amount of available parasite DNA was not suitable for amplification. Another hypothesis is that circulating parasites from Cambodia are not suited for the selection of molecular markers that were developed on samples from other countries, with different transmission dynamics and intensity. As orders and shipments had lengthy delays detailed analyses are still ongoing.

Name	ICEMR Asia-Pacific
Support	NIH
Project duration	2017-2024
External collaborator	WEHI (Pr. Ivo MULLER), Burnett Institute (Pr. Leanne ROBINSON), IP (Dr. Michael WHITE)

Evaluation of point of care G6PD test. Southeast Asia is a main focus of human G6PD deletion. This genetic trait triggers severe adverse events following administration of certain drugs, 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 population. The existing methods used to measure G6PD activity and adapted to field conditions so far only include qualitative ones (spot test, RDT). The qualitative aspect is an important limitation that restricts valid results to male. To safely administer PQ to females, there was a need for quantitative field implementable assays. In this context we are therefore evaluating novel G6PD field tests (quantitative) and are determining whether these devices can safely spot G6PD deficient individuals. More than 1,400 individuals were screened with G6PD point of care (POC) and the results compared with spectrophotometric gold standard methodology. A manuscript now in preparation that presents the definitive conclusions of this study will be submitted in 2022.

Name	G6PD POC evaluation
Support	MMV/NIH
Project duration	2020-2022
External collaborator	CDC (Dr. Jimmee Wang), PMI (Dr. Michael Thigpen), CNM (Dr. Lek Dysoley)

Vaccinal approaches in *P. vivax* control. Thanks to the development of in vitro short-term cultures in the MMEU in the past years, we are now able to evaluate blood-stage vaccine candidates to determine how they block invasion of parasites in erythrocytes. We previously showed that plasmodium vivax duffy binding protein (PvDBP) gene amplification led to a marked decrease in susceptibility of *P.vivax* isolates to neutralization by anti-PvDBP antibodies. These data, based on in vitro experiments, suggest that this gene amplification is an immune evasion mechanism for *Pv* to evade host humoral anti-PvDBP antibodies. To confirm those in vitro observations and determine how they translate into in vivo protection for the parasites, we started an intra-population and intra-host analysis of the association between this gene amplification and the capacity of parasites to infect immune individuals. These analyses are ongoing and they will be crucial to determine how likely a PvDBP-based vaccine against *Pv* (currently the most advanced vaccine candidate in Phase II clinical trials) will select for PvDBP-amplified parasites. We are also interested in evaluating the in vitro blocking efficacy of other *P. vivax* targets, PvRBP2b, described as a key ligand for RBC invasion but also reported as a highly immunogenic protein and excellent marker of exposure, as well as PvAMA and PvRBP2a. We have completed the analysis for PvRBP2b and have demonstrated that none of the antibodies targeting this parasite protein allow for in vitro neutralization. Similarly, while blocking the receptor of PvRBP2b on the surface of the immature erythrocyte (the transferrin receptor) allows the inhibition of PvRBP2b-RBC binding, we have shown that it does not prevent the in vitro invasion of parasites. Our data suggest that PvRBP2b is dispensable for parasite invasion and might not be worth pursuing as a vaccine candidate by itself. We are now determining if combining both targets PvRBP2b and PvDBP might improve the neutralization of parasites by anti-PvDBP in particular of isolates with multiple

copies of the PvDBP gene refractory to in vitro neutralization. Our work on PvAMA1 and PvRBP2a will start in the coming weeks. Finally, to identify blood-stage vaccine targets that could lead to universal protection we have initiated a study to determine the relevance of targeting PvDBP for parasites able to invade Duffy negative individuals, theoretically lacking the Duffy receptor on the surface of their RBC despite being infected by *P. vivax*. Altogether this work will pave the way for evidence-based vaccine candidate selection.

Name	RBC invasion blocking
Support	NIH
Project duration	2020-2025
External collaborator	Case Western Reserve University (CWRU) (Chris L King), WEHI (James Beeson, Wai-Hong Tham), UNCC (Eugenia Lo)

Optimal drug regimen for *P. vivax* radical cure. PQ represents the gold standard molecule used for the *P. vivax* radical cure. Beside the hematotoxicity of this drug on G6PD- subject, there is no consensus on the posology to adopt. Moreover, the individual drug efficacy is poorly characterized notably because of the difficulty proposing suitable follow-up design. Understanding these aspects is critical for the elimination of *P. vivax*. To address these different points, we have developed a collaborative project with UMD aiming to determine the most effective regimen of PQ. To avoid bias due to reinfection, we are performing a follow up of enrolled patients after a relocation in a non-transmission area. In addition to the therapeutic aspect the secondary objective of this project is to better understand the molecular signature of parasite relapses. This project has started this year and 25 patients have been enrolled so far. The results at this stage are partial and will be presented in a future report.

Name	PQRC
Support	NIH
Project duration	2020-2025
External collaborator	UMD (Dr. David SERRE)

Innovative methods for eliminating *P. vivax*. The conclusions obtained through the comprehensive analysis of malaria epidemiology open for consideration of possible next intervention design to eliminate malaria. These data lead IPC to conclude that P. falciparum is on the verge of elimination but a reservoir of P. vivax remains. This reservoir is represented by populations at risk of exposure, immune and who rarely present parasite density sufficient for testing positive on a rapid diagnostic test (RDT). Therefore, a rational approach is to propose a test and treat strategy focused on the population most at risk of being in contact with the parasite recently. Because of its inconstant presence in the blood, the methods aiming for direct detection (PCR) of the parasite are not sufficiently resolutive. Instead we will propose a serology-based methodology aiming at the characterization of recently exposed individuals followed by a radical cure treatment. Our objective is to provide evidence about the feasibility and the efficacy of this strategy in rural Cambodia. Our study design was to select three control villages and three intervention villages. A serology-based test and treat (SEROTAT) intervention will be conducted in three target villages based on measurement of immunoglobuline titles against nine P. vivax antigens followed by administration of 14 days of PQ in case of positivity. The measurement of the prevalence is done from cross sectional surveys (CSS) and molecular detection of malaria parasites in the inhabitants. This first approach will be focused on adult males

to maximize the possible effect of the intervention and for logistical reasons.Our first CSS data showed that despite the continuous reduction in reported cases, the prevalence of P. vivax remains important in our target population and comparable to the rate observed in 2018, 2019 and 2020. This year the first part of the intervention will be launched and the final outcomes from this project will be available in 2023.

Name	RAI3 SEROTAT
Support	Global Fund
Project duration	2020-2023
External collaborator	WEHI (Pr. Ivo MULLER), CNM (Dr. Lek DYSOLEY)

4.1.3. Research Programs – Outlook for 2022

Research Plan Overview

The research plan for the MMEU is structured around the following axes, all ultimately revolving around elimination of malaria:

- dynamics of malaria in Cambodia;
- therapeutic options and drug resistance (multi-centric);
- biology of *P. vivax* and novel approaches for tailored interventions.

Dynamics of malaria in Cambodia

ICEMR program **Funding source: NIH**

A longitudinal cohort was constituted and cross-sectional surveys were conducted in Mondulkiri, eastern Cambodia. Additional cross-sectional surveys will be performed in the same area. 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 magnitude of malaria exposure in the population. Entomological investigations will continue to evaluate the distribution and behavior of malaria vectors in the study area. All of these lab-based data will be associated with epidemiological information. We will also investigate parasite population structure through an amplicon sequencing approach. All this information should bring new insights on malaria epidemiology and will enable a better tailoring of specific control measures. In addition, we will provide additional metrics in terms of malaria prevalence in the frame of the RAI3 intervention project (see below).

Therapeutic & Drug resistance

Drug resistance epidemiology **Funding: WHO**

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 remains highly relevant. The drug resistance epidemiology in GMS will remain as emergence of new strains could jeopardize malaria control measures while investigation in other sites (Africa, Pacific, Central Asia) will increase as early emergence of drug resistance has been factually identified. Funding has been secured to perform these activities.

Drug candidates **Funding: MMV/UGA, MMV**

The antimalarial therapeutic approaches still present some important gaps that need to be filled. These are 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* is now emerging in Africa. Therefore, our efforts toward the identification of molecules suitable to these situations will be maintained. The funding necessary to perform these activities is already in place.

Therapeutic efficacy of primaquine **Funding: NIH**

To date amino-8-quinoline are the only drugs that provide effective antiparasitic response against hypnozoite. Among those, primaquine is the only registered drug in Cambodia for this purpose. So far, there has been no rigorous assessment of the therapeutic efficacy nor data on globally recommended regimens in Cambodia. In November 2021 we launched a randomized PQ efficacy trial to determine whether the recommended regimen could support *P. vivax* control in Cambodia. This trial will include 260 patients. In addition to this clinical trial we are also aiming to better decipher parasite relapses chronology and mechanisms. Funding has been acquired to perform these activities.

Biology of P. vivax and novel approaches for tailored interventions

SEROTAT **Funding: Global Fund**

While *P. falciparum* is on the verge of elimination in Cambodia, *P. vivax* appears to be more stubborn to the measures implemented. The experience gained out of the ICEMR project indicates that a vast proportion of individuals living in endemic areas and undertaking "at risk" activities are carriers of hypnozoites. These infections are mainly silent for several months but these individuals represent a reservoir for further transmission. We launched an operational study in 2022 aiming to detect and treat these carriers. The method will be sero-diagnosis coupled with PQ therapeutics (serological test and treat). This is a feasibility study to determine if a roll out of this methodology would make sense in Cambodia. Funding is in place to perform these activities.

Receptor-ligand interactions involved in red blood cell invasion **Funding: NIH**

Understanding the molecular mechanisms involved in the invasion of *P. vivax* into erythrocyte will be critical to develop a blood-stage vaccine against this species. Thanks to our capacity to perform in vitro short-term cultures of *P. vivax*, in collaboration with colleagues from Case Western Reserve University and WEHI, we are determining which parasite proteins can be targeted by antibodies to allow for neutralization. Our work also aims at determining how parasites evade host immunity targeting such candidates to identify early enough any pitfalls during this preclinical vaccine development process. We currently focus our work on PvDBP, PvRBP2b, PvRBP2b and PvAMA1. Also, in collaboration with colleagues from University of North Carolina, Charlotte, and from Ethiopia, we are launching a project that aims to identify the mechanisms enabling *P. vivax* to invade Duffy negative red blood cells, an erythrocyte phenotype common in Africa where increasing evidence of *P. vivax* infections are reported. Funding has been secured to perform these activities.

Factors involved in *P. vivax* growth **Funding: NIH**

We know very little on the factors influencing the growth of *P. vivax* in its human hosts. We are starting a project that aims at deciphering the contribution of human factors (specifically G6PD deficiency and HbE

hemoglobinopathy) and of parasite genotypes on the erythrocyte invasion and development of *P. vivax*. Using a combination of scRNA-seq, genotyping and in vitro phenotype, we aim at better understanding how the parasite develops in its host. Funding is in place to perform these activities.

4.1.4. Support to National Authorities

The IPC malaria unit works in support of the Cambodian Ministry of Health. Specifically, our unit is a main collaborator and a technical partner to the Cambodia National Malaria Control Program (NMCP) managed by the National Center for Parasitology, Entomology and Malaria Control (CNM). Our unit offers its support to drug efficacy studies that are conducted yearly in Cambodia.

4.1.5. Teaching and Training

PhD students:
Kutub Ashraf: *P. vivax* liver stage (defense in June 2021)
Anais Pepey: Ecology of malaria transmission (defense in June 2022)

Teaching:
Benoit Witkowski provided a 1.5 hour lecture to first year Master's students at the joint program offered by the Cambodian University of Health Sciences (UHS) and Université de Paris-Saclay, coordinated the drug resistance module for second year master's students at the same program, and provided 3 hour lectures on malaria drug resistance and introduction of chemoresistance at the second year master's level.

Jean Popovici provided a 1.5 hour lecture on parasitology first year Master's students at the joint program offered by UHS and Université de Paris-Saclay, a 4.5 hour tutorial for the same students on article analysis, a 6 hour tutorial on parasitology-mycology during the year. He also provided 1.5 hours on antigenic variation in plasmodium parasites for second year master's students.

Amelie Vantaux coordinated the module on host/bacterial and parasitic pathogen intereractions to first year master's students at UHC/Saclay.

4.1.6. Publication List 2021

NOTE

The name of authors from the Institut Pasteur du Cambodge are underlined.
Publications in a journal without impact factor are listed separately and identified at the end of the list
** equal contribution, first author / ** equal contribution, last author*

1. ***Anopheles ecology, genetics and malaria transmission in northern Cambodia***
Amélie Vantaux, Michelle M. Riehle, Eakpor Piv, Elise J. Farley, Sophy Chy, Saorin Kim, Anneli G. Corbett, Rachel L. Fehrman, Anais Pepey, Karin Eiglmeier, Dysoley Lek, Sovannaroth Siv, Ivo Mueller, Kenneth D. Vernick**, Benoit Witkowski**
Sci Rep. 2021;11(1):6458. DOI: 10.1038/s41598-021-85628-1

2. ***Artemisinin-independent inhibitory activity of Artemisia sp. infusions against different Plasmodium stages including relapse-causing hypnozoites***
Kutub Ashraf*, Shahin Tajeri*, Christophe-Sébastien Arnold, Nadia Amanzougaghene, Jean-François Franetich, Amélie Vantaux, Valérie Soulard, Mallaury Bordessoulles, Guillaume Cazals, Teun Bousema, Geert-Jan van Gemert, Roger Le Grand, Nathalie Dereuddre-Bosquet, Jean-Christophe Barale, Benoit Witkowski, Georges Snounou, Romain Duval, Cyrille Y. Botté, Dominique Mazier.
Life Science Alliance. Life Science Alliance; 2021;5(3). DOI: 10.26508/lsa.202101237

3. ***Characterization of the Tubovesicular Network in Plasmodium vivax Liver Stage Hypnozoites and Schizonts***
Kayla Sylvester, Steven P. Maher, Dora Posfai, Michael K. Tran, McKenna C. Crawford, Amélie Vantaux, Benoît Witkowski, Dennis E. Kyle, Emily R. Derbyshire.
Frontiers in Cellular and Infection Microbiology; 2021. DOI: 10.3389/fcimb.2021.687019

4. ***Choosing interventions to eliminate forest malaria: preliminary results of two operational research studies inside Cambodian forests***
Amber Kunkel, Chea Nguon, Sophea Iv, Srean Chhim, Dom Peov, Phanith Kong, Saorin Kim, Sarun Im, Mark Debackere, Nimol Khim, Jean Popovici, Sreynet Srun, Amélie Vantaux, Jean-Olivier Guintran, Benoit Witkowski, Patrice Piola.
Malar J. 2021;20(1):51. DOI: 10.1186/s12936-020-03572-3

5. ***Cross-resistance of the chloroquine-derivative AQ-13 with amodiaquine in Cambodian Plasmodium falciparum isolates***
Flore Nardella, Mélissa Mairet-Khedim, Camille Roesch, Steven P Maher, Sopheakvatey Ke, Rithea Leang, Didier Leroy, Benoit Witkowski.
Journal of Antimicrobial Chemotherapy. 2021;76(10):25658. DOI: 10.1093/jac/dkab219

6. ***Efficacy of dihydroartemisinin/piperaquine in patients with non-complicated Plasmodium falciparum malaria in Yaoundé, Cameroon***
Mélissa Mairet-Khedim, Sandrine Nsango, Christelle Ngou, Sandie Menard, Camille Roesch, Nimol Khim, Sreynet Srun, Xavier Iriart, Thomas Lanot, Laure Otam, Francis Abega, Lawrence Ayong, Isabelle Morlais, Peggy Gandia, Benoit Witkowski, Antoine Berry.
Journal of Antimicrobial Chemotherapy. 2021;76(11):303744. DOI: 10.1093/jac/dkab281

7. ***Probing the distinct chemosensitivity of Plasmodium vivax liver stage parasites and demonstration of 8-aminoquinoline radical cure activity in vitro***
Steven P. Maher*, Amélie Vantaux*, Victor Chaumeau, Adeline C. Y. Chua, Caitlin A. Cooper, Chiara Andolina, Julie Péneau, Mélanie Rouillier, Zaira Rizopoulos, Sivchheng Phal, Eakpor Piv, Chantrea Vong, Sreyvouch Phen, Chansophea Chhin, Baura Tat, Sivkeng Ouk, Bros Doeurk, Saorin Kim, Sangrawee Suriyakan, Praphan Kittiphanakun, Nana Akua Awuku, Amy J. Conway, Rays H. Y. Jiang, Bruce Russell, Pablo Bifani, Brice Campo, François Nosten, Benoît Witkowski, Dennis E. Kyle.
Sci Rep. 2021;11(1):19905. DOI: 10.1038/s41598-021-99152-9

8. ***Procainamide-SAHA Fused Inhibitors of hHDAC6 Tackle Multidrug-Resistant Malaria Parasites.***
Flore Nardella, Ludovic Halby, Irina Dobrescu, Johanna Viluma, Corentin Bon, Aurélie Claes, Véronique Cadet-Daniel, Ambre Tafit, Camille Roesch, Elie Hammam, Diane Erdmann, Melissa Mairet-Khedim, Roger Peronet, Salah Mecheri, Benoit Witkowski, Artur Scherf, Paola B. Arimondo.
J Med Chem. 2021;64(14):1040317. DOI: 10.1021/acs.jmedchem.1c00821

4.2. Epidemiology & Public Health Unit

4.2.1. Functional Structure

The epidemiology and public health (EPH) unit, conducts operational epidemiological research studies on major public health challenges in Cambodia. In 2021, the unit was composed of 45 people, was headed by Dr. Patrice Piola and organized around four individual but complementary groups (Figure 6). The four groups are:

- **The Community Epidemiology Group (CEG)**, led by Dr. Sowath Ly, who has extensive experience in community epidemiology research projects on rabies, dengue, avian influenza and investigations of epidemics and health alert situations ;
- **The Clinical Research Group**, led by Dr. Laurence Borand, which has been piloting clinical trials and contributing to guide international prevention, diagnosis and treatment strategies for vulnerable populations with HIV, tuberculosis, hepatitis and some vaccine preventable and emerging infectious diseases.
- **The One Health Group**, led by Dr. Véronique Chevalier, is part of a collaboration between CIRAD and IPC and enabling to tackle the human and animal components of disease transmission and prevention in a constructive and coordinated approach. Its focus is on zoonotic and vector-borne diseases.
- **The Forest Malaria Group**, led by Dr Patrice PIOLA in charge of the research project on residual transmission of forest malaria. Research projects conducted by the epidemiology unit are carried out in close collaboration with other IPC research units, depending on the subject.

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 Cambodian CDC-MoH, the National Immunization Program (NIP), the National Animal Health and Production Research Institute (NAHPRI), the Council for the Development of Cambodia (CDC), and the National Center for Parasitology, Entomology and Malaria Control (CNM) to a name a few. The unit's projects would not be possible without the interest and contribution of several reference hospitals in Phnom Penh and across the country, including the Calmette Hospital, the National Maternal Child Health Center (NMCH), 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 Avenir, Gillings Public Health Fellowship, the World Health Organization, UNITAID, L'Initiative-Expertise France, and the Agence Française de Développement (AFD).

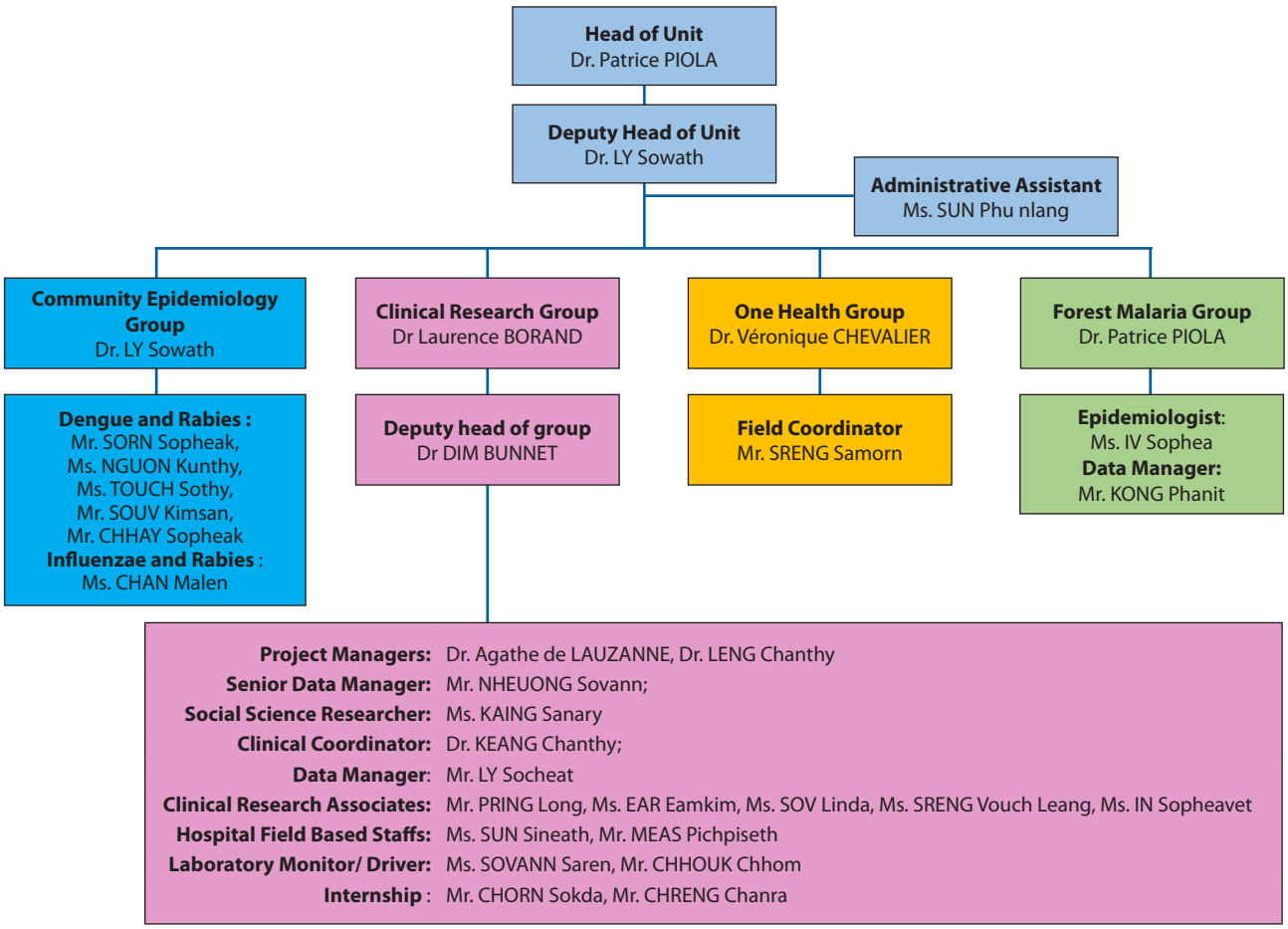


Figure 6. Epidemiology and Public Health unit flowchart, 2021

4.2.2. Research Programs - Major Achievements In 2021

Axis 1: Viral Diseases

Rabies

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

The aim of the study is to evaluate the protective antibody response survival time, following a cohort of approximately 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	Institut Pasteur du Cambodge: (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.

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.

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

Rabies - One Health

An extensive door-to-door longitudinal survey in Battambang and Kandal was carried out to estimate dog population demographic parameters, identify dog ownership determinants, and analyze dog management practices. The estimated yearly cumulative bite incidences, 3.1% and 2.3% in Battambang and Kandal respectively, were found among the highest in the world.

Collaborations	V. Chevalier. In coll with IPC, ANSES Maisons Alfort, Davis University
Funding	Swedish Research Council, Région Occitanie, Davis University (2019-2022)

Arboviruses

Japanese encephalitis

We assessed the capacity of the epidemiological system to be invaded by JEV and sustain virus transmission in villages in 3 districts. We highlighted that JEV could invade a multi-host system with no pigs, reinforcing the assumption of poultry acting as reservoirs. The annual human exposure probability appeared linearly correlated with dog seroprevalence, suggesting that dog seroprevalence would be a good proxy for human exposure.

Collaborations	V. Chevalier. In coll with IPC, ANSES Maisons Alfort
Funding	French Ministry of Agriculture

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 and alvino Maestru- Netto (Immunology Unit), Veasna Duong (Virology Unit),Olivier Ségéral, Jayavarman VII Hospital
Funding	ANRS-MIE - IPC

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.

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

Coronaviruses

ECOMORE 2 Top-Up COVID

The Top-Up COVID project is a complementary component of the Ecomore 2 project, which provided a significant support to Ecomore 2 partner institutes, as well as Pasteur Network institutes in Vietnam (Institut Pasteur in Nha Trang and Institut Pasteur in Ho Chi Minh), that were on the front line in the fight against COVID-19. This support provided by the epidemiology unit and the group geo-health helped the institutes to implement surveillance activities at national or regional scale and to implement the epidemic response.

Collaborations	IPC – Virology (Veasna Duong, Narjis Boukli), IPC - Epidemiology (Sowath Ly, Sopheak Sorn), IRD - GeoHealth (Vincent Herbreteau, Florian Girond, Lucas Longour)
Funding	AFD, 2020-2022 (complementary to ECOMORE 2)

ZooCov

ZooCov project "Toward an integrated surveillance of potentially zoonotic Beta Coronaviruses in the wild animal value chains of Cambodia" aimed to collect information on wildlife trade and live animal market networks, as well as practices and perception of bushmeat trade and consumption at the human/wildlife interface in Stung Treng and Mondulkiri provinces. Wild animals and human samples are investigated. Next steps will be the identification of risk factors of exposure of humans to BetaCove.

Collaborations	V. Chevalier. In collaboration with IPC, IRD, WCS (Wildlife Conservation Society), International Development Enterprises (IDE), Hong Kong University
Funding	ANR, Région Occitanie, Pasteur Foundation (2020-2022)

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 ≤ 100 cells/µL)

Mortality in people entering late into HIV care with a tuberculosis (TB) co-infection is high. The objective of the DATURA clinical trial is to estimate the impact of an intensified initial phase of tuberculosis (TB) treatment on mortality at 48 weeks among HIV-infected adults and hospitalized adolescents for TB with CD4 ≤ 100 cells/µL in comparison with the standard TB regimen.

Collaborations	Team leaders: Laurence Borand (Clinical Research Group), NCHADS, Cambodia-China Friendship Preah Kossamak Hospital, Khmer-Soviet Friendship Hospital, Calmette Hospital, NCHADS Clinic and Laboratory,
Funding	ANRS-MIE (2021-2024)

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

Previous investigations showed 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 (2020-2021)

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 diagnosis 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 & L'Initiative-Expertise France (2019-2021)

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) by assessing the impact of an alternative treatment intervention- 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. The Identification of barriers to TPT is completed. Recruitment and follow-up of patients is ongoing as is the intervention phase.

Hepatitis

TA-PROHM - ANRS 12345: Tenofovir as prevention of hepatitis B transmission for mothers

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
Funding	ANRS-MIE (2017-2021)

Pertussis immunization programs in low income countries (PERILIC)

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 on (1) the assessment of the serological status to diphtheria toxin in low and middle-income countries and (2) the characterization of the main relevant genes carried by B. pertussis strains collected in Cambodia.

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	Fondation Total (2017-2022)

Water and health risks in cambodia (Wat-Health)

Wat-Health aims at studying the exposure and vulnerability of rural populations to the most notable health hazards related to floods (leptospirosis, melioidosis, mosquito-borne diseases, exposure to pesticides), conducting investigations in the Mekong Delta. In 2021, the GeoHealth group worked to describe the land use and land cover with very high resolution satellite images. In 2022, the LBM and Epi Units will conduct a serological and survey to assess the prevalence of leptospirosis and melioidosis in the local population and explore the risk factors associated with these two diseases.

Collaborations	Wat-Health coordination: Sylvain Massuel (IRD - UMR G-EAU); KAP survey: Vincent Herbreteau (IRD – Epidemiology Unit) and Sowath Ly (Epidemiology Unit); IRD (G-EAU, MIVEGEC, Espace-Dev), IPC (LBM, EPI, Entomo), ITC, RUA
Funding	Fonds de Soutien pour les Projets Innovants (FSPI), 2021-2022

A Hospital-based case-control study to identify risk factors of leptospirosis and to improve post-disaster management of emerging diseases (ECOMORE 2 - WP Myanmar)

This study helped to develop the diagnosis of leptospirosis in hospitals within the Yangon agglomeration of Myanmar. An hospital-based case-control study allowed to explore socio-demographic and environmental risk factors of leptospirosis. The collaboration stopped due to difficulties with the political situation but data analysis restarted in 2021. In 2021, the GeoHealth group developed an online application for the prediction of suitable environments for Leptospirosis.

Collaborations	Team leader: Patrice Piola (Epidemiology Unit), NHL Myanmar (Htay Htay Tin, Khin Nyein Zan, May July), Institut Pasteur de Nouvelle Calédonie (Cyrille GOARANT), IRD-Espace-Dev (Vincent Herbreteau, Sylvaine Jégo, Lucas Longour)
Funding	ECOMORE 2 (AFD 2017-2022) – WP Myanmar

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 a diagnostic tool. The epidemiology unit was involved 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.

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	Fonds de Soutien pour les Projets Innovants (FSPI) – 2020-2022

Parasitological Diseases

Blocking malaria transmission in vulnerable forest populations through forest malaria workers: a key for malaria elimination in Cambodia

The main reservoirs of parasites in Cambodia are inside its forests. This study done in collaboration with MMEU aims at an in-depth understanding of malaria transmission inside three forests totaling 200km^2 (Year 1: 2019-2020) followed by an intervention (Year 2: 2020-2021) to eliminate in-forest malaria. Malaria cases notified in neighboring health centers were monitored to estimate the effectiveness of the intervention.

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	L'Initiative Canal 2: 17SANIN205 – 2019-2021

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, through continuous in-forest active mass screening and treatment (MSATs with RDTs) and continuous passive detection. Preliminary results showed a very low sensitivity of RDTs among forest goers, contributing to the decision to implement the IPTfg mentioned in the above study.

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 (2019-2021)

Cambodian forest people: anthropological study of an often-marginalized society important to malaria elimination

The main objectives of the study were to analyze the effectiveness of the Workers (FMWs) program and to investigate the perception of forest goers (FGs) regarding our forest malaria intervention. Main findings showed that forest population in Mondulkiri, Stung Treng and Kratie Provinces, is strongly influenced by a social hierarchy. The study highlighted the difficulty to access healthcare service in remote areas and the need to adapt our program to forest population traditional lifestyles and beliefs.

Collaborations	Team leaders: Téphanie Sieng, Patrice Piola (Epidemiology Unit). Malaria Consortium (MC), Partners for Development (PfD), National Center for Parasitology, Entomology and Malaria Control, Institut de Recherche pour le Développement (Frédéric Bourdier)
Funding	L'Initiative Canal 2: 17SANIN205 Resistance to Artemisinin Initiative 2 – Operational Research QSE-M-UNOPS (2019-2021)

4.2.3. Research Programs - Outlook for 2022

BCOming "biodiversity conservation to mitigate the risks of emerging infectious diseases" is a 6 million Euros project coordinated by CIRAD which aims to better understand the relation between biodiversity loss in biodiversity hotspots and disease emergence in order to prevent and react to future pandemics. It will be implemented in three tropical biodiversity hotspots with different environmental and socio-cultural settings in Southeast Asia (Cambodia), West Africa (Ivory Coast and Guinea) and the Caribbean (Guadeloupe) and within these, the sites will cover a range of ecosystems along a gradient of biodiversity from urbanized sites to pristine forests. In Cambodia, the project will help to understand and prevent the emergence of SARS-CoV-2-related coronaviruses that have recently been detected in cave-roosting rhinolophid bats in the northern part of the country.

Collaborations	V. Chevalier In coll. with CIRAD, IPC, IRD, Centre de Recherche et de Formation en Infectiologie de Guinée (CERFIG), Avia-GIS, Institut national de la recherche pour l'agriculture, l'alimentation et l'environnement (INRAE), NatureMetrics, Fauna & Flora International, Mekong Region Futures Institute, Université de Liège, International Development Enterprises (IDE), Universiteit Antwerpen, Europa Media
Funding	EU (2022-2026)

AFRICAM is a project developed in the framework of the Preventing Zoonotic Disease Emergence (PREZODE) initiative coordinated by CIRAD and IRD. It takes place in four African countries and in Cambodia. The main objectives of the project will be (1) to study the zoonotic risk at different interfaces between human, animal and environment taking into account the climatic and environmental dynamics; (2) implement activities to reduce the risk of emergence and (3) strengthen the existing surveillance systems toward a One Health and integrated surveillance. The epidemiology unit will handle different activities within the project and conduct a serological investigation in the Battambang study sites (three villages from Battambang cities to the Prek Toal Reserve).

Collaborations	Team Leader: Anne-Laure Bañuls (IRD - MIVEGEC), L. Sowath, H Guis, V. Herbreteau and C. Flamand in collaboration with IRD, CIRAD, IPC, Agronomes et vétérinaires sans frontières (AVSF), International Development Enterprises (IDE), ITC, Wildlife Conservation Society (WCS), Battambang Hospital.
Funding	AFD, 2022-2025

DogZooSea is a one-year project aiming to assess the potential role of dogs as sentinels of human exposure to arboviruses in two rural villages in Cambodia by comparing exposure and measuring seroconversion or reversion in paired dog owners and their dog. A similar approach will be carried out in Thailand and Indonesia as part of the SeaDogSea project.

Collaborations	Team Leader: Sowath Ly is co-PI, Helene Guis is co-I with colleagues of CIRAD and IPC. In coll with CIRAD, IPC, University of Kasetsart (Thailand).
Funding	French Ministry of Foreign Affairs French Embassy (FSPI OH)- 2022

RAMSES (Resistance to AntiMicrobials: Socio-Economic and regulatory factors influencing emergence and dissemination in the South) is part of an initiative of the AVIESAN "AMR South" network and aims to explore socio-economic and regulatory factors influencing AMR emergence and dissemination, understand the links between these factors and identify levers for improved AMR surveillance and control actions, in low- and middle- income countries (LMICs). It will focus on the socio-economic factors that shape the circulation of antimicrobials in society and the tools available to manage risks in humans, animals and the environment. The project will take place in Burkina Faso, Cambodia, Ivory Coast and Madagascar.

Collaborations	Scientific coordinators: Alexandre Hobeika (CIRAD), Adèle Kacou N'Douba (University of Abidjan) Country coordinator: Sowath Ly (IPC)
Funding	French Ministry of Foreign Affairs French Embassy (FSPI OH)

OHARAT (One health anthropological approach to rat-related knowledge and practices in Cambodia and beyond in Southeast Asia)

By analyzing the interconnection between people, animals, plants, and their shared environment, this one-year project aims to document how sociocultural local knowledge is linked and articulated with scientific knowledge. These investigations provide us a strong fieldwork to analyze the social representation of rats (rural-urban perceptions, differences between sites and between species), zoonoses and health practices by conducting in-depth ethnographic and multi-field research.

Collaborations	Meriem M'Zoughi (Center for Khmer Studies, IRD), Vincent Herbreteau (IRD)
Funding	Appel à Projets du FSPI One Health in South-East Asia (OHSEA, Fonds de Soutien pour les Projets Innovants), 2022

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, was reported to increase from 11% to 20% in a median time of 5.8 years. 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). Olivier Ségéral, NCHADS and OI/ART sites, Jayavarman VII Hospital, Kantha Bopha 1 and 2 Hospitals
Funding	ANRS-MIE (2022-2024)

4.2.4. Support to National Authorities

The following summarizes key support to Cambodian national authorities during 2021:

- V. Chevalier was a member of the Technical Zoonotic Working Group
- L. Borand is a member of the Cambodian 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
- B. Dim 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
- S. Ly's and V. Herbreteau's teams provided support in data management for Covid-19 lab testing at IPC and assured the transfer of quality data to the CDC-MoH.

4.2.5. Teaching and Training

PhD Students:

- Mrs. IV Sophea (University Paul Sabatier-Toulouse III from 2019-2023-Thesis Title: Blocking Malaria Transmission in Vulnerable Populations of the Forest, by the Forest Malaria Workers: Key to Eliminating Malaria in Cambodia).
- H. Ladreyt. Co-supervision with Dr. Benoit Durand (ANSES Maisons Alfort). « Modeling of Japanese encephalitis transmission in Cambodia ». Defense in 2021.
- J. Baron. Co-supervision with B. Martinez Lopez (Université de Davis University, Californie) (memory of understanding CIRAD/IPC/Davis)). «Evaluation of rabies burden in Cambodia and transmission modelling. Defense in 2021.

Masters Students:

- Dr. DIM Bunnet (LSHTM Clinical Trials-2019-2024).
- Mr. NHEUONG Sovann (UHS-Epidemiology from 2020-2023).
- Ms. SOV Linda (Master of Science in Epidemiology at NIPH, Cambodia, 2020-2022).
- Aude Tralci. Master II GIMAT. Characterization of human population at the human wildlife interface and risk factors of exposition to Beta Coronaviruses, Cambodia. Defense in 2021.

