70 ans years 1953-2023

Institut Pasteur du Cambodge

Annual Report 2023

PHNOM PENH

INSTITUT PASTEUR DU CAMBODGE 1953 - 2023

PASTEUR NETWORK

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1 List of Acronyms

A*STAR	Agency for Science, Technology and Research, in Singapore
AB	Antibody
ACREME	Australian Centre of Research Excellence in Malaria Elimination
ADE	Antibody Dependent Enhancement
AFD	Agence Française pour le Développement (French Development Agency)
AFRIMS	Armed Forces Research Institute of the Medical Sciences
AHF	AIDS Healthcare Foundation
AIRD	Agence Inter-Etablissements de Recherche pour le Développement
AIV	Avian Influenza Virus
AMR	Antimicrobial Resistance
ANRS	Agence Nationale de Recherche sur le SIDA (National Agency for AIDS Research)
ANRS-MIE	Agence Nationale de Recherche sur le SIDA, les Hépatites et les Maladies Infectieuses et Emergentes (French National Agency for AIDS and Hepatitis Research)
Anti-EDIII Ab	Anti-Envelope Domain III Abs
ANU	Australian National University
ART	Antiretroviral Therapy
AUF	Agence Universitaire de la Francophonie
AVSF	Agronomes et Vétérinaires Sans Frontières
BA	Bachelor of Arts
BCG	Bacillus Calmette-Guerin
BCOMING	Biodiversity Conservation to Mitigate the Risks of Emerging Infectious Diseases
BCR	B-Cell Receptor
BMA	Bone Marrow Aspirate
BSC	Biological Safety Cabinet
BSL3	Biosafety Level 3
BSLII	Biosafety Level 2
BTRP	Biological Threat Reduction Program
CADT	Cambodian Academy of Digital Technology
CANARIES	Consortium of Animal Market Networks to Assess Risk of Emerging Infectious Diseases through Enhanced Surveillance
CAP	Community-Acquired Pneumonia
CAPRED	Cambodia Australia Partnership for Resilient Economic Development
CBRN	Chemical, Biological, Radiological and Nuclear
CCHFV	Crimean-Congo Haemorrhagic Fever Virus
CCHFVACIM	Crimean-Congo Haemorrhagic Fever Vaccine and Immunotherapy
CDC	Center for Disease Control and Prevention in Cambodia
CEIRR	Centers for Excellence in Influenza Research and Response
CENAT	National Center for Tuberculosis and Leprosy Control
CFO	Chief Financial Officer
CHIKV	Chikungunya Virus
CILM	Centre d'Infectiologie Lao Christophe Mérieux
CIN	Cervical Intraepithelial Neoplasia
CIRAD	Centre de Coopération Internationale en Recherche Agronomique pour le Développement (French Agricultural Research Centre for International Development)

CLPD	Chronic Lymphoproliferative Disorder
CNM	National Center for Parasitology, Entomology, and Malaria Control (Cambodia)
COFRAC	French Accreditation Committee (Comité Français d'Accréditation)
COI	Cytochrome C Oxidase Subunit 1
Co-PI	Co-Principal Investigator
COVID-19	Coronavirus Disease 2019
CRAB	Carbapenem-Resistant A. Baumannii
CRE	Carbapenem-Resistant Enterobacteriaceae
CREID	Centers for Research in Infectious Diseases
CVRP	Comprehensive Viral Research Panel
CWRU	Case Western Reserve University (USA)
DAA	Direct Acting Antiviral
DATURA	Determination of Adequate Tuberculosis Regimen in Adults and Adolescents
DBP	Duffy Binding Protein
DC	Distribution Centre
DENTHOM	Study of Dengue and Dengue-Like Illnesses in Kampong Thom Province, Cambodia
DENV	Dengue Viruses
DFAT	Direct Fluorescent Antibody Test
DHF	Dengue Hemorrhagic Fever
DNA	Deoxyribonucleic Acid
DSS	Dengue Shock Syndrome
DTG	Dolutegravir
ECACC	European Collection of Authenticated Cell Cultures
ECOMORE	Economic Development, Ecosystem Modifications and Emerging Infectious Diseases Risk Evaluation
ECTS	European Credit Transfer and Accumulation System
EDE	E-Dimer Epitope
EIC	Education, Information and Communication
ELISA	Enzyme-linked Immunosorbent Assay
EOC	Emergency Operational Center
EPHU	Epidemiology and Public Health Unit
ERIG	Equine Rabies Immunoglobulins
ES	Environmental Sampling
ESBL	Extended Spectrum Beta Lactamase
ESBL-PE	Extended Spectrum Beta-Lactamase Producing Enterobacteriaceae
ETP	Electronic Transfer of Prescriptions (or Equivalent Temps Plein)
EU	The European Union
EV71	Enterovirus 71
FAO	Food and Agriculture Organization of the United Nations
FAVNT	Fluorescent Antibody Virus Neutralization Test
FCS	Food Contact Surface
FL	Fusion Loop
FSPI-R	French Ministry of Europe and Foreign Affairs through the Solidarity Funds for Innovative Projects
G6PD	Glucose 6-Phosphate Dehydrogenase
GCRF	UK Global Challenges Research Fund
GDAPH	General Directorate of Animal Health and Production