Student Internships:

- Mr. CHORN Sokda (Bachelor Geography and Land Management at RUPP; 2017-2021-Thesis Title: Impact of Chikungunya to Pregnancy Women at IPC).
- Mr. CHRENG Chanra (Bachelor Geography and Land Management at RUPP from 2017-2021)
- Mr. NHEOM Phanut (Bachelor Geography and Land Management at RUPP; 2018-Ongoing).
- Mr. Nathan CLAVEAU (Master Biodiversité-Ecologie-Evolution, parcours EPI, Université de Montpellier), 03/2021 - 06/2021. "Analyse spatiale de l'antibiorésistance à l'interface Homme/ Animal/ Environnement dans la province de Battambang au Cambodge. (ARCAHE Project)"

4.2.6. Publications List

NOTE

The name of authors from the Institut Pasteur du Cambodge are underlined.
Publications in a journal without impact factor are listed separately and identified at the end of the list
** equal contribution, first author / ** equal contribution, last author*

1. ***Antibody fucosylation predicts disease severity in secondary dengue infection***
   Stylianos Bournazos, Hoa Thi My Vo, Veasna Duong, Heidi Auerswald, Sowath Ly, Anavaj Sakuntabhai, Philippe Dussart, Tineke Cantaert**, Jeffrey V. Ravetch**
   Science. American Association for the Advancement of Science; 2021;372(6546):11025. DOI: 10.1126/science.abc7303

2. ***Circulation of Bordetella pertussis in vaccinated Cambodian children: a transversal serological study***
   Gaelle Noel*, Laurence Borand*, Chanthy Leng, Chanthy Keang, Chhunly Botr, Bunnet Dim, Alexandra Kerleguer, Yik S. Peng, Navin Sreng, Vichit Ork, Mohand Ait-Ahmed, Nicole Guiso, Fabien Taieb.
   Int J Infect Dis. 2021; DOI: 10.1016/j.ijid.2021.03.054

3. ***"Health in" and "Health of" Social-Ecological Systems: A Practical Framework for the Management of Healthy and Resilient Agricultural and Natural Ecosystems***
   Michel De Garine-Wichatitsky, Aurélie Binot, John Ward, Alexandre Caron, Arthur Perrotton, Helen Ross, Hoa Tran Quoc, Hugo Valls-Fox, Iain J. Gordon, Panomsak Promburom, Rico Ancog, Richard Anthony Kock, Serge Morand, Véronique Chevalier, Will Allen, Waraphon Phimpraphai, Raphaël Duboz, Pierre Echaubard.
   Frontiers in Public Health 2021; 8:616328. DOI: 10.3389/fpubh.2020.616328

4. ***Host-Feeding Preference and Diel Activity of Mosquito Vectors of the Japanese Encephalitis Virus in Rural Cambodia***
   Sébastien Boyer, Benoit Durand, Sony Yean, Cécile Brengues, Pierre-Olivier Maquart, Didier Fontenille, Véronique Chevalier.
   Pathogens. 2021;10(3):376. DOI: 10.3390/pathogens10030376

5. ***Impact of systematic early tuberculosis detection using Xpert MTB/RIF Ultra in children with severe pneumonia in high tuberculosis burden countries (TB-Speed pneumonia): a stepped wedge cluster randomized trial***
   Aurélia Vessière, Hélène Font, Delphine Gabillard, Laurence Adonis-Koffi, Laurence Borand, Chishala Chabala, Celso Khosa, Sandra Mavale, Raoul Moh, Veronica Mulenga, Juliet Mwanga-Amumpere, Jean-Voisin Taguebue, Mao Tan Eang, Christophe Delacourt, James A. Seddon, Manon Lounnas, Sylvain Godreuil, Eric Wobudeya, Maryline Bonnet, Olivier Marcy.
   BMC Pediatr. 2021;21(1):136. DOI: 10.1186/s12887-021-02576-5

6. ***In-field Evaluation of SD Bioline HBsAg Whole Blood Rapid Test in Pregnant Women in Cambodia: the ANRS 12345 TA PROHM Study***
   Olivier Segeral, Wathanakpiseychoupoan Phirum, Ousa Khan, Hyna Chea, Saren Sovann, Sovann Nhoeung, Kearena Chhim, Song Yin, Bunnet Dim, Chantana Yay, Denis Laurent, Samsorphea Chhun, Laurence Borand.
   Diagn Microbiol Infect Dis. 2021;101(2):115452. DOI: 10.1016/j.diagmicrobio.2021.115452

7. ***Large scale dog population demography, dog management and bite risk factors analysis: A crucial step towards rabies control in Cambodia***
   Véronique Chevalier, Holl Davun, Sopheak Sorn, Pitou Ly, Vutha Pov, Sowath Ly.
   PLoS One. 2021;16(7):e0254192. DOI: 10.1371/journal.pone.0254192

8. ***Longitudinal monitoring in Cambodia suggests higher circulation of alpha and betacoronaviruses in juvenile and immature bats of three species***
   Julien Cappelle, Neil Furey, Thavry Hoem, Tey Putita Ou, Thona Lim, Vibol Hul, Oudam Heng, Véronique Chevalier, Philippe Dussart, Veasna Duong.
   Sci Rep. 2021;11(1):24145. DOI: 10.1038/s41598-021-03169-z

9. ***Malaria in Cambodia: A Retrospective Analysis of a Changing Epidemiology 2006–2019***
   Srean Chhim, Patrice Piola, Tambri Housen, Vincent Herbreteau, Bunkea Tol.
   Int J Environ Res Public Health. 2021;18(4). DOI: 10.3390/ijerph18041960

10. ***Mosquito Vector Competence for Japanese Encephalitis Virus***
    Heidi Auerswald, Pierre-Olivier Maquart, Véronique Chevalier, Sebastien Boyer.
    Viruses. 2021;13(6):1154. DOI: 10.3390/v13061154

11. ***Novel anti-malarial drug strategies to prevent artemisinin partner drug resistance: A model-based analysis***
    Amber Kunkel, Michael White, Patrice Piola.
    PLoS Comput Biol. 2021;17(3):e1008850. DOI: 10.1371/journal.pcbi.1008850

12. ***Phenotypic and genetic characterization of MERS coronaviruses from Africa to understand their zoonotic potential***
Ziqi Zhou, Kenrie P. Y. Hui, Ray T. Y. So, Huibin Lv, Ranawaka A. P. M. Perera, Daniel K. W. Chu, Esayas Gelaye, Harry Oyas, Obadiah Njagi, Takele Abayneh, Wilson Kuria, Elias Walelign, Rinah Wanglia, Ihab El Masry, Sophie Von Dobschuetz, Wantanee Kalpravidh, Véronique Chevalier, Eve Miguel, Ouafaa Fassi-Fihri, Amadou Trarore, Weiwen Liang, Yanqun Wang, John M. Nicholls, Jincun Zhao, Michael C. W. Chan, Leo L. M. Poon, Chris Ka Pun Mok, Malik Peiris.
Proc Natl Acad Sci U S A. 2021;118(25):e2103984118. DOI: 10.1073/pnas.2103984118

13. ***Severe bacterial neonatal infections in Madagascar, Senegal, and Cambodia: A multicentric community-based cohort study***
Bich-Tram Huynh, Elsa Kermorvant-Duchemin, Rattanak Chheang, Frederique Randrianirina, Abdoulaye Seck, Elisoa Hariniaina Ratsima, Zafitsara Zo Andrianirina, Jean-Baptiste Diouf, Armya Youssouf Abdou, Sophie Goyet, Véronique Ngo, Siyin Lach, Long Pring, Touch Sok, Michael Padget, Fatoumata Diene Sarr, Laurence Borand, Benoit Garin, Jean-Marc Collard, Perlinot Herindrainy, Agathe de Lauzanne, Muriel Vray, Elisabeth Delarocque-Astagneau, Didier Guillemot, On behalf of the BIRDY study Group.
PLOS Medicine. Public Library of Science; 2021;18(9):e1003681. DOI: 10.1371/journal.pmed.1003681

14. ***Tuberculosis Diagnosis in HIV-Infected Children: Comparison of the 2012 and 2015 Clinical Case Definitions for Classification of Intrathoracic Tuberculosis Disease***
Olivier Marcy, Sophie Goyet, Laurence Borand, Philippe Msellati, Vibol Ung, Mathurin Tejiokem, Giang Do Chau, Francis Ateba-Ndongo, Abdoul Salam Ouedraogo, Bunnet Dim, Paul Perez, Julien Asselineau, Guislaine Carcelain, Stéphane Blanche, Christophe Delacourt, Sylvain Godreuil.
Journal of the Pediatric Infectious Diseases Society. 2021;piab113. DOI: 10.1093/jpids/piab113

Abstracts and Presentations

1. **Feasibility and yield of systematic tuberculosis molecular testing in children with severe pneumonia in countries with high tuberculosis incidence.** 52nd Union World Conference on Lung Health, 19-22 October 2021, virtual conference. Aurélia Vessière, Manon Lounas, Hélène Font, Laurence Borand, Raoul Moh, Chishala Chabala, Jean-Voisin Taguebue, Celso Khosa, Eric Wobudeya, Olivier Marcy for the TB-Speed Pneumonia Study Group.
2. **Impact of systematic TB detection using Xpert Ultra on nasopharyngeal aspirate and stool samples on mortality in children with severe pneumonia.**TBScience 2021 – Virtual Event, 52nd Union World Conference on Lung Health, 19-22 October 2021. Olivier Marcy, Hélène Font, Aurélia Vessière, Raoul Moh, Laurence Borand, Jean-Voisin Taguebue, Chishala Chabala, Celso Khosa, Eric Wobudeya, Maryline Bonnet for the TB-Speed Pneumonia Study Group.
3. **Acceptability of nasopharyngeal aspirate and stool sample collection for tuberculosis diagnosis in children with severe pneumonia – parents' and healthcare workers' perspective.** 52nd Union World Conference on Lung Health, 19-22 October 2021, virtual conference. Bandana Bhatta, Aurelia Vessière, Laurence Borand, Raoul Moh, Celso Khosa, Chishala Chabala, Eric Wobudeya, Maryline Bonnet, Olivier Marcy, Joanna Orne-Gliemann, for the TB-Speed Pneumonia Study Group.
4. **French Embassy studies sponsored by Initiative 5% : progress as at 01/2021, 21/01/2022.** Laurence Borand
5. USAID meeting – **Presentation of TB-speed** 17/03/2021. Laurence Borand
6. **Progress of current TB-related research** – TB-Speed; 17/06/202. Laurence Borand
7. ANRS Scientific Days – **TB-Speed presentation**; 28/09/202. Laurence Borand
8. **French Embassy Studies sponsored by Initiative 5% : progress as at 12/2021, 07/12/202.** Laurence Borand
9. **Workshop on Annual Operational Plan for TB Control 2022** –,Siem Reap. Laurence Borand

4.2.7. Outlook

At 3 years

According to the current priorities and research activities, it seems important to reconsider the interest of the malaria group within the unit and to integrate two additional groups in its flow chart:

- The "Methodology and Data Analysis" Group, which will be coordinated by Dr. Claude Flamand, the current Head of the epidemiology and public health unit, which aims to develop and implement the methodological tools necessary for the design of studies, analysis and interpretation of the data resulting from the research work carried out. This group should be composed of at least one biostatistics engineer and one postdoc specialized in biostatistical approaches and/or mathematical modeling;
- The "GeoHealth" group, coordinated by Dr. Vincent Herbreteau, seconded from the IRD, which specializes in the study of the effects of the environment and geographical factors on human health. While this group has collaborated with the IPC teams as an independent group, its integration into the epidemiology unit should allow the development of innovative and ambitious research programs, bringing to the unit a useful expertise for the research activities conducted within the unit.

Particular emphasis will be placed on training and upgrading the skills of the team members, notably in clinical research, data management as well as biostatistical, informatics and geomatic methods and tools.

In the context of the PREZODE and Ecomore-3 initiatives, it will be fundamental to extend regional collaborations in order to favor the implementation of multi-centre collaborative projects. Finally, given the priority public health issues in Cambodia, it will also be interesting to reflect on the interest of extending the research themes to non-communicable diseases (respiratory diseases, cardiovascular diseases, cancer, etc.);

At 5 years

The next few years present the opportunity to encourage university and academic training of team members (Master's degree, PhD) as well as preparing them to directly conduct research after having acquired significant experience. It is also important to attract talented Cambodian researchers and international postdoctoral scientists and to reinforce support to national health authorities through relevant research findings to build local capacity. .

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 (1) immune responses during flavivirus infections, (2) identification of new immune-related biomarkers for infectious diseases, and (3) immunological assessment of rabies post-exposure prophylaxis. Moreover, we are hosting a transversal single cell analysis platform.

4.3.1. Functional Structure

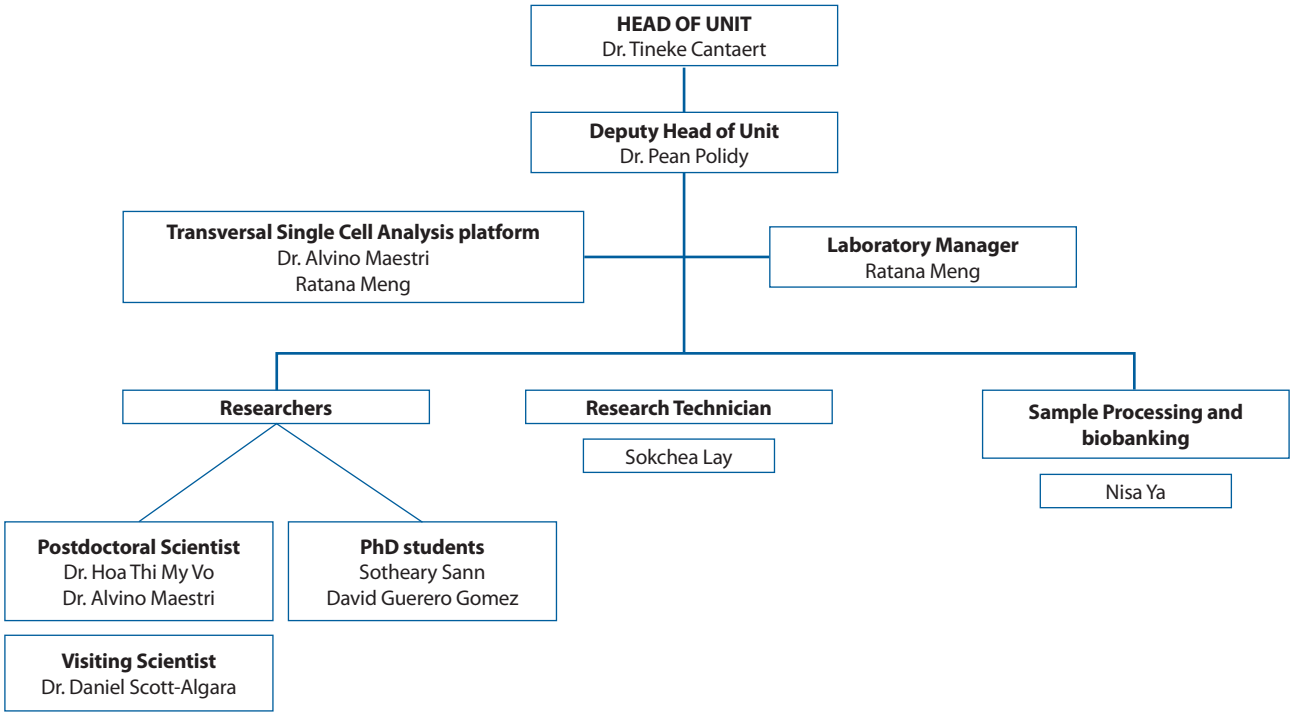


Figure7. Immunology Unit Organogram

4.3.2. Research Programs – Major Achievements in 2021

Axis 1: Immune responses During Flavivirus Infection

Dengue viruses infect up to 390 million individuals each year, of which 500,000 cases require hospitalization. Since 2012, dengue has been the most important vector-borne viral disease of humans and likely more important than malaria globally in terms of morbidity and economic impact. The mosquito vectors, *Aedes aegypti* and *Aedes albopictus* both thrive well in populated urbanized areas, contributing to the spread of dengue. Disease outcome after infection varies greatly between individuals. 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 Humoral immune responses to dengue virus

Although antiviral antibodies generally confer protective functions, antibodies against dengue virus are associated with enhanced disease susceptibility. Antibodies can mediate dengue virus infection of leukocytes via Fcγ receptors, likely contributing to dengue disease pathogenesis. To determine if this mechanism accounts for variable disease severity, we examined Fab and Fc structures of anti-dengue antibodies from patients before and after infection and with variable disease outcomes. Neither antibody titers nor neutralizing activity correlated with disease severity in dengue-infected populations. Rather, dengue infection induced a specific increase in immunoglobulin G1 fucosylation, and the levels of afucosylated IgG1 were predictive of dengue disease severity. Moreover, increased IgG1 fucosylation levels correlated with increased hematocrit and decreased platelet counts, 2 hallmarks of dengue disease severity. (Bournazos et al, Science 2021)

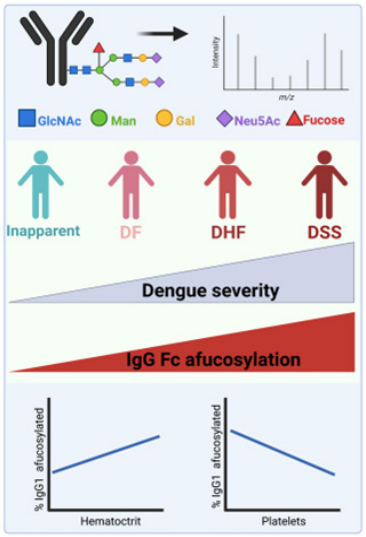


Figure 8. Abundance of IGG1 fucosylation is associated with disease severity after denv infection.

Collaborations	Virology Unit, IP Cambodia (Duong V), Epidemiology and Public Health Unit, IP Cambodia (Ly S), Rockefeller University (Ravetch J, Bournazos S)
Funding	HHMI/Wellcome International Research Scholars Program (208710/Z/17/Z) 2017-2022

Aim 1.2 Immunity to *Aedes* mosquito saliva

When a mosquito inserts its proboscis and probes for blood, the mosquito ejects a salivary mix of vasodilators, anticoagulants, immunomodulatory and anti-hemostatic components into both the epidermis and the dermis (reviewed in Cantaert T and Manning J, Vaccines, 2019). However, little is known about skin immunity to mosquito saliva. We assessed the cutaneous innate and adaptive immune responses via controlled *Aedes aegypti* feedings in humans living in an Aedes-endemic country. Gene expression profiling and immunophenotyping revealed induction of neutrophil degranulation and recruitment of skin-resident dendritic cells and M2-macrophages. As the immune reaction progressed over time, T cell priming and regulatory pathways were upregulated along with a shift to a Th2-driven response and CD8+ T cell activation. In accordance, participants' bitten skin cells produced less pro-inflammatory cytokines when stimulated by *Aedes aegypti* salivary gland extract. These results identify key immune genes, cell types, and pathways in the human response to mosquito bites that can be leveraged to develop novel therapeutics and vector-targeted vaccine candidates to arboviral diseases. (Guerero Gomez, Vo et al, submitted).

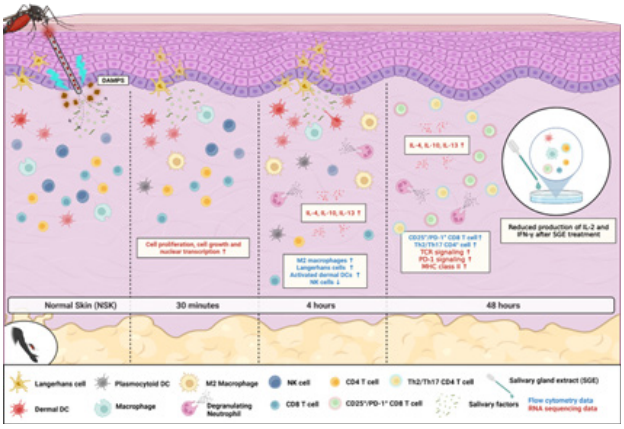


Figure 9. Summary of changes occurring in normal human skin of endemic volunteers after a bite with an Aedes mosquito

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

Aim 2.1 *Agent National de Recherche sur le SIDA/les hépatites virales et les maladies infectieuses d'émergence* (ANRS) No12358: "*Micro- ribonucleic acid* (MicroRNA or miRNAs) as prediction and/or prognostic markers of IRIS (immune reconstitution inflammatory syndrome) in TB/HIV co-infected patient (miRBoo)"

MicroRNAs 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. In this study, we 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 27 selected miRNAs with the clinical evolution and the occurrence of IRIS. We found that the combination of at least two or three microRNA markers (MiRBio0004, MiRBio0001 and MirBio0008) can be used as the biomarker to differentiate IRIS from non IRIS before any treatment initiation and predict the occurrence of IRIS. These results were approved for the protection of Industrial property and classified in the category of invention " *l'article L611-7.1 du code de Propriété Intellectuelle et de l'Accord d'Entreprise*" (Ref. NT/SC/NaT No 01213). Inventor Daniel Scott (IP Paris), Co-inventor : Pean Polidy (IPC), Laurence Borand (IPC), Dim Bunnet (IPC), Meng Ratana (IPC), and Yoann Madec (IP Paris).

Collaborations	Daniel Scott (IP-Paris-France), Yoann Madec (IP-Paris-France), Laurence Borand (IPC-Cambodia), Srey Pichsovannary (Center of Hope, Cambodia)
Funding	ANRS-MIE/INSERM, 2017-2019

Aim 2.2 ANRS No 12394: "Lowering InterLeukin-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 Interleukin-1 receptor antagonist (IL-1Ra) plasma concentrations dropped dramatically after two months of TB treatment. The objective of this proof-of-concept study is to demonstrate that IL-1Ra concentrations significantly decrease earlier within two weeks following TB treatment initiation in adults with documented TB. In parallel, we are assessing two other biomarkers: Interferon gamma induced protein -10 (IP-10) and sCD163. The plasma of 22 TB+HIV- and 6 HIV+TB patients at day 0 , week 1, week 2 , week 4 and week 8 after anti-tuberculosis drugs treatment were analyzed. We have confirmed a significant decrease in IL-1Ra and IP 10 levels at week two. Interestingly, the decrease in IL-1Ra and IP 10 levels from week one after the treatment was similar. The major difficulty is the recruitment of HIV-infected patients for our study. In Cambodia, we were able to include only 6 of the 30 planned patients in the study.

Collaborations	Laurence Weiss (Univ. Paris 7 Diderot, Paris, France), Raoul Moh (PACCI, Ivory Coast), Laurence Borand (IPC-Cambodia), Srey Pichsovannary (Center of Hope , Cambodia)
Funding	ANRS-MIE/INSERM, 2019-2021

Axis 3: Vaccine Responses to Rabies Virus Vaccination

The World Health Organization endorsed a new shortened protocol of post exposure prophylaxis (PEP) in the April 2018 guidelines. 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.

Collaborations	Virology Unit, IP Cambodia (Duong V), Epidemiology and public Health Unit, IP Cambodia (Ly S), Vaccination center (Peng Y)
Funding	IP Cambodia internal project, 2019-2023

Axis 4: Investigation of Adaptive Immune Responses in SARS-CoV-2 Infected Patients

The duration of humoral and cellular immune memory following SARS-CoV-2 infection in populations in least developed countries remains understudied but is key to overcoming the current SARS-CoV-2 pandemic. Sixty-four Cambodian individuals with laboratory-confirmed infection with asymptomatic or mild/moderate clinical presentation were evaluated for spike-binding and neutralizing antibodies and antibody effector functions during acute phase of infection and at 6-9 months follow-up. Antigen-specific B cells, CD4+ and CD8+ T cells were characterized, and T cells were interrogated for functionality at late convalescence. Anti-spike antibody titers decreased over time, but effector functions mediated by Spike-specific antibodies remained stable. Spike-specific B cells could be detected in late convalescence in the activated memory B cell compartment and are mostly IgG+. CD4+ and CD8+ T cell immune memory was maintained to S and membrane (M) protein. Asymptomatic infection resulted in decreased antibody-dependent cytotoxicity and frequency of SARS-CoV-2-specific CD4+ T cells at late convalescence. Whereas anti-spike antibodies correlated with spike-specific B cells, there was no correlation between T cell response and humoral immune memory. Hence, all aspects of a protective immune response are maintained up to nine months after SARS-CoV-2 infection and in the absence of re-infection. (Vo, Maestri et al, Frontiers Immunol 2021).

Collaborations	Virology Unit, IP Cambodia (V. Duong), Epidemiology and Public Health Unit , IP Cambodia, Virus and Immunity Unit, IP Paris (T. Bruel)
Funding	Institut Pasteur « COVID-19 urgence » - 2020-2021

4.3.3. Research Programs – Outlook 2022

Axis 1: Immune responses During Flavivirus Infection

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

In this project, we aim to understand the mechanism of the generation and protection of the humoral immune response in very mild dengue cases, compared to hospitalized dengue cases with classical dengue fever and hospitalized cases with severe dengue on a single cell level. We will evaluate the development, quality and quantity of the humoral responses in detail. To accomplish this goal, we work on the following specific aims: 1/ Generation of dengue-derived antigens from clinical field isolates ; 2/ Determination of antibody affinity for four key-dengue 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/ single cell RNA sequencing of CD19+ B cells in different dengue-infected patient groups.

Collaborations	Virology Unit, IPC (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, IP Paris (M. Hasan)
Funding	PTR (PTR 2019-212) Janssen Horizon grant:2019-2022

Aim 1.2 Humoral immune responses to dengue virus (DENV) infection

Other than virus neutralization, many other 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 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. Four in vitro cell-based assays have been optimized in the immunology unit to evaluate antibody-effector functions of IgG generated during dengue infection: antibody-dependent enhancement assay, antibody dependent cytotoxicity assay, antibody dependent phagocytosis assays, and complement dependent cytotoxicity. 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, IP Cambodia (V. Duong) ; Virus and Immunity Unit, IP Paris (T. Bruel)
Funding	HHMI/Wellcome International Research Scholars Program (208710/Z/17/Z) ; PTR 2019-2022 , 2017-2022

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 unclear, 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). In this project, we aim to understand the contribution of homotypic and heterotypic dengue- specific T cells to disease development or protection after infection. Regulatory T cells (Tregs) 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.

Aim 1.4 Immunity to Aedes mosquito saliva

To extend our observations in humans (see above, major achievements), we aim to investigate the effect of mosquito saliva on the immune response to dengue. We will compare dengue 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 (dendritic cells and keratinocytes) to dengue in the presence of *Aedes aegypti* saliva.

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

Aim 1.5 Pasteur Institute Centers of Research in Emerging Infectious Diseases Network (PICREID)

IP Cambodia is part of the NIH funded consortium PICREID. 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. 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 arbovirus infections 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 a 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	Virology Unit, IPC (V. Duong), Epidemiology and Public Health Unit, IPC (S. Ly), Entomology Unit, IP Cambodia (S. Boyer), Functional Genetics of Infectious Diseases Unit (A. Sakuntabhai)
Funding	NIH PIC REID (1U01AI151758 – 01): 2020-2025

Axis 2: Biomarkers of Infectious Diseases

Aim 2.1 Microrna as potential biomarkers of HIV/IRIS

We follow up on the project ANRS No12358, where we found that the combination of at least 2 or 3 microRNA (e.g. MiRBio0004, MiRBio0001 and MirBio0008) can differentiate IRIS from non-IRIS patients before any

treatment. In the current project, we would like to confirm our results in a large patient cohort (n=660 biobank of previous Camelia study).

Collaborations	Daniel Scott (IP Paris , France), Polidy Pean (IPC, Cambodia), Laurence Borand (IPC, Cambodia),
Funding	DARRI, IP Paris, France: 2022-2023

Aim 2.2 Microrna as potential biomarkers of infectious diseases

MiRNs 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, that has been validated and set up in our lab, allows 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 2022 are (1) a grant proposal to Programme Transversal de Recherche 2022 (PTR) on microRNA in dengue (in collaboration with Dr. Carolina SCAGNOLARI, IP Rome, submitted), (2) biomarkers associated with tuberculosis severity in HIV and/or non-HIV infected patients (ANRS-MIE, discussions ongoing) (3) biomarkers associated with the clinical outcome of HBV MTCT (ANRS-MIE, discussions ongoing) (4) biomarkers for guiding and monitoring antimicrobial treatment (pilot study) (IPC funding, June 2022), and (5) biomarkers of Schistosomiasis infection, in collaboration with CNM/MOH (funder not yet 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 recruitment is continuing from 2020.

Collaborations	Virology Unit, IP Cambodia (V. Duong), Epidemiology and Public Health Unit, IP Cambodia (S. Ly/L. Borand), Vaccination center, IP Cambodia (Y. Peng)
Funding	Internal IP Cambodia project: 2020-2023

4.3.4. Support to National Authorities

Tineke Cantaert, PhD and Policy Pean, MD, PhD are both members of the steering committee for the international master's degree program on infectiology and coordinators of the immunology module used in master's degree year. The degree program is offered jointly by the (University of Health Sciences, Phnom Penh, Cambodia and Universite Paris Saclay, Paris, France).

Polidy Pean is a member of the 10-person COVID-19 national experts team nominated by the Ministry of Health. The team reviews the scientific literature related to COVID-19 vaccines in order to provide recommendations to the Ministry of Health/Government's COVID-19 task force.

4.3.5. Teaching and Training

PhD students:

- SANN Sotheary: University of Hasselt, Belgium (2019-2023). Visits to UHasselt are covered by a BOF/ BILA grant of the Flemish Government
- GUERRERO GOMEZ David: University of Montpellier, France (2020-2022)

Internship/Master students:

- HENG Borita: Royal University of Phnom Penh. Bachelor thesis Bioengineering.

1. Tineke Cantaert, PhD and Policy Pean, MD, PhD:
   - CANTAERT Tineke, Master Infectious Diseases, University Paris-Saclay-University of Health Sciences, 10 hours
   - PEAN Polidy, Master Infectious Diseases, University Paris-Saclay-University of Health Sciences, 10 hours

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

4.3.6. Outlook

The strengths of the immunology unit in infectious diseases research is dependent on the commitment and quality of our workforce, our resources (e.g. biobanking, state of the art technology-including maintenance contracts, biosafety level II laboratories), strong collaborations with other IPC Units and longstanding collaborations with excellent researchers worldwide. This is exemplified by our high quality research output. Our challenges are lack of laboratory space to expand our activities, turnover of skilled staff, difficulty attracting specialized international researchers, limited opportunities for training and development for our local scientists, limited capacity in -omics and big dataset analysis, an unstable funding situation and lack of collaborative projects with local universities. In 2021 and 2022, multiple innovative grant proposals have been/will be submitted aiming to (1) secure our current research lines (Axes 1-3) and (2) expand our research to new subjects with novel collaborating partners (eg. Parasite immunology, type 2 mediated immune diseases). This will guide our research programs for the upcoming 5 years. We also aim to attract a project manager to facilitate all multi-partner collaborations in a timely fashion. Next, we keep aiming to create an educational learning environment for students, PhD students and postdoctoral researchers. We will enroll one additionall Cambodian student to the PhD program this year (Universite de Paris-Saclay). We also emphasize collaborative work in an environment of cultural diversity.

4.3.7. Publications List 2021

Awards and Grants Approved in 2021

Program "Maturation" for Diagnosis Application (Direction des applications de la recherche et des relations industrielles - DARRI-IP Paris): " A set of plasmatic miRNA as predictive biomarkers of IRIS (Immune Reconstitution Inflammatory Syndrome) in HIV TB co-infected patients" Awardees : Daniel Scott (Program leader, IP Paris) and Polidy Pean (PI , IPC). Funded by IP Paris (50,000 Euros)

NOTE

The name of authors from the Institut Pasteur du Cambodge are underlined.
Publications in a journal without impact factor are listed separately and identified at the end of the list
** equal contribution, first author / ** equal contribution, last author*

1. ***Editorial: Balanced and Unbalanced Immune Response to Dengue Virus in Disease Protection and Pathogenesis***
   Tineke Cantaert, Nolwenn Jouvenet, Sean A. Diehl.
   Frontiers in Immunology. 2022;13. DOI: 10.3389/fimmu.2022.835731

2. ***Robust and Functional Immune Memory Up to 9 Months After SARS-CoV-2 Infection: A Southeast Asian Longitudinal Cohort***
   Hoa Thi My Vo*, Alvino Maestri*, Heidi Auerswald, Sopheak Sorn, Sokchea Lay, Heng Seng, Sotheary Sann, Nisa Ya, Polidy Pean, Philippe Dussart, Olivier Schwartz, Sovann Ly, Timothée Bruel, Sowath Ly, Veasna Duong, Erik A. Karlsson**, Tineke Cantaert**.
   Frontiers in Immunology. 2022;13. DOI: 10.3389/fimmu.2022.817905

3. ***Antibody fucosylation predicts disease severity in secondary dengue infection***
   Stylianos Bournazos, Hoa Thi My Vo, Veasna Duong, Heidi Auerswald, Sowath Ly, Anavaj Sakuntabhai, Philippe Dussart, Tineke Cantaert**, Jeffrey V. Ravetch**
   Science. American Association for the Advancement of Science; 2021;372(6546):11025. DOI: 10.1126/science.abc7303

4. ***Antibody-independent functions of B cells during viral infections***
   Vinit Upasani, Izabela Rodenhuis-Zybert, Tineke Cantaert.
   PLoS Pathog. 2021;17(7):e1009708. DOI: 10.1371/journal.ppat.1009708

5. ***Differential levels of IFNa subtypes in autoimmunity and viral infection***
   Vincent Bondet, Mathieu P. Rodero, Céline Posseme, Pierre Bost, Jérémie Decalf, Liis Haljasmägi, Nassima Bekaddour, Gillian I. Rice, Vinit Upasani, Jean-Philippe Herbeuval, John A. Reynolds, Tracy A. Briggs, Ian N. Bruce, Claudia Mauri, David Isenberg, Madhvi Menon, David Hunt, Benno Schwikowski, Xavier Mariette, Stanislas Pol, Flore Rozenberg, Tineke Cantaert, J. Eric Gottenberg, Kai Kisand, Darragh Duffy.
   Cytokine. 2021;144:155533. DOI: 10.1016/j.cyto.2021.155533

6. ***Direct Infection of B Cells by Dengue Virus Modulates B Cell Responses in a Cambodian Pediatric Cohort***
   Vinit Upasani, Hoa Thi My Vo, Heidi Auerswald, Denis Laurent, Sothy Heng, Veasna Duong, Izabela A. Rodenhuis-Zybert, Philippe Dussart, Tineke Cantaert.
   Front Immunol. 2021;11. DOI: 10.3389/fimmu.2020.594813

4.4. Virology

4.4.1. Functional Structure

IPC's virology unit celebrated its 25th anniversary on December 14 2021. The unit's activities in 2021 were directed toward biomedical research, the surveillance and monitoring of infectious diseases and support to public health emergencies (Figure 10). These activities comprise 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 48 staff including seven PhDs, two PhD candidates, four master's degree holders, two medical doctors and two pharmacists. A number of staff was recruited to maintain the COVID-19 testing activity in support of the Ministry of Health.

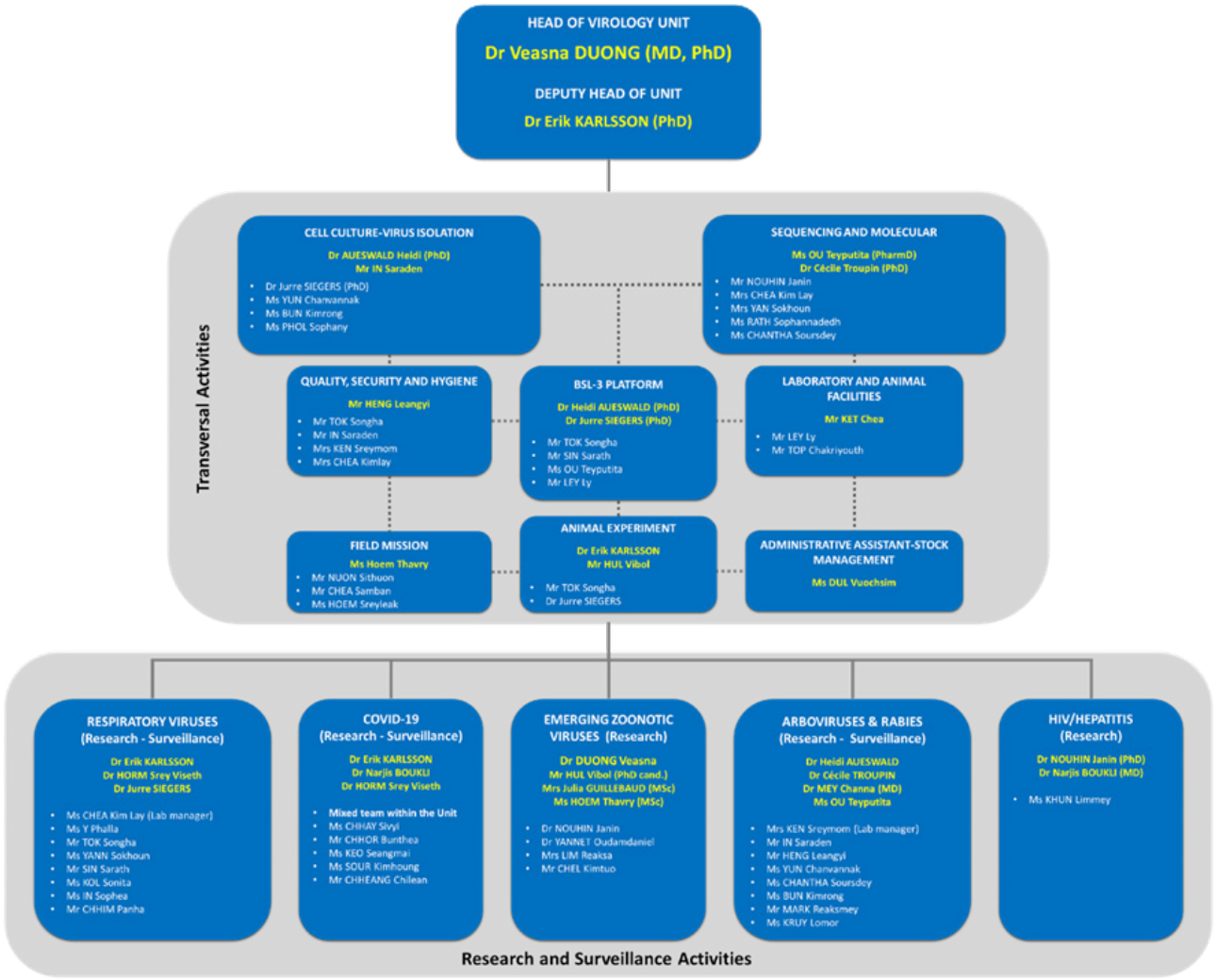


Figure 10. Organogram of virology unit and main research and surveillance activities.

Within each of these components, the virology unit has developed numerous research programmes. Most of these programmes are conducted in collaboration with other units at IPC: epidemiology and public health unit (EPH), entomology unit and immunology unit as well as with other collaborators across the globe. These programs focus on infectious diseases of concern to the Cambodian population.

4.4.2. Research Programs – Major Achievements in 2021

AXIS 1: Arboviral Diseases

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 unpredictability of a chikungunya outbreak occurring 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, we lack comprehensive understanding of the duration of CHIKV viremia in patients and the risk of transmission in the household setting. This information would help improve understanding of the clinical feasibility of potential new treatment. The main objectives were to characterize the kinetic viraemia during the acute phase of CHIKV infection and to determine the risk of CHIKV infection in household members of an index case.

The study is ongoing and is being carried out in Kampong Thom Province, having started in December 2020 and is expected to conclude in October 2022. Patients will be recruited at Kampong Thom Provincial Referral Hospital, two other district referral hospitals in Baray and Staung Districts, Kampong Thom Province. The study was then expanded countrywide due to a reduction in new cases reported in these two provinces. Therefore, Jayavarman VII Hospital in Siem Reap and Kuntha Bopha Hospital in Phnom Penh were included to increase sample availability.. Household members of CHIKV confirmed cases are also enrolled. Blood samples are collected for virological tests (viral detection by RT-PCR and IgM/IgG detection by ELISA assays). As of April 2022, 23 viremic cases were enrolled with 73 household members of CHIKV confirmed index cases. The serology tests on household member's samples is ongoing.

Collaborations	Institut Pasteur du Cambodge: Virology unit (V. Duong); Epidemiology-Public Health unit (S. Ly); Immunology unit (T. Cantaert); **Evotec** (Hugh Watson)
Funding	Evotec International GmbH: December 2020 to October 2022

DenThom: Study of dengue-like illness in Kampong Thom Province, Cambodia. The primary objective of this NIH-funded project within the CREID network is to study the prevalence and incidence of dengue and dengue-like illness in Cambodia by implementing a novel study site in Kampong Thom, a province in the center of Cambodia that is a major transport axis, and where information on dengue transmission and circulation is lacking. The full description of this project is detailed in the report by the immunology unit. The virology unit is involved in the implementation of the project in the field in collaboration with the Epi-Public Health unit and in the diagnosis of arboviruses using serological and molecular tools in humans and mosquitoes. Due to COVID-19 crisis, the field activities were delayed but will start in May 2022.

Collaborations	Institut Pasteur du Cambodge: Virology unit (V. Duong); Epidemiology-Public Health unit (S. Ly); Immunology unit (T. Cantaert); Entomology Unit (S. Boyer) IP, Paris (A. Sakuntabhai)
Funding	NIH collaborative agreement (1-IPC-NIH-U01-AS-2020): August 2020 – May 2025

AXIS 2: Respiratory Viruses

Avian influenza surveillance in key Live Bird Markets (LBMs). In 2017, in collaboration with the FAO and National Animal Health and Production Research Institute (NAHPRI), we sought to establish avian influenza virus 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 and 2020 study periods, we collected 5,120 poultry samples from 2,560 birds (paired tracheal and cloacal swabs). 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. Surveillance was restarted in fall 2021 (after some delays due to COVID-19) and we again aim to collect 5,120 poultry samples from 2,560 birds (paired tracheal and cloacal swabs) between 2021-2022. In 2021, we were able to collect 2,560 samples from 1,280 birds with an overall positivity rate of 25%.

Analysis is complete for the 2017-2020 sessions and final collections, analysis, and sequencing of the 2021-2022 sessions is underway and several manuscripts are in preparation (see below). This collaboration is planned to continue for the 2022-2023 season.

Sequencing of avian influenza samples to investigate outbreaks and the diversity of influenza viruses circulating in Cambodian poultry. We have been conducting LBM surveillance in the Orussey Market in Phnom Penh since 2011. This is intended 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 have been sequenced and phylogenetic, antigenic, and molecular risk assessment are underway in collaboration with WHOCC in Melbourne, Hong Kong University, and other partners. Recently in 2021, IPC has worked with partners at Johns Hopkins University Applied Physics Laboratory to use a novel multi-segment, barcoded PCR for sequencing on Oxford Nanopore Technologies. We have successfully established this pipeline and are internally sequencing 24-48 influenza samples/week from AIV surveillance.

Detection of A/H5 clade 2.3.4.4b viruses in Cambodian live bird markets. In recent years, A/H5Nx clade 2.3.4.4b highly pathogenic avian influenza viruses (HPAIv) have spread globally, causing severe outbreaks in Africa, Europe, Asia, and most recently, North America. In addition to a number of LPAIv, multiple A/H5 subtypes and clades continue to circulate in Cambodia. H5N1 clade 2.3.2.1c viruses were still detected regularly into 2022. H5N6 clade 2.3.4.4g and 2.3.4.4h viruses were identified in Cambodian LBMs from late 2018 through 2020. During active surveillance in late 2021, duck samples from Phnom Penh, Kandal and Takeo markets tested positive for the H5 HA gene but were negative for neuraminidase (NA) subtype N1 by real time RT-PCR. Further analysis revealed these samples to be subtype H5N8 and further characterization

by full genome sequencing confirmed these samples were positive for HPAI H5N8 and correspond to H5 clade 2.3.4.4b (Figure 11). The detection of these novel lineage viruses, association with outbreaks in poultry throughout the world, 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.

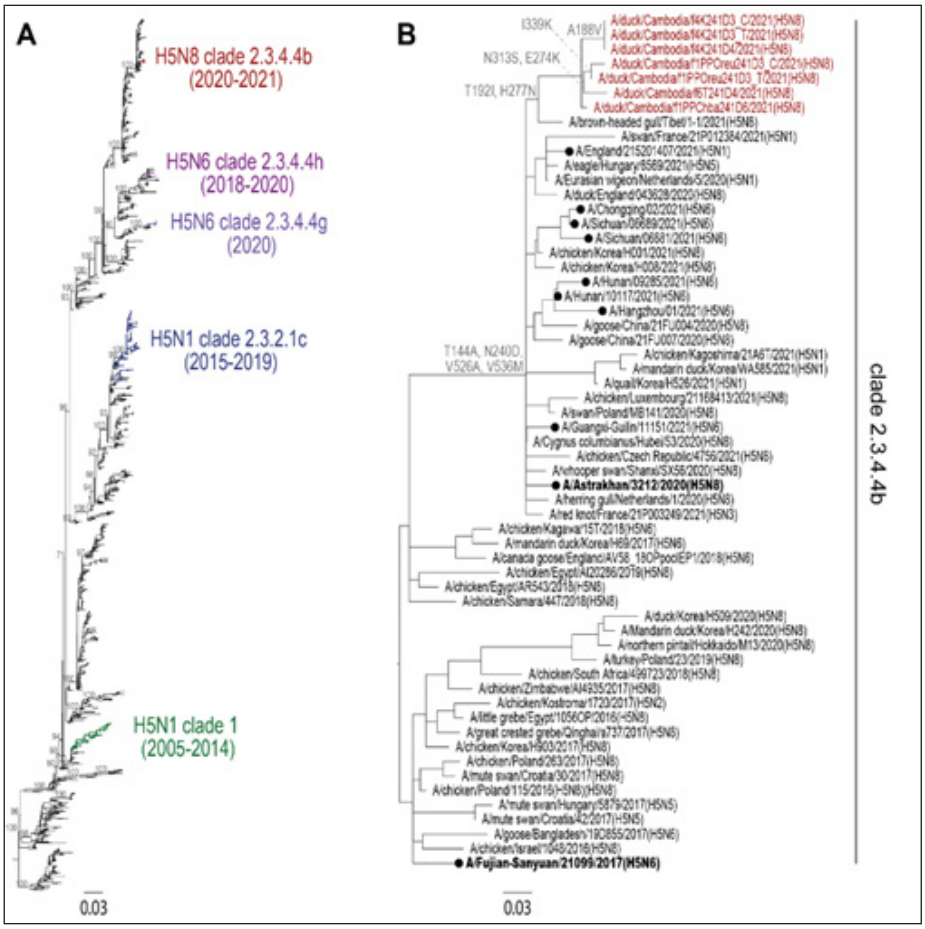


Figure 11. ***Maximum likelihood phylogeny of the HA genes of H5 viruses in Cambodia.*** *Scale bars represent nucleotide substitutions per site.* **A**, *All HPAI H5Nx clades detected in Cambodia.* **B**, *H5N8 clade 2.3.4.4b. Candidate vaccine viruses for clade 2.3.4.4b are shown in bold. Amino acid mutations are indicated at select nodes. Tip shapes indicate cases of human infection with avian H5Nx viruses*

Testing and response to A/H5N1 outbreaks in poultry and wild birds. In 2021, IPC was involved in the investigation of two outbreaks associated with a poultry mortality event in Battambang Province in January 2021 and with a wild bird mortality in Prey Veng in May 2021. The samples associated with the two outbreak events were sent to the IPC virology unit for sequencing following testing at the NAHPRI and were confirmed to be positive for subtype A/H5. Sequencing and initial analysis of wild bird sample (A/Asian openbill story/f052401/2021) reveals all genes are closely related to A/H5N1, Clade 2.3.2.1c viruses from Vietnam collected in domestic poultry in 2019 (Figure 11). Similar viruses have been detected in Cambodian domestic poultry between 2017 to 2019 and the virus closely resembles the virus that caused the outbreak in domestic poultry in January 2021 in Battambang (A/chicken/Cambodia/f0114254/2021).

Continued detection of endemic A/H7 viruses in Cambodian live bird markets. 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 Subregion 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. 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 paper detailing these initial findings was published in 2019.

Throughout the 2020 and 2021 seasons, 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-2022.

Continued detection of endemic A/H9 viruses in Cambodian live bird markets. 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 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 to 2019. The majority of Cambodian A/H9N2 viruses detected after 2015 belong to two genotypes, P and V.

One Health investigation on a case of avian influenza A(H9N2) virus infection in Cambodia, 2021. On February 26, 2021, an otherwise healthy 3-year-old male living in Prasat Bakong District, Siem Reap presented to an outpatient clinic on February 24th. A nasopharyngeal sample was obtained as part of influenza-like illness (ILI) sentinel surveillance, and tested positive for influenza A virus at the National Institute of Public Health (NIPH) but was un-subtypable for human seasonal (H1, H3, B) or for avian (H5 and H7) influenza virus. The sample was then transported to the National Influenza Centre at IPC, where it tested positive for avian influenza A(H9N2) virus by conventional and qRT-PCR. A joint One Health investigation was undertaken March 8-11th by the Cambodian Centers for Disease Control (CDC), Ministry of Health, NAHPRI, and provincial divisions with support from the United States CDC, the WHO, the Food and Agriculture Organization of the United Nations (FAO), and IPC (Figure 12). One A(H9N2) virus was detected from a chicken at the infected child's house. Subsequent sequence analysis revealed close genetic distances between the HAs of the human and chicken viruses which supports a possible link to a recent shared "ancestor," suggesting that the source of the exposure of the child was from household chickens. The pandemic potential of these viruses remains to be assessed. The results of this investigation were published in 2021.

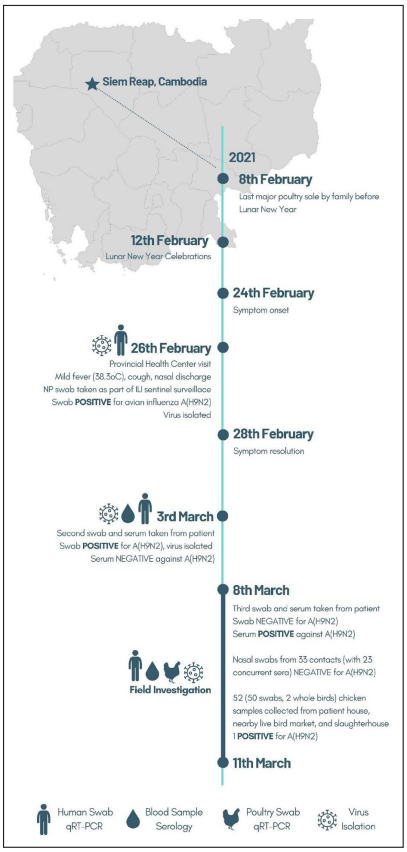


Figure 12. One Health investigation on a case of avian influenza A(H9N2) virus infection in Siem Reap, Cambodia

Longitudinal serological surveillance for AIV infection in poultry workers. Following a serosurvey 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 holidays) 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, four more sessions were planned for collection in the same locations; however, due to issues surrounding the COVID-19 pandemic, only 2 sessions were implemented. Four further sessions were planned for 2021-2022; however, again due to issues surrounding the COVID-19 pandemic, no samples were collected in 2021 but sample collection is still planned for 2022.

Swine influenza viruses

While swine production in Cambodia was traditionally characterized by backyard, small-scale farming efforts, larger confinement farms have increased in number in recent years. 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 (IAV) diversity, epidemiology, prevalence, and disease dynamics in Cambodia, virological surveillance in swine from backyard farms in Cambodia was undertaken 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. A/H1 and A/H3N2 swine lineages were detected as well as A/H5 and H9N2 in Cambodian swine, which is concerning for zoonotic potential. In 2020, swine samples were sent to collaborators at Duke University-National University of Singapore (NUS) for attempts at full genome sequencing to understand the viruses detected in these animals and have been completed.

Further swine work is funded through The National Institute of Allergy and Infectious Diseases (NIAID) funded Centers for Excellence in Influenza Research and Response (CEIRR) for 2021-2028 and is under further consideration for funding from other sources. In 2021, we were successfully awarded project funding through UPenn CEIRR/Royal Veterinary College (RVC) and established swine and human surveillance and began ethical clearance for the studies. Sampling and analysis will commence in 2022.

Collaborations	Institut Pasteur du Cambodge: Virology unit (E. Karlsson), EPH Unit (S. Ly, M. Chan) MoH/CCDC, NHPRI/GDP, FAO, WHO CC Melbourne/Peter Doherty Institute, Duke-NUS, WHO, RVC/UPenn, AAHL/CSIRO, London School of Tropical Medicine and Hygiene, UHS, CEIRRA
Funding	US-DHHS, FAO, NIH/NIAID/CEIRR, 2021-2028

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 have started 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. Funding for this study was approved in November 2021 and sampling commenced in three locations with 981 samples from wild birds and 1,336 samples from poultry already collected. Sampling, analysis, and the project will extend into 2022.

Collaborations	Institut Pasteur du Cambodge: Virology unit (E. Karlsson) NHPRI/GDP, MAFF, MoE, FAO, WCS
Funding	Defense Threat Reduction Agency (DTRA), FAO, 2021-2022

AXIS 3: Zoonoses

HEPAR: Rodents as Reservoirs for hepatitis E Virus (Hev), arenavirus and other rodent-borne viruses and risk assessment of infection in humans in CAMBODIA. The study objective remains to investigate HEV, arenavirus and other rodent-borne virus infections in rodents and estimate the seroprevalence of those rodent-borne virus infections in humans 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 and Preah Sihanouk Provinces was carried out. Rodent trapping sessions and blood sampling and data collection on humans exposed to rodents were conducted during rainy and dry seasons over a two-year period (June 2020-March/April 2022). In total, 750 rodents were trapped (336 and 414 during the rainy and dry season, respectively). Four genera were morphologically identified (Rattus, Bandicota, Suncus and Mus).

Various pools of organs have been tested for HEP-C, HEV-A and ARV using molecular detection methods. HEV-C was detected and confirmed by Sanger sequencing in 16 specimens (2.1%). HEV-C were detected in R. norvegicus (n=14) and R. rattus (n=2) and all positive rodents were from Phnom Penh markets. ARV was detected in 36 specimens (4.8%), of which five were detected in Phnom Penh markets, five in Kampong Cham sites, and 26 in Preah Sihanouk sites. Thirty-five were detected among R. norvegicus, one in R. exulans individually. Sequencing of PCR products identified them as Cardamones virus.

In total, 788 individuals working in markets in Phnom Penh, and living in communities rodents were captured trapping during the first session in the dry season of 2020 were enrolled in the study.In total, 788 individuals working in markets in Phnom Penh, and communities living in villages or sites were recruited for rodent trapping during the first session in the dry season of 2020. Follow-up sampling sessions are ongoing at the time of this report (due to the sanitary situation in Cambodia). Commercial ELISA kits for the detection of antibodies (IgG) against HEV (Wantai BioPharm) allowed the confirmation of exposure to HEV in 188 participants (23.8%): 124/304 (40.8%), 23/196 (11.7%) and 41/288 (14.2%) in Phnom Penh, Kampong Cham and Sihanouk sites, respectively.

An in-house IgG ELISA against nucleoprotein (NP) of arenavirus, Cardamone's variant of Wenzhou Virus (WENV), was used to screen human serum samples. One hundred out of 788 (12.7%) serum samples were positive for IgG antibodies with a higher percentage in urban area (Phnom Penh; 21.7%) compared to rural sites (Kampong Cham; 9.2% and Preah Sihanouk; 5.5%).

Whole genome sequencing of positive samples is being developed using metagenomics and ampliseq sequencing techniques. Development of sero neutralization assay for confirmation of positive samples by ELISA is ongoing.

Collaborations	Institut Pasteur du Cambodge: Virology Unit (Veasna DUONG, Julia GUILLEBAUD, Oudamdaniel YANNETH, Vibol HUL and Thavry HOEM); Epidemiology-Public Health Unit (Sowath LY) Aix-Marseille Université (X. de Lamballerie and B. Coutard),Ministry of Agriculture, Forestry and Fisheries: National Animal Health and Production Research Institute; Forestry Administration,Ministry of Health: Cambodian CDC
Funding	Internal project (Institut Pasteur du Cambodge), January 2020-June 2022

PICREID: Hantavirus detection and characterization in rodents and human seroprevalence study in Cambodia. Hantaviruses are widespread across the world. In Asia, there is a lot of evidence of the circulation of these viruses in the eastern part of the continent (especially in Far-East Russia, China, and Republic of Korea). In Cambodia, we reported for the first time the presence of Seoul ortho hantavirus (SEOV)and Thailand hantavirus (THAIV) in 1998. 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 little data of serological evidence of infection in humans are available in Cambodia.

The present study is an ancillary study of the HEPAR project in which rodent (n = 750) and human (n = 788) samples were collected. The study was conducted in two phases. Firstly, it involved developments of serological (ELISA, neutralization assay, multiplex microsphere immuno-assay) and molecular (qRT-PCR and NGS) tools for detection and characterization of the hantavirus. Secondly, the developed tools were applied to characterize hantavirus in rodents and to evaluate seroprevalence of hantavirus in humans for subsequent analysis on factors associated with hantavirus infection and/or exposure.

The presence of hantavirus was detected in 26/750 (3.5%) of rodents. Most of the infected rodents (25/26) were from urban areas. Analysis of partial hantavirus L gene sequences revealed that 24 rodents were infected with SEOV, one rodent with Thottapalayam virus and another one with Hantaan ortho hantavirus. In collaboration with IP Paris, the whole genome sequence of SEOV was obtained from one sample. In humans, the overall rate of positive anti-Hantavirus IgG was 8.6% (68/788) and 10 (1.3%) out of 788 participants had positive anti-hantavirus IgM. Remaining work on NGS to obtain additional whole genome sequences of hantavirus are ongoing at both IP Paris and IPC. Statistical analysis to identify factors associated with hantavirus infection and/or exposure in humans is expected to be completed by July 2022.

Collaboration	Institut Pasteur du Cambodge: Virology Unit (J. NOUHIN, V. HUL, J. GUILLEBAUD, S. Ken, L. KHUN, R. LIM, H. AUERSWALD, and V. DUONG) Institut Pasteur Paris: French National Reference Center for Hantaviruses (J.M. Reynes); Urgent Response to Biological Threats laboratory (J.C. Manuguera; J. Vanhomwegen and V. Caro) and A. Sakuntbhai
Funds	PICREID-Network Pilot Research Program (7-312-0217530-66440L under NIAID Award Number 1U01AI151378-01):May 2021 – April 2022

ZooCoV: Toward an integrated surveillance of potential zoonotic Beta Coronaviruses in the wild animal value chains of 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 spillover at the human/animal interface. Data on knowledge on animal reservoirs, as well as on cultural, sociological, and ecological factors driving Beta-CoVs spread to humans and emergence are limited. 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 bushmeat trade and consumption at the human/wildlife interface, and 4) develop a flexible and integrated early-detection system of viral spillover 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 bushmeat trade survey in the human population using quantitative surveys during rainy and dry seasons in 2021. All individual serum (n=901) have been tested for the detection of antibodies against SARS-CoV-2 using in-house ELISA and Foci Reduction Neutralization Test (FRNT). No sample was detected with anti-SARS-CoV-2 IgG, however, 265 displayed a positive or equivocal result for CoV antibodies. Luminex technology was used to characterize the immune response of participants to various CoVs (in collaboration with the TRANSVIHMI unit, IRD). Analyses are ongoing at the time of this report.

Concurrent to human surveys, wildlife sampling was implemented in both provinces, either with active sessions (bats and rodents sampling), opportunistic sessions, or through collaborations with conservation NGOs. In total, 2,138 individuals/specimens from bats, rodents and other mammals were tested for the presence of CoV using molecular techniques (real time RT-PCR for SARS-CoV targeting E and N genes, conventional PCR for Pan-CoV detection). Overall, 21 specimens (1.0%) tested positive and were confirmed by sequencing for CoVs in rodents (2.5%, 4/159), in bats (0.9%, 3/568), and in bat feces (1.3%, 14/1,005). Additionally, 14 specimens are still under sequencing confirmation.

Collaborations	Institut Pasteur du Cambodge: Virology unit (Veasna DUONG, Julia GUILLEBAUD, Oudamdaniel YANNETH, Vibol HUL and Thavry HOEM); Epidemiology-Public Health unit (Sowath LY) CIRAD (V. Chevalier); IRD (Martin Peeters); Wildlife Conservation Society; Flora and Fauna International; Jahoo NGO 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 Foundation:June 2020 – January 2022

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 multisectoral, multi-level approach to integrating programs, policies, legislation, and research to achieve better One Health outcomes.

CANARIES continued in 2021, 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 consortium as a whole is actively writing manuscripts, grants, and other collaborative efforts, and a second consortium meeting is being planned for 2022.

Collaborations	Institut Pasteur du Cambodge: Virology unit (E. Karlsson)
Funding	DTRA – UKGCRF: 2019-2022

COVID-19

SARS-CoV-2 Serology. Upon initial isolation of the SARS-CoV-2 at the beginning of March, 2020, several in-house serological assays were implemented: immunofluorescence assay, indirect enzyme-linked immunosorbent assay (ELISA), plaque and foci reduction neutralization test (PRNT and FRNT), and multiplex microsphere-based immunoassay (MIA). The ELISA and PRNT/FRNT were used to analyze the COVID-19 surveillance samples received at IPC. Additionally, the performance of ELISA, PRNT and MIA was compared to commercial electro-chemiluminescence immunoassays, revealing that in-house ELISA and PRNT performed similarly well than the commercial assays. The FRONT was also used to demonstrate that 70% of the investigated Cambodian cohort had neutralizing antibodies after the acute infection, persisting in 56 % of the people 6-9 months after infection. The successful isolation of α- and o-VoC viruses was used to adapt the FRONT accordingly.

Collaboration	Institut Pasteur du Cambodge: Virology Unit (Heidi AUERSWALD, Philippe DUSSART and Veasna DUONG) and Immunology Unit (Tineke CANTAERT, Hoa Thi My VO, Alvino MAESTRI), Institut Pasteur (Jessica VANHOMWEGEN, Jean-Claude MANUGUERRA)
Funds	Internal funding, URGENCE COVID-19 fundraising campaign of Institut Pasteur: May 2020 – December 2021

In vitro evaluation of inhibitory effects of Artemisia annua and A. afra extracts on SARS-CoV-2 (CovArt-RIIP). Recently antiviral effects of Artemisia spp. were reported. Artemisinin (ART) can be found in A. annua and to a lesser extent A. afra. Extracts from the latter have shown dose-dependent inhibition of SARS-CoV-2 in previous studies. This study aims to investigate in vitro inhibitory effects of Artemisia extracts on Cambodian SARS-CoV-2 isolates and is complementary to the CovArt project at IP Paris. The recent investigations revealed a 10-fold higher SARS-CoV-2 inhibition by A. afra extracts then observed for A. annua (Figure 13). Therefore, the inhibitory effect appears to be ART-independent. Further investigations whether the Artemisia extracts affect the virus entry or the replication are underway.

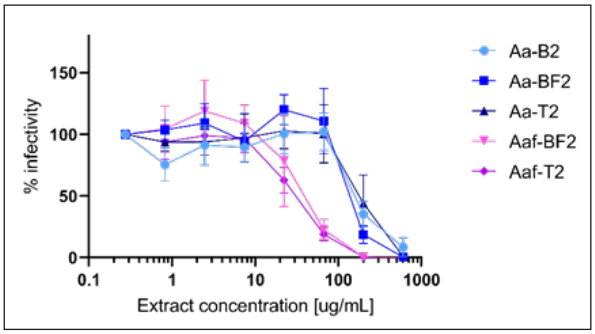


Figure 13. Kinetics of inhibitory effects of Artemisia annua and A. afra extracts on SARS-CoV-2

Collaboration	Institut Pasteur du Cambodge: Virology Unit (Heidi AUERSWALD and Veasna DUONG) Institut Pasteur (Jean-Christophe BARALE, Maël BESSAUD)
Funds	URGENCE COVID-19 fundraising campaign of Institut Pasteur: September 2020 – August 2021

Investigation of in vitro host-pathogen interaction between coronaviruses and bat cells. The immortalized kidney cell line from Blyth's horseshoe bat, Rhinolophus lepidus, (Rhileki) was demonstrated to be susceptible and permissive for SARS-CoV-2. This is the first report indicating this Rhinolophus species as a potential host/reservoir for SARS-CoV-2-related viruses. The Rhileki cell lines were also used in isolation trials of SARS-CoV-2-like and swine acute diarrhea syndrome coronavirus (SADS) from Cambodian surveillance bat samples. However, the isolation attempts were not successful so far and will be extended to other bat cell lines (e.g. TB1 Lu purchased from ECACC) and primary bat cells.

Collaboration	Institut Pasteur du Cambodge : Virology Unit (Erik KARLSSON, Heidi AUERSWALD and Veasna DUONG)Duke-NUS Medical School Singapore (Gavin J.D. SMITH)
Funds	Virology Unit Internal funding: 2021-2022

Risk assessment of the impact of the COVID-19 pandemic on tour guides becoming poultry farmers and live bird sellers. The current COVID-19 pandemic has dealt a devastating blow to the tourism industry in Cambodia, especially in Siem Reap's Angkor Wat UNESCO World Heritage Center. Tourist arrivals to Cambodia were down by 98.1% in 2020-2021 and are only now slowly starting to recover. In Siem Reap, visitors to Angkor in Q3 2020 were down 98% compared to the same time in 2019, dropping from 418,070 visitors in 2019 to only 8401 in 2020. Due to the pandemic, it is estimated that as many as 30,000 to 40,000 jobs have been lost in Siem Reap alone based on the number of closed hotels, restaurants, bars, shops, and spas as well as job loss for more than 6,000 licensed tour guides operating in Siem Reap. In response to the need for income and sustenance, it is rumored that a number of tour guides have turned to small-scale farming to support a subsistence living during the crisis, including chicken farming. Many of them have not received any formal training in zoonotic risk. Therefore, increases in poultry farming, especially in individuals who may lack training in good farming practices and preventative capabilities, potentially increasing avian influenza virus risk and circulation. This semi-quantitative cohort study seeks to understand the impact of COVID-19 on tour guides (and farmers), resultant food insecurity, and knowledge, attitude, and practice in biosafety and biosecurity in regards to avian influenza risk. The survey was started in 2021 and continues into 2022.

AXIS 4: HIV, Human papillomavirus and viral hepatitis

Human Papillomavirus E6 and E7 coding gene variations and their possible association with occurrence of cervical intraepithelial neoplasia. Cervical cancer is the fourth most common cancer among women worldwide, with an estimated 604,127 new cases in 2020. Nearly 90% of the 341,831 deaths worldwide occurred in low and middle income countries, and among them 58.5% were in Asia. Therefore, cervical intraepithelial neoplasia (CIN) and cervical cancers remain a public health concern in most of the countries. Cervical cancer is caused by sexually acquired infection with certain types of human papillomavirus (HPV), notably genotypes 16 and 18. A few studies have suggested associations between oncogenic potential of HPV and viral genetic variabilities including E6 and E7 coding regions that play important roles in cervical carcinogenesis.

The main objective of our study is to describe and characterize HPV diversity circulating in HIV-infected women in Cambodia using the NGS approach. Secondarily, we will assess the associations between variants in HPV E6 and E7 coding genes and CIN status.

The expected outcomes are 1) acquisition of HPV full-length genome sequencing competency, 2) description of HPV diversity in HIV-infected Cambodian women, and 3) data of clinical significance and relationships between variation of HPV E6 and E7 coding regions and the occurrence of CIN.

Collaboration	Institut Pasteur du Cambodge: Virology Unit (J. NOUHIN, N. BOUKLI, J. GUILLEBAUD, and L. KHUN) and Sequencing Platform (N. KHIM and V. HEANG) Calmette Hospital (S. LIMSRENG, A. KORN) University of Health Sciences (S. KIM, S. MOEUNG) ANRS (O. SEGERAL) IRD (P. De BEAUDRAP)
Funds	IPC internal budget: November 2021 - August 2022

Construction of virus replicon to characterize genotype-6 hepatitis C virus (HCV) drug resistance. Despite the efficacy of direct-acting antivirals (DAA), HCV remains a major concern for public health in the absence of a preventive vaccine and the 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 the NGS approach. 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 that 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. 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.

Collaboration	Institut Pasteur du Cambodge: Virology Unit (J. NOUHIN, H. AUERSWALD, and V. DUONG) Institut Pasteur Shanghai (J. Zhong)
Funds	IPC internal budget:June 2021 - May 2022

Axis 5: Rabies and other viruses

Immunogenicity assessment of subjects receiving rabies post-exposure prophylaxis in Cambodia. This study aimed to compare different post-exposure prophylaxis (PEP) vaccination strategies in a real life setting of individuals attending IPC's rabies vaccination center in Phnom Penh. The antibody kinetics in patients were evaluated following either the one-week, 3-dose intradermal regimen (ID IPC regimen) or the two-week, 4-dose intramuscular regimen (IM). Fluorescent Antibody Virus Neutralization Test (FAVNT) determined the titer of functional (neutralizing) antibodies. Overall, 210 study participants remained

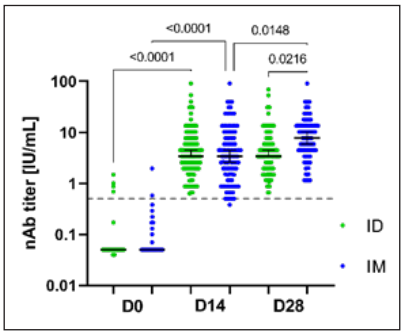


Figure 14. Rabies neutralization antibodies kinetic in patients after 3-dose intradermal regimen

throughout the course of the study and were included in the analysis. All, except of one subject in the IM group, seroconverted after receiving three vaccination doses (Figure 14). The additional dose in the IM regimen led to an additional increase of the antibodies

Collaboration	Institut Pasteur du Cambodge: Epidemiology and Public Health Unit (Sowath LY), Immunology Unit (Tineke CANTAERT) and Virology Unit (Heidi AUERSWALD and Veasna DUONG)
Funds	Sanofi Pasteur: June 2020 – June 2022

Capacity and capability development for ante mortem rabies diagnostic at National Animal Health and Production Research Institute (NAHPRI). In a twinning approach IPC will enable NAHPRI to perform rabies virus diagnosis on animal brain samples. The assessment of necessary equipment, material and reagents was performed early in 2022 and the training of NAHPRI personnel will be done in Q2 2022. Afterwards, confirmatory testing and sample sharing on request will ensure the quality of testing at NAHPRI.

Collaboration	Institut Pasteur du Cambodge: Virology Unit (Heidi AUERSWALD, Veasna DUONG and Rabies team) Ministry of Agriculture, Forestry and Fisheries: National Animal Health and Production Research Institute (Sothyra TUM) GIZ
Funds	German cooperation (GIZ): November 2021- March 2024

Cross-cutting activities

Strengthening the platform for characterization of emerging viruses. A live-cell imaging system (IncuCyte S3, Sartorius) was purchased and installed within the BSL3 facility to strengthen the capacity isolation and characterization of novel and emerging pathogens. This system allows easier and higher throughput virus isolation as well as virus inhibitor and host range testing. It was already used for the isolation of SARS-CoV-2 o-VoC, artemisia inhibition experiments (see CovArt project), attempts on the isolation of bat SARS-CoV-2-like and SADS viruses, and the isolation of arboviruses and insect-specific viruses from Cambodian mosquito samples. In 2022, the platform will be further used for isolation trials, virus characterization and neutralization of hantaviruses, hepatitis E virus type C, and Cambodian arenaviruses. To further scale up the capacity for virus isolation and host range testing, several human and animal cell lines were purchased from the European Collection of Authenticated Cell Cultures (ECACC), and efforts are underway to establish a platform for human and animal primary cells. Additionally, the capacity for serological testing was increased by the purchase of a multimode reader (Cytation5, BioTek) and a suspension array system (BioPlex 200, BioRad), enabling high-throughput testing of antigens (serology) and viruses (diagnostic and characterization). Further multiplex microsphere-based assays (MIA) are planned for the investigation of antibodies against bat- and rat-borne viruses in human and animal samples (zoonosis and surveillance projects). The Cytation5 reader was set-up for influenza inhibitor testing and will be further used for fluorescence- or luminescence-based virus characterization and immunoassays.

Collaboration	-
Funds	German cooperation (GIZ): April 2002 to December 2021

4.4.3. Research Programs – Outlook for 2022

Arboviral Diseases

CHIKV: Genetic and phenotypic characterization of CHIKV from 2020 outbreak in Cambodia. Chikungunya virus 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 2020 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 the E gene and potentially other mutations throughout the genome might play an important role in the 2020 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	Institut Pasteur du Cambodge: Virology unit (V. Duong) ; Entomology Unit (S. Boyer) University of Texas Medical Branch (Scott WEAVER)
Funding	Pending

DogZooSEA and SEAdogSEA

An investigation of dog-borne diseases in Southeast Asia through a survey will investigate the perceptions and practices of villagers regarding dog ecology and epidemiology, as well as dog distribution and population dynamics. Additional serum samples of dogs and their owners will be collected to determine the prevalence of arboviruses (dengue, Japanese encephalitis, West Nile) by a neutralization test at IPC. This will also establish the potential of dogs as sentinels for arboviruses. The dog samples will also be analyzed for rabies antibodies to investigate non-lethal exposure to canine rabies. To further strengthen the laboratory capacity for zoonotic diseases in the region, IPC will organize and host a training on arbovirus neutralization methods in Q3 2022.

Collaboration	Institut Pasteur du Cambodge: Virology Unit (Veasna DUONG and Heidi AUERSWALD), Epidemiology and Public Health Unit (Sowath LY) Kasetsart University Bangkok (Anamika KRITIYAKAN) Gadjah Mada University Yogyakarta (Wayan T ARTAMA)
Funds	CIRAD: February 2022- March 2023

Virulence evolution of Japanese encephalitis virus along the axis of pigs, mosquitoes, and humans in Asia.

- Name of the awarding body: Swedish Council
- Name(s) of grant holder(s): Uppsala University, Sweden (Jiaxin LING) and Institut Pasteur du Cambodge: Virology Unit (Veasna DUONG and Heidi AUERSWALD)
- Objective: The proposal aims to decipher JEV virulence evolution features in Vietnam and Cambodia: 1. how JEV evolves along the host axis of pigs, mosquitoes, and humans; and 2. how the changes of the viral genome are linked to the virulence evolution.
- Funding: NA
- Duration: 2023-2026
- Status: PENDING for response

Seasonal and avian influenza viruses

Avian and human influenza surveillance activities in Cambodia and surveillance using novel collection and sequencing techniques.

- Name of the awarding body: FAO/USAID
- Name(s) of grant holder(s): IPC (Erik KARLSSON)
- Objective: Continued surveillance and novel technology deployment in Live Bird Markets and other locations in Cambodia to detect and respond to Avian Influenza Virus
- Funding: USD199,000
- Duration: 2022-2023
- Status: PENDING signing Letter of Agreement

CEIRR Pilot Project: Capacity building for in vitro influenza diagnostics.

- Name of the awarding body: CEIRR/NIAID
- Name(s) of grant holder(s): IPC (Erik KARLSSON, Heidi AUERSWALD), St. Jude Children's Research Hospital (Stacey SCHULTZ-CHERRY)
- Objective: Capacity building for use of the new cell culture facility to utilize human and novel chicken cell lines for future risk assessment, diagnostics development, and pandemic preparedness.
- Funding: USD375,000
- Duration: 2022-2024
- Status: PENDING – Submitted March 2022

Assessing the transmission, carrying capacity, and pathogenesis of avian influenza virus associated with native chicken breeds in the Greater Mekong Subregion.

- Name of the awarding body: Wellcome Trust
- Name(s) of grant holder(s): IPC (Erik KARLSSON), Cambridge (Jim KAUFMAN), Ben-Gurion University of the Negev (Tomer HERTZ)
- Objective: Assessment of immune responses, transmission, and infectivity of AIV in native chicken breeds in the Greater Mekong Region to understand circulation, reassortment, zoonotic risk, and

preventive mechanisms
- Funding: £3,000,000
- Duration: 2023-2026
- Status: PENDING – To be submitted April 2022

Pilot implementation of SILAB LIMs system in Cambodia for use in AIV surveillance.
- Name of the awarding body: FAO
- Name(s) of grant holder(s): Theramo (IPC implementer – Erik KARLSSON)
- Objective: Implementation and piloting of a ISO accredited LIMS system in the AIV laboratory to investigate utility and data sharing and to facilitate accreditation
- Funding: n/a
- Duration: 2022
- Status: Started March 2022

ZOONOSES (including SARS-CoV-2 in bats)

HANTA-FRAKAM Project: Improving our Knowledge of Hantavirus Circulation in France and Cambodia
- Name of the awarding body: PTR-Institut Pasteur Paris
- Name(s) of grant holder(s): Virology Unit (J. NOUHIN, V. HUL, J. GUILLEBAUD, S. KEN, and V. DUONG) and Epidemiology and Public Health Unit (S. LY, S. SORN, and K. NGUON) and Institut Pasteur Paris (J.-M. REYNES, J. VANHOMWEGEN, V. CARO, and V. SAUVAGE)
- Objective: The proposed study aims to 1) improve serological and molecular assays for detection and characterization of hantavirus, 2) conduct molecular epidemiology analysis of hantavirus infection in rodents and humans for better understanding their circulation in France and Cambodia, 3) assess disease burden and risk factors associated with hantavirus infection in humans in Cambodia, and 4) study natural history of Thailand virus infection in rodent models.
- Funding: €249,830.00
- Duration: 2022-2024
- Status: Submitted to Institut Pasteur PTR 2022

Biodiversity Conservation to mitigate the risks of emergING infectious diseases (BCOMING).
- Name of the awarding body: EU (HORIZON-CL6-2021-BIODIV-01-11)
- Name(s) of grant holder(s): Julien Cappelle
- Objective: The project aim will be 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: 6 million €
- Duration: 2022-2026
- Status: kick-off meeting will be held in France in July 2022.