GF-AAS	Graphite Furnace Atomic Absorption Spectroscopy
GIZ	German Agency for International Cooperation
GIZMAT	Gestion Intégrée des Zoonoses et des Maladies Animales Tropicales
GMS	Greater Mekong Subregion
GO	Grants Office
HA	Hemagluttinin
HACCP	Hazard Analysis Critical Control Points
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
Hep A	Hepatitis A Virus
HEPEDIAC	Study of DAA Treatment for Children and Adolescents with Active HCV Infection in Cambodia
HHMI	Howard Hughes Medical Institute Inc
HIB	Haemophilus Influenzae Type B
HIV	Human Immunodeficiency Virus
HPAI	Highly Pathogenic Avian Influenza
HPV	Human Papillomavirus
HSeQM	Hygiene, Security, Environment, Quality and Maintenance
IAEA	International Atomic Energy Agency
IAS	International Accreditation Service
IAV	Swine Influenza A Virus
ICEMR	International Center of Excellence in Malaria Research
ICER	International Center for Excellency in Research
ID	Intradermal Injections
IDE	International Development Enterprises
iDES	Integrated Drug Efficacy Surveillance
IDR	Intradermal Reaction
IF	Impact Factor
IFI	Invasive Fungal Infections
IgG	Immunoglobulin G
IGHB	Immunoglobulin Hepatitis B
IGRA	Interferon-Gamma Release Assays
ILI	Influenza-Like Illness
IM	Intramuscular
INSERM	Institut National de la Santé et de la Recherche Médicale (France)
INSTI	Integrase Strand Transfer Inhibitor
IPC	Institut Pasteur du Cambodge
IPIN	Institut Pasteur International Network
IPL	Institut Pasteur du Laos
IPNC	Institut Pasteur New Caledonia
IPP	Institut Pasteur in Paris
IPTf	Intermittent Preventive Treatment for Forest Goers
IQC	Internal Quality Control
IRD	Institut de Recherche pour le Développement (Institute for Research and Development- France)
IRIS	Immune Reconstitution Inflammatory Syndrome
ISO	International Organization for Standardization
IT	Information Technology

ITC	Institute of Technology of Cambodia
IVC	International Vaccination Center
IVI	International Vaccine Initiative
IZS	Istituto Zooprofilattico Sperimentale in Teramo, Italy
JEV	Japanese Encephalitis Virus
KAP	Knowledge, Attitudes and Practices
KBH	Kantha Bopha Hospital
KOICA	Korea International Cooperation Agency
LBC	Liquid-Based Cytology
LBM	Live Bird Market
LEFS	Laboratory of Environment and Food Safety
LIMS	Laboratory Information Management System
LIS	Laboratory Information System
LMICs	Low-and-Middle-Income Countries
LMI-DRISAOH	Laboratoire Mixte International - Drug Resistance in Southesat Asia: A One Health Approach to Tackle AMR Spread
LMVR	Laboratory of Malaria and Vector Research
LPAI	Low Pathogenic Avian Influenza
LTBI	Latent Tuberculosis Infection
mAbs	Monoclonal Antibodies
MAFF	Ministry of Agriculture, Forestry, and Fisheries (Cambodia)
MBL	Medical Biology Laboratory
MCC	Maximum Clade Credibility
MCTC	Mother-To-Child Transmission
MCV	Meningococcal Vaccine
MD	Medical Doctor
MD USA	Maryland, United States of America
MEAE	Ministère de l'Europe et des Affaires Etrangères (French Ministry for Europe and Foreign Affairs)
MESRI	Ministère de l'Enseignement Supérieur, de la Recherche, et de l'Innovation. (French Ministry for Higher Education, Research and Innovation)
MIA	Multiplex Immunoassay
MIC	Minimum Inhibitory Concentration
MIVEGEC	Maladies Infectieuses et Vecteurs Écologie, Génétiques, Évolution et Contrôle (IRD, France)
MMR	Measles, Mumps and Rubella
MMV	Medicines for Malaria Venture
MoH	Ministry of Health of Cambodia
Mpox	Monkeypox (Viral Disease)
MRU	Malaria Research Unit
MS	Multiple Sclerosis
MTA	Material Transfer Agreement
MTBC	Mycobacterium Tuberculosis Complex
NAAT EQA	Nucleic Acid Amplification Testing External Quality Assurance
NABM	Nomenclature des Actes de Biologie Médicale
NAbs	Neutralizing Antibodies
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
NFCS	Non-Direct Food Contact Surface
NGS	Next Generation Sequencing
NIAID	National Institute of Allergy and Infectious Diseases
NIC	National Influenza Center
NIH	National Institutes of Health
NIHE	National Institute of Hygiene and Epidemiology
NIP	National Immunization Program
NIPH	National Institute for Public Health
NMCHC	National Maternal Child Health Center
NMCP	National Malaria Control Program
NNRTI	Non-Nucleoside Reverse Transcriptase inhibitor
NPH	National Pediatric Hospital
NPIs	Non-Pharmaceutical Interventions
NS1	Nonstructural Protein 1
NTM	Nontuberculous Mycobacterial
NTU	Nanyang Technological University
NUS	National University of Singapore
OH4CAM	One Health 4 Cambodia
OPTICAM	Optimizing Latent Tuberculosis Treatment Initiation in Cambodia among People Living with HIV
OUCRU	Oxford University Clinical Research
PB	Peripheral Venous Blood
PBMCs	Peripheral Blood Mononuclear Cells
PCR	Polymerase Chain Reaction
PDR	People's Democratic Republic
PEP	Post Exposure Prophylaxis
PfD	Partners for Development
PhD	Doctor of Philosophy (degree)
PHD	Provincial Health Department
PHEIC	Public Health Emergency of International Concern
PICREID	Pasteur Institute – Center for Research for Emerging Infectious Diseases
PIU	Pasteur International Research Unit
PIV	Parainfluenza Virus
PLHIV	People Living with HIV
PN	Pasteur Network
PNG	Papua New Guinea
PP	Phnom Penh
PPR	Epidemic Preparedness and Response
PPSV	Pneumococcal Polysaccharide Vaccine
PQ	Primaquine
PREZODE	Preventing Zoonotic Disease Emergence
PTR	Programme Transversal de Recherche
Pv	Plasmodium vivax
RAMSES	Resistance to Antimicrobials: Socio-Economic and Regulatory Factors Influencing Emergence and Dissemination in the South