PREZODE-AFRICAM.

- Name of the awarding body: French Development Agency (AFD)
- Name(s) of grant holder(s): IRD and CIRAD
- Objective: Study the risks of emergence of zoonotic diseases impacted by the hydrological dynamics, climate and environment in diversified ecosystems representing key animal/human/environment interfaces. Implement activities to reduce the emergence of zoonotic risks and strengthen, in coordination with local and national partners, the existing surveillance systems towards integrated surveillance OH.
- Funding: 1.8 million €
- Duration: 2022-2024
- Status: Research activities planning is ongoing.

Understanding of SARS-CoV-2 related virus distribution at the human-animal interface in Southeast Asia.

- Name of the awarding body: Defense Threat Reduction Agency (DTRA)
- Name(s) of grant holder(s): IPC (Veasna Duong), Thai Red Cross Emerging Infectious Diseases Health Science Center (Supaporn Wacharapluesadee), and DUKE-NUS (Linfa Wang)
- Objective: The project will establish the bat research network in Southeast Asia region (Cambodia and Thailand), which aims to understand more on the origin of SARS-CoV-2 and diversity of SARS-CoV-2 related coronaviruses for a better preparedness of the next pandemic.
- Funding: NA
- Status: Pending for response

In the Air Tonight: Metagenomic Pathogen Discovery as Tools in Pathogen Surveillance.

- Name of the awarding body: EID-SEARCH CREID/NIAID
- Name(s) of grant holder(s): IPC (Jurre SIEGERS, Vireak HENG; Mentor: Erik KARLSSON)
- Objective: Assessment of the utility of air sampling at the animal-human interface coupled with metagenomic viral sequencing to advance and expand pathogen surveillance at points of high risk
- Funding: USD150,000
- Duration: 2022-2023
- Status: Awarded, pending signatures

Coronavirus surveillance in bats in Cambodia and genetic analysis using novel sequencing techniques.

- Name of the awarding body: FAO/USAID
- Name(s) of grant holder(s): Erik KARLSSON
- Objective: Surveillance for coronaviruses and other bat-borne pathogens using environmental samples and novel field technologies to inform future surveillance efforts and expand/advance surveillance techniques
- Funding: USD80,000
- Duration: 2022
- Status: PENDING signing Letter of Agreement

Understanding the Original Variant of Concern: In vitro Studies of SARS-CoV-2 in Bats and the Consequences of Reverse Spillover.

- Name of the awarding body: Wellcome Trust
- Name(s) of grant holder(s): IPC (Erik KARLSSON), Cambridge (Jim KAUFMAN), Duke-NUS (Gavin SMITH)
- Objective: Capacity building for sequencing bat genomes, immune responses, and generation of primary bat cell lines linked to a scientific exploration of what may make Rhinophilid species adept at carrying SARS-CoV-2-related viruses and the potential impacts of reverse zoonoses.
- Funding: £3,000,000
- Duration: 2022-2025
- Status: PENDING – To be submitted July 2022

HIV/Hepatitis

HIV/HEPATITIS AND ASSOCIATED DISEASES

HIV Drug Resistance in the Era of Dolutegravir-Based First-Line Regimen in Cambodia. Following the updated WHO guidelines, Cambodia recommends dolutegravir (DTG), a second-generation integrase (IN) strand transfer inhibitor treatment as a preferred first-line regimen for HIV-infected patients. Since its introduction, cases of DTG resistance mutations started to emerge. Moreover, because of diversity in populations, clinical practices and health infrastructures, the outcomes of a DTG-based regimen in a "real-world" setting, especially in low- and middle-income countries should differ from the clinical trials.

The main objective of the present study is to investigate the evolution of HIV drug resistance in Cambodia, among antiretroviral (ARV)-naïve adults receiving DTG-based first line regimen, through the implementation of a prospective observational cohort study and the use of NGS approach to perform HIV drug resistance testing.

Seven hundred ARV-naïve adults (≥18 years) initiating DTG-based first line regimen in the framework of the HIV/AIDS national program will be included over a period of one year and followed up during a period of 36 months. HIV drug resistance will be assessed in HIV reverse transcriptase and IN genes for patients experiencing viral failure on specimens collected at failure and baseline time points.

Collaboration	Institut Pasteur du Cambodge: Virology Unit (J. NOUHIN and N. BOUKLI) National Center for HIV/AIDS, Dermatology and STD (V. OUK, B. NGAUV, C. MOM, and S. Samreth) Stanford University (R. W. SHAFER)
Funds	Pending – Submitted to NIH in Dec. 2021 and Expertise France 5% Initiative in Jan. 2022. Duration as yet unknown

4.4.4. Support to National Authorities

National Dengue Control Program in Cambodia (NDPD)

As part of a collaboration with the WHO and NDCP, the virology unit laboratory received samples from six provincial hospitals and the National Pediatric Hospital in Phnom Penh and from a clinical laboratory within IPC. Results from the monitoring of hemorrhagic syndromes are reported weekly or monthly to the various monitoring programme participants (Director of the NDCP, hospital physicians, etc.).

All four DENV serotypes are co-circulating in Cambodia with changing dominant serotypes. DENV-3 was the main serotype during the major outbreak in 2007 followed by DENV-2 in subsequent years until 2010. DENV-1 became the main serotype detected from 2011 to 2015, and 2016 was marked by an increased detection of DENV-2. Five years after the DENV-3 outbreak, Cambodia experienced another large outbreak in 2012 caused by DENV-1. We continued to detect mainly DENV-2 in 2017-2018, while DENV-1 was still present in the country at a lower level. The year 2019 was marked by another major dengue outbreak by DENV-1 with more than 68,000 hospitalized cases and 48 deaths reported throughout the country. In comparison, in 2018, 24,684 cases and 23 deaths were reported (Figure 15). Since 2020, DENV-2 has returned to be the dominant serotype with a sharp decrease of reported cases countrywide.

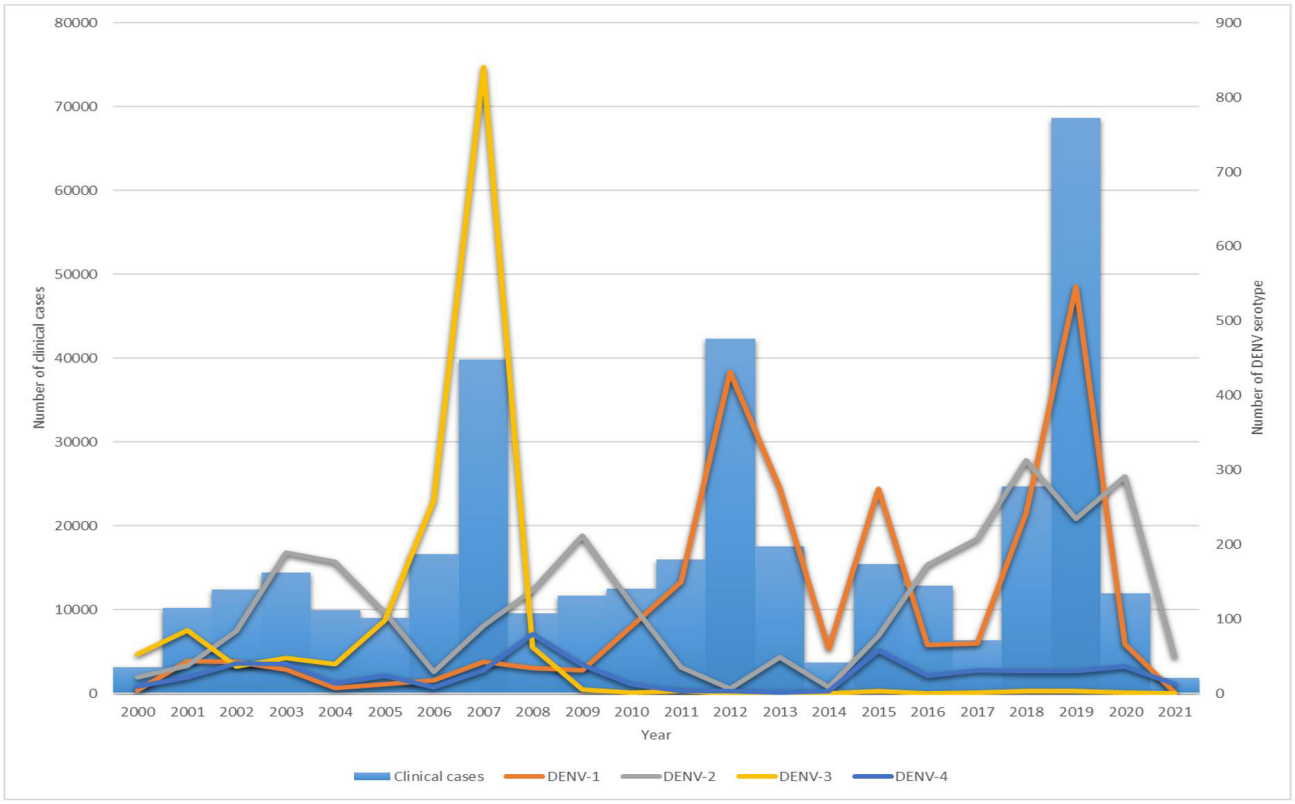


Figure 15. Dengue virus serotypes circulation in Cambodia and number of dengue clinical cases reported.

Diagnostics for rabies infections

Rabies remains a major public health concern in Cambodia. IPC's Virology Unit has been involved in the diagnosis of rabies infections using a fluorescein-conjugated antibody specific for rabies virus nucleoprotein (Fluorescent Antibody Test – FAT) for almost two decades. This test is routinely performed on samples obtained from suspected rabies infected animals, specifically fresh Ammon's horn or brain samples.

The average number of animals brought to IPC for rabies diagnosis was 201 with a positivity rate at 54.2% betweem 2000 to 2021. In 2019, the number of dog brains tested (n=125) decreased as we received 28% fewer samples than in 2018. However, the percentage of samples testing positive for rabies remains high at 68.8% (n=86). Similarly, due to COVID-19, we received only 164 and 175 specimen in 2020 and 2021, respectively, but the positivity rate was higher compared to previous years at 70% and 77% in 2020 and 2021 respectively (Figure 16).

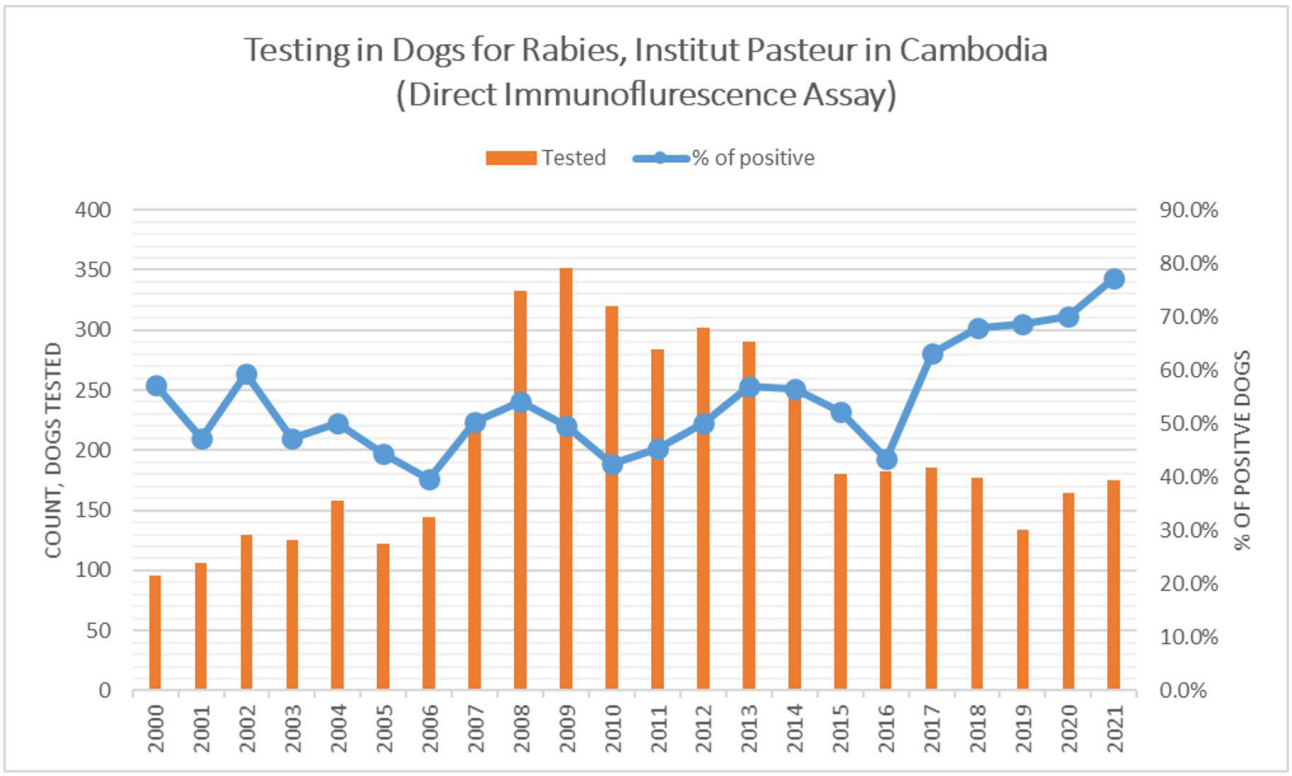


Figure 16. Testing in Dogs for Rabies, Institut Pasteur in Cambodia by Direct Immunofluorescence Assay

Cambodian National Influenza Center, WHO Regional H5 Reference Laboratory, and WHO Global COVID-19 Laboratory

IPC's Virology Unit has been Cambodia's National Influenza Center (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. 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 Referral Laboratory. All support to Cambodian National Authorities and International Partners will continue in 2022 and beyond as described above for 2021.

Seasonal human respiratory virus surveillance (Influenza-like Illness and Severe Acute Respiratory Illness)

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: Kampot, Battambang, Kampong Cham, Mondulkiri, Svay Rieng, Angkor Children's Hospital (Siem Reap) and the National Pediatric Hospital (Phnom Penh). Each hospital randomly collects clinical samples from a maximum of 5 ILI patients per week. Samples are first analyzed by NIPH and are then sent to IPC for

confirmation. Samples are also received from other institutions in Cambodia which have public health and research activities on influenza, such as the National Institute of Public Health (NIPH), the Naval Army Medical Research Unit (NAMRU-2; now defunct), and the Armed Forces Research Institute of the Medical Sciences (AFRIMS).

Human seasonal influenza

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 March-June, and peak 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 SARS-CoV-2 infection has shown a remarkable reduction in the rates of infection of many respiratory diseases despite continued, or even increased, testing for influenza in some countries.

Once international border restrictions were eased on 11th June 2020, Cambodia experienced an outbreak of influenza A(H3N2), that circulated in several provinces from July through to November 2020 including clustered detections in closed/semi-closed systems (prisons/pagodas) and also spreading in the general community (Figure 17). 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.

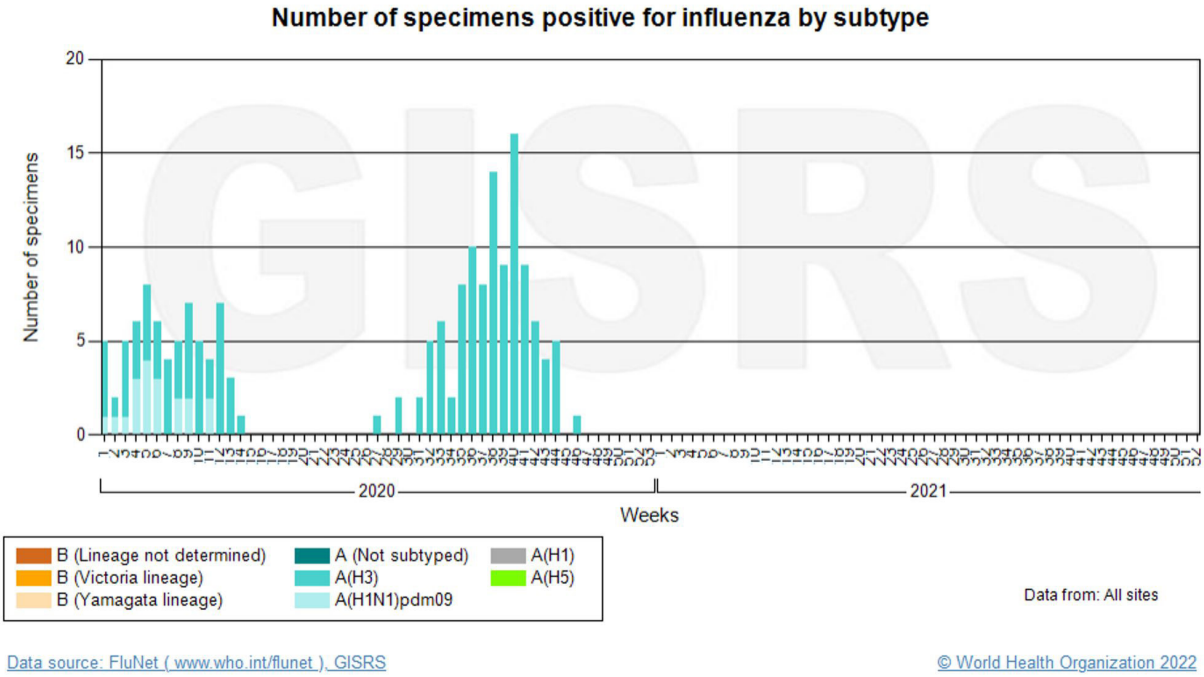


Figure 17. Number of influenza cases detected in sentinel and outbreak samples 2020-2021. No influenza cases were detected in Cambodia in 2021.

One paper detailing the initial A(H3N2) outbreaks in mid to late 2020 was published and a second which details the genetic and antigenic findings from the entire outbreak was also published in 2021.
Following the outbreak in 2020, IPC began to follow suggestions from WHO Guidelines to maintain influenza surveillance, especially with the exceptionally low numbers of influenza infections detected worldwide in 2020 and 2021. Out of 4,421 samples from symptomatic patients presenting with influenza-like illness or SARI during COVID-19 screening tested in 2021, no human seasonal influenza was detected in Cambodia. IPC continues to monitor all samples for influenza into 2022 as cases begin to rise globally.

Respiratory syncytial virus and parainfluenza virus

While the dangers of avian influenza and SARS-CoV-2 are well established, the prevalence and etiology of other respiratory pathogens such as parainfluenza virus (PIV), respiratory syncytial virus (RSV), adenovirus and rhinovirus have not been well studied. RSV is particularly important as it is the leading cause of respiratory infection-associated hospitalization of children aged <5 years in industrialized countries. New WHO guidelines are establishing an enhanced global influenza surveillance and response system (GISRS+) system which focuses on including RSV surveillance into existing sentinel systems. As per surveillance decisions, IPC began prospectively screening all samples from symptomatic children <5 years of age in August 2021 for RSV and PIV. Unlike influenza, RSV and PIV were detected in Cambodian children, with a small outbreak of PIV-2 in October and RSV-A in late October, early November 2021 (Figure 18). RSV-B and PIV-3 were also detected sporadically.

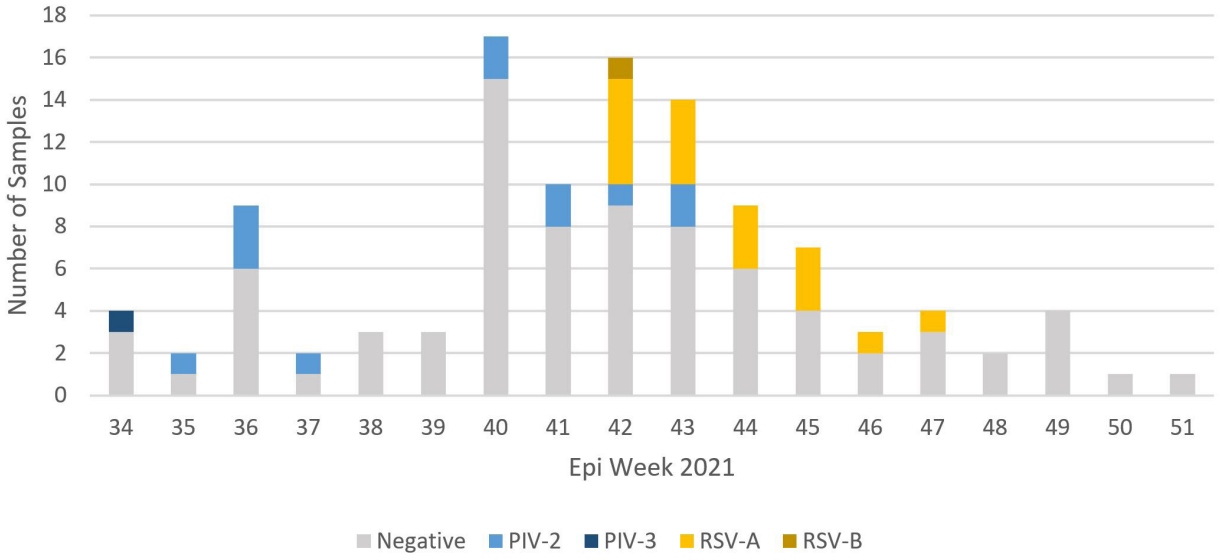


Figure18. Detection of RSV and PIV in samples from SARS-CoV-2 negative symptomatic children <5 years of age in 2021.

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 screening processes for individuals should any cases be detected in Cambodia. Through IPC's previous involvement in the Pandemic Preparedness for Global Health (PREDICT)

program, IPC was quickly able to mobilize and prepare to begin testing 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 19). On January 27th, IPC confirmed the first COVID-19 case (a traveler from Wuhan) in Cambodia. In April 2020, the work done at IPC in response to the global COVID outbreak was recognized by recognizing the virology unit as a WHO COVID-19 global reference center. In addition, IPC continues working closely with the Cambodian CDC, 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. **By December 31st 2021, IPC tested over 918,800 samples for SARS-CoV-2 by RT-PCR** and identified (or confirmed when first identified by NPHL at NIPH or one of the regional laboratories) tens of thousands of positive cases as part of surveillance and response, including the ongoing community transmission events (Figure 19).

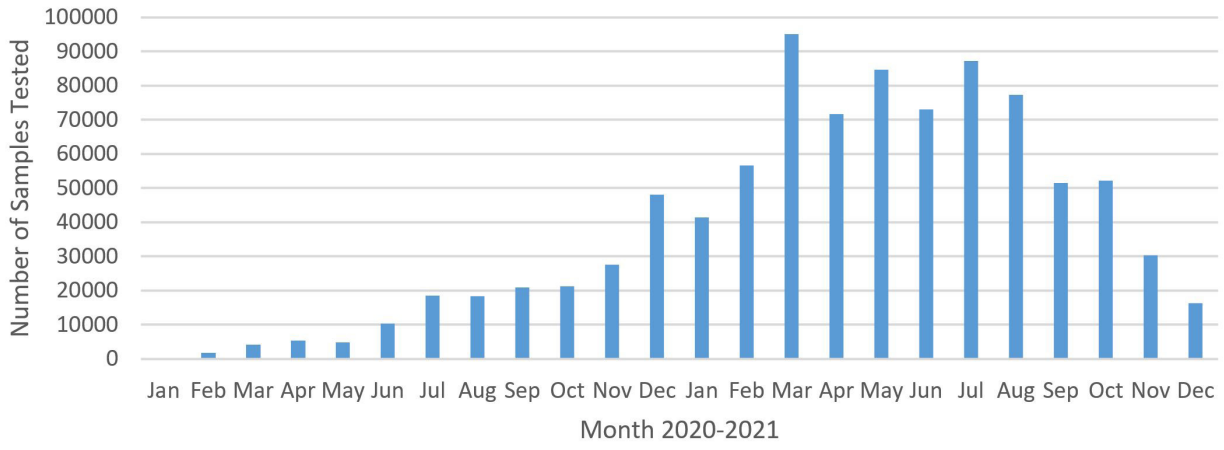


Figure 19. Number of samples tested for SARS-CoV-2 by month at Virology Unit, IPC between 2020 and 2021.

Testing and use of molecular detection kits (RT-PCR assays) for detection of VoCs in Cambodia

Even before COVID-19 was declared a public health emergency of international concern (PHEIC), IPC was designated by the government of Cambodia to validate and verify all novel and incoming assays for SARS-CoV-2 before use in the Kingdom. While sequencing remains the "gold standard" for SARS-CoV-2 variant detection, with the global spread of COVID-19 different variants of concern (VoC), numerous commercial kits for the molecular detection of VoC lineages of SARS-CoV-2 have become available on the market touting quick, sensitive, and specific detection of different VoC in suspected cases. In 2021, IPC validated five of these kits, and this data is frequently shared with the global network. In addition, technology transfer has been achieved with NIPH, Sihanoukville, and Ket Mealea laboratories as beneficiaries to help ensure testing for all VoC is widely available in the country. A publication detailing this work is expected in early mid-2022.

Based on the validation/verification and usability of the VoC RT-PCR kits tested, IPC decided to employ the KogeneBiotech PowerChek™ SARS-CoV-2 S-gene mutation kit in daily routine genomic surveillance. IPC aims to test 92 samples/day (644/week) in routine testing of new cases in Cambodia. These samples are chosen based on availability of what was tested the previous day and selected, to the extent possible from

across provinces and sample types as possible. In addition, IPC does daily VoC testing for the Siem Reap and Battambang laboratories and confirmatory testing for NIPH and KTML laboratories as they also employ these kits in daily testing. As of the end of December 2021, IPC has tested over 14,880 samples for VoC by RT-PCR to monitor Variants of Concern (VoC) circulating in and entering Cambodia (Figure 20).

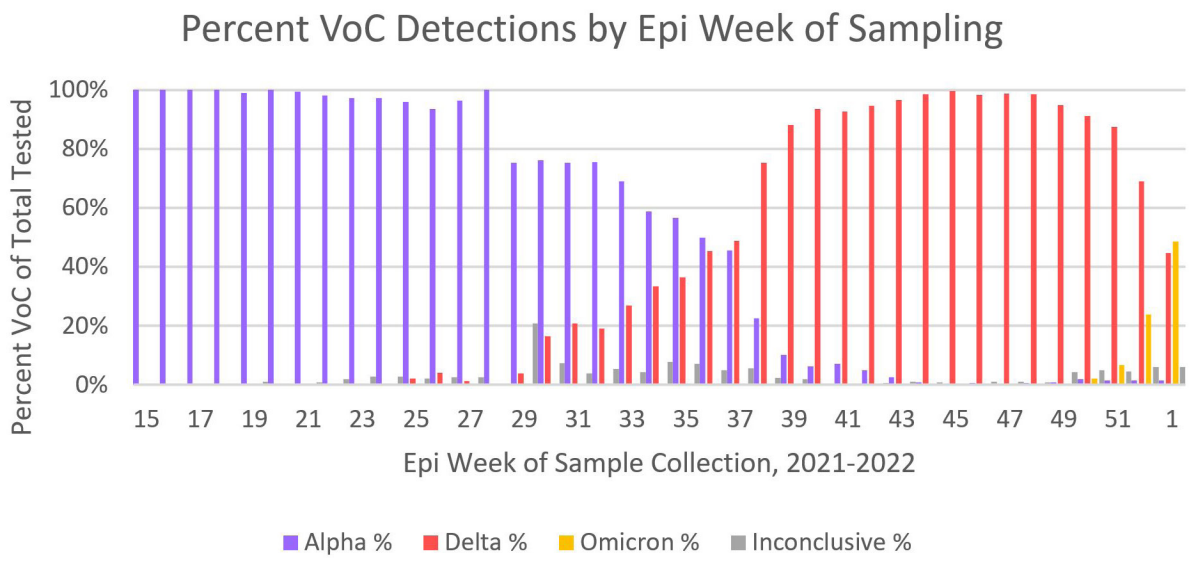


Figure 20. Detection of VOC in community samples in Cambodia by epidemiological week from April, 2021. Due to declining sample numbers after week 50 of 2021, percentages may not reflect true prevalence in the community.

SARS-CoV-2 Sequencing

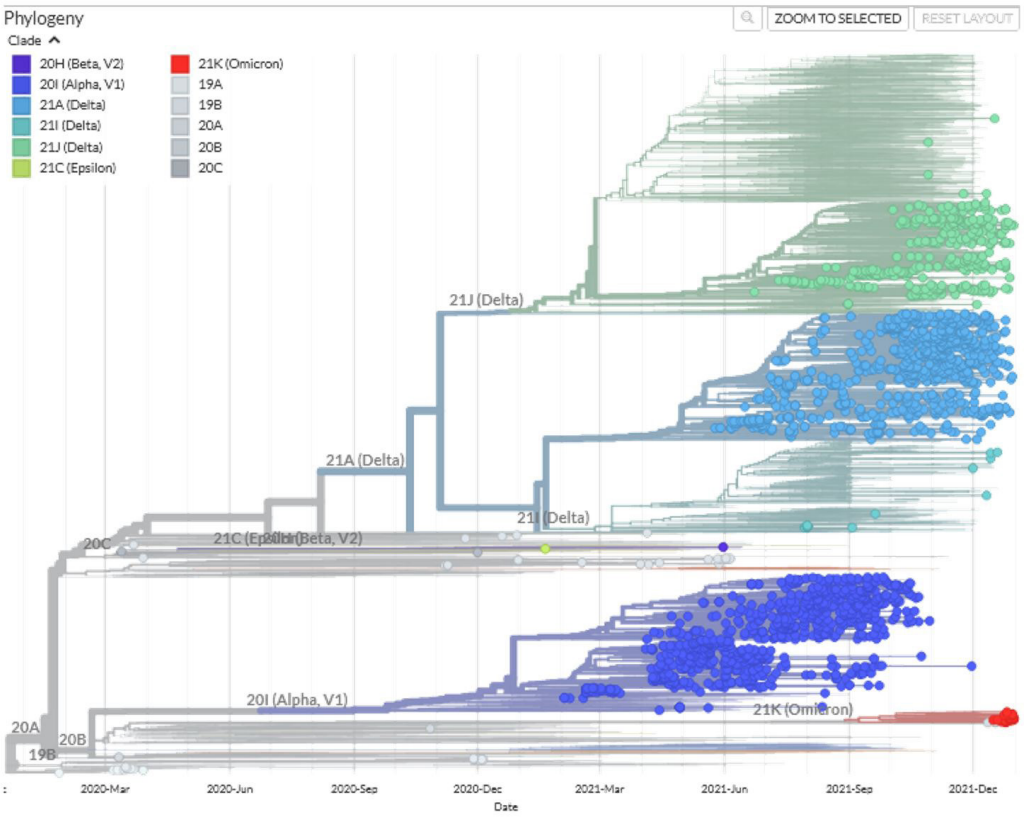


Figure 21. Timescale phylogeny generated by in-house IPC Nextstrain build of SARS-CoV-2 sequences between Jan 27th 2020 and early January 2022. Cambodian sequences are highlighted.

Network multiplex PCR primers set v3[1], v4, and MIDNIGHT[2] protocols on Oxford Nanopore GridION/MinION technology, in part from collaboration with partners at IP-Paris. This technique has successfully been employed by IPC to sequence SARS-CoV-2 samples with low viral load (Ct = <30) and is utilized weekly to sequence a limited number of samples to help with the COVID-19 response efforts and monitoring clusters and community spread.

Between January 2020 and the end of December 2021, IPC was able to sequence 2,041 samples (1.70% of total reported cases at that time) and submitted to GISAID[3] (Figure 21). Further work continues to monitor mutation rates in vaccinated versus unvaccinated individuals, transmission dynamics, and phylogeography of viral spread. Two manuscripts were drafted regarding sequencing SARS-CoV-2 sequencing in Cambodia.

Viral Isolation and Titration

Having a BSL-3 level facility and experience in isolating numerous types of viruses, IPC was quickly able establish viral isolation and tittering (both TCID50 and PFU) for COVID-19 in Cambodia. At the current time, 129 viral isolates (Wuhan-like, Alpha, and Omicron) are available from patients identified in Cambodia. We have as yet been unsuccessful in isolating Delta VoC. This strain biobank is vital for our continued validation and technical improvement work but also for establishing serological assays for seroepidemiological surveys and contact tracing efforts. Further isolation attempts are made on all possibly viable samples as available.

4.4.5. Teaching and Training

One of IPC's main missions is to provide teaching and training activities. The virology unit has been proactive in the training of students and staff from national and international institutions and government partners in the fields of surveillance and research.

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 NIPH and SARS-CoV-2 molecular diagnostic to personals from other regional COVID-19 laboratories based in Siem Reap, Battambang, Pailin, Oudarmeachey and others.

The unit hosted one second-year master's student from Université Libre de Bruxelles, one first-year master's student in Infectiology at University of Health Sciences (UHS), and one bachelor's student from Sun Yat-sen University.

Ms. AITCHEDJI Camille, Faculty of Medicine of Université Libre de Bruxelles, as part of her master's program in biomedical science, spent 12 weeks with IPC from October to December 2021 working on the detection and characterization of Hantavirus in rodents. Mrs. Yan Sokhoun, a first year student in the master's of infectious diseases program at the UHS, spent seven weeks between March and April 2021 working on the characterization of influenza virus in poultry from live bird markets. Finally, Mr. Chhouk Khemara, a bachelor's student in biomedical engineering at Sun Yat-sen University in Guangzhou China, spent 6 months working on detection of avian influenza viruses in poultry at the Orussey live bird market in Cambodia during the Pchum Ben festival.

[1] https://artic.network/ncov-2019
[2] https://nanoporetech.com/about-us/news/oxford-nanopore-provides-updated-protocols-rapid-scalable-and-low-cost-sequencing
[3] https://www.gisiad.org/

Two virology staff registered in PhD programs in the field of virology. Mr. HUL Vibol, is in the third year of his PhD program (2019-2022) at AIX-Marseille University, where his thesis is on the characterization of two novel arenaviruses and their seroprevalence in Cambodia. Ms. Ou Tey Putita started PhD studies in 2021 and is expected to complete the degree by 2024. The topic being studied is diversity and characterization of coronavirus and SARS-CoV-related viruses circulating in bats in Cambodia.

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

4.4.6. Outlook

At 3 years

In 2021, the virology unit expanded exponentially. Increases occurred not only in size, but also number of activities, covering a wide range of research in the laboratory and in the field. Utilization of more advanced skills have started being the norm, such as next generation sequencing and cell culture. To sustain and further build on this expansion, several critical short-term plans to improve technical skills, to bring in senior researchers including post-docs or visiting scientists, to extend collaboration, and to improve laboratory management are already in process. It is also crucial for the unit to publish the huge amount of data generated, which has been significantly delayed by the urgent response to the COVID-19 pandemic. Within 3 years, we expect that the following will be true.

- Capacity and capability regarding in vitro work, including a greatly expanded biorepository of immortalized and primary cell lines will be greatly improved;
- Skills in next generation sequencing (NGS) and metagenomics sequencing, full integration of sequencing capacity with mini-Platform will continue to improve;
- Bioinformatics analysis expertise will be fully established and providing full support to the unit;
- Luminex assays implementation will be fully active to support surveillance and research programs;
- Collaboration within IP-Paris, across the IPIN, and global partners will continue to strengthen;
- Research programs in connection with basic science for mechanistic studies will continue to develop;
- A postdoctoral student in bioinformatics will be recruited;
- Through research programs continue to bring postdoctoral fellows and visiting scientist to complement our staff;
- The Unit will be integrated and will enroll more students in Masters and PhD programs (both Cambodian students attending foreign universities and foreign students conducting research in the virology unit in collaboration with universities);
- The laboratory database system will be improved;
- IPC will have prepared and received accreditation for ISO17025 at the national reference laboratory activities.

At 5 years

In the long term, the unit will continue to increase activity in research programs on zoonotic viruses and One Health, and expects to become a reference laboratory for zoonotic research and emerging infectious diseases.

It is hoped that with the capacity and capability built through the COVID-19 response, national Cambodian labs will be able to take on more of the burden of daily surveillance activities while the unit conducts more research-based activities to enhance risk mitigation and response. Indeed, the virology unit has long been a center of excellence for influenza and avian influenza research in Cambodia, as well as in the region, and will continue to contribute to other laboratories in training and technical support. It is expected that the current efforts to conduct advanced in vitro work will be further developed in the unit, eventually becoming a cell culture hub for virus-host interaction studies, including an extensive biorepository of novel tools, and will attract more collaboration in basic research.

4.4.7. Publications List – 2021

NOTE

The name of authors from the Institut Pasteur du Cambodge are underlined.
Publications in a journal without impact factor are listed separately and identified at the end of the list
** equal contribution, first author / ** equal contribution, last author*

1. ***An influenza A (H3N2) virus outbreak in the Kingdom of Cambodia during the COVID-19 pandemic of 2020***
   L. Y. Sovann, B. Sar, V. Kab, S. Yann, M. Kinzer, P. Raftery, R. Albalak, S. Patel, P. Long Hay, H. Seng, S. Um, S. Chin, D. Chau, A. Khalakdina, E. Karlsson, S. J. Olsen, J. A. Mott.
   International Journal of Infectious Diseases. 2021;103:352 7. DOI: 10.1016/j.ijid.2020.11.178

2. ***A Novel SARS-CoV-2 Related Coronavirus in Bats from Cambodia***
   Delaune Deborah*, Vibol Hul*, Erik A. Karlsson*, Alexandre Hassanin, Tey Putita Ou, Artem Baidaliuk, Fabiana Gámbaro, Matthieu Prot, Vuong Tan Tu, Sokha Chea, Lucy Keatts, Jonna Mazet, Christine K. Johnson, Philippe Buchy, Philippe Dussart, Tracey Goldstein, Etienne Simon-Lorière**, and Veasna Duong**
   Nat Communications ; 12,1 (2021), 6563 ; Doi:10.1038/s41467-021-26809-4

3. ***Antibody fucosylation predicts disease severity in secondary dengue infection***
   Stylianos Bournazos, Hoa Thi My Vo, Veasna Duong, Heidi Auerswald, Sowath Ly, Anavaj Sakuntabhai, Philippe Dussart, Tineke Cantaert**, Jeffrey V. Ravetch**
   Science. American Association for the Advancement of Science; 2021;372(6546):11025. DOI: 10.1126/science.abc7303

4. ***Antigenic evolution of dengue viruses over 20 years***
   Leah C. Katzelnick, Ana Coello Escoto, Angkana T. Huang, Bernardo Garcia-Carreras, Nayeem Chowdhury, Irina Maljkovic Berry, Chris Chavez, Philippe Buchy, Veasna Duong, Philippe Dussart, Gregory Gromowski, Louis Macareo, Butsaya Thaisomboonsuk, Stefan Fernandez, Derek J. Smith, Richard Jarman, Stephen S. Whitehead, Henrik Salje, Derek A. T. Cummings.
   Science. American Association for the Advancement of Science; 2021; DOI: 10.1126/science.abk0058

5. ***Assessment of inactivation procedures for SARS-CoV-2***
   Heidi Auerswald, Sokhoun Yann, Sokha Dul, Saraden In, Philippe Dussart, Nicholas J. Martin, Erik A. Karlsson, Jose A. Garcia-Rivera.
   J Gen Virol. 2021; DOI: 10.1099/jgv.0.001539

6. ***Decoding the RNA viromes in rodent lungs provides new insight into the origin and evolutionary patterns of rodent-borne pathogens in Mainland Southeast Asia***
   Zhiqiang Wu, Yelin Han, Bo Liu, Hongying Li, Guangjian Zhu, Alice Latinne, Jie Dong, Lilin Sun, Haoxiang Su, Liguo Liu, Jiang Du, Siyu Zhou, Mingxing Chen, Anamika Kritiyakan, Sathaporn Jittapalapong, Kittipong Chaisiri, Phillipe Buchy, Veasna Duong, Jian Yang, Jinyong Jiang, Xiang Xu, Hongning Zhou, Fan Yang, David M. Irwin, Serge Morand, Peter Daszak, Jianwei Wang, Qi Jin
   Microbiome. 2021; 9**,** 18. DOI: 10.1186/s40168-020-00965-z

7. ***Direct Infection of B Cells by Dengue Virus Modulates B Cell Responses in a Cambodian Pediatric Cohort***
   Vinit Upasani, Hoa Thi My Vo, Heidi Auerswald, Denis Laurent, Sothy Heng, Veasna Duong, Izabela A. Rodenhuis-Zybert, Philippe Dussart, Tineke Cantaert.
   Front Immunol. 2021;11. DOI: 10.3389/fimmu.2020.594813

8. ***Genetic and Antigenic Characterization of an Influenza A(H3N2) Outbreak in Cambodia and the Greater Mekong Subregion during the COVID-19 Pandemic, 2020.***
   Jurre Y. Siegers, Vijaykrishna Dhanasekaran, Ruopeng Xie, Yi-Mo Deng, Sarika Patel, Vanra Ieng, Jean Moselen, Heidi Peck, Ammar Aziz, Borann Sarr, Savuth Chin, Seng Heng, Asheena Khalakdina, Michael Kinzer, Darapheak Chau, Philomena Raftery, Veasna Duong, Ly Sovann, Ian G. Barr**, Erik A. Karlsson**
   J Virol. 2021;95(24):e01267-21. DOI: 10.1128/JVI.01267-21

9. ***Human Infection with Avian Influenza A(H9N2) Virus, Cambodia, February 2021***
Samnang Um, Jurre Y. Siegers, Borann Sar, Savuth Chin, Sarika Patel, Seng Bunnary, Makara Hak, Sothy Sor, Oum Sokhen, Seng Heng, Darapheak Chau, Tum Sothyra, Asheena Khalakdina, Joshua A. Mott, Sonja J. Olsen, Filip Claes, Ly Sovann, Erik A. Karlsson.
Emerg Infect Dis. 2021;27(10):2742 5. DOI: 10.3201/eid2710.211039

10. ***Kinetics of the SARS-CoV-2 antibody response and serological estimation of time since infection***
Stéphane Pelleau, Tom Woudenberg, Jason Rosado, Françoise Donnadieu, Laura Garcia, Thomas Obadia, Soazic Gardais, Yasmine Elgharbawy, Aurelie Velay, Maria Gonzalez, Jacques Yves Nizou, Nizar Khelil, Konstantinos Zannis, Charlotte Cockram, Sarah Hélène Merkling, Annalisa Meola, Solen Kerneis, Benjamin Terrier, Jerome de Seze, Delphine Planas, Olivier Schwartz, François Dejardin, Stéphane Petres, Cassandre von Platen, Sandrine Fernandes Pellerin, Laurence Arowas, Louise Perrin de Facci, Darragh Duffy, Clíona Ní Cheallaigh, Jean Dunne, Niall Conlon, Liam Townsend, Veasna Duong, Heidi Auerswald, Laurie Pinaud, Laura Tondeur, Marija Backovic, Bruno Hoen, Arnaud Fontanet, Ivo Mueller, Samira Fafi-Kremer, Timothée Bruel, Michael White.
J Infect Dis. 2021;jiab375. DOI: 10.1093/infdis/jiab375

11. ***Longitudinal monitoring in Cambodia suggests higher circulation of alpha and betacoronaviruses in juvenile and immature bats of three species***
Julien Cappelle, Neil Furey, Thavry Hoem, Tey Putita Ou, Thona Lim, Vibol Hul, Oudam Heng, Véronique Chevalier, Philippe Dussart, Veasna Duong.
Sci Rep. 2021;11(1):24145. DOI: 10.1038/s41598-021-03169-z

12. ***Mosquito Vector Competence for Japanese Encephalitis Virus***
Heidi Auerswald, Pierre-Olivier Maquart, Véronique Chevalier, Sebastien Boyer.
Viruses. 2021;13(6):1154. DOI: 10.3390/v13061154

13. ***Neutralization of Dengue Virus Serotypes by Sera from Dengue-Infected Individuals Is Preferentially Directed to Heterologous Serotypes and Not against the Autologous Serotype Present in Acute Infection***
Heidi Auerswald, Simone Kann, Leonard Klepsch, Janne Hülsemann, Ines Rudnik, Sebastian Schreiber, Philippe Buchy, Michael Schreiber.
Viruses. 2021;13(10):1957. DOI: 10.3390/v13101957

14. ***Potential role of vector-mediated natural selection in dengue virus genotype/lineage replacements in two epidemiologically contrasted settings***
Olivia O'Connor, Tey Putita Ou, Fabien Aubry, Stéphanie Dabo, Sylvie Russet, Dominique Girault, Saraden In, Marine Minier, Sebastian Lequime, Thavry Hoem, Sébastien Boyer, Philippe Dussart, Nicolas Pocquet, Valérie Burtet-Sarramegna, Louis Lambrechts**, Veasna Duong**, Myrielle Dupont-Rouzeyrol**
Emerging Microbes & Infections. Taylor & Francis; 2021;10(1):134657. DOI: 10.1080/22221751.2021.1944789

15. ***Ranking the risk of animal-to-human spillover for newly discovered viruses***
Zoë L. Grange, Tracey Goldstein, Christine K. Johnson, Simon Anthony, Kirsten Gilardi, Peter Daszak, Kevin J. Olival, Tammie O'Rourke, Suzan Murray, Sarah H. Olson, Eri Togami, Gema Vidal, Expert Panel, Predict Consortium, Jonna A. K. Mazet.
PNAS. National Academy of Sciences; 2021;118(15). DOI: 10.1073/pnas.2002324118

16. ***Replication Variance of African and Asian Lineage Zika Virus Strains in Different Cell Lines, Mosquitoes and Mice***
Tey Putita Ou*, Heidi Auerswald*, Saraden In, Borin Peng, Senglong Pang, Sébastien Boyer, Rithy Choeung, Myrielle Dupont-Rouzeyrol, Philippe Dussart**, Veasna Duong**
Microorganisms. Multidisciplinary Digital Publishing Institute; 2021;9(6):1250. DOI: 10.3390/microorganisms9061250

17. ***The continuing search for the origins of SARS-CoV-2***
Erik A. Karlsson, Veasna Duong.
Cell. Elsevier; 2021;184(17):43734. DOI: 10.1016/j.cell.2021.07.035

18. ***The Ecology and Evolution of Japanese Encephalitis Virus***
Peter Mulvey, Veasna Duong, Sebastien Boyer, Graham Burgess, David T. Williams, Philippe Dussart, Paul F. Horwood.
Pathogens. Multidisciplinary Digital Publishing Institute; 2021;10(12):1534. DOI: 10.3390/pathogens10121534

Publications in a journal without impact factor

19. ***Socializing One Health: an innovative strategy to investigate social and behavioral risks of emerging viral threats***
Karen Saylors, David J. Wolking, Emily Hagan, Stephanie Martinez, Leilani Francisco, Jason Euren, Sarah H. Olson, Maureen Miller, Amanda E. Fine, Nga Nguyen Thi Thanh, Phuc Tran Minh, Jusuf D. Kalengkongan, PREDICT Consortium (Veasna Duong, Philippe Dussart), Tina Kusumaningrum, Alice Latinne, Joko Pamungkas, Dodi Safari, Suryo Saputro, Djeneba Bamba, Kalpy Julien Coulibaly, Mireille Dosso, Anne Laudisoit, Kouassi Manzan N'guettia Jean, Shusmita Dutta, Ariful Islam, Shahanaj Shano, Mwokozi I. Mwanzalila, Ian P. Trupin, Aiah Gbakima, James Bangura, Sylvester T. Yondah, Dibesh Karmacharya, Rima D. Shrestha,Marcelle Annie Matsida Kamta, Mohamed Moctar Mouliom Mouiche, Hilarion Moukala Ndolo, Fabien Roch Niama, Dionne Onikrotin, Peter Daszak, Christine K. Johnson and Jonna A. K. Mazet
One Health Outlook. 2021;3(1):11. DOI: 10.1186/s42522-021-00036-9

4.5. Medical and Veterinary Entomology

This year, the Medical and Veterinary Entomology Unit saw a continued increase in its personnel to 15 from 12 in 2020 and nine in 2019. The number of publications also continued its increase from six in 2019 to 11 in 2020 and 14 in 2021.

4.5.1. Functional Structure

The Medical Entomology Unit was officially created on 1 October 2018 (N/Réf: N°413/IPC/DIR/2018) coinciding with the recruitment of Sebastien Boyer by Institut Pasteur in Paris for a permanent position. As of March 2022, the unit has fifteen members, an increase of three over the previous year.

A key change in the department in 2021 was the recruitment of a second post-doctoral scientist, Dr. Antsa Rakotonirina. She arrived to develop the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Maldi-Tof) database and molecular competence within the team.

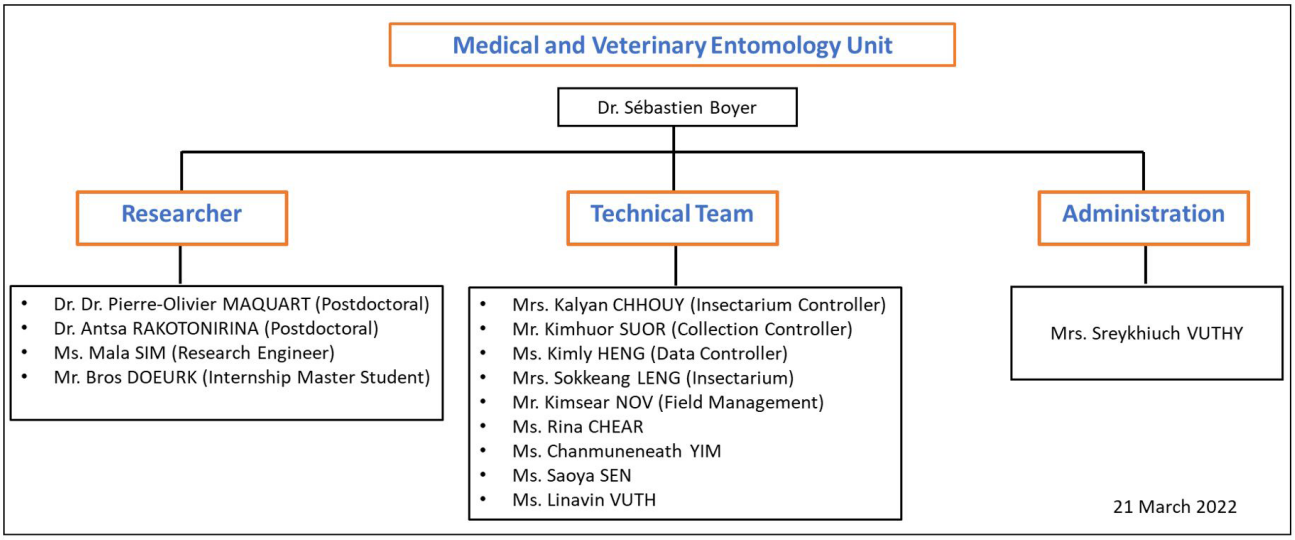


Figure22. Medical and Veterinary Entomology Unit Orgonogram

4.5.2. Major Achievements in 2021

Field Missions in 2021

During 2021 the medical and veterinary entomology unit undertook 35 field missions in Cambodia and five sampling missions in Phnom Penh City for a total of 393 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*). The objective of the project is to determine if a successful integrated vector management system in localized areas could decrease the incidence of dengue virus in communities in rural and peri-urban areas. The project will officially finish in December 2022. In 2021, there was a technical audit and a steering committee review.

Collaborations	Epidemiology and Virology Unit (IPC)IRD (GeoHealth) Institut Pasteur du Laos Institut Pasteur, Paris Ministry of Health Ministry of Youth, Education and Sport Ministry of Agriculture, Fisheries and Food
Funding	Agence Française pour le Développement (AFD) CZZ 2146 01:2016-2022

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-Cambodia.

In 2021, the last two project field missions took place during the dry season. The two sites listed below were sampled in 2021:

- Prek Toal Wildlife Sanctuary, located in Battambang Province is recognized as an important migration spot for migratory birds in Southeast Asia;
- 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).

One article was published on the mosquito biodiversity in Prek Toal (Maquart et al. 2021) and one studying the mosquitoes in mangrove areas in Koh Kong Province is under review. Another article on the factors explaining the distribution of different species in four different forests in Cambodia is in progress.

Finally, the mosquitoes collected in Cambodia (and in other countries) were sent to Institut Pasteur in Paris to determine the presence of viruses in the different species. New viruses were found in these mosquitoes and collaborators are currently analyzing and describing them. A first article was written and is currently under review on the use of next-generation sequencing (NGS) use on mosquitoes.

In addition, one specific new virus was discovered in four different mosquito species, and six different viruses were found in one mosquito species. This particular mosquito species represents about 60% of all mosquitoes collected in Cambodia, highlighting the importance of this discovery.

Collaborations	Virology Unit (IPC) Institut Pasteur Paris Ministry of Environment
Funding	DARPA - HR001118S0017:2017-2021

FSPI (Fonds de Solidarité pour les Projets Innovants) Project: Surveillance and Prevention of Emerging Viruses in Cambodia and the Region

The objective of this project was 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 wanted to understand these issues through the mosquito vector, because of the in-depth knowledge we had of it, 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 intended through this project, to train entomologists in the field by providing training in medical entomology, and to train virologists for the national reference center for surveillance of arboviruses in Cambodia.

After twenty missions were completed in 2020 that covered five different areas in as many different provinces (Pailin, Preah Vihear, Kampong Saom, Battambang, Kampong Thom), four last missions were done in 2021 (Kampong Thom, Battambang and Pursat in February and Preah Vihear in March).

The final technical and financial reports were sent to the French Foreign Ministry for Europe (MEAE) in March 2021. Within the lifespan of the project, two videos were produced: one video described the objectives of the project, the second one described our technical unit. The videos are both available on the Youtube channel of Institut Pasteur du Cambodge.

The four articles below are in progress and more may follow:

- Factors influencing the distribution of mosquito species along an ecological gradient from forest to the city;
- Description of new viruses and evolutionary relationship with mosquito species;
- Distribution of virus within mosquito species within an ecological gradient from forest to the city;
- Potential arboviruses cultivated on both mosquitos and human cells.

A part of these analyses are in progress. Some of them are being done by our collaborators at Institut Pasteur in Paris, and some parts by a potential PhD student in our unit.

Collaborations	Institut Pasteur Paris Ministry of Health Wildlife Conservation Society IRD (GeoHealth)
Funding	FSPI 2019-17: 2019-2021

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 is not yet fully known. A study on the dynamics of dengue vectors in Phnom Penh was undertaken in 2019 and 2020. The relative abundance of the different species is being analyzed against 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 *Ae. aegypti* and 2,771 Ae. 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.

Based on this internal IPC project, a collaborative project based on our design and sampling was designed with Michael C. Fontaine from MiVEGEC (IRD). This project aimed to explain and date the arrival of *Ae. albopictus*. This project (MUSE INVALBO) was financed in 2021.The characterization of *Ae. albopictus* invasivity in Phnom Penh will be completed in 2021. The project objective is to determine the invasion methods 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 its evolution to determine the manners of 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.

Several articles were written with this data. One article was published in 2021 on the presence of Ae. albopictus-that had not previously been detected-in Phnom Penh. Two other articles were submitted on (1) the predicted distribution of these arbovirus vector species in Southeast Asia, based in part from these data, and (2) on the invasive methods of mosquitoes. These two articles were written in collaboration with IRD (Bonin et al.) and Belgium (Da Re). Another article is in development on the factors explaining the distribution of dengue vector species in Phnom Penh. Data analysis of the INVALBO project is planned this year by the team headed by Michael C. Fontaine.

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

Collaborations	Ministry of Cults and Religion, Cambodia Ministry of Tourism, Cambodia Mivegec, IRD, France Entropie, IRD, Univ Réunion, CNRS, Ifremer, Nouvelle Calédonie UCLouvain, Belgium
Funding	IPC:2019-2022

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

The PICREID project has as its 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 hosts and adaptive responses of emerging infectious diseases.

The mosquito component aims to study the dynamics of the main dengue virus vector species in Kampong Thom Province (Ae. albopictus and Ae. 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 Institute Pasteur in Paris.

In 2021, 12 field missions were planned and 11 were done. The planned April 2021 mission could not be conducted due to Covid restrictions. In total, we collected 37,824 mosquitoes that year. Of the collected mosquitoes, 12,084 were sent to IP Paris for virus discovery.

Collaborations	Immunology, Epidemiology and Virology Units (IPC) Institut Pasteur Paris Ministry of Health IRD (GeoHealth)
Funding	NIH-U01AI151758-01:2020-2024

What Health Project (FSPI project)

This project was accepted in 2021,started in April 2021, and is expected to end in December 2022. It studies the anthropological effects on mosquito/virus complex changes. The WatERWealth Project will help determine the link between flooding and mosquito populations in Cambodia, related to pollutants and sanitary risk. More particularly for our unit, the 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.

In 2021, six monthly entomological missions were done between July and the end of December 2021. In total, 1,047 mosquitoes were caught with 15 light-traps used for three consecutive days every month. Larvae were also collected for identification of breeding sites of the most representative species and to initiate bioassays to determine the insecticide resistance at this specific location.

Collaborations	Ambassade de France au Cambodge, Cambodia IRD (Ecoland, GeoHealth) Ministry of Agriculture, Cambodia Institut Technologique du Cambodge (ITC), Cambodia Royal University of Agriculture (RUA), Cambodia Institut Pasteur de Nouvelle Calédonie (IPNC), Cambodia
Funding	NIH-U01AI151758-01:2021-2022

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 (four populations) were resistant to the three insecticides currently used in this country. The results were shared with the Ministry of Health (National Center for Parasitology, Entomology and Malaria Control). These results were also presented at six national and four international conferences.

Bioassays that were commenced at the end of 2020 were completed in 2021. In total, 17 insecticides (larvicides and adulticides) were tested with *Ae. aegypti* from Phnom Penh. Results were published in 2021.

Identification of mosquitoes

A particularly intensive work was undertaken in 2021 by the Malaria Molecular Epidemiology Unit to identify 20,237 mosquitoes. It collected mosquitoes for three days every month in 15 different sites in Mondulkiri Province. Our unit identified all the mosquitoes, and also found some species never before described yet in Cambodia. All these data will be analyzed in 2022 by a year 2 Master's student from the Royal University of Phnom Penh.

Based on all previous and current projects and the description of mosquito specimens, an intensive review was initiated in 2020 and concluded in 2021 with the publication of the checklist of mosquito species in Cambodia. This reference document for Cambodia was sent to all our national partners.

Since 2020, we have made efforts to develop a method for 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 2021, with the recruitment of Dr. Rakotonirina, we continue to improve the database of mosquito reference spectra with MALDI-TOF. In parallel, 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 was also strengthened under the supervision of Dr. Rakotonirina.

Finally, the phylogeographical and phylogenetic study of Culex vishnui mosquito species complex in Cambodia was assessed. This work is currently under review.

Collaborations	Virology Unit, IPC, Cambodia Medical Laboratory, IPC, Cambodia Malaria Molecular Epidemiology Unit, IPC, Cambodia
Funding	NIH-U01AI151758-01

4.5.3. Research Programs- Outlook for 2022

In 2021, seven projects were written and submitted to funding bodies.

Biodiversity Conservation to Mitigate the Risks of Emerging Infectious Diseases (BCOMING)
The project will analyze mechanisms underlying the impact of biodiversity on the risk of infectious disease emergence and aims to define tools of context-adapted biodiversity conservation and restoration strategies to reduce zoonotic risk. Surveillance strategies and pathogen detection would be implemented.

Biodiversity loss in hotspots of biodiversity is, among other socio-ecological factors, key to understanding, preventing and reacting to future pandemics. Despite this knowledge, the current COVID-19 crisis highlights the limitations of the implementation of One Health approaches. A main limitation is the lack of context-adapted solutions that stakeholders can easily implement in the field. To overcome this, BECOMING will build on past international projects to co-construct innovations with all stakeholders of biodiversity hotspots to reduce the risk of infectious disease emergence through biodiversity conservation and disease surveillance strategies.

The project activities will be implemented in Europe and three tropical biodiversity hotspots in Southeast Asia, West Africa and the Caribbean. BECOMING will lead to a better understanding of the mechanisms underlying the impact of biodiversity on the risk of infectious disease emergence. Participatory tools developed will facilitate the design of context-adapted biodiversity conservation and restoration strategies that reduce zoonotic risk. The surveillance strategies and pathogen detection tools developed will improve the capacity to detect emerging epidemics and arrest them before they turn into pandemics. The consortium constitutes a strong multi-actor group of partners with a history of successful cooperation including academics from biomedical, environmental and social sciences, private companies, NGOs, local and international stakeholders who bring together the wide range of disciplines and expertise required to reach all the expected outcomes of the proposal. The embedding of BECOMING in the Prezode Initiative will help to scale up the project innovations and disseminate cutting-edge socio-economic environmental strategies.

Preventing Zoonotic Diseases Emergence (PREZODE)
Within the Prezode Initiative, Cambodia is involved in the AfriCa project (2022-2024) in which four African countries (Cameroon, Guinea, Madagascar, Senegal) and one Asian country (Cambodia) are involved. The AfriCan project will aim to (1) study the risks of emergence of zoonotic diseases impacted by the hydrological dynamics, climate and environment in diversified ecosystems representing key animal/human/environment interfaces and (2) implement activities to reduce the emergence of zoonotic risks and strengthen, in coordination with local and national partners, the existing surveillance systems towards integrated surveillance One Health.

The overall project has three main components: (1) the risk assessment for zoonotic disease emergence, (2) environmental and climate influence, and (3) the prevention strategies for risk reduction of zoonotic emergence and the reinforcement of surveillance towards an integrated One Health system.

Protection of Karst and Cave Ecosystems to Prevent Future Pandemics (IKI – Karst)

The biodiversity of karst areas in Southeast Asia is the highest worldwide, with an exceptional number of globally threatened karst and cave restricted species, often endemic to single sites. This includes a very high diversity of bat species, critically endangered pangolins and primates. Karst/cave ecosystems remain under-represented in Southeast Asia's protected area network. Limestone quarrying for the cement industry threatens the karst landscape and essential habitats for at-risk species. Unmanaged cave tourism and guano collection disturbs cave habitats, directly impacting cave invertebrates and bats, as well as creating the risk of zoonosis. The hunting and wildlife trades further exacerbate the risk of zoonotic spillover. Bat colonies in Stung Treng Karst host a virus very similar to COVID-19. Protecting karst/cave ecosystems through protected area designation, while preventing human-wildlife contact through improved cave management will prevent future pandemics.

One objective is to improve the knowledge of biodiversity and zoonotic diseases in karst/cave areas with biodiversity surveys of karst/cave areas, including zoonotic diseases in wildlife, in particular in cave bats to improve the protection and management of karst and cave areas. Also, we aim to strengthen management of new karst/cave protected areas with participatory protected area gazetting, management planning and collaborative specific, measurable, attainable, relevant, time-bound) (SMART) patrolling to reduce hunting and wildlife trade. Finally, we expect to improve the management of caves to mitigate the spillover of zoonotic diseases with the development and implementation of guidelines for cave tourism and bat guano collection, cave management planning and zonation, training and capacity building for cave managers, guides and guano collectors.

Proof of Concept: Detection of Dengue and Chikungunya Viruses in Mosquitoes using MALDI-TOF and Artificial Intelligence

Pioneering studies over the last decades have demonstrated the reliability of MALDI-TOF MS to identify mosquito species and detect the microorganisms they carry. Current advances in artificial intelligence could complement and enhance performance of the MALDI-TOF MS to analyze mosquito spectra.

In this study, we will investigate, for the first time, the use of MALDI-TOF MS coupled with artificial intelligence to detect arboviruses in mosquitoes. For this purpose, Aedes aegypti and Aedes albopictus from the laboratory will be used. Mosquitoes will be exposed to an infectious blood meal (dengue and chikungunya viruses) in a biosafety level P3 (Pathogen level 3) laboratory. In parallel, other Aedes aegypti and Aedes albopictus will be fed with uninfected blood. At day ten post blood feeding, mosquito saliva will be collected. All mosquitoes will subsequently be killed and dissected. Mosquito abdomens will be used for RNA extraction and RT-PCR while the protein extraction and MALDI-TOF analysis will be carried out using saliva, legs and thorax individually.

The obtained results will show if we can detect an arbovirus infection (chikungunya and/or dengue) in different mosquito species. If so, we will be able to compare the signals and observe their specificity by virusand/or mosquito species in order to determine if the most important signals come from the multiplication of viruses or protein production from mosquitoes .

Anticipating the Emergence of Novel Arboviruses in Cambodia: Development of a Rapid Diagnostic Tool (postdoctoral PIC REID call)

Arbovirus emergence is generally initiated by spillover of the pathogen from wildlife into urban areas where it then reaches humans and mosquitoes. Cambodia is a possible hotspot for the emergence of new arboviruses. Indeed, the country hosts a great diversity of mosquito species including mosquitoes feeding on both animals and humans. The country is also particularly affected by deforestation and urbanization, leading to a constantly shifting interface between anthropic areas and forests.

These circumstances promote contact between humans and sylvatic mosquitoes, and therefore potential spillover events. In addition, recent studies underscored the high diversity of viruses within Cambodian mosquitoes. Consequently, spatial and temporal distribution of certain viruses carried by mosquitoes from Cambodia and their potential to emerge as pathogens should be studied. This information is crucial and the implementation of a rapid detection method is of interest due to their potential to emerge as novel arbovirus with public health implications.

The objectives of this project are: (1) to determine the ability of viruses to replicate in cell lines of both mosquito vectors and vertebrate hosts, and (2) to develop a diagnostic tool based on MALDI-TOF coupled with artificial intelligence for rapid and low-cost detection of potential new arboviruses in mosquitoes. The results of this project will allow us to study and potentially help predict the risk of emergence of new arboviruses in Cambodia. Health authorities could also use our results to make decisions to prevent and cope with future outbreaks.

Mosquito database Maldi-Tof mass spectrophotometry (MODEM)

The objectives of this research program are (1) to create and/or collect mosquito spectra from different regions of the world (2) to improve the identification of mosquito species with MALDI-TOF MS with advanced bioinformatic tools and (3) to create a shared MALDI-TOF MS database and initiate discussion regarding a possible open access spectra library including more mosquito species.

The first objective of this project is to create and collect mosquito spectra from different regions of the world using different MALDI-TOF MS instruments. Five institutes belonging to four continents from the Institut Pasteur International Network will be involved. Three cosmopolitan mosquito species vectors of arboviruses will be collected from the field. MALDI-TOF MS spectra will be created. For each mosquito used to create MSP, sequencing will be performed to confirm the species identification. The deliverable of this first objective is the compilation of mosquito spectra from different countries.

The second objective of this project is to improve the identification of mosquito species with MALDI-TOF MSwith advanced bioinformatic tools. Mosquito spectra will be used to improve data analysis and in fine mosquito species identification. The pre-processing of spectra consists in identification and joining of isotopic peaks. A normalization of spectra will also be performed in order to improve the estimate of the abundances and make them comparable in different acquisitions. The computing of average spectra for replicates of the same sample. This step will be performed in order to remove single analysis artifacts and strengthen the quality and representativeness of the resulting MSP. The alignment of MSPs that will be realized in order

to verify the reproducibility of signals among samples belonging to the same species. In a second step, a convolutional neural network algorithm allowing the comparison of mosquito spectra to the MSP is planned to be performed.

The third objective of this project is to create a shared MALDI-TOF MS database and initiate discussion about an open access spectra library including more mosquito species. When the algorithms allowing the improvement of mosquito species identification will have been developed and if the results are reproducible, we will create a common MALDI-TOF MS database composed of the MSPs of the mosquito species within the framework of PIBnet.

Southeast Asia Tick Determination Key (SEA TICKET)

This project aims to develop a determination key for tick species for all the countries in Southeast Asia. This project will initiate and develop veterinary entomology in Cambodia. The main partners are the Royal University of Agriculture and the Ministry of Agriculture. This project was proposed to a regional Southeast Asia FSPI, based in Thailand.

We propose to create and develop a complete and updated key for tick species identification for Southeast Asia that will be freely distributed to all public and private actors. In the medium term, the identification key created in the present project will be used to perform an inventory of tick species present in Cambodia and the Lao People's Democratic Republic (PDR), diversity and distribution to share knowledge with local veterinary and public health services and universities, to build capacities to monitor TBD in humans and livestock at the national level. It will also allow the development of several planned research projects on TBD in Southeast Asia in a One Health approach.

In parallel to the creation of the tick identification key and its dissemination throughout Southeast Asia, we will also initiate the implementation of MALDI-TOF databases using ticks collected in the field in Cambodia and Lao PDR (with the involvement of veterinary and farm actors). One database created from one leg of each collected tick specimen will be developed for tick species identification. In future One Health projects, the MALDI-TOF method will be further used for pathogen discovery.

Establishment of Veterinary Entomology in Cambodia (EVECA)

As for the medical entomology that was absent within the research landscape in Cambodia, our 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, 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. The main partners are the Royal University of Agriculture and Ministry of Agriculture. This project was submitted at MEAE with the French Embassy in Cambodia.

4.5.4. Support to National Authorities

The Medical and Veterinary Entomology Unit is working with five different ministries: Ministry of Health, Ministry of Education, Youth and Sport, and Ministry of Agriculture, Forestry and Fisheries, Ministry of Environment and Ministry of Cults and Religion.

At the request of the Ministry of Agriculture, Forestry and Fisheries, and with the help of Royal University of Agriculture, two projects were written and submitted to establish and develop veterinary entomology in Cambodia.

In 2021, an article highlighting the resistance of Aedes aegypti to several insecticides and proposing an alternative to the use of current insecticides was directly proposed to the CNM.Feedback is being awaited.

4.5.5. Teaching and Training

Mentorship

Master's Students

- Ms. VORN Sovatey, Cambodian student, International Joint Master's year two of Infectious Disease Study: Biology of Infectious Diseases, Master 1.
  "Interspecific competition between two dengue-vector species in Phnom Penh"
- Ms. DUSADEEPONG Rutaiwan, Thaï student, International Joint Master's year two of Infectious Disease Study: Biology of Infectious Diseases, Master 2.
  "Determination of the number of mosquito species within Culex Vishnui Complex in Cambodia"

Teaching

Since 2020, Sebastien Boyer has been responsible for the module "Vector Borne Diseases and Vector Transmission" within 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; significant time was required to prepare and complete it. The teaching module consists of 8 lectures of 1.5 hours. The exams and corrections were also done by our team.

Trainings

In 2021, all the newly recruited staff followed the Aptitude training online course on laboratory security and good laboratory practices.

Calmet & Yersin Grant

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

Collaborations	Institut Pasteur, Paris
Funding	Calmette & Yersin Post doc Grant, IP Paris (2021-2022)

4.5.6. Outlook

At 3 years

- Defense of two PhD students in Medical and Veterinary Entomology
- New insectarium and new space (laboratory and office)
- Change of Head of Unit
- Develop mechanistic research on transmission
- Ecomore 3

At 5 years

- Future research will depend of the new Head of Unit
- Strengthening Veterinary and Medical Entomology thematics
- Initiate works on Triatomine bugs in Cambodia
- Consolidate vector biology on bats ectoparasites,

4.5.7. Publications List 2021

NOTE

The name of authors from the Institut Pasteur du Cambodge are underlined.
Publications in a journal without impact factor are listed separately and identified at the end of the list
** equal contribution, first author / ** equal contribution, last author*

1. ***A review of Capezoum Adlbauer, 2003 (Coleoptera: Cerambycidae) with the description of two new species from the Succulent Karoo ecosystem in South-Africa***
   Pierre-Olivier Maquart, Francesco Vitali, Riana Bate.
   Zootaxa. 2021;4915(4):559 66. DOI: 10.11646/zootaxa.4915.4.6

2. ***Checklist of the mosquito fauna (Diptera, Culicidae) of Cambodia***
   Pierre-Olivier Maquart, Didier Fontenille, Nil Rahola, Sony Yean, Sébastien Boyer.
   Parasite. EDP Sciences; 2021;28:60. DOI: 10.1051/parasite/2021056

3. ***High Rickettsial Diversity in Rodents and Their Ectoparasites From the Central Highlands of Madagascar***
   Fanohinjanaharinirina Rasoamalala, Mamionah N. J. Parany, Soloandry Rahajandraibe, Malala N. Rakotomanga, Tojo Ramihangihajason, Voahangy Soarimalala, Sébastien Boyer, Minoarisoa Rajerison, Beza Ramasindrazana.
   J Med Entomol. 2021;tjab207. DOI: 10.1093/jme/tjab207

4. ***Host-Feeding Preference and Diel Activity of Mosquito Vectors of the Japanese Encephalitis Virus in Rural Cambodia***
   Sébastien Boyer, Benoit Durand, Sony Yean, Cécile Brengues, Pierre-Olivier Maquart, Didier Fontenille, Véronique Chevalier.
   Pathogens. 2021;10(3):376. DOI: 10.3390/pathogens10030376

5. ***Potential role of vector-mediated natural selection in dengue virus genotype/lineage replacements in two epidemiologically contrasted settings***
   Olivia O'Connor, Tey Putita Ou, Fabien Aubry, Stéphanie Dabo, Sylvie Russet, Dominique Girault, Saraden In, Marine Minier, Sebastian Lequime, Thavry Hoem, Sébastien Boyer, Philippe Dussart, Nicolas Pocquet, Valérie Burtet-Sarramegna, Louis Lambrechts**, Veasna Duong**, Myrielle Dupont-Rouzeyrol**
   Emerging Microbes & Infections. Taylor & Francis; 2021;10(1):1346 57. DOI: 10.1080/22221751.2021.1944789

6. ***Recent and massive invasion of Aedes (Stegomyia) albopictus (Skuse, 1894) in Phnom Penh, Cambodia***
   P. O. Maquart, D. Fontenille, S. Boyer.
   Parasites & Vectors. 2021;14(1):113. DOI: 10.1186/s13071-021-04633-5

7. ***Replication Variance of African and Asian Lineage Zika Virus Strains in Different Cell Lines, Mosquitoes and Mice***
   Tey Putita Ou*, Heidi Auerswald*, Saraden In, Borin Peng, Senglong Pang, Sébastien Boyer, Rithy Choeung, Myrielle Dupont-Rouzeyrol, Philippe Dussart**, Veasna Duong**
   Microorganisms. Multidisciplinary Digital Publishing Institute; 2021;9(6):1250. DOI: 10.3390/microorganisms9061250

8. ***Mosquito diversity (Diptera: Culicidae) and medical importance, in a bird sanctuary inside the flooded forest of Prek Toal, Cambodia***
Pierre-Olivier Maquart, Chea Sokha, Sébastien Boyer.
Journal of Asia-Pacific Entomology. 2021;24(4):1221 7. DOI: 10.1016/j.aspen.2021.08.001

9. ***Mosquito Vector Competence for Japanese Encephalitis Virus***
Heidi Auerswald, Pierre-Olivier Maquart, Véronique Chevalier, Sebastien Boyer.
Viruses. 2021;13(6):1154. DOI: 10.3390/v13061154

10. ***The Ecology and Evolution of Japanese Encephalitis Virus***
Peter Mulvey, Veasna Duong, Sebastien Boyer, Graham Burgess, David T. Williams, Philippe Dussart, Paul F. Horwood.
Pathogens. Multidisciplinary Digital Publishing Institute; 2021;10(12):1534. DOI: 10.3390/pathogens10121534

Publications in a journal without impact factor

11. ***Description of a new species of Conobrium (Coleoptera, Cerambycidae, Obriini) from São Tomé and Principe***
Alain Coache, Francesco Vitali, Maquart Pierre-Olivier.
Faunitaxys 2021;9:1 3.