RDT	Rapid Diagnostic Test
RFFIT	Rapid Fluorescent Focus Inhibition Test
RIG	Rabies Immunoglobulin
RITM	Research Institute of Tropical Medicine in Manila
RNA	Ribonucleic Acid
RPC	Rabies Prevention Centers
RSV	Respiratory Syncytial Virus
RUA	Royal University of Agriculture
RVC	Royal Veterinary College
SAB	Scientific Advisory Board
SADS	Swine Acute Diarrhea Syndrome Coronavirus
SARI	Severe Acute Respiratory Illness
SARS-CoV-2	Severe Acute Respiratory Illness, COVID-19- 2
SATREPS	Science and Technology Research Partnership for Sustainable Development
scRNAseq	Single Cell RNA Sequencing
SDSV	Structure et Dynamique des Systèmes Vivants
SE	South East
SEA TICKEY	Southeast Asia Tick Identification Key
SGE	Salivary Gland Extracts
SHC	Social Health Science Clinic
SHCH	Sihanouk Hospital Center of Hope
SOP	Standard Operating Procedures
TB	Tuberculosis
TCR	T Cell Receptor
Tdap	Tetanus, Diphtheria, Pertussis
TEMVS	Targeted Enriched Viral Metagenomic Sequencing
TES	Therapeutic Efficacy Studies
TPT	Tuberculosis Preventive Treatment
TST	Tuberculin Skin Tests
TWG	Technical Working Group
UHS	University of Health Sciences
UMB	University of Maryland, Baltimore
UNICEF	United Nations International Children's Emergency Fund
UPS	Université Paris-Saclay in France
USA	United States of America
USF	University of South Florida in USA
USTH	University of Science and Technology of Hanoi
VACC	Vaccination Service
VCCT	Voluntary Confidential Counselling and Testing for HIV
VECAM	Veterinary Entomology in Cambodia
VOCs	Variants of Concern
WCS	Wildlife Conservation Society
WEHI	Walter and Eliza Hall Institute of Medical Research
WGS	Whole Genome Sequencing
WHO	World Health Organization
WHOCC	WHO Collaborating Center on Influenza - Melbourne Australia
WOAH	World Organization for Animal Health
ZIKV	Zika Virus

2 The IPC in 2023 at a Glance : The Director's Remarks

The Institut Pasteur du Cambodge (IPC), created in December 1953, is a non-profit research institution operating under the high patronage of the Cambodian Ministry of Health (MoH). Our mission is to contribute to the prevention and treatment of infectious diseases through research, public health activities, and training. This report presents the Institute's activities in 2023, a year marked by the celebration of the IPC's 70th anniversary.

As of 31 December 2023, the IPC's on-site staff represented 16 different nationalities. Scientific activities are carried out by more than 35 scientists, each holding at least a PhD or a doctorate degree in medicine, veterinary medicine or pharmacy and another PhD or master's degree.