12. ***Expansion of the range of Eupatorus siamensis (Castelnau, 1867) (Coleoptera: Scarabaeidae: Dynastinae) in Cambodia***
Pierre-Olivier MAQUART, SIN Sopha, DOEURK Bros, CHHORN Soksan, Sébastien BOYER, PHAUK Sophany
Cambodian Journal of Natural History. 2021;76.

13. ***Report of carnivorous plants (Droseraceae, Lentibulariaceae and Nepenthaceae) from seasonally dry savannahs in Ratanakiri Province, Cambodia***
Pierre-Olivier MAQUART, François Sockhom MEY, CHHUOY Kalyan, HENG Kimly, CHHUM Moeun, SUOR Kimhuor, Sébastien BOYER
Cambodian Journal of Natural History.

14. ***The Clytini fauna of Benin and Togo, with the description of a new species from Benin (Coleoptera, Cerambycidae)***
Pierre-Olivier Maquart, Alain Coache, Francesco Vitali', Laurent Péru; Bernard'Rainon, Denis Richard, Pierre, Juhel
Bulletin de la Société entomologique de France. DOI: 10.32475/bsef_2210

4.6. Laboratory of Environment and Food Safety

The Laboratory of 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

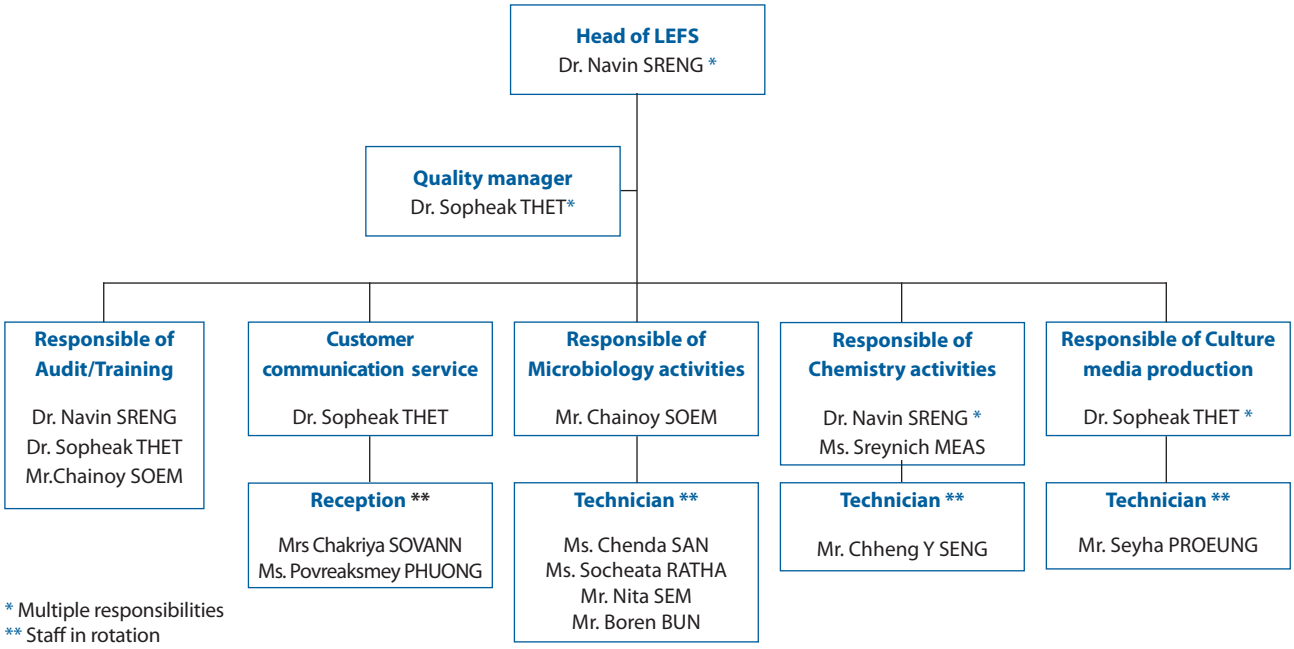


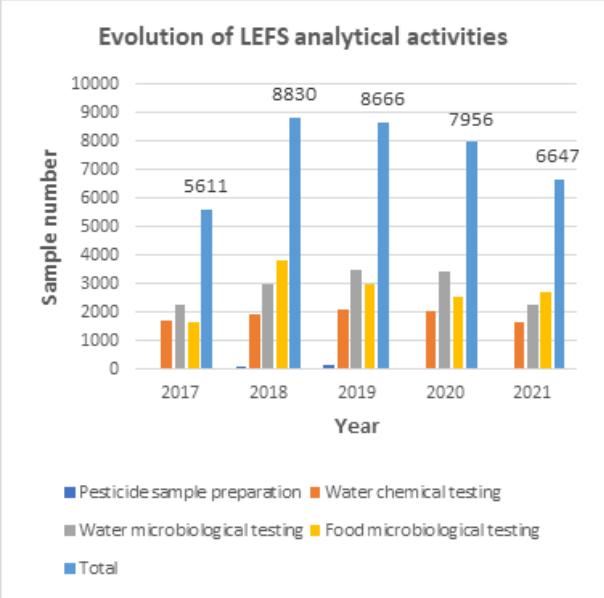
Figure 23. Laboratory of Environment and Food Safety organogram

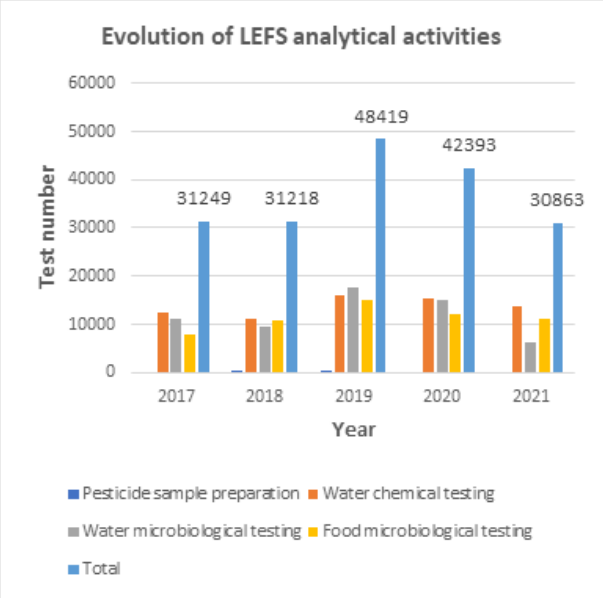
Changes occurred to the team composition in 2021; four new technicians and one technical manager for water physico-chemistry were recruited. Three of these four technicians replaced staff members who resigned.

4.6.2. Services Activities 2021

During 2021, the laboratory tested 6,647 samples comprising 2,731 samples of food, 2,280 water samples for microbiology testing and 1,636 water samples for chemical testing. Four requests for training were received. Unfortunately, due to the ongoing Covid-19 pandemic, we could provide only one training for trainers on food safety and personal hygiene.

Compared to 2020, the number of total samples tested decreased by 16 percent. The year 2021 has been a difficult one due to the continuing of the Covid-19 pandemic. Entry restrictions drastically reduced the number of tourists to Cambodia, and many hotels and restaurants suspended operations or closed permanently. This resulted in a decrease in the number of samples analyzed at LEFS. With the number of international flights also declining, one of our largest customers suspended these requests for pesticide sample preparation choosing instead to send them to New Zealand for testing. The decrease of water microbiological testing number may be due to the application of new regulations for drinking water.
The analytical activities (sample numbers and test numbers) over the last five years are shown in the table and figure below:





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

- Fifty-one percent of food samples (1,393/2,731) were reported to conform to standards/customer requirement although seven percent (92/1,393) were unsatisfactory;
- The unsatisfactory results were due to *Salmonella* contamination (12%), and high levels of hygiene indicators such as Coliforms bacteria (25%), *E.coli* (21%) and Enterobacteriaceae (5%). Ninety % of *Salmonella* positive food samples were raw meat.
- Sixty-three percent of water samples for microbiology testing (1,434/2,280) for which test results were completed, 25% (362/1,434) were unfit for human consumption because of fecal contamination.
- Thirty-four percent of ice cube samples (85/249) served in the restaurants and bars were found to be contaminated by fecal bacteria as coliforms and *E.coli*.

Quality Management System

Table 3. Quality Management Documents in LEFS

Documents	Verified and Validated in 2021	In application in 2021 (Total)
Procedure	**22**	**27**
Standard Operating Procedure	**39**	**111**
Form	**25**	**194**

LEFS has set itself the objective of obtaining ISO 17025 accreditation towards 2022. For this, the drafting of various quality documents has been initiated, some of which have been validated and implemented in 2020 while others were validated in 2021. All are in application in 2021.

4.6.3. Research Programs - Major Achievements in 2021

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 Southeast Asia where very high levels of ABR prevalence are reported, as is the case in Cambodia. 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 Institut de Recherche pour le Développement (IRD), CIRAD and two major hospitals in Cambodia, Calmette (Phnom Penh) and Battambang (Province in Cambodia's Northwest), 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:

1. To estimate the prevalence of antibiotic resistance in a key Phnom Penh hospital, Calmette Hospital, and in Battambang Provincial Referral Hospital, due to the elevated number of community infections in that province.
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.

Table 4. Bacteria detected and their resistance phenotypes

	ESBL	ESBL & Hcase	Hcase	ESBL & CPE	FQ-RS	ESBL & FQ-Rs	ESBL & Non FQ-Rs	Non FQ-Rs	MRSA	Total
Food	**73**	**1**	**7**		**2**	**2**	**3**	**17**	**2**	**107**
E. coli	34	1	3							38
K. pneumoniae	32		2							34
Salmonella spp.					2	2	3	17		24
C. freundii	3		2							5
E. cloacae	4									4
S. aureus									2	2
Soil	**57**	**3**	**12**					**12**		**84**
E. coli	39	2	6							47
K. pneumoniae	13		4							17
Salmonella spp.								12		12
E. cloacae	5	1	2							8
Waste Water	**49**		**8**					**11**	**1**	**69**
K. pneumoniae	21		4							25
E. coli	20		4							24
Salmonella spp.								11		11
E. cloacae	8									8
S. aureus									1	1
Water	**39**	**2**	**4**	**1**				**10**		**56**
K. pneumoniae	19		1	1						21
E. coli	13	1	2							16
Salmonella spp.								10		10
E. cloacae	7	1	1							9
Total	218	6	31	1	2	2	3	50	3	316

FQ-Rs : Resistant to Fluoroquinolones
CPE : Carbapenemase
Hcase : High-level Cephalosporinase
MRSA : Methicillin-resistant Staphylococcus aureus

Twenty-six patients hospitalized at the Battambang Hospital were included in the project for which 372 food and environmental samples were collected: 125 food samples, 80 water samples, 95 soil samples, and 72 wastewater samples. Table 4 shows preliminary results on bacteria detected in different sample types and their resistance phenotypes. In total, 316 strains of bacteria were detected: Enterobacteriaceae (256), *Salmonella* (57), and S. *aureus*. The most commonly detected Enterobacteriaceae are E. coli (49%) and *K. pneumoniae* (38%) and are ESBL in the majority of cases. A strain of *K. pneumoniae* found in water contains both ESBL and carbapenemase resistance phenotypes. The majority of *Salmonella* detected are sensitive to fluoroquinolones. Two strains of *Salmonella* are resistant to fluoroquinolones only, two are resistant to both fluoroquinolones and ESBL and two are resistant only to ESBL. Three strains of Methicillin-resistant S. *aureus* are detected: two in food and one in wastewater.

The results shown above are preliminary results of the resistance phenotyping of the bacteria detected. In-depth genetic studies will be developed.

Collaborations	Institut Pasteur du Cambodge: LBM (S. CHENG and G. DELVALLEZ), LEFS (N. SRENG), Epidemiology and Public Health (P. PIOLA) Institute of Research for Development (IRD) (A.-L. BANULS) CIRAD (V. CHEVALIER)
Funding	Fonds de solidarité pour les projets innovants, French Ministry for Europe and Foreign Affairs (n° FSPI 2020-14): 2020 - 2021

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 contribute significantly 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.

LEFS is mainly involved in part 1 of the project 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.

Based on literature review and clinical data of children under 15 with diarrheal diseases over the past 24 months from the medical biology laboratory of the Institut Pasteur du Cambodge, the provincial hospital Battambang Provincial Hospital and Angkor Hospital for Children in Siem Reap, E. coli and Salmonella were selected as the two important bacterial pathogens associated with vegetable-borne diseases.
Due to the Covid-19 pandemic and the travel restrictions in Cambodia in 2021, sample collection has been postponed to 2022. A pilot mission was carried out in February 2022 and analysis is in progress.

Collaborations	Institut Pasteur du Cambodge (N. SRENG), Kansas State University (J. VIPHAM), Purdue University (P. EBNER) Institute of Technology of Cambodia (C. PENG), Royal University of Agriculture (R. CHRUN), World Vegetable Center (S. RAMASAMY): 2020 - 2024
Funding	Feed the Future Innovation Lab for Food Safety, U.S. Agency for International Development (USAID) (n° A21-0346-S002)

4.6.4. 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, Ministry of Agriculture, Forestry and Fisheries as well as some private sector laboratories.

In 2021, as part of a national monitoring program, the Ministry of Health sent IPC 830 samples through sampling campaigns of industrial foods imported from different countries.

4.6.5. Teaching and Training

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

Table 5. Internship Students at LEFS in 2021

University	Number of students	Scholar Year	Period (Month)	Date
University of Health Sciences (UHS)	4	Year 4	1	04/01/2021 - 26/01/2021 01/02/2021 - 02/03/2021
University of Health Sciences (UHS)	2	Year 2	1.5	22/02/2021 – 09/04/2021

4.6.6. 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;
- Develop increasing cooperation with internal and external partners for research projects.

4.7. Medical Biology Laboratory

4.7.1. Functional Structure

The medical biology laboratory (LBM) activities comprise two major components as shown in figure 24.

The LBM provides a platform for comprehensive biological analyses. A panel of approximately 150 tests is offered to public/private hospitals and clinics, non-governmental organizations (NGOs), and walk-in patients. The laboratory is divided into six different sectors: (1) the reception area, (2) the sampling area, (3) the microbiology laboratory, the (4) mycobacteriology laboratory, (5) the blood biology laboratory and (6) the molecular biology platform. In December 2021, the LBM renewed its ISO 15189 accreditation by the Comité Français d'Accréditation (COFRAC) for the third time, with 62.9% of the activity under the scope of accreditation.

The LBM also hosts a bacteriology research group in collaboration with the Institut de Recherche pour le Développement (IRD) within the framework of the LMI DRISA (International Joint Laboratory "Drug Resistance in Southeast Asia") with a dedicated team of researchers and technicians. Research projects focus on the diagnosis, surveillance, molecular epidemiology and phylogenomic characterization of several pathogenic bacteria of particular public health importance (global antimicrobial resistance surveillance system (GLASS) AMR pathogens, tuberculosis, melioidosis, leptospirosis…).

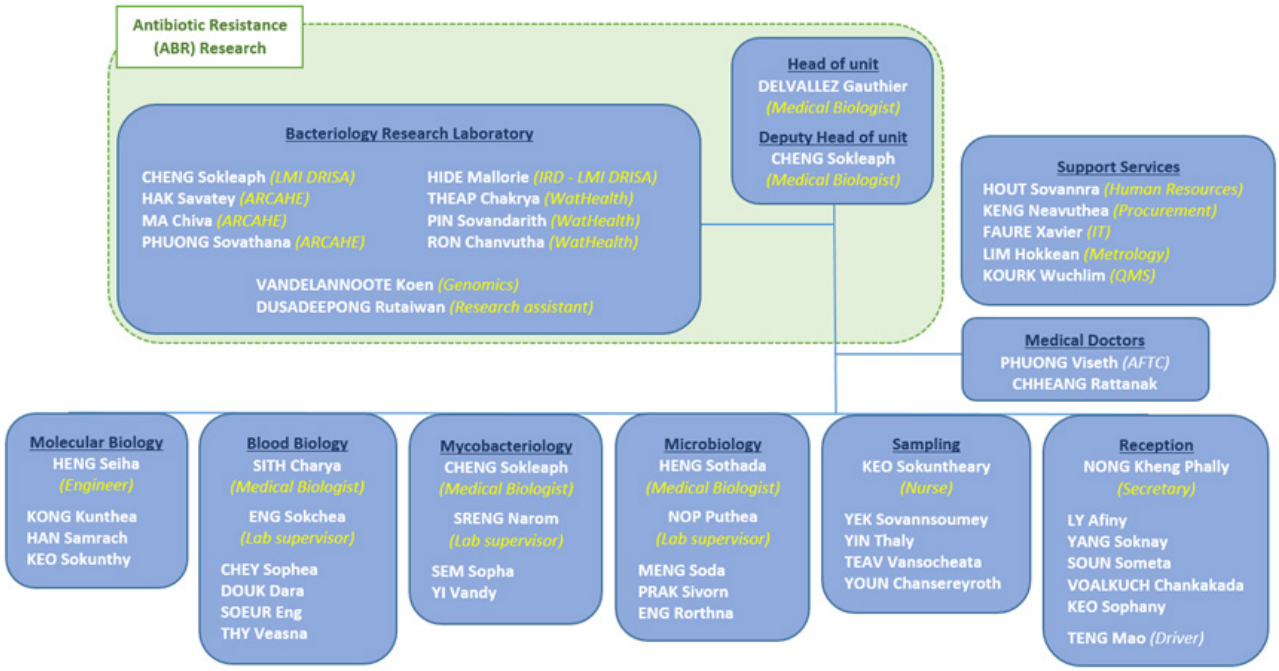


Figure24. Organogram- Medical Biology Laboratory (December 31th 2021)

4.7.2. Service Activities

Our 2021 activities were strongly affected by the various events surrounding the COVID-19 pandemic. COVID-19 qPCR represented 61.5% of 2021 laboratory activities, but there was a decline in service activities for all other sectors. LBM performed 110,.000 analyses in 2021, representing a total of 37,969 patient files

(26.121 internal and 11.848 external customers) and an activity estimated at 6 million B in the Nomenclature of Medical Biology Acts (NABM) (adapted to the LBM). Figure 25 illustrates the trend of our activities over the last 15 years. We observe a regular decrease in activity over these years due in part to the establishment of new private laboratories in Phnom Penh and the set-up of internal laboratories in health centers that were previously sending their samples to LBM. We also note two peaks of activity in the last decade. The first one in 2016/2017 corresponds to the activities of a research project on the hepatitis C virus funded by the Foundation for Innovative New Diagnostics (FIND), and the second one in 2021 aligns to the COVID-19 pandemic.

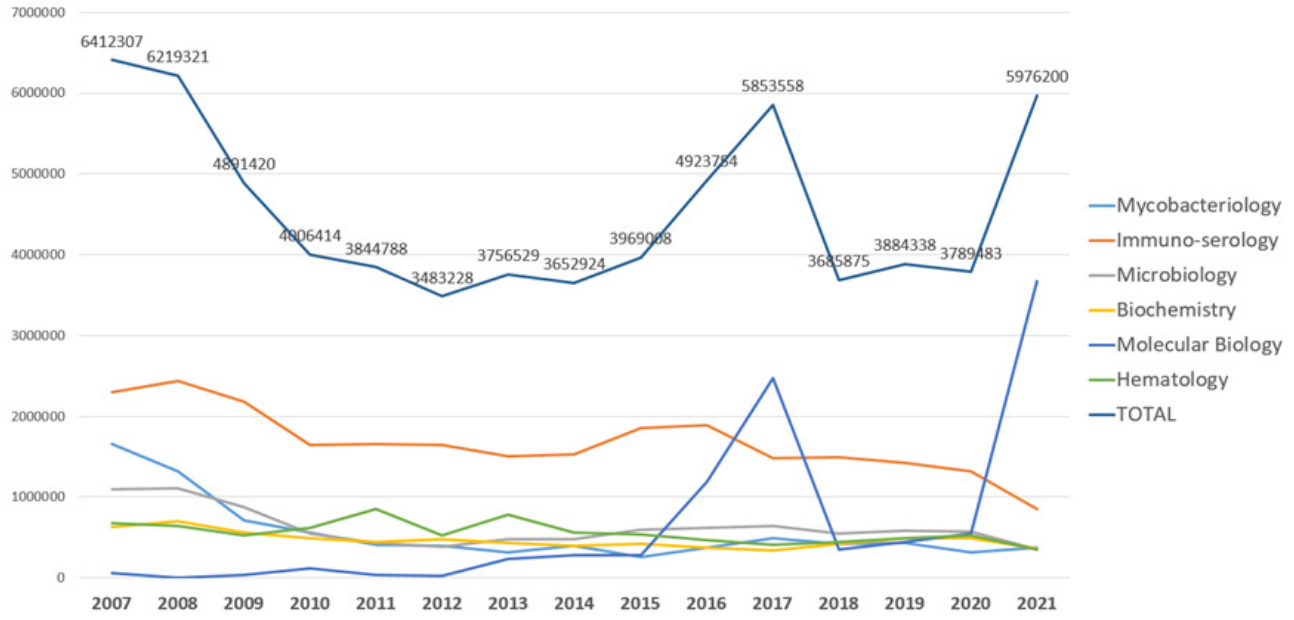


Figure 25. Trends in LBM activities expressed in B

During 2021, the LBM further developed its technical capacity by acquiring a GeneXpert GX-XVI machine that allows it to perform several additional molecular diagnostic tests. These include a PCR for high-risk Human Papillomavirus (hr-HPV), *Chlamydia trachomatis* / *Neisseria gonorrhoeae* screening, SARS-CoV-2 screening as a second confirmation test after Cobas 4800 (ROCHE), and BCR-ABL transcript quantification for the follow-up of Chronic Myeloid Leukemia (CML) patients. The blood biology laboratory also implemented a COVID-19 serology test targeting the S protein and an automatic assay inferring the dosage of both rheumatoid factors (RF) and anti-streptolysin-O (ASLO) which replaces the previous manual agglutination test.

4.7.2.1. HIV

The Anonymous Free Testing Center (AFTC)

In 2021, 333 patients consulted the AFTC and benefited from a free HIV consultation and screening (Figure 26). The HIV-positive population represents 43.2% of the consultations. This high rate of positivity is due to the fact that most patients that consulted the AFTC were referred by other health centers for a free confirmation of a positive HIV antibody rapid test administered elsewhere. Of the 144 positive patients, 80 were referred by NGOs (Men's Health Cambodia, Reproductive Health Association of Cambodia, Chouk Sor Clinic) working with most at-risk populations such as entertainment workers, men who have sex with men and transgender persons. In addition, 13 patients were referred by National Hospitals (Calmette Hospital, Ang Duong

Hospital, Preah Kossamak Hospital, Khmer–Soviet Friendship Hospital, etc). In recent years, we have noted a decrease of AFTC activity, triggered by the opening of additional HIV testing centers that are focused on the most at-risk populations. On the other hand, the AFTC remains a trusted laboratory for these health centers to confirm their results.

HIV nominative serology (LBM patients)

In 2021, the seropositivity rate of LBM patients remained stable at 5.0% (Figure 26). The prevalence is higher than the general population's because of the 63 positive samples tested in 2021, 38 samples were sent by other private laboratories to confirm their positive results.

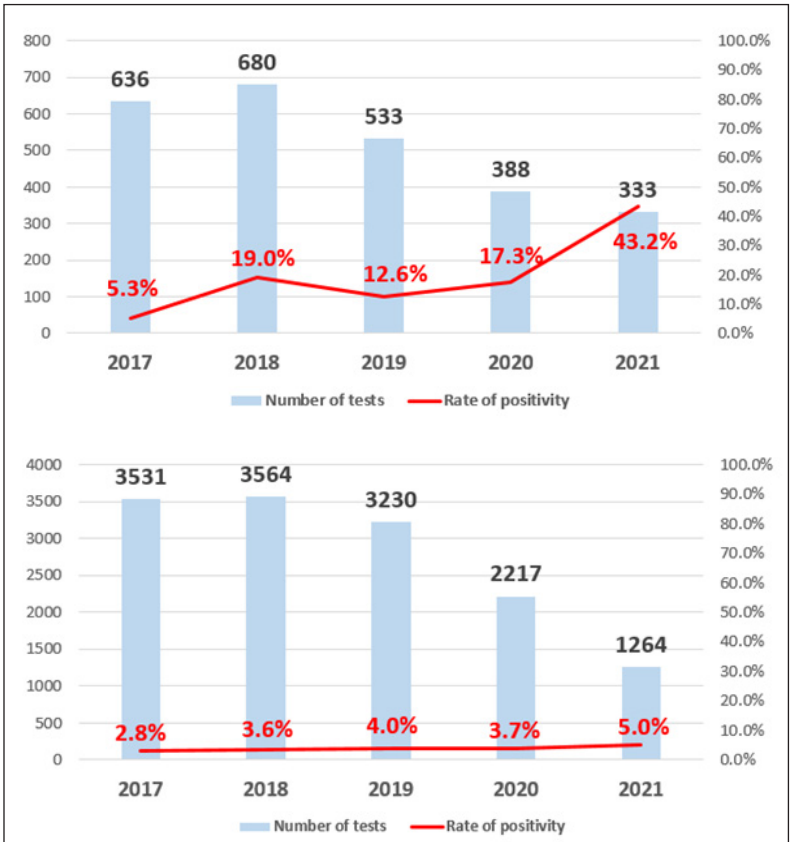


Figure 26. HIV serology and rate of positivity at LBM and AFTC

During the two years of the COVID-19 pandemic (2020 and 2021), we observed a 63.3% decrease in the number of HIV serology screenings.

4.7.2.2. Bacteriology

Like other sectors, bacteriology was strongly impacted by COVID-19 with a sharp 40% drop in the number of bacteriological analyses. The rate of extended-spectrum-beta-lactamase (ESBL) remains stable in comparison with 2020 (28%), but we observed a 6% increase of Carbapenemase-producing *Enterobacteriaceae* (CPE), mainly within *Escherichia coli* species. Of particular note was LBM's first detection of KPC carbapenemase. The rate of Methicillin-resistant *Staphylococcus aureus* (MRSA) and Carbapenem-resistant *Acinetobacter baumannii* (CRAB) are variable. To date, no resistance to vancomycin has been detected *in Enterococcus faecium*. The resistance ratio of *Neisseria gonorrhoeae* to third-generation cephalosporins (3GC) has dramatically increased (50%). Still, the number of isolated strains remains limited due to the implementation of molecular diagnostic testing. The resistance profile of *Salmonella* paratyphi A isolates did not evolve with the absence of resistance to 3GC but a constant resistance to quinolones. Finally, 22 strains of *Burkholderia pseudomallei* were isolated (Table 6).

Table 6. Bacteriological data in 2021

Bacteria of particular public health importance	2020	2021
Samples for bacteriological culture	4114	2508
Positive cultures with AST	1466 (35.6%)	967 (38.6%)
Extended spectrum beta-lactamase (ESBL)		
Enterobacteriaceae	200/701 (28.5%)	143/503 (28.4%)
Escherichia coli	155/366 (42.3%)	107/254 (42.1%)
Klebsiella pneumoniae	31/206 (15.0%)	25/127 (19.7%)
Others	14/129 (10.9%)	11/122 (9.0%)
Carbapenemase-producing Enterobacteriaceae (CPE)		
Enterobacteriaceae	26/701 (3.7%)	30/503 (6.0%)
Escherichia coli	12/366 (3.3%)	21/254 (8.3%)
Klebsiella pneumoniae	9/206 (4.4%)	7/127 (5.5%)
Others	5/129 (3.9%)	2/122 (1.6%)
Carbapenemase type	NDM and OXA-48	NDM, OXA-48 and KPC
Staphylococcus aureus		
Methicillin-resistant Staphylococcus aureus (MRSA)	93/238 (39.1%)	43/139 (30.9%)
Acinetobacter baumanii		
Carbapenem-resistant Acinetobacter baumannii (CRAB)	9/20 (45.0%)	9/17 (52.9%)
Enterococcus faecium		
Vancomycin-Resistant Enterococci (VRE)	0/12 (0%)	0/6 (0%)
Neisseria gonorrhoeae		
Resistance to 3GC	0/11 (0%)	3/6 (50.0%)
Salmonella paratyphi A (Blood culture)		
Resistance to 3GC	0/37 (0%)	0/10 (0%)
Resistance to nalidixic acid	37/37 (100%)	10/10 (100%)
Burkholderia pseudomallei	14	22

As an alternative to the classic bacterial culture for the diagnosis of sexually transmitted infections (STIs), in 2021 LBM performed 155 *Chlamydia trachomatis/Neisseria gonorrhoeae* PCRs on vaginal, urine, urethral, throat, and anal samples. Of these, 9.7% (n = 15) were positive for *Chlamydia trachomatis*, 4.5% (n = 7) for *Neisseria gonorrhoeae*, while we detected three co-infections.

4.7.2.3. COVID-19

In 2021, the LBM carried out almost 17,000 COVID-19 qPCRs (Figure 27). Up until April 2021, our activities were limited to producing negative travel certificates and performing end-of-quarantine testing for Type-A and Type-B Visa holders upon arrival in Cambodia. However, from April 2021 on, LBM got the authorization to produce negative travel certificates for the general public. Finally, as of June 2021, agreements were signed with several private clinics that are authorized to manage COVID-19 patients. Over the year, certificates for travelers represented 87.0% of COVID-19 activities while private clinics represented 7.0%. The other requests included end-of-quarantine tests, tests required for official visits (royal family, ministries…) and IPC staff screening. The positivity rate was 2.3% for travel certificate testing (with a peak at 14% in December 2021) and 39.0% for other samples.

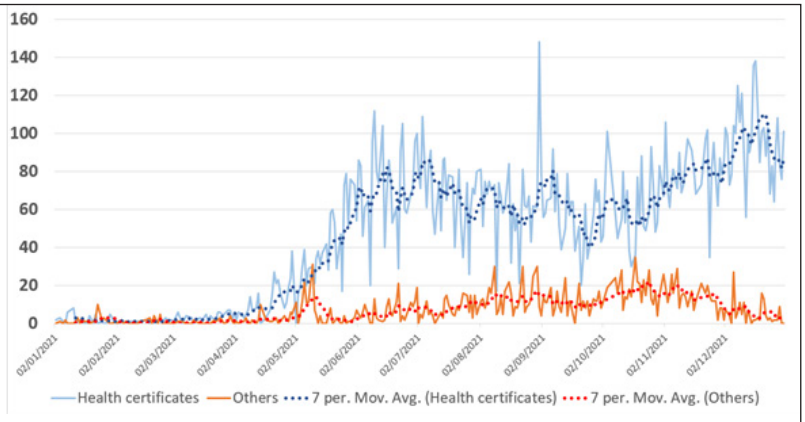


Figure 27. Evolution of the COVID-19 qPCR requests in 2021

4.7.2.4. Tuberculosis

Similarly, mycobacteriology activities continued to be affected by the COVID-19 pandemic, even though the situation improved slightly compared to the previous year. The total number of analyses regarding mycobacteriology was 5,832 in 2021, representing an increase of 11.3% compared to the number of analyses in 2020 (5,242). This increase is due to the uptake of health screening activities for travelers during the year. In 2021, 828 Xpert MTB/RIF Ultra tests were performed for rapid diagnosis of TB, of which 24.9% were mycobacterium tuberculosis (MTB) positive, and 2.0% of positive samples were resistant to Rifampicin. Regarding resistance to second-line anti-TB drugs, the trend in the resistance rate is presented in Figure 28 in 2021

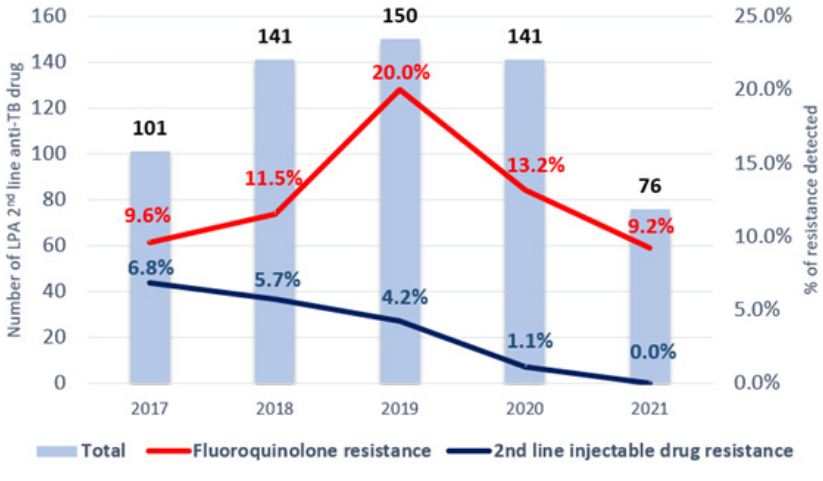


Figure 28. Trends in resistance to second-line anti-TB drugs

4.7.3. Major Achievements in 2021

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

Through a collaboration with IRD, since August 2018, we have been hosting an engineer specialized in molecular biology, Dr. Mallorie HIDE. She has been based in LBM for four years within the framework of the LMI DRISA. She previously worked on molecular epidemiology and drug resistance of *Mycobacterium tuberculosis, Enterobacteriaceae* and *Burkholderia pseudomallei*. She 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.

Collaborations	Team Leader: S. CHENG (LBM) NIHE Vietnam, USTH Vietnam, OUCRU Vietnam, CILM Laos, ITC Cambodia, IRD (MIVEGEC, MARBEC, TRANSVIHMI)
Funding	IRD (2016-2020) : 200 k€.

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 in humans, animals, or in the environment. The main objective of the project is to explore the transmission of antibiotic-resistant bacteria at the interface between humans, animals and the environment in Cambodia. To achieve the objectives, the study has been subdivided into three 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 (Northwest 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, the 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. Final global data analysis will allow IPC to assess drug resistant bacteria genetic diversity and to identify potential cross-contamination between humans, animals and their environment, to define any flow of resistant 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 the "One Health" concept.

This project is ongoing until May 2022. The patient recruitment (WP1) in the two hospitals and the sample collection campaign at the patients' surrounding environments in Battambang Province ended on 31 December 2021 with a total of 355 patients recruited and 1,869 samples collected from the patients' close environments (including 980 animal samples, 601 environmental samples, and 288 food and water samples). The pilot genomic NGS study (WP3) was performed on 25 samples (4 bacterial isolates, 2 animal, 11 environmental, and 8 food and water samples) to validate the genomic DNA extraction and sequencing protocol as well as to validate the bioinformatic analysis. The NGS is in progress for 539 selected bacteria isolates and 751 samples from patients' environments.

Collaborations	Team Leaders: S. CHENG (LBM) and AL. BANULS (IRD) ,MIVEGEC Unit, IRD (M. HIDE) ESPACE-DEV unit, IRD (V. HERBRETEAU) Medical Biology Laboratory (G. DELVALLEZ), Epidemiology and Public Health Unit (P. PIOLA and C. FLAMANT), Calmette Hospital (S. BORY), Battambang Provincial Referral Hospital (S. CHIEK), CIRAD (V. CHEVALIER), Laboratory of Environment and Food Safety, IPC (N. SRENG), General Directorate of Animal Health and Production (GDAPH), MAFF (S. SAN)
Funding	Team Leaders: S. CHENG (LBM) and AL. BANULS (IRD), MIVEGEC Unit, IRD (M. HIDE) ESPACE-DEV unit, IRD (V. HERBRETEAU) Medical Biology Laboratory (G. DELVALLEZ), Epidemiology and Public Health Unit (P. PIOLA and C. FLAMANT), Calmette Hospital (S. BORY), Battambang provincial Referral Hospital (S. CHIEK), CIRAD (V. CHEVALIER), Laboratory of Environment and Food Safety, IPC (N. SRENG), General Directorate of Animal Health and Production (GDAPH), MAFF (S. SAN)

FSPI Wat-Health 2021-2022

The Water and Health Risks in Cambodia (Wat-Health – 600k€) research project aims to define the exposure and vulnerability of rural populations to the most critical health hazards related to floods in the Koh Thum District. The study is based on the premise that, as in other countries of the region, water-related hazards such as contaminants, bacterial and vector-borne diseases represent a major potential health threat. Investigations are conducted at a pilot site in the Mekong Delta, located between Phnom Penh and the border with Vietnam. The overall scientific goal is to determine how changes in river flood patterns affect the distribution of pollutants and lead to changes in the biodiversity of water-related pathogens and disease vectors, with ultimate effects on health, agricultural production and the environment. LBM is coordinating the "water-related bacteria" activities focused on the detection of bacteria (especially *Leptospira* and *Burkholderia*) in water and soil samples. Monthly field sampling has been performed at 22 sampling sites since July, 2021 and the 9 missions allowed us to identify the presence of *Leptospira* and *Burkholderia* in Koh Thum. Sampling will continue until the summer of 2022 and *Leptospira/Burkholderia* species will be identified by molecular experiments.

Collaborations	Team Leaders: M. HIDE and G. DELVALLEZ Medical Biology Laboratory (S. CHENG, S. HAN, S. MENG) MIVEGEC Unit, IRD, LMI DRISA (A. BANULS) G-EAU (S. MASSUEL, JP VENOT) ESPACE-DEV, IRD (V. HERBRETEAU), Entomology unit (S. BOYER) ITC (Cambodian PI) Institut Pasteur in New Caledonia (C. GOARANT) RUA, CNRS
Funding	Fonds de solidarité pour les projets innovants (FSPI 2020-2021) : 240k€ for LBM French Embassy in Cambodia, French Ministry for Europe and Foreign Affairs

Establishing microbial AMR genomics in Phnom Penh through genomic snapshot surveys of multidrug-resistant Gram-negative bacteremia

The rising prevalence of antimicrobial resistance (AMR) represents a threat to public health globally, as emerging AMR mechanisms and multidrug-resistant pathogens compromise treatment of microbial infections. In Cambodia, the detection of AMR has been traditionally reliant on culture-based antimicrobial susceptibility testing (AST). Even though AST provides direct visual evidence of how a bacterium will interact with an antimicrobial, it generally provides little or no data regarding resistance mechanisms. Whole-genome sequencing (WGS), provides genome-wide information at the single nucleotide level that can be

used to identify the presence and mechanisms of AMR, as well as pathogen identity, virulence, and ancestry. Microbial genomics is currently revolutionizing the diagnosis, surveillance and control of AMR. This is why this study aims to combine rapid advances in genomics with public health epidemiology and best practice bioinformatics to set up a new paradigm for AMR control in Phnom Penh.