The IPC's activities encompass four main categories: (i) biomedical research with a specialization in infectious diseases, (ii) support and capacity building for public health in Cambodia and the Greater Mekong Subregion, (iii) the provision of health services (laboratory, vaccination), and (iv) training and education. The IPC focuses on infectious diseases and on public health challenges and issues, which include illnesses related to arboviruses, respiratory viruses, rabies, malaria, antimicrobial resistance in microorganisms, and zoonoses, among others. These complex scientific matters, particularly those that involve pathogens with complex life cycles that can involve humans, mammals, and arthropods — studied with a One Health approach — could not be effectively addressed without complementarity between the Institute's units and specialists (entomologists, doctors, veterinary scientists, immunologists, epidemiologists, mammalogists and others) or without its high-level technical platforms, including a Biosafety Level-3 (BSL3) laboratory and an animal research facility.

Research activities done in 2023 were featured in 49 articles published by scientists affiliated to IPC, appearing in peer-reviewed international journals with impact factors (IF) greater than 0. Among them are 18 as first or last author and 36 with an IF greater than or equal to 4.

The IPC's three WHO reference centres carried out public health activities alongside and in support of MoH teams, including: (i) monitoring human and avian influenza viruses (six human cases of highly pathogenic avian influenza virus H5N1), (ii) monitoring SARS-CoV2 variants, and (iii) the virological confirmation of a case of monkeypox virus infection (an imported case from Thailand).

Post-exposure rabies management activities decreased slightly in 2023, by -4,1% compared to 2022, with the management of 60,476 patients in our 3 rabies prevention centres. The risks related to rabies indeed remain high in Cambodia: 74 % of the 220 animals tested for rabies virus at the Virology Unit were positive.

The IPC plays a major role in the training of university students. Its scientists participate in curricula offered by local universities, including the University of Health Sciences (UHS) in Phnom Penh. Additionally, the IPC itself welcomes many students for internships and practical experiences. During 2023, 131 students interned at the IPC. This is higher than the 73 welcomed in 2022, a year which saw a progressive return to normalcy after the COVID-19 pandemic. Among the 131 students, 95 were Cambodian nationals, while the others were French, American, Bangladeshi, Colombian, Cameroonian, Dutch, Pakistani, Kenyan, Italian, and Ugandan.

An ambitious capacity-strengthening policy for young Cambodians was put in place in 2022 (for young talents at the IPC), allowing them to carry out their doctorate studies at the IPC (scholarships for non-

IPC students and continued salaries for IPC personnel). This policy continued throughout 2023. As a result, the IPC hosted sixteen doctoral students in the last quarter of 2023, including eight Cambodian nationals.

Our Health Service activities in 2023 compared well to those of 2022: (i) our Medical Biology Laboratory's activities decreased by 25.5 % (6.2 vs 8 million of "B"), but if we exclude COVID-19 tests, the activity level increased by 53.1 % (5.9 vs 3.8 million of "B"); (ii) a 0.3 % increase for the total number of tests performed by for the Laboratory of Environment and Food Safety; and (iii) a 24 % increase for the total number of injections (including vaccinations and immunoglobulins) provided by the International Vaccination Centre.

2023 Highlights

Human Resources

- Dr Tineke Cantaert, from the Immunology Unit, was promoted to Research Director among the Institut Pasteur's Staff Scientists in April 2023.
- Dr Sébastien Boyer, from the Medical and Veterinary Entomology Unit, was promoted to Research Director among the Institut Pasteur's Staff Scientists in April 2023.
- Dr Claude Flamand, from the Epidemiology and Public Health Unit, was promoted to Senior Research Manager among the Institut Pasteur's Staff Scientists in April 2023.
- Dr Erik Karlsson, from the Virology Unit, was integrated as Senior Research Manager among the Institut Pasteur's Staff Scientists in April 2023.
- Dr Ou Teyputita, from the Virology Unit, was integrated as Research Assistant into the "Pasteur Network's Scientific Staff" in August 2023.

1st International Symposium on Ticks and Tick-borne Diseases in Southeast Asia (22–23 June 2023)

On 22 and 23 June 2023, the Royal University of Agriculture (RUA) hosted the *1st International Symposium on Ticks and Tick-borne Diseases in Southeast Asia* in Phnom Penh, Cambodia. The symposium was organized by the IPC as part of the Veterinary Entomology in Cambodia (VECAM) project, funded by the French Ministry of Foreign Affairs and supported by the French Embassy and its *Fonds de Solidarité pour les Projets Innovants*.

This meeting encouraged international-level scientific exchanges with the objective of combating tick-borne diseases in Southeast Asia. This unique initiative provided an opportunity for all of those working in the field of ticks and their related diseases to meet and to work toward the developing a strong network for ASEAN countries. With 73 delegates from more than 10 nations, the symposium provided an extensive platform to discuss existing knowledge on ticks and tick-borne diseases, offering hope that these diseases can be tackled while taking climate change and One Health into account.

Workshops on Biosecurity Organized Through the EU-funded Project 81 (25–29 September 2023)

The IPC hosted two workshops entitled *Laboratory Handling and Management of High Consequence Pathogens*, from September 25th to 29th. These workshops, organized through the EU-funded "Project 81" - *BIOSEC Enhanced Biosecurity in Southeast Asia*, and conducted by an expert team from the UK's Health Security Agency, were specifically designed to elevate biosecurity practices in Southeast Asia. In the inaugural workshop titled *Safe Use of Class-III Biological Safety Cabinets*, experts provided invaluable guidance on the secure handling of high-consequence pathogens within Class-III Biological Safety Cabinets. These cabinets assume a pivotal role in ensuring the safety of researchers engaged in work with dangerous microorganisms.

The second workshop, titled *Maintenance & Servicing of Biological Safety Cabinets* was dedicated to the critical aspects of maintaining and servicing Biological Safety Cabinets, an essential procedure for averting containment breaches. Field experts shared indispensable insights during this session. The knowledge and skills acquired during these training courses will significantly enhance laboratory safety and pathogen management, further advancing biosafety and biosecurity.
While the IPC possesses substantial expertise and experience in safety enclosure protocols, the proposal made by the experts of Project 81 to conduct two workshops on the subject has proven to be a valuable opportunity for the IPC to contribute to the broader mission of elevating biosecurity standards in the region.

Course on Flow Cytometry and its Applications

From 4 to 8 September 2023, the IPC's Immunology Unit organized a course on flow cytometry and its applications. Sixteen international students, including students from eight different Pasteur Institutes, have been selected to participate. The week-long course combined both theoretical and practical sessions to teach students the various applications of flow cytometry. The course was funded by the Pasteur Network, the Wellcome Trust, the NIH's PICREID project and DKSH.

Providing Expertise to the Ministry of Health Regarding Emerging Diseases

Highly pathogenic avian influenza (HPAI), subtype A/H5N1, has been endemic in Cambodia since 2004, with 67 reported human cases (CFR 63.6 %) and 65 reported poultry outbreaks up to 2024. Two cases of low pathogenicity A/H9N2 occurred in Siem Reap province in 2021 and 2022, respectively. Avian influenza (AIV) continues to circulate in Cambodia, especially during holiday periods. Longitudinal, active surveillance coordinated between the IPC and the National Animal Health and Production Institute (NAHPRI) with support from the FAO, has shown that A/H5N1 viruses were still detected regularly in 2023. In 2023, the IPC's Virology Unit helped to respond to 6 cases of A/H5N1 clade 2.3.2.1c in humans, the first detected in the country since 2014. The Virology Unit remains an integral part of the AIV monitoring and surveillance network in Cambodia.

The monkeypox virus (MPXV) is a zoonotic orthopoxvirus that causes a smallpox-like disease (mpox) in humans. It was first discovered in captive monkeys in 1958, with the first human case reported in 1970 in the Democratic Republic of the Congo. Historically, MPXV has been endemic in Central and West Africa, with sporadic outbreaks typically linked to animal-to-human transmission from rodents or other wildlife. However, in 2022 there was an unprecedented global outbreak of mpox. As of 7 February 2024, over 93,000 cases have been reported in over 100 countries. In 2023, the IPC's Virology Unit has been assisting the Ministry of Health with genomic surveillance of MPXV in the country, with 2 cases detected in December 2023. These insights were critical to understand the circulation of the virus in the country. Further work continues to shed light on the transmission and risks of MPXV in Cambodia.

Toward a New Rabies Prevention Centre in Kampong Cham

As part of its mission against rabies, and to increase the population's accessibility to post-exposure prophylaxis (PEP), it was decided to build a new anti-rabies treatment centre in Kampong Cham to replace the one operating on temporary premises within the Kampong Cham Hospital since 2019.
An agreement was signed with the director of the Provincial Health Department of Kampong Cham Province in April 2023. The first stone was laid in December 2023, and the new centre should be inaugurated in May 2024.

70th anniversary of the IPC (1953-2023).

In 2023, the Institut Pasteur du Cambodge celebrated its 70 years since its establishment in Cambodia. Various events were organized throughout the year as part of this celebration: conferences, a reception at the French Embassy, a booklet and a film retracing the history of the Institute, souvenirs, and a gala dinner offered to all IPC staff (see the specific chapter of this report).

Study of the "IPC-2030 Project" for the Renovation of the IPC's Campus

A study of the "IPC 2030" campus renovation project was conducted in depth. In 2023, and with the help of IPP experts (Mr. Pascal TENEGAL, Head of the Real Estate and Technical Department), the IPC selected *2 B Concept Consulting* for a mission of AMO & AMOE on the IPC's campus modernization (June 2023 – March 2024). This study was supplemented by a feasibility study carried out by Bloom Architecture.