We are planning to organize retrospective snapshot surveys of a number of high-priority AMR Gram-negative pathogens causing bloodstream infections in Cambodia. We believe high-throughput WGS methods will facilitate the sequencing of large, geographically representative collections of disease isolates. Our planned retrospective genomic snapshot surveys will be the first to comprehensively characterize the AMR gene repertoire, the resistance mechanisms, and the relatedness of high-priority AMR pathogens in Cambodia. Genomic characterization will make it possible to define AMR with much greater precision compared to phenotypic categorization. As a result we believe the study will provide insights that can help contain AMR and protect Cambodian public health.

Collaborations	Team Leader: K. VANDELANNOOTE (LBM-IPC) Medical Biology Laboratory (R. DUSADEEPONG, G. DELVALLEZ, S. CHENG) The Doherty Institute (T. STINEAR, B. HOWDEN, D. INGLE) Institut Pasteur (P. GLASER)
Funding	The Doherty Institute, Institut Pasteur, Institut Pasteur du Cambodge (200.000 USD)

Public Health Alliance for Genomic Epidemiology (PHA4GE)

The LBM has been working with partnering institutions to build genomic AMR surveillance capacity for Cambodia. We have been exploring various bioinformatic pipelines capable of analyzing genomic AMR data in an interoperable manner. An awarded PHA4GE sub-grant supported us in exploring the PHA4GE AMR gene detection output standard for exchanging AMR genomic surveillance results between IPC and the Microbiological Diagnostic Unit Public Health Laboratory (MDU PHL) at the Peter Doherty Institute, who have implemented pathogen genomics for public health in the state of Victoria (Australia). We didn't experience any issues during the implementation of the "hAMRonization" activity and successfully performed a series of exchange exercises with MDU-PHL. During these exercises we didn't identify any discrepant results. We did notify the developers of a number of minor points that could be better described in the hAMRonization documentation. We plan to continue using hAMRonization when comparing output from different AMR gene detection tools during exchanges with other institutes.

Collaborations	Team Leader: K. VANDELANNOOTE (LBM-IPC) Medical Biology Laboratory (R. DUSADEEPONG, G. DELVALLEZ) The Doherty Institute (K. HORAN, T. HOANG, T. STINEAR)
Funding	Public Health Alliance for Genomic Epidemiology (PHA4GE): 20.000 USD

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 and in-contact staff members 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. 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 a 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. The project is ongoing. The sequencing of 102 *M. tuberculosis* isolates using the NGS is in progress at Murdoch University, Australia.

Collaborations	Team Leader: S. CHENG (LBM) Free the Bears (K. OFFICER), Murdoch University (B. JACKSON)
Funding	Murdoch University

New Antimicrobial Peptides

The general objective of the study is to evaluate the antibacterial activity of a new set of eight synthetic antimicrobial peptides (AMP) against clinical isolates of relevant GLASS bacteria 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 status (ESBL, CPE, MRSA, colistin and fluoroquinolone resistance) were evaluated starting in July 2021 as part of an internship linked to a master's degree in medical biology.

The specific objectives are (1) to set up antibacterial activity testing using the broth microdilution method for the eight AMP on E. coli reference strain (ATCC 25922) and (2) to measure the susceptibility of relevant resistant GLASS bacteria against the eight new AMP by comparing with a sensitive bacterial strain.

Collaborations	Team Leader: M. HIDE Medical Biology Laboratory (R. Chheang, S. Cheng) MIVEGEC Unit, IRD, LMI DRISA (A. Banuls)
Funding	LMI DRISA

Pediatric Melioidosis in Cambodia

In order to investigate the epidemiology of melioidosis in Cambodia, we will study an exhaustive sample of 122 isolates from pediatric melioidosis collected between June and 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 Medical Biology Laboratory (K. Vandelanotte, R. Dusadeepong, G. Delvallez, S. Cheng) MIVEGEC Unit, IRD, LMI DRISA (A. Banuls) GeoHealth-IRD (V. Herbreteau Kantha Bopha Children's Hospitals (D. Laurent)
Funding	LMI DRISA

4.7.4. RESEARCH PROGRAMS

LMI-DRISAOH "DRUG RESISTANCE IN SOUTHEAST ASIA: A ONE HEALTH APPROACH TO TACKLE AMR SPREAD"

This is a continuing project from LMI-DRISA that was set up in 2016 as part of a regional effort with Vietnam, Cambodia and Lao PDR. The main objective of the LMI is to share competencies and best practices between different academic and non-academic institutions in the three mentioned countries and France in order to study the mechanisms and factors that influence the emergence and the transmission of drug resistance and their implications for public health in Southeast Asia. To address this complex situation, the second phase of this 5-year project (2020-2025) 'LMI-DRISAOH' aims to integrate a 'One Health' approach to unravel what is truly happening for antimicrobial resistance emergence and spread at the interface of humans, animals and the environment.

Collaborations	Team Leader: S. Cheng (LBM), T.K.O. Nguyen (USTH), Q.H. Nguyen (USTH) and A.L. Banuls (IRD) MIVEGEC Unit, IRD (M. Hide) Medical Biology Laboratory, IPC (G. Delvallez) Centre d'Infectiologie Lao Christophe Mérieux (CILM) Fondation Mérieux, National Institute of Hygiene and Epidemiology (NIHE) University of Science and Technology of Hanoi (USTH) Oxford University Clinical research (OUCRU)
Funding	IRD (2020-2025) : under evaluation

AFRICAM

Cambodia is a country at high risk for the emergence or re-emergence of infectious pathogens of zoonotic origin. Anthropic modifications (e.g. deforestation, urbanization, industrial activities, agriculture, tourism) are taking place at a rapid pace, and these changes dramatically alter natural ecosystems while creating conditions favoring pathogen emergence, including diseases related to food or water (diarrhea-causing bacteria (including their drug resistance characteristics), hepatitis A, typhoid, leptospirosis, melioidosis), vector-induced diseases (dengue, chikungunya, Japanese encephalitis) or other (re-)emerging zoonotic diseases (rabies, coronavirus, Nipah virus, avian or porcine influenza). Therefore, Cambodia has a strong commitment to integrating the "One Health" concept in initiatives related to infectious and especially to zoonotic diseases and thus improve the coordination between human, animal and environmental health sectors. The AFRICAM Project is coled by teams from France and Cambodia (including the LMI-DRISA) and aims (1) to study zoonotic risks in Cambodia and correlate them with external parameters related to hydrological, climatic or environmental dynamics and (2) to implement activities to mitigate emergence risks and reinforce national surveillance towards a "One Health" surveillance system.

Collaborations	PI: A.L. Banu;s (IRD) IPC (LBM, Virology, Epidemiology and Public Health Unit, Medical Entomology) IRD, CIRAD, AVSF (Agronomes et Vétérinaires Sans Frontières), IDE (Powering entrepreneurs to end poverty), WCS (Wildlife Conservation Society), Battambang Hospital, Institute of Technology in Cambodia (ITC), MIVEGEC, Espace-Dev;).
Funding	AFD

CIRCUS

This project was built and funded as part of the Antimicrobial Resistance South (AMR SUD) consortium of teams from France, Burkina Faso, Ivory Coast and Madagascar coordinated by Christian Lienhardt (TRANSVIMI, IRD) supported by AVIESAN. Similar to ARCAHE, this project aims to use a One Health approach to describe the transmission of multidrug-resistant (MDR) enterobacteriaceae in rural, peri-urban and urban areas. To do so, it is using both microbiological characterization of clinical pathogens and meta-genomics analysis of environmental samples. But while ARCAHE was mostly targeting rural areas, CIRCUS implementation in Cambodia will be focused on peri-urban areas and will include additional experiments and target groups. The data obtained will allow us not only to understand better AMR transmission in Cambodia through the One Health perspective but also to compare these results obtained with similar methodologies in other developing countries (Burkina Faso, Ivory Coast and Madagascar).

Collaborations	PIs: N. Guessemd (IP in Ivory Coast), A. Salam, E. Cardinal (Cirad), A.L. Banuls (IRD) Team Leader for Cambodia: S. Cheng (LBM) Other partners to be identified
Funding	AVIESAN AMR-SUD ANR "Antibiorésistance: comprendre, innover, agir (AMR) : 700k€

Antimicrobial Resistance Circulation along the Mekong and its Delta - ARCIMED

The ARCIMED Project was selected for support in December 2021 for the FSPI call 'OHSEA' ('One Health in Practice in Southeast Asia'), co-led by IRD, CIRAD and CNRS and aiming to support emerging One Health initiatives. The main objective of this pilot project is to gain insights into the emergence, transmission and dynamics of resistance determinants along the Mekong River in Laos, Cambodia and Vietnam. To do so, we plan to describe the presence and dynamics of AMR determinants over time and correlate it with external parameters such as the hydrology of the river, the season (dry/wet), the physicochemical properties of water, the presence of other pollutants and the anthropic activities taking place in the proximity of sampling sites. So far, partners from Laos, Cambodia, Vietnam and France have joined the project for this first phase; we aim to invite additional partners and ultimately develop an ambitious collaborative One Health proposal including all six Mekong River countries to study the role played by this river in the emergence and spread of AMR in Southeast Asia, from land to sea.

Collaborations	PI: Nguyen Quang Huy (USTH) Team Leaders for Cambodia: S. Cheng (LBM) and C. Pheng (ITC) IRD (MIVEGEC, TRANSVIHMI, MARBEC, Espace-Dev), Institute of Technology of Cambodia (ITC), National University Of Laos
Funding	FSPI OHSEA : 22 500€

The Emergence of Tuberculosis at the Human-Elephant Interface: ELAOS

In Southeast Asia, tuberculosis represents a significant public health threat and is also considered an emerging disease in wildlife. Amongst others, TB is threatening Asian elephants and other animals like sun bears, building on other threats such as shrinking habitats, poaching, human conflicts and other diseases. Moreover, many captive elephants still live in constant proximity with villagers, especially their mahout (person owning the elephant), and there is, therefore, a non-negligible risk of disease transmission in both directions, including TB. This has already been documented among keepers and elephants in zoos and sanctuaries across the world. Therefore, the project ELAOS, a collaboration between teams from Laos, Cambodia and France, aims to

develop a reliable, non-invasive diagnostic method to detect and characterize TB and antibiotic resistance in elephant feces. By adopting a One Health approach, the goal is to determine the prevalence and molecular characteristics of mycobacterium tuberculosis among captive elephants, their mahouts and domestic animals, and to evaluate the risk of TB emergence at the human-animal interface in Laos. Later, the objective is to extend the study to a broader domesticated and wild elephant population. Ultimately, establishing this disease detection method would be valuable to better understand TB prevalence, spread and potential for zoonotic transmission among humans and animals in Southeast Asia.

Collaborations	PIs: S. Locatellil (MIVEGEC-IRD), P. Paboriboune (CILM), S. Cheng (LBM), A.B.L. Perez (ECC), S. Sayasone (LTPHI) Team leader for Cambodia: S. CHENG (LBM)
Funding	FSPI OHSEA : 22 500€

4.7.5. Support to National Authorities

- With the National Center for HIV/AIDS, Dermatology and Sexually Transmitted Diseases (NCHADS) of Cambodia, follow-up of HIV seropositivity;
- Participation in CENAT's Technical Working Group on Laboratory including the development of technical procedures guideline;
- Participation in CENAT's Technical Working Group on multi-drug resistant tuberculosis;
- Participation in technical working group on AMR with Ministry of Health;
- Provide technical support on quality with the Ministry of Health.

4.7.6. Teaching and Training

Continuing Professional Training and Development for Staff

- Partook in the Colloque Human Papillomavirus (ANRS);
- ISO 15189:2012 Certified Auditor Training (Punyam Academy)
- ISO 17025:2017 Certified Auditor Training (Punyam Academy)
- Quality Management in Medical Biology Laboratory (MOOC)
- Pre-Intermediate General English (Australian Centre for Education)
- Advanced Writing and Speaking (Australian Centre for Education)
- MiSeq training (Bio-Active Co., Ltd)
- E-International Public Health Management Development Program (ITEC, IPHMDP, Ministry of External Affairs, Government of India)

Internships

One of IPC's main missions is to contribute to teaching and training activities. The LBM has been proactive in the training of laboratory technicians and pharmacists from partner institutions in the fields of medical biology including hematology, immuno-serology, biochemistry, microbiology and molecular biology. In 2021, LBM received students for internship from the following organizations:

- Pharmacists (University of Health Sciences)
- Laboratory technicians (University of Health Sciences)

- UHS Master's of Infectious Disease (Infectiologie), Master's 1: POK Chendavatey
- UHS Master's Degree in Medical Biology: CHHEANG Rattanak

Infectiology Master's Courses at University of Health Sciences, Phnom Penh

- Resistance mechanisms to anti-infectious agents: antibiotics and resistance to antibiotics

4.7.7. Outlook

Implementation of Flow-cytometric Immunophenotyping

For several years, flow cytometric immunophenotyping (FCI) has become an indispensable tool for the diagnosis, classification, staging, and monitoring of hematological malignancies, such as acute leukemia and lymphoma. FCI makes it possible to determine the appearance of malignant cells, and to specify the level of differentiation. Currently in Cambodia the diagnosis of hematological malignancies is based only on medical imaging, cytology and immunohistochemistry, rendering medical management complicated. In collaboration with the immunology unit of IPC and the Kantha Bopha Hospital, one of the major aims of LBM for the coming years is the implementation of FCI to improve the diagnosis of hematological malignancies and allow appropriate treatment. An internal IPC budget (approximately 15.000 dollars) has been allocated to this project.

Laboratory Reorganization

Faced with the increased availability of the local laboratory services and the corresponding reduction of income from this service, it seems necessary to review LBM's business model. A change in analyses prices in alignment with the NABM is planned for 2022 to more optimally set prices for laboratory analyses. Although the LBM is still benefits from its long-standing good reputation (Pasteur, Accreditation NF EN ISO 15189) and the quality of its analyses, the growing increase in competition with increasingly well-equipped laboratories in Phnom Penh that are offering in-demand analysis panels has decreased demand for LBM services. In parallel with the implementation of the new pricing scheme, extended opening days could be useful and may be considered (e.g. opening 7 days a week).

Diversification of Analyses Offered

In order to increase its added value, the LBM plans to diversify its catalog of analyses by offering tests not carried out anywhere else in Cambodia. This includes the continued strengthening of our molecular biology platform. After the implementation of several molecular diagnostics over the last two years (HPV PCR, *Chlamydia trachomatis / Neisseria gonorrhoeae* PCR, COVID-19 PCR, BCR-ABL transcript), we plan to implement various molecular diagnostics for arboviruses (PCR Triplex Dengue/Zika/Chikungunya) in 2022. The implementation of these molecular assays to our list of analyses would be a major asset for the management of a possible future epidemic similar to the dengue epidemic of 2019 and the 2020-2021 chikungunya epidemic. We also plan to reinvest the income earned from COVID-19 PCR analysis in the purchase of new machines for the implementation of immuno-allergology and auto-immunity analyses.

Research activities

To strengthen research activities, LBM wishes to identify a team of permanent staff within the laboratory dedicated to research projects in addition to staff recruited for within specific research projects. This would lessen the effects of frequent arrivals and departures of personnel recruited by specific research projects, and the establishment of a permanent team will make it possible to consistently have trained local staff and to maintain the technical expertise as well as strengthen the capacity of the local staff. This action would also help increase the output of scientific publications.

4.7.8. Research Activities

NOTE

The name of authors from the Institut Pasteur du Cambodge are underlined.
Publications in a journal without impact factor are listed separately and identified at the end of the list
** equal contribution, first author / ** equal contribution, last author*

1. ***Circulation of Bordetella pertussis in vaccinated Cambodian children: a transversal serological study***
   Gaelle Noel*, Laurence Borand*, Chanthy Leng, Chanthy Keang, Chhunly Botr, Bunnet Dim, Alexandra Kerleguer, Yik S. Peng, Navin Sreng, Vichit Ork, Mohand Ait-Ahmed, Nicole Guiso, Fabien Taieb.
   Int J Infect Dis. 2021; DOI: 10.1016/j.ijid.2021.03.054

2. ***Dynamics of G6PD activity in patients receiving weekly primaquine for therapy of Plasmodium vivax malaria***
   Walter R. J. Taylor, Saorin Kim, Sim Kheng, Sinoun Muth, Pety Tor, Eva Christophel, Mavuto Mukaka, Alexandra Kerleguer, Lucio Luzzatto, J. Kevin Baird**, Didier Menard**
   PLOS Neglected Tropical Diseases. Public Library of Science; 2021;15(9):e0009690. DOI: 10.1371/journal.pntd.0009690

3. ***Resistance to Second-Line Anti-TB Drugs in Cambodia: A Phenotypic and Genetic Study***
   Sokleaph Cheng, Mallorie Hide, Sok Heng Pheng, Alexandra Kerléguer, Gauthier Delvallez, Sophan Sam, Tan Eang Mao, Thi Van Anh Nguyen, Anne-Laure Bañuls.
   Infect Drug Resist. 2021;14:1089 104. DOI: 10.2147/IDR.S289907

4. ***Severe bacterial neonatal infections in Madagascar, Senegal, and Cambodia: A multicentric community-based cohort study***
   Bich-Tram Huynh, Elsa Kermorvant-Duchemin, Rattanak Chheang, Frederique Randrianirina, Abdoulaye Seck, Elisoa Hariniaina Ratsima, Zafitsara Zo Andrianirina, Jean-Baptiste Diouf, Armya Youssouf Abdou, Sophie Goyet, Véronique Ngo, Siyin Lach, Long Pring, Touch Sok, Michael Padget, Fatoumata Diene Sarr, Laurence Borand, Benoit Garin, Jean-Marc Collard, Perlinot Herindrainy, Agathe de Lauzanne, Muriel Vray, Elisabeth Delarocque-Astagneau, Didier Guillemot, On behalf of the BIRDY study Group.
   PLOS Medicine. Public Library of Science; 2021;18(9):e1003681. DOI: 10.1371/journal.pmed.1003681

4.8. Vaccination Service

The Vaccination Service at the Institut Pasteur du Cambodge (IPC) is composed of one International Vaccination Center (IVC) 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 Service has 23 team members based in Phnom Penh and two provinces: nine medical doctors (including the head of the Vaccination Service and three medical supervisors), ten nurses, two administration staff, one data manager, and one person in charge of hygiene.

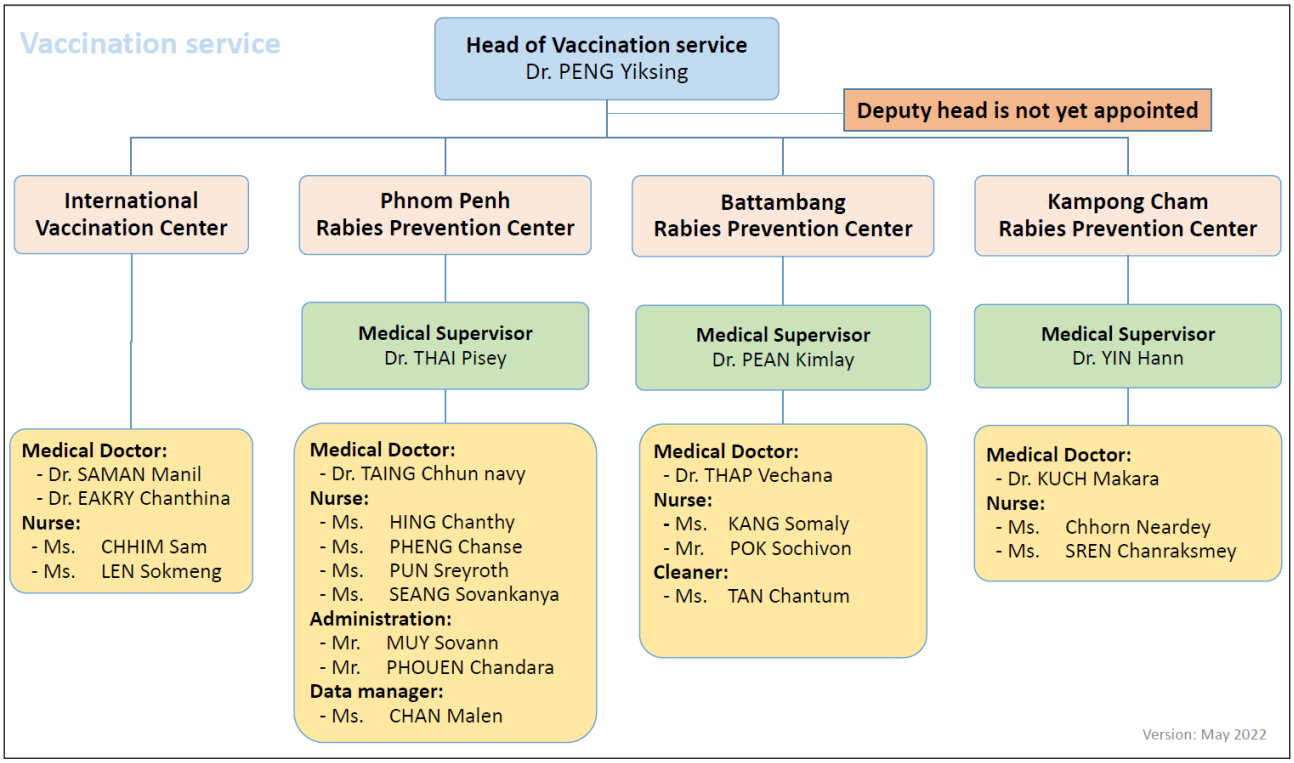


Figure 29. Vaccination Service Organogram

4.8.2 Rabies Prevention Centers

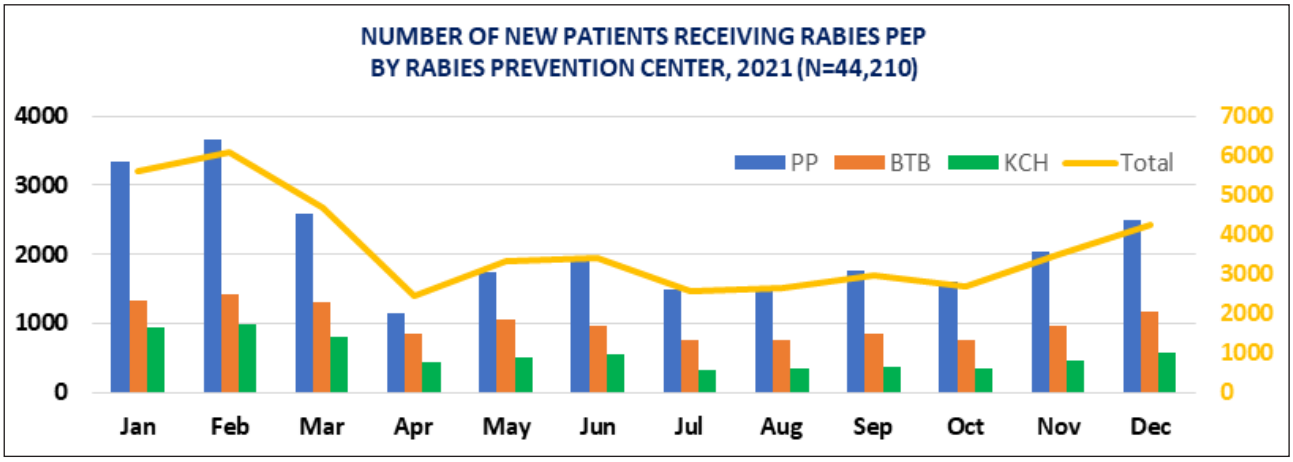
The three rabies prevention centers employ 18 full-time staff under the leadership of the Vaccination Center Head. These centers provide post-exposure prophylaxis (PEP) against rabies including the administration of Equine Rabies Immunoglobulins (ERIG) at an affordable price to the public, as the treatment 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 one, which consists of three 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.

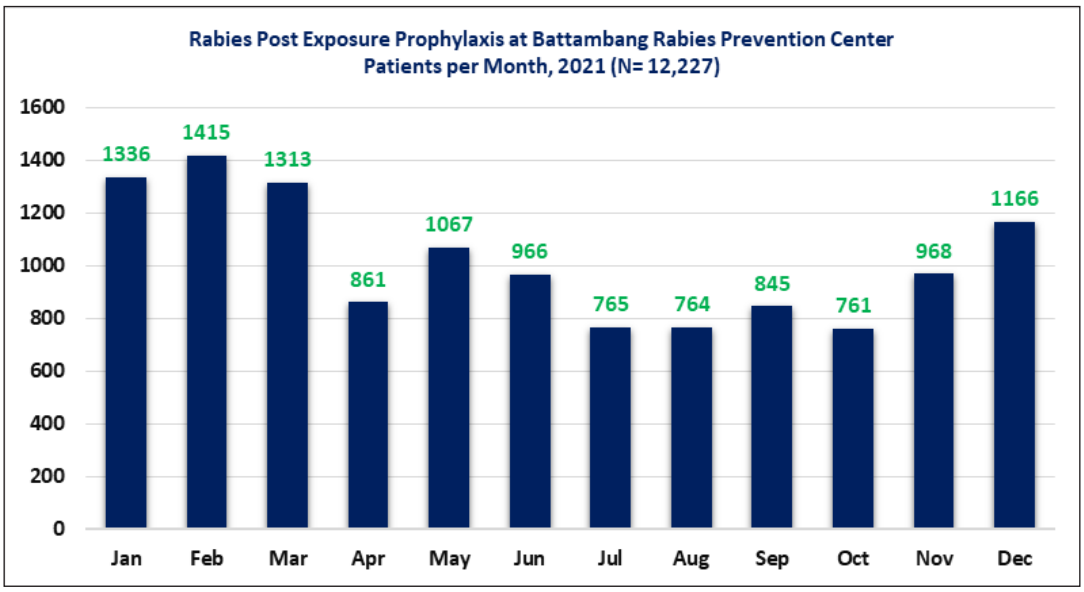
Rabies Prevention Centers Activities in 2021:

- Provided rabies post-exposure prophylaxis to 44,210 patients.
- 25,327 patients received rabies PEP at the Rabies Prevention Center in Phnom Penh
- 12,227 patients received rabies PEP at the Rabies Prevention Center in Battambang
- 6,656 patients received rabies PEP at the Rabies Prevention Center in Kampong Cham
- RPC's reach declined by 23% compared to 2020, the activity was seriously impacted by COVID-19.
- A total of 174 animal heads were tested by immunofluorescence for rabies virus at the virology unit.
- 134 samples (77%) were positive for rabies (132 dogs, 2 cats).
- This information is regularly communicated to MoH and MoH,CDC, and WHO.

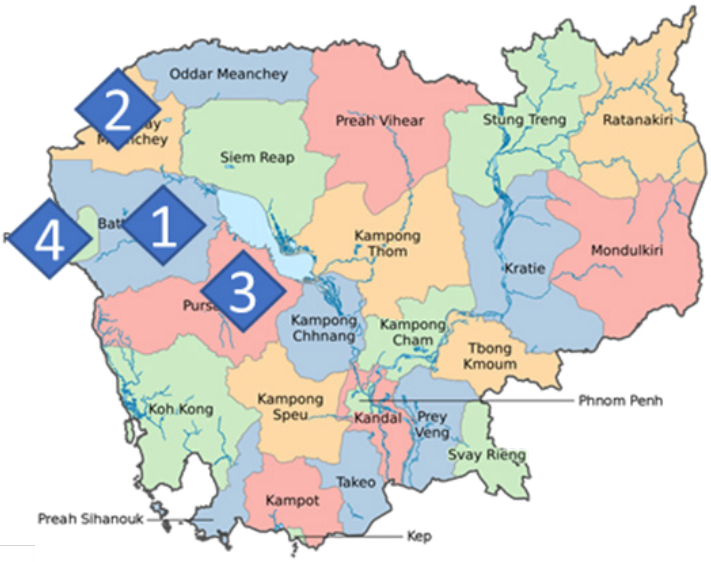


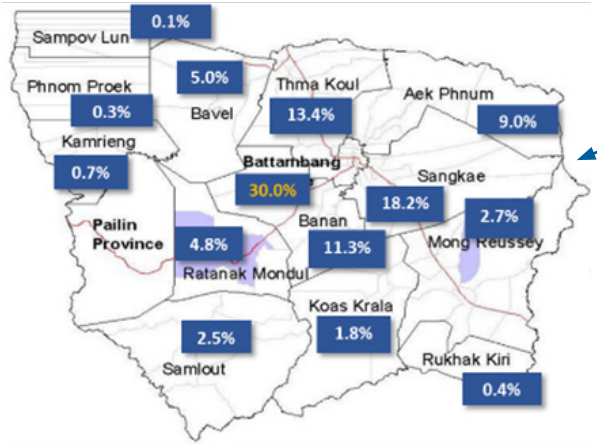
4.8.2.1. Battambang Rabies Prevention Center

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 neighboring provinces.



Provinces	%
1. Battambang	**91,2%**
2. Banteay Meanchey	5.6%
3. Pursat	1.8%
4. Pailin	0,8%
Other	0,6%



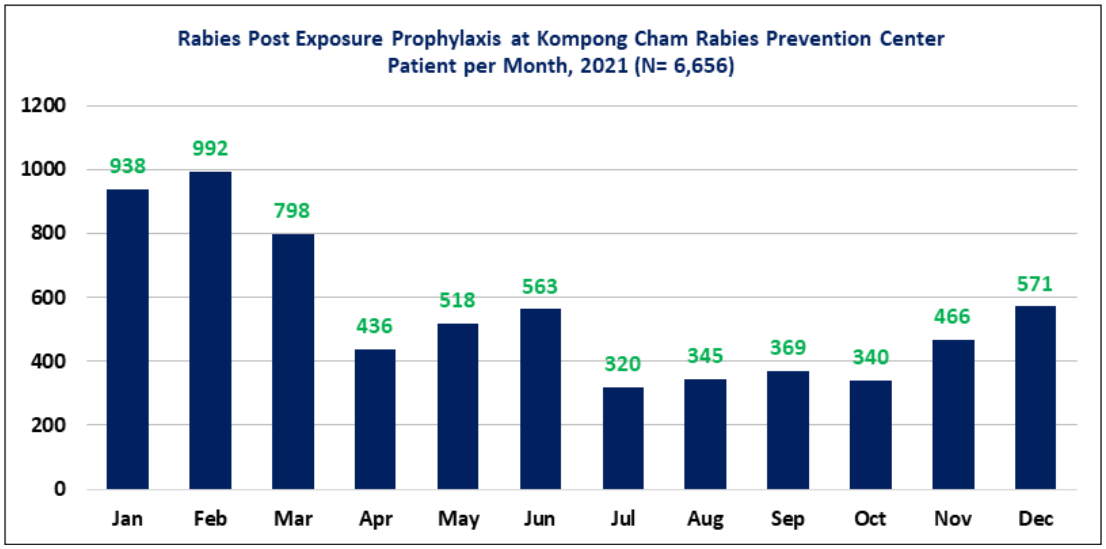


Origin of Patients within Battambang Province (N= 11,146)

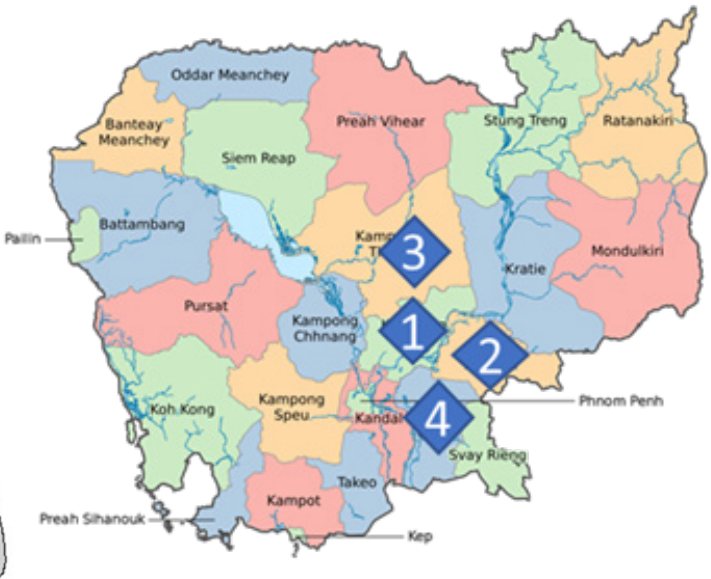
Battambang Rabies Prevention Center

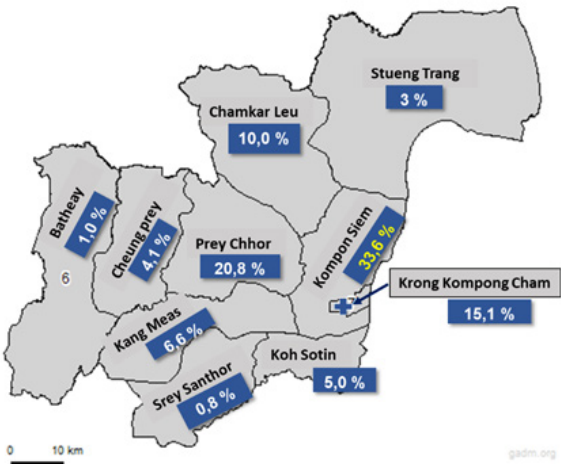
4.8.2.2. Kampong Cham Rabies Prevention Center

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	**76.6%**
2. Tboung Khmom	18.9%
3. Kampong Thom	1.4%
4. Prey Veng	1.9%
Other	1.2%





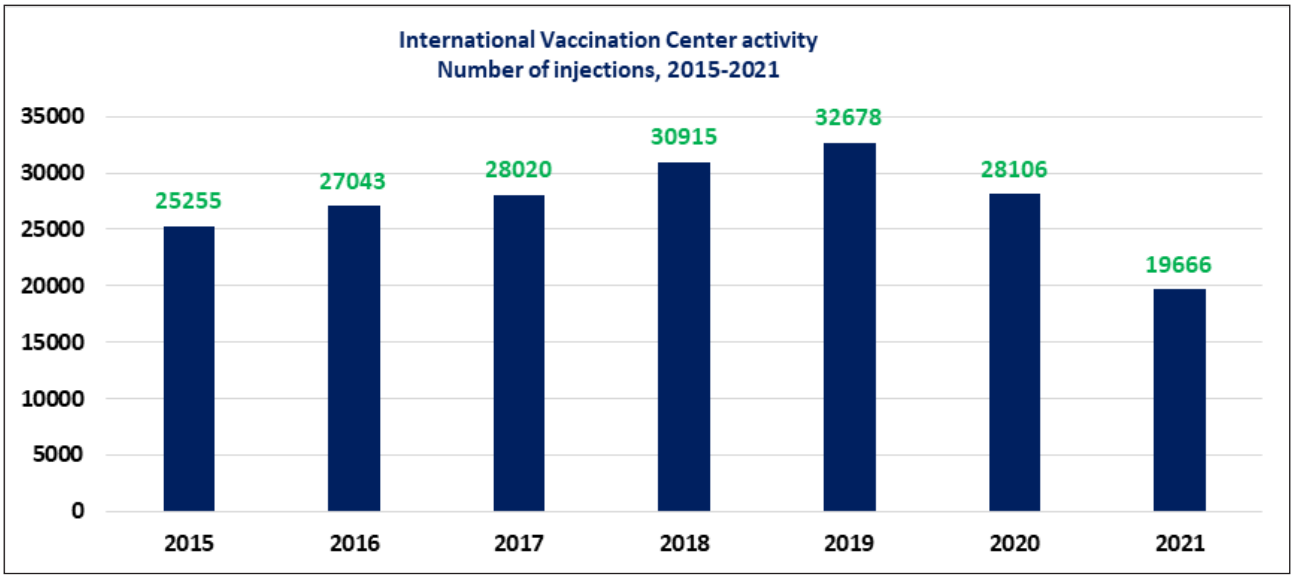
Origin of Patients within Kampong Cham Province (N= 5,095)

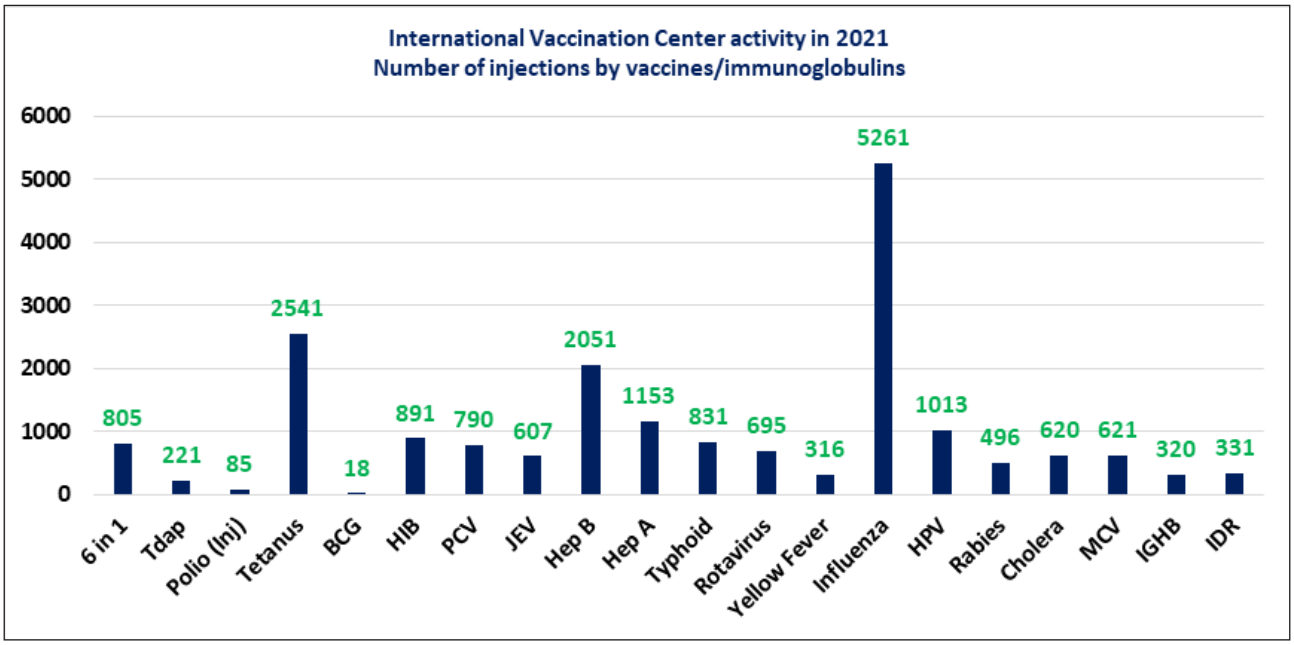
Kampong Cham Rabies Prevention Center

4.8.3. International Vaccination Center

The International Vaccination 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 are available at the International Vaccination Center. We maintain international standards with qualified products, proper cold chain management, high-quality control, and professionalism.