UHS–IPC Scientific Seminars

A joint initiative was launched by the University of Health Sciences (UHS) and the Institut Pasteur du Cambodge (IPC), aiming to introduce and maintain a series of scientific seminars involving researchers from both institutions. The main objective is to promote interaction and collaboration between the UHS and the IPC. Seminar sessions are scheduled every two months at both institutions, with alternating venues and speakers, to present and discuss scientific studies and projects. The inaugural session of this seminar series was held on 16 Feb 2023 at the UHS' Campus 1, in the presence of H.E. Prof. Saphonn Vonthanak, rector of the UHS, and Prof. André Spiegel, director of the IPC, who delivered their opening remarks and provided guidance. In 2023, four seminar sessions were held, including two hosted sessions and studies presented by our scientific colleagues at the UHS. Different topics related to pneumococcal colonization, rabies, HIV, hepatitis C, and hepatitis B were covered.

Creation of a Grant Office

In recent years, the IPC's development has been accompanied by an increasing number of scientists and of grants requested and obtained from conditional donors (NIH, Wellcome Trust, Bill & Melinda Gates Foundation, etc.). The time and effort required to monitor calls for projects, to submit, implement and coordinate projects, both administratively and financially, increasingly impedes our scientists' work, often reducing the amount of time they can devote to research. Hence, it appears necessary to support them in these tasks by creating a new structure adapted to this mission within the IPC, which would be called the "Grants Office" (GO).

Under the director's authority, the GO's manager will be charged with the creation of this new entity at the IPC and with the implementation of the GO's various activities, with the support and guidance of the Grant Office at the Institut Pasteur in Paris.

After the withdrawal of a first candidate who had been selected, the recruitment process made it possible to recruit a person with extensive experience of this type of function and who had been able to exercise it in a similar environment at an Institute within the Pasteur Network. She assumed her position on 1 January 2024.

In Conclusion

In 2023, the first truly "post-COVID-19" year, the Institut Pasteur du Cambodge demonstrated its effectiveness and commitment in the fight against infectious diseases for the benefit of the Cambodian population, in close partnership with the Ministry of health, particularly due to its expertise with viruses (avian flu virus, monkey pox, SARS Cov2) and rabies post-exposure prophylaxis. Sadly, these important activities are based on an economic model that is too dependent on revenues and on the mobilization of external donors. More than ever, our Institute needs the commitment of the Institut Pasteur in Paris and of the Royal Government of Cambodia to develop.

The IPC is making continuous progress, and I would like to thank all of our personnel for their efforts and their commitment to provide excellent work and allow our institute to fulfil its mission for a better public health.

Dr André SPIEGEL
Professeur agrégé du Val-de-Grâce
Director of the *Institut Pasteur du Cambodge*

3 Institut Pasteur du Cambodge in 2023

3.1 Overview of the Institute

Statutes and Operational Systems

The IPC, created in December 1953, is a non-profit research institution operating under the high patronage of the Cambodian Ministry of Health (MoH). The IPC's statutes and operational systems are codified in the agreement signed between the Royal Government of Cambodia and the Institut Pasteur in Paris in 1992, and were since modified through two amendments. The 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. The institute's guidance also falls within the purview of the Institut Pasteur in Paris, France, as regards its scientific and technical approaches and plans. The institute is part of the Pasteur Network (PN), which is a worldwide network of 33 member institutes united by Pasteurian values, which contribute to the improvement of global health.

Governance

The Institut Pasteur du Cambodge is led by a director and is monitored by a Liaison Council. The IPC's director is appointed by the director general of the Institut Pasteur in Paris, France, in consultation with the Kingdom of Cambodia's MoH. The IPC's deputy director is nominated 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 biology or public health.

The Institute's activities are reviewed on an annual basis by the Liaison Council presided by His Excellency the Minister of Health of Cambodia. The council is composed of ten high-ranking members from 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 sector (WHO, UNICEF) round out the membership.

The IPC's scientific activities are also reviewed every two or three years by the Scientific Advisory Board, whose last session was held in early February 2021. Our scientific strategy is then adapted, based on the recommendations from both the Liaison Council and the Scientific Advisory Board.

In addition, at the beginning of each year, the Chief Financial Officer (CFO) sends the consolidated financial statements of the previous year to the Institut Pasteur (International Division). An external financial audit is also performed in April of each year.

3.2 Structure

The organizational chart appears at the end of this report, in Annex 1.

The Institute is composed of:

- A management unit comprising the director, the deputy director, and the chief financial officer.
- Administrative, financial and logistical services.
- Five research units: Malaria, Epidemiology and Public Health, Immunology, Medical and Veterinary Entomology, Virology, and a 4-year group, the Genomic and Antibiotic Resistance Research Group.
- Health services, including a Medical Biology Laboratory (MBL), a Laboratory of Environment and Food Safety (LEFS), and a vaccination service (VACC) with three Rabies Prevention Centers and an International Vaccination Centre.

- Public health laboratories, comprising 3 WHO reference centres hosted by the Virology Unit (the National Influenza Centre in Cambodia, the WHO's regional H5 reference laboratory, and the WHO's COVID-19 global referral laboratory.
- A Voluntary Confidential Counselling and Testing for HIV (VCCT) service, and rabies centres at three different sites that provide pre- and post-exposure prophylaxis at a fee.
- Four technical platforms: (i) a BSL-3 Laboratory, (ii) a biobank, (iii) a sequencing platform, and (iv) a single-cell analysis platform.

3.3 Human Resources

As of 31 December 2023, the Institute had a team of 293 personnel of 16 different nationalities, with 90 % being Cambodian nationals:

- 283 with IPC contracts (56 of which are civil servants attached to the MoH);
- 10 Cambodian scientists (2 research directors, 7 research fellows, and 1 research assistant);
- 8 Cambodian PhD students;
- 38 expatriates, including:
    - 10 on institutional contracts: one MEAE, one Expertise-France (20 % ETP), seven from Institut Pasteur in Paris (IPP), and one from the French Agricultural Research and International Cooperation Organization (CIRAD); and
    - 28 on IPC contracts, including 4 scientific seniors, 1 IT engineer, 10 postdocs, 8 PhD Students, and 5 Master-2.

Diversity and Leadership

The Institute prioritizes gender balance and equity; 55 % of staff members are women. The professional development of scientific leaders and other national professionals is a matter of great importance to the IPC. Of the 16 management positions (3 directors, 8 research or service unit heads and 5 service support managers), 12 are occupied by men (75 %) and 8 (50 %) by Cambodian nationals.

3.4 Finances and investments

3.4.1 Finances

The majority of the IPC's revenue comes from binding research contracts funded by donors (34 %), from the services offered by IPC (48 %), including those preventing and treating rabies, and from a subsidy from the Ministère de l'Enseignement Supérieur, de la Recherche, et de l'Innovation (the 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 it provides a significant contribution in the form of a tax and customs exemptions. Details of the different revenue streams are shown in table 1, and the figures 1 and 2 below show a breakdown of the funding by country and by donor in 2023. The share of income attributable to service activities mainly originates from our International Vaccination and Medical Biology Laboratory. The other revenues mainly come from foreign exchange gains and income from financial investments.

A bad debt with the MoH was written off in 2023 for €1.3 M. However, discussions with the MoH are still underway to find a solution.

	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
Research contracts	46%	52%	60%	58%	59%	56%	56%	54%	40%	22%	28%	**34%**
Health services	29%	25%	21%	23%	25%	29%	30%	34%	51%	71%	47%	**48%**
MESRI grant	20%	17%	14%	12%	11%	11%	11%	10%	6%	3%	5%	**4%**
Other revenues	5%	5%	6%	7%	5%	5%	2%	2%	2%	5%	20%	**14%**
	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	**100%**

Table 1: Revenue breakdown by source type (2012–2023)

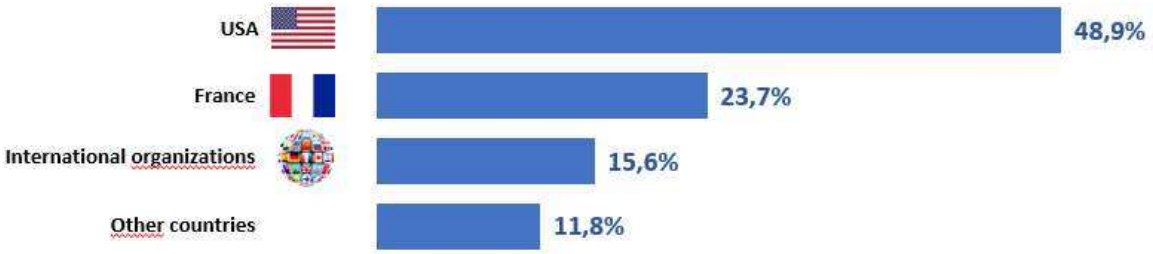


Figure 1: Breakdown of the research funds received by country

Figure 2: Breakdown of the research funds received by donor

3.4.2 Equipment Purchases

Total equipment purchases exceeded €648,000 in 2023, more than 85 % of which were financed by the Institut Pasteur du Cambodge's own funds.

The main purchases are as follows:

- A Steriplus machine for treatment of biomedical waste with infectious risks (€132,000), donated by the European Union,
- A new 750 kVA electric generator (€82,000), paid for with IPC funds,
- A liquid nitrogen generator (€71,000), paid for with IPC funds,
- A Ford pickup (€35,000), paid for with IPC funds,
- A new truck that will house a mobile laboratory (€24,000), paid for with IPC funds.

3.5 Study of the "IPC-2030 Project" for the Renovation of the IPC's Campus

The dynamic and ever-growing Institut Pasteur du Cambodge (IPC) has seen its workforce increase greatly since its establishment on Monivong Boulevard in 1994 (65 people in 1994, and 300 people at the end of 2023), and its facilities have come to occupy the campus' entire surface, or approximately 9000 m2, with one- or two-story buildings. In order to maintain and strengthen the position of the

Pasteur Institute as a leading institution in the field of biomedical research in Cambodia and in the Southeast Asian region, the Institute must increase its research and innovation capabilities by developing research infrastructures to accommodate more researchers and broaden its fields of activity.