In 2021, a total of 19,666 injections (including the immunoglobulins) were done as part of 12,221 vaccine protocols. IVC's activity decreased by 30% compared to 2020, again, the reason being COVID-19 restricting movements and reducing incomes and resulting in a shortage of vaccines.





4.8.4. Support to National Authorities

Contribution to the fight against rabies in Cambodia

The Vaccination Center supports the Ministry of Health in Cambodia in fighting against rabies through various key activities, including education, information, communication, and providing rabies PEP to the public at an affordable price.

The Vaccination Center collaborates with several national authorities and international agencies (MoH US-CDC, Institut Pasteur in Paris, WHO, GDAPH, FAO, CIRAD, Mission Rabies and others) to contribute to research studies, especially on rabies.

4.8.5. 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 eliminating rabies by 2030. In order to achieve this milestone two main action plans to fight rabies have been developed by IPC in collaboration with the Ministry of Health:

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

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.

4.8.6. Publication List 2021

NOTE

The name of authors from the Institut Pasteur du Cambodge are underlined.
Publications in a journal without impact factor are listed separately and identified at the end of the list
** equal contribution, first author / ** equal contribution, last author*

1. ***Circulation of Bordetella pertussis in vaccinated Cambodian children: a transversal serological study***
Gaelle Noel*, Laurence Borand*, Chanthy Leng, Chanthy Keang, Chhunly Botr, Bunnet Dim, Alexandra Kerleguer, Yik S. Peng, Navin Sreng, Vichit Ork, Mohand Ait-Ahmed, Nicole Guiso, Fabien Taieb.
Int J Infect Dis. 2021; DOI: 10.1016/j.ijid.2021.03.054

4.9. Technical Platforms

4.9.1. Biobank

4.9.1.1. Background

IPC Biobanking was initiated in 2015 by an internship student in Management of Biobanking (Ms. Sara Cashillo). In 2017, the development of biobank software was initiated by Mr. Stephane Grenier and Ms. LIM Pisey with a local developer namely DEV KHMER S.A.R.L. In 2018, the biobank software was fully developed and it was launched for use under the management of Ms. LIM Pisey as a Biobank Manager. In 2019 and 2020, users met a problem in the export function. After this issue was fixed by DEV, users still encountered this kind of issue. In 2020, the biobank software was re-developed and until now, it is still ongoing and is expected to be fully developed and put for use within May 2022.

4.9.1.2. Functional Structure

The biobank section is under the HSeQM service/Administration unit. It has a cross-cutting role to support the research and testing laboratories of IPC with different material including documents such as standard operating procedures (SOPs) and software to monitor the samples. Key responsibilities and functions are as described below.

- Biobank software → manage biobank software, work with developer, handle the complaint from users, solve problems/errors raised by users relating to the software;
- Sample labels → print labels for users (biobank template + QR code);
- Material transfer agreement (MTA) → review MTA
- Nagoya protocol → prepare documents, contact with Ministry of Environment (MOE).

Current users of the biobank and the virology and the epidemiology and public health units.

All samples are currently stored in the freezers at -80°C of which we now have 44 units and in the future, some samples will be stored in liquid Nitrogen (N2); IPC has its own liquid nitrogen generator for daily production. All these freezers are installed in a purpose-designed room with air conditioners working permanently. This room is only accessed by authorized persons and entry is strictly controlled. The freezers are constantly monitored through the use of the monitoring of the Oceasoft system whose use is dedicated to this purpose alone.

4.9.1.3. Biobank Database (9 Projects)

There are currently nine research projects with approximately 55,634 samples stored in the databases as per the detail in table below:

Collection/Project	No. of samples	Nature of samples	Storage
Medical Biology Laboratory			
Aspergilloma in Cambodia	3,063	Serum	Freezer -80oC (HSM-04) Freezer code: 439
Virology			
ECOMORE 2	6,425	Serum	Freezer -80oC (HSM-04) Freezer code:
Immuno PEP follow up 2019	239	Serum	Freezer -80oC (VIR-02) Freezer code: 1324
RAB00056 IM/ID (Sanofi study)	27	Serum	Freezer -80oC (VIR-02) Freezer code: 1324
Rabies surveillance	14,069	Brain tissue Ammon's horn Spinal bulb	Freezer -80oC (VIR-02) Freezer code: 1324
Rodents as Reservoir for Hepatitis E Virus (HEV), Arenavirus and Other Rodent-borne Viruses and risk assessment of infection in human in Cambodia	1,940	Kidneys, Swab, Urine, Heart, Lung, Liver, Ectoparasite, Blood clot, blood serum, Pool organ	N/A
Clinical research			
Lowering Interleukin-1 Receptor Antagonist Concentrations after TB Treatment Onset: A proof of concept study in Cambodia and Ivory	304	Plasma Blood Red blood cell	Freezer -80oC (HSM-04) Freezer code: LILAC-TB
TB-Speed Output 2 - Severe pneumonia	1,812	Plasma Stool Whole blood	Freezer -80oC (HSM-04) Freezer code: 1551
Tenofovir As PRevention Of Hepatitis b Mother-to-child transmission	27,738	Plasma Blood Red blood cell	Freezer -80oC (HSM-04) Freezer code: 1475
Zika Sentinel Surveillance in prenatal care visit and maternity ward in Calmette Hospital (Phnom Penh, Cambodia)	17	Serum Urine Blood	Freezer -80oC (HSM-04) Freezer code: 439
Total samples	**55,634**		

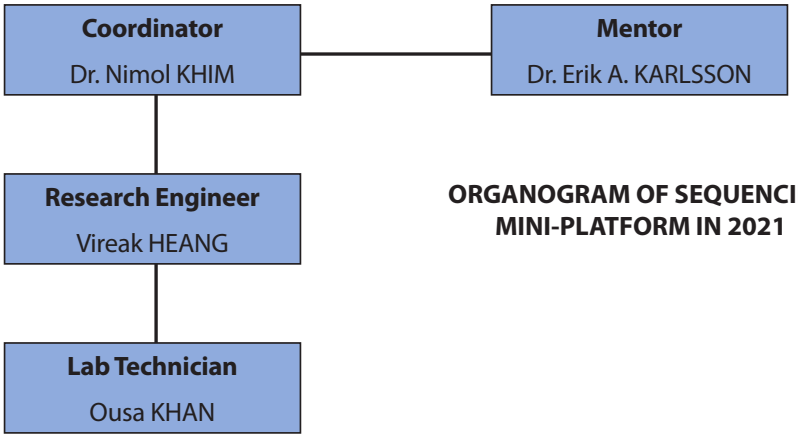
4.9.1.4. Action Plan

The biobank activities should be centralized under the responsibility of 01 person who is taking care of input and output of samples. The SOPs for management of Biobank are being written.

4.9.2. Sequencing Mini-Platform

4.9.2.1. Functional Structure

In April 2021, Institut Pasteur du Cambodge launched a mini-platform for sequencing with support from a number of stakeholders including Agence Française de Développement (AFD), MediLabSecure, the United Kingdom Embassy, Wellcome Trust, and the World Health Organization. This mini-platform is intended to enhance operational research and public health response at IPC, and is composed of one coordination Principal Investigator (20% of time, KHIM Nimol, Deputy Head of Malaria Molecular Epidemiology Unit), one Mentor PI (10% of time, Erik A. KARLSSON, Deputy Head of Virology Unit), one research engineer and one laboratory technician. The laboratory, which was the initial step in creation, is also coordinated by other researchers within the institution such as Cécile TROUPIN, Virology Unit, and Koen VANDELANNOOTE, Medical Biology Laboratory.



ORGANOGRAM OF SEQUENCING MINI-PLATFORM IN 2021

4.9.2.2. Research Programs – Major Achievements in 2021

Activities focused on amplicon-based and metagenomic sequencing. These include wet lab procedures, general data clean-up and management, as well as project advice and integration for all IPC researchers. A consultation and request form has been created to facilitate and welcome any scientists/ collaborations to strengthen and sustain our laboratory capabilities.

During 2021, this sequencer has been used for two research and internal collaboration projects:

1. In collaboration with the virology unit, whole genome sequencing was performed on 96 COVID-19 specimens, sequenced on MiSeq in our laboratory. The Illumina V3 600 cycles kit effectively sequenced paired-end reads for most of the samples tested, providing 90% of genomic coverage. All raw data (.fastq files) and 96-consensus sequences generated by Geneious software were transmitted to the Principal Investigator (Virology Unit) for further bioinformatics analysis.

Collaborations	Virology Unit, Sequencing mini-Platform, IPC
Funding	United Kingdom Embassy Grant, World Health Organization

2. Amplicon deep sequencing of five molecular markers *ama1-D3, cpp, cpmp, csp and msp7* from a set of 96 Plasmodium falciparum were also been sequenced with this MiSeq system in partnership with the malaria molecular epidemiology unit. Ninety percent of the samples tested were successfully sequenced using paired-end reads Illumina V3 600 cycles kit. All raw data (.fastq files) and 96-consensus sequences produced by Geneious software have been forwarded to PI, malaria molecular epidemiology unit, to be used in further bioinformatics research.

Collaborations	Malaria Molecular Epidemiology Unit, Sequencing mini-Platform, IPC
Funding	World Health Organization, IPC Internal collaboration projects

4.9.2.3. Research Programs – Outlook for 2022

1. A proposal entitled **"In the Air Tonight: Metagenomic Pathogen Discovery as Tools in Pathogen Surveillance"** to the Centers for Research in Emerging Infectious Diseases (CREID) lead by the mini-Platform research engineer (Mr. Vireak Heng) and a postdoctoral researcher in the virology unit (Dr. Jurre SIEGERS) was successfully accepted for a one year pilot project grant starting in June 2022 through EID-SEARCH with strong support from Mentor PI Dr. E. Karlsson. This funded pilot project will greatly increase capacity and capabilities in metagenomic viral discovery, and increase the visibility of the platform and the engineering within high-profile NGS networks.

2. In partnership with MMEU, amplicon **deep sequencing of five molecular markers *ama1-D3, cpp, cpmp, csp and msp7* from other sets of 200 *Plasmodium falciparum*** have also been/ will be sequenced with this MiSeq system this year. This project investigated the utility and benefits of amplicon deep sequencing for genotyping of antimalarial drug efficacy in Plasmodium falciparum infection.

3. In collaboration with the virology unit, the sequencer will be used for study protocol entitled **"Human papillomavirus E6 and E7 coding gene variations and their possible association with occurrence of cervical intraepithelial neoplasia".**

4. Discussions and project plans are also in progress for usage of the mini-Platform/sequencer for studying hantaviruses and coronaviruses, as well as **grant submissions for studying Major Histocompatibility Complex** from bats and chickens to understand disease risk.

4.9.2.4. Outlook (Two to Five years)

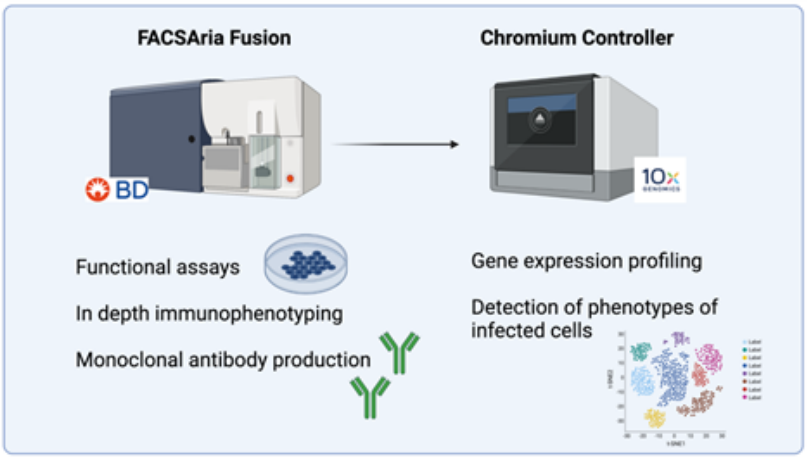
As the platform is newly established, IPC PIs will need to be encouraged and motivated to fully utilize (and fund) the platform in upcoming projects and grants, apart from sending samples to other institutions or collaborators. In order to persuade IPC PIs to use this platform as the first line for sequencing capacity, more effort is needed.

It is our vision that this platform will become increasingly important in the near future not only for advancement of scientific progress at IPC, but also for other projects within Cambodia as it continually becomes increasingly difficult to export genetic material around the world due to roadblocks and political accords such as the Nagoya Protocol. In addition, genomic surveillance and NGS are becoming commonplace in many public health activities, making this mini-platform critical to maintain in the daily fight against this pandemic and preventing the next.

As a next step, bioinformatics and IT infrastructure support is critical for sequencing data analysis, and the creation of a BioInformatics Working Group aims to support and strengthen this. in the various Units as well as at the sequencing mini-platform,and provide capacity and capability training for Cambodia and beyond.

4.9.3. Single Cell Analysis Platform

Host-pathogen interactions are complex and biomedical research has evolved to interrogate multiple parameters at the same time using different -omics approaches. Single cell analysis and functional assays on purified cell populations have become established methodologies to study cell and pathogen heterogeneity. This platform allows us to investigate complex pathogen-host interactions to the single cell level directly on site in a low/middle income country. The availability of this equipment and patient cohorts in the same location allows us to advance our basic research on infectious diseases of major importance in Cambodia.

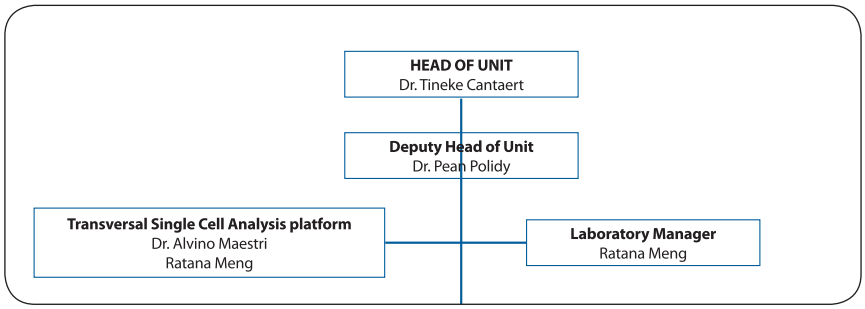


In 2020, we purchased and initiated the use of a new 4 laser, 18-color single cell sorter (FACSAria Fusion III). Funding was provided by Wellcome Trust and GIZ (multi user equipment grant, PI: Cantaert Tineke, Co-investigators: Jean Popovici, Benoit Witkowski and Erik Karlsson). Moreover, we purchased and implemented a 10x genomics chromium controller for single cell RNA sequencing, funded by the NIH PICREID grant (country PI: Cantaert Tineke). Both pieces of equipment are in a BSLII+ biosafety environment.

The platform is open to researchers both within and outside of IPC, research entities and universities in Cambodia on a collaborative basis. The platform provides expertise in the experimental design and a dedicated research engineer will perform the experiments. No user fees will be charged but the collaborative partners should purchase the reagents and consumables needed for any experiment.

4.9.3.1. Functional Structure

The platform is integrated within the Immunology Unit.



4.9.3.2. Research Programs – Major Achievements in 2021

In 2021, the platform was used for the following research projects:

1. Understanding the role of regulatory T cells in dengue virus infection;
2. Evaluation of the skin immune response to mosquito saliva (Guerero-Gomez, Vo et al, manuscript submitted) in a collaborative project with the NIH (Dr. Jessica Manning);
3. Understanding the mechanisms of plasmodium vivax receptor-ligand interactions involved in reticulocyte invasion;
4. Assessment of long-term immunity to SARS-CoV-2 (Vo, Maestri et al, Frontiers Immunol 2022).

4.9.3.3. Outlook 2022

1. Assessment of a protective immune response to plasmodium vivax;
2. Mechanistic understanding of the involvement of the T-cell response in the development of chronic chikungunya disease;
3. Generation and analysis of monoclonal anti-dengue antibodies.

The platform can be used to study interactions between host and pathogens of major health importance in Cambodia. The platform can also help in the design, planning and execution of the experiments. With the expansion of biomedical research activities at IPC, we will initiate research projects with various units such as the virology unit, the medical entomology unit and the medical biology laboratory. Within Cambodia, we aim to set up novel collaborations with universities and other research entities. Moreover, the platform aims to support research programs in Cambodia and in the region through workshops and training. It will help increase the visibility of IPC internationally and attract foreign scientists. We will train Internship and thesis students from various Universities in state-of-the-art techniques.

5. Conclusion

The year 2021 was characterized by COVID-19 epidemic, whose evolution resulted in intense activity in response to local events. This involved and affected all of IPC and created challenges due to shifting but ever-present constraints (limitations on movement and travel, difficulties with obtaining supplies, etc.) that required flexibility and perseverance to address and overcome.

On December 14, 2021, IPC detected the Omicron variant of SARS-COV 2 through sequencing. This new, extremely contagious variant caused a new epidemic wave, but in most cases the clinical symptoms were relatively mild.

The Institut Pasteur du Cambodge once again proved its capacity and commitment to- in close partnership with the Ministry of Health- combating infectious disease to the benefit of the Cambodian people.

Under extremely challenging conditions, IPC was able to effectively carry out its mission, and it warrants using this opportunity to thank all of the constituent teams for their demonstrated professionalism, hard work, and sense of duty.

We at IPC are confident that despite the many challenges and work that remains undone, given the quality of our teams and our commitment to working with our partners, and the Ministry of Health and its constituent departments, we will be successful in meeting our objectives and goals as defined earlier in this report.

L'année 2021 a été marquées par l'épidémie de Covid19 génératrice d'une activité intense de la part de différentes équipes de l'IPC et de difficultés liées à différentes contraintes (déplacement, approvisionnement,) qui ont dû être surmontées.

Le 14 décembre les équipes de l'IPC ont détecté par séquençage le variant Omicron du SARS-COV2. Ce nouveau variant très contagieux a entraîné une nouvelle vague épidémique mais dont les formes cliniques étaient le plus souvent relativement modérées.

L'Institut Pasteur du Cambodge a fait la preuve une fois de plus de son efficacité et de son engagement dans la lutte contre les maladies infectieuses au profit de la santé des populations du Cambodge en partenariat étroit et au côté du Ministère de la Santé.

Dans un contexte difficile l'IPC a pu assurer ses missions et il convient ici de remercier les équipes pour leur professionnalisme, leur ardeur au travail, leur sens du devoir.

Même si beaucoup de choses restent à faire et les objectifs pour les années à venir ont été fixés plus haut dans les premières pages de ce document, nous pouvons être confiants compte tenu de la qualité de nos équipes si nous savons nous appuyer sur nos partenaires traditionnels aux premiers rang desquels le Ministère de la Santé et ses différents établissements.

6. Scientific Publications 2021

NOTE

The name of authors from the Institut Pasteur du Cambodge are underlined.
Publications in a journal without impact factor are listed separately and identified at the end of the list
** equal contribution, first author / ** equal contribution, last author*

1. ***An influenza A (H3N2) virus outbreak in the Kingdom of Cambodia during the COVID-19 pandemic of 2020***
   L. Y. Sovann, B. Sar, V. Kab, S. Yann, M. Kinzer, P. Raftery, R. Albalak, S. Patel, P. Long Hay, H. Seng, S. Um, S. Chin, D. Chau, A. Khalakdina, E. Karlsson, S. J. Olsen, J. A. Mott.
   International Journal of Infectious Diseases. 2021;103:352 ; DOI: 10.1016/j.ijid.2020.11.178

2. ***A Novel SARS-CoV-2 Related Coronavirus in Bats from Cambodia***
   Delaune Deborah*, Vibol Hul*, Erik A. Karlsson*, Alexandre Hassanin, Tey Putita Ou, Artem Baidaliuk, Fabiana Gámbaro, Matthieu Prot, Vuong Tan Tu, Sokha Chea, Lucy Keatts, Jonna Mazet, Christine K. Johnson, Philippe Buchy, Philippe Dussart, Tracey Goldstein, Etienne Simon-Lorière**, and Veasna Duong**
   Nat Communications ; 12,1 (2021), 6563 ; Doi:10.1038/s41467-021-26809-4

3. ***A review of Capezoum Adlbauer, 2003 (Coleoptera: Cerambycidae) with the description of two new species from the Succulent Karoo ecosystem in South-Africa***
   Pierre-Olivier Maquart, Francesco Vitali, Riana Bate.
   Zootaxa. 2021;4915(4):559 -566. DOI:10.11646/zootaxa.4915.4.6.

4. ***Anopheles ecology, genetics and malaria transmission in northern Cambodia***
   Amélie Vantaux, Michelle M. Riehle, Eakpor Piv, Elise J. Farley, Sophy Chy, Saorin Kim, Anneli G. Corbett, Rachel L. Fehrman, Anais Pepey, Karin Eiglmeier, Dysoley Lek, Sovannaroth Siv, Ivo Mueller, Kenneth D. Vernick**, Benoit Witkowski**
   Sci Rep. 2021;11(1):6458. DOI: 10.1038/s41598-021-85628-1

5. ***Antibody fucosylation predicts disease severity in secondary dengue infection***
   Stylianos Bournazos, Hoa Thi My Vo, Veasna Duong, Heidi Auerswald, Sowath Ly, Anavaj Sakuntabhai, Philippe Dussart, Tineke Cantaert**, Jeffrey V. Ravetch**
   Science. American Association for the Advancement of Science; 2021;372(6546):11025. DOI: 10.1126/science.abc7303

6. ***Antibody-independent functions of B cells during viral infections***
   Vinit Upasani, Izabela Rodenhuis-Zybert, Tineke Cantaert.
   PLoS Pathog. 2021;17(7):e1009708. DOI: 10.1371/journal.ppat.1009708

7. ***Antigenic evolution of dengue viruses over 20 years***
   Leah C. Katzelnick, Ana Coello Escoto, Angkana T. Huang, Bernardo Garcia-Carreras, Nayeem Chowdhury, Irina Maljkovic Berry, Chris Chavez, Philippe Buchy, Veasna Duong, Philippe Dussart, Gregory Gromowski, Louis Macareo, Butsaya Thaisomboonsuk, Stefan Fernandez, Derek J. Smith, Richard Jarman, Stephen S. Whitehead, Henrik Salje, Derek A. T. Cummings.
   Science. American Association for the Advancement of Science; 2021; DOI: 10.1126/science.abk0058

8. ***Artemisinin-independent inhibitory activity of Artemisia sp. infusions against different Plasmodium stages including relapse-causing hypnozoites***
   Kutub Ashraf*, Shahin Tajeri*, Christophe-Sébastien Arnold, Nadia Amanzougaghene, Jean-François Franetich, Amélie Vantaux, Valérie Soulard, Mallaury Bordessoulles, Guillaume Cazals, Teun Bousema, Geert-Jan van Gemert, Roger Le Grand, Nathalie Dereuddre-Bosquet, Jean-Christophe Barale, Benoit Witkowski, Georges Snounou, Romain Duval, Cyrille Y. Botté, Dominique Mazier.
   Life Science Alliance. Life Science Alliance; 2021;5(3). DOI: 10.26508/lsa.202101237

9. ***Assessment of inactivation procedures for SARS-CoV-2***
   Heidi Auerswald, Sokhoun Yann, Sokha Dul, Saraden In, Philippe Dussart, Nicholas J. Martin, Erik A. Karlsson, Jose A. Garcia-Rivera.
   J Gen Virol. 2021; DOI: 10.1099/jgv.0.001539

10. ***Characterization of the Tubovesicular Network in Plasmodium vivax Liver Stage Hypnozoites and Schizonts***
Kayla Sylvester, Steven P. Maher, Dora Posfai, Michael K. Tran, McKenna C. Crawford, Amélie Vantaux, Benoît Witkowski, Dennis E. Kyle, Emily R. Derbyshire.
Frontiers in Cellular and Infection Microbiology; 2021. DOI: 10.3389/fcimb.2021.687019

11. ***Checklist of the mosquito fauna (Diptera, Culicidae) of Cambodia***
Pierre-Olivier Maquart, Didier Fontenille, Nil Rahola, Sony Yean, Sébastien Boyer.
Parasite. EDP Sciences; 2021;28:60. DOI: 10.1051/parasite/2021056

12. ***Choosing interventions to eliminate forest malaria: preliminary results of two operational research studies inside Cambodian forests***
Amber Kunkel, Chea Nguon, Sophea Iv, Srean Chhim, Dom Peov, Phanith Kong, Saorin Kim, Sarun Im, Mark Debackere, Nimol Khim, Jean Popovici, Sreynet Srun, Amélie Vantaux, Jean-Olivier Guintran, Benoit Witkowski, Patrice Piola.
Malar J. 2021;20(1):51. DOI: 10.1186/s12936-020-03572-3

13. ***Circulation of Bordetella pertussis in vaccinated Cambodian children: a transversal serological study***
Gaelle Noel*, Laurence Borand*, Chanthy Leng, Chanthy Keang, Chhunly Botr, Bunnet Dim, Alexandra Kerleguer, Yik S. Peng, Navin Sreng, Vichit Ork, Mohand Ait-Ahmed, Nicole Guiso, Fabien Taieb.
Int J Infect Dis. 2021; DOI: 10.1016/j.ijid.2021.03.054

14. ***Cross-resistance of the chloroquine-derivative AQ-13 with amodiaquine in Cambodian Plasmodium falciparum isolates***
Flore Nardella, Mélissa Mairet-Khedim, Camille Roesch, Steven P Maher, Sopheakvatey Ke, Rithea Leang, Didier Leroy, Benoit Witkowski.
Journal of Antimicrobial Chemotherapy. 2021;76(10):25658. DOI: 10.1093/jac/dkab219

15. ***Decoding the RNA viromes in rodent lungs provides new insight into the origin and evolutionary patterns of rodent-borne pathogens in Mainland Southeast Asia***
Zhiqiang Wu, Yelin Han, Bo Liu, Hongying Li, Guangjian Zhu, Alice Latinne, Jie Dong, Lilin Sun, Haoxiang Su, Liguo Liu, Jiang Du, Siyu Zhou, Mingxing Chen, Anamika Kritiyakan, Sathaporn Jittapalapong, Kittipong Chaisiri, Phillipe Buchy, Veasna Duong, Jian Yang, Jinyong Jiang, Xiang Xu, Hongning Zhou, Fan Yang, David M. Irwin, Serge Morand, Peter Daszak, Jianwei Wang, Qi Jin
Microbiome. 2021; 9**,** 18. DOI: 10.1186/s40168-020-00965-z\

16. ***Development of weight and age-based dosing of daily primaquine for radical cure of vivax malaria***
Walter Robert Taylor, Richard M. Hoglund, Pimnara Peerawaranun, Thuy Nhien Nguyen, Tran Tinh Hien, Arnaud Tarantola, Lorenz von Seidlein, Rupam Tripura, Thomas J. Peto, Arjen M. Dondorp, Jordi Landier, Francois H.Nosten, Frank Smithuis, Koukeo Phommasone, Mayfong Mayxay, Soy Ty Kheang, Chy Say, Kak Neeraj, Leang Rithea, Lek Dysoley, Sim Kheng, Sinoun Muth, Arantxa Roca-Feltrer, Mark Debackere, Rick M. Fairhurst, Ngak Song, Philippe Buchy, Didier Menard, Nicholas J. White, Joel Tarning, Mavuto Mukaka.
Malaria Journal. 2021;20(1):366. DOI: 10.1186/s12936-021-03886-w

17. ***Differential levels of IFNa subtypes in autoimmunity and viral infection***
Vincent Bondet, Mathieu P. Rodero, Céline Posseme, Pierre Bost, Jérémie Decalf, Liis Haljasmägi, Nassima Bekaddour, Gillian I. Rice, Vinit Upasani, Jean-Philippe Herbeuval, John A. Reynolds, Tracy A. Briggs, Ian N. Bruce, Claudia Mauri, David Isenberg, Madhvi Menon, David Hunt, Benno Schwikowski, Xavier Mariette, Stanislas Pol, Flore Rozenberg, Tineke Cantaert, J. Eric Gottenberg, Kai Kisand, Darragh Duffy.
Cytokine. 2021;144:155533. DOI: 10.1016/j.cyto.2021.155533

18. ***Direct Infection of B Cells by Dengue Virus Modulates B Cell Responses in a Cambodian Pediatric Cohort***
Vinit Upasani, Hoa Thi My Vo, Heidi Auerswald, Denis Laurent, Sothy Heng, Veasna Duong, Izabela A. Rodenhuis-Zybert, Philippe Dussart, Tineke Cantaert.
Front Immunol. 2021;11. DOI: 10.3389/fimmu.2020.594813

19. ***Dynamics of G6PD activity in patients receiving weekly primaquine for therapy of Plasmodium vivax malaria***
Walter R. J. Taylor, Saorin Kim, Sim Kheng, Sinoun Muth, Pety Tor, Eva Christophel, Mavuto Mukaka, Alexandra Kerleguer, Lucio Luzzatto, J. Kevin Baird**, Didier Menard**
PLOS Neglected Tropical Diseases. Public Library of Science; 2021;15(9):e0009690. DOI: 10.1371/journal.pntd.0009690

20. ***Efficacy of dihydroartemisinin/piperaquine in patients with non-complicated Plasmodium falciparum malaria in Yaoundé, Cameroon***
Mélissa Mairet-Khedim, Sandrine Nsango, Christelle Ngou, Sandie Menard, Camille Roesch, Nimol Khim, Sreynet Srun, Xavier Iriart, Thomas Lanot, Laure Otam, Francis Abega, Lawrence Ayong, Isabelle Morlais, Peggy Gandia, Benoit Witkowski, Antoine Berry.
Journal of Antimicrobial Chemotherapy. 2021;76(11):303744. DOI: 10.1093/jac/dkab281

21. ***Genetic and Antigenic Characterization of an Influenza A(H3N2) Outbreak in Cambodia and the Greater Mekong Subregion during the COVID-19 Pandemic, 2020.***
Jurre Y. Siegers, Vijaykrishna Dhanasekaran, Ruopeng Xie, Yi-Mo Deng, Sarika Patel, Vanra Ieng, Jean Moselen, Heidi Peck, Ammar Aziz, Borann Sarr, Savuth Chin, Seng Heng, Asheena Khalakdina, Michael Kinzer, Darapheak Chau, Philomena Raftery, Veasna Duong, Ly Sovann, Ian G. Barr**, Erik A. Karlsson**
J Virol. 2021;95(24):e01267-21. DOI: 10.1128/JVI.01267-21

22. ***"Health in" and "Health of" Social-Ecological Systems: A Practical Framework for the Management of Healthy and Resilient Agricultural and Natural Ecosystems.***
Michel De Garine-Wichatitsky, Aurélie Binot, John Ward, Alexandre Caron, Arthur Perrotton, Helen Ross, Hoa Tran Quoc, Hugo Valls-Fox, Iain J. Gordon, Panomsak Promburom, Rico Ancog, Richard Anthony Kock, Serge Morand, Véronique Chevalier, Will Allen, Waraphon Phimpraphai, Raphaël Duboz, Pierre Echaubard.
Frontiers in Public Health 2021; 8:616328. DOI: 10.3389/fpubh.2020.616328

23. ***High Rickettsial Diversity in Rodents and Their Ectoparasites From the Central Highlands of Madagascar***
Fanohinjanaharinirina Rasoamalala, Mamionah N. J. Parany, Soloandry Rahajandraibe, Malala N. Rakotomanga, Tojo Ramihangihajason, Voahangy Soarimalala, Sébastien Boyer, Minoarisoa Rajerison, Beza Ramasindrazana.
J Med Entomol. 2021;tjab207. DOI: 10.1093/jme/tjab207

24. ***Host-Feeding Preference and Diel Activity of Mosquito Vectors of the Japanese Encephalitis Virus in Rural Cambodia***
Sébastien Boyer, Benoit Durand, Sony Yean, Cécile Brengues, Pierre-Olivier Maquart, Didier Fontenille, Véronique Chevalier.
Pathogens. 2021;10(3):376. DOI: 10.3390/pathogens10030376

25. ***Human Infection with Avian Influenza A(H9N2) Virus, Cambodia, February 2021***
Samnang Um, Jurre Y. Siegers, Borann Sar, Savuth Chin, Sarika Patel, Seng Bunnary, Makara Hak, Sothy Sor, Oum Sokhen, Seng Heng, Darapheak Chau, Tum Sothyra, Asheena Khalakdina, Joshua A. Mott, Sonja J. Olsen, Filip Claes, Ly Sovann, Erik A. Karlsson.
Emerg Infect Dis. 2021;27(10):27425. DOI: 10.3201/eid2710.211039

26. ***Impact of systematic early tuberculosis detection using Xpert MTB/RIF Ultra in children with severe pneumonia in high tuberculosis burden countries (TB-Speed pneumonia): a stepped wedge cluster randomized trial***
Aurélia Vessière, Hélène Font, Delphine Gabillard, Laurence Adonis-Koffi, Laurence Borand, Chishala Chabala, Celso Khosa, Sandra Mavale, Raoul Moh, Veronica Mulenga, Juliet Mwanga-Amumpere, Jean-Voisin Taguebue, Mao Tan Eang, Christophe Delacourt, James A. Seddon, Manon Lounnas, Sylvain Godreuil, Eric Wobudeya, Maryline Bonnet, Olivier Marcy.
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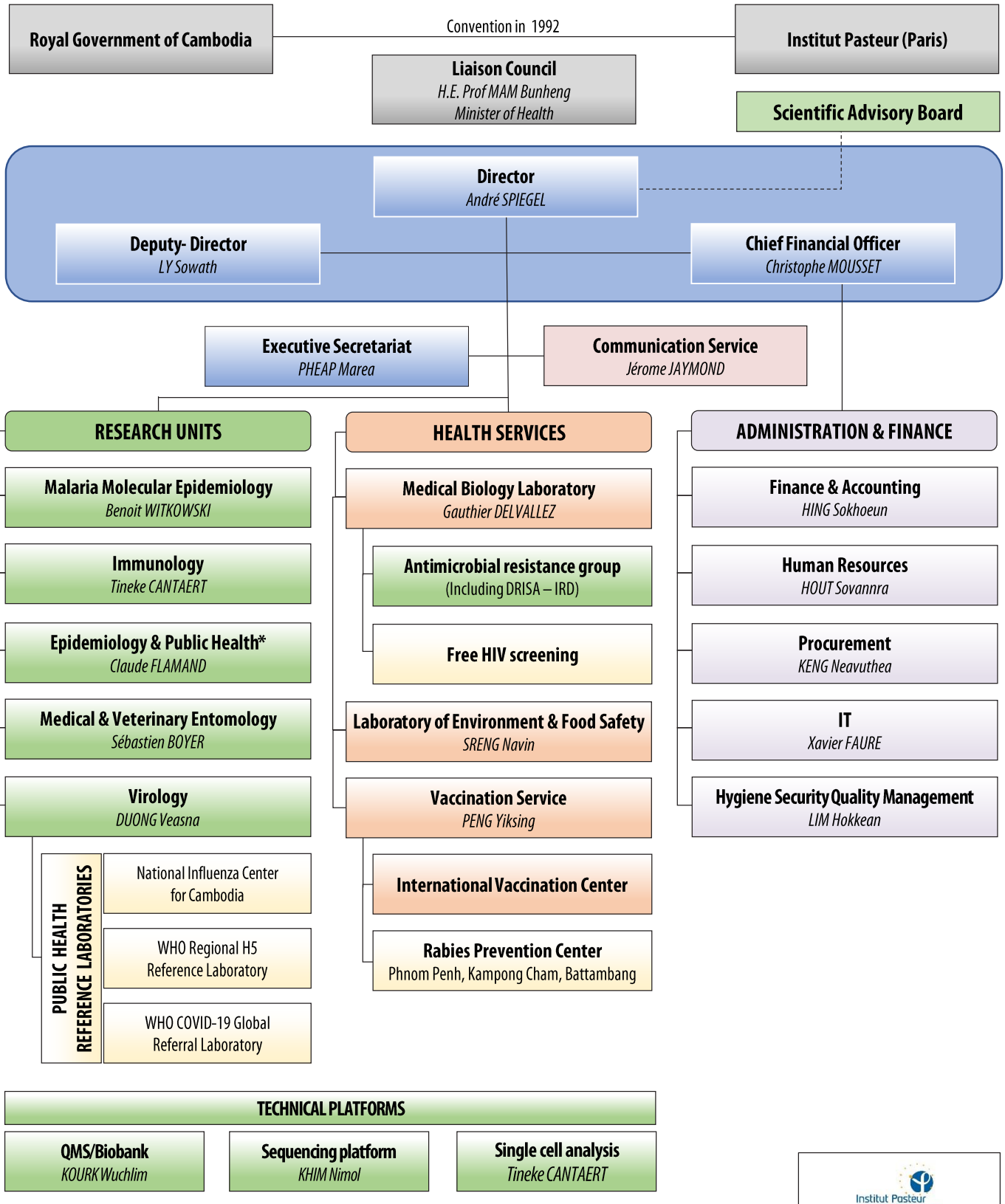
7. Annex

ORGANIZATIONAL CHART OF THE INSTITUT PASTEUR DU CAMBODGE



*Including **Clinical Research Group**, **Geo Health Group** (IRD/IPC), **One Health Group** (CIRAD/IPC)

Institut Pasteur du Cambodge

Reference: Organizational Chart
Date: 3rd May 2022