The essential factor limiting its development is the absence of free floor space enabling the construction of new laboratories. In 2022, the Bloom Architecture firm was commissioned to identify solutions allowing an increase in laboratory surface area (raised heights, etc.). The conclusions of this study indicate that the original foundations of the buildings and the evolution of urban planning rules do not allow the laboratories to be raised by one or two floors. Consequently, in a long-term vision, one or more of the buildings would have to be completely reconstructed. This study highlighted the site's potential, envisioning the construction of two new buildings, an increase of parking space, a new access road at the rear of the site, new traffic patterns, more green spaces and buildings promoting sustainable development.

In 2023, and with the help of IPP experts (Mr. Pascal Tenegal, Head of the Real Estate and Technical Department), the IPC selected *2 B Concept Consulting* for a mission of AMO & AMOE on the IPC's campus modernization (June 2023 – March 2024). This study was supplemented by a feasibility study carried out by Bloom Architecture.

The proposed project's highlights include: (i) a 100 % increase in surface area for laboratories and offices (12,057 m^2 vs 6,080 m^2), (ii) a 20 % footprint reduction (3,740 m^2 vs 4,460 m^2), (iii) green spaces for cross-service socializing, (iv) the construction of new training venues (modular conference room) and (v) building designs better-suited for the tropical climate. This ambitious project will require the backing and support of IP Paris and of the Cambodian MoH.

The IPC's campus, 2024

The new campus, 2030

An artist's view of the main building

Figure 3: View of the new Campus (Bloom Architecture)

3.6 Publications in 2023

The IPC's research and public health activities are detailed in later sections. A summary of these is presented in figures 5 and 6 below. Research activities done in 2023 comprise 49 articles published by scientists affiliated to IPC and appearing in peer-reviewed international journals with impact factors (IF) higher than 0. Among them are 18 (37 %) as first or last author and 36 (74 %) with an IF greater than or equal to 4 (figures 4 & 5).

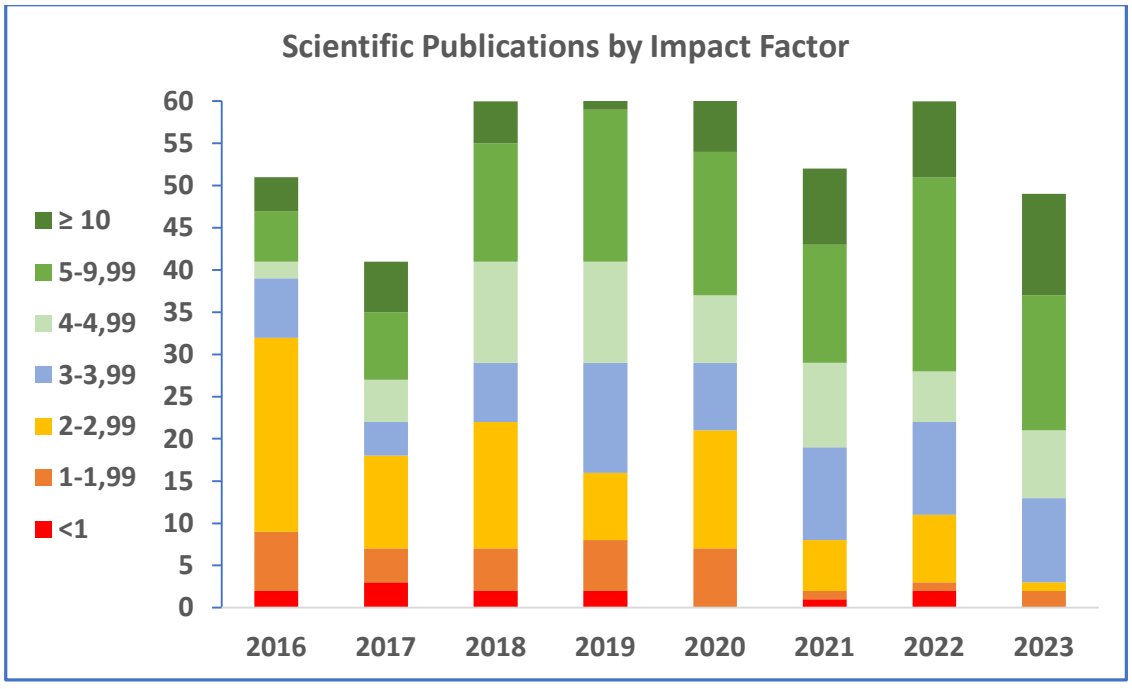


Figure 4: Scientific publications, sorted by impact factor of the journal (2016-2023)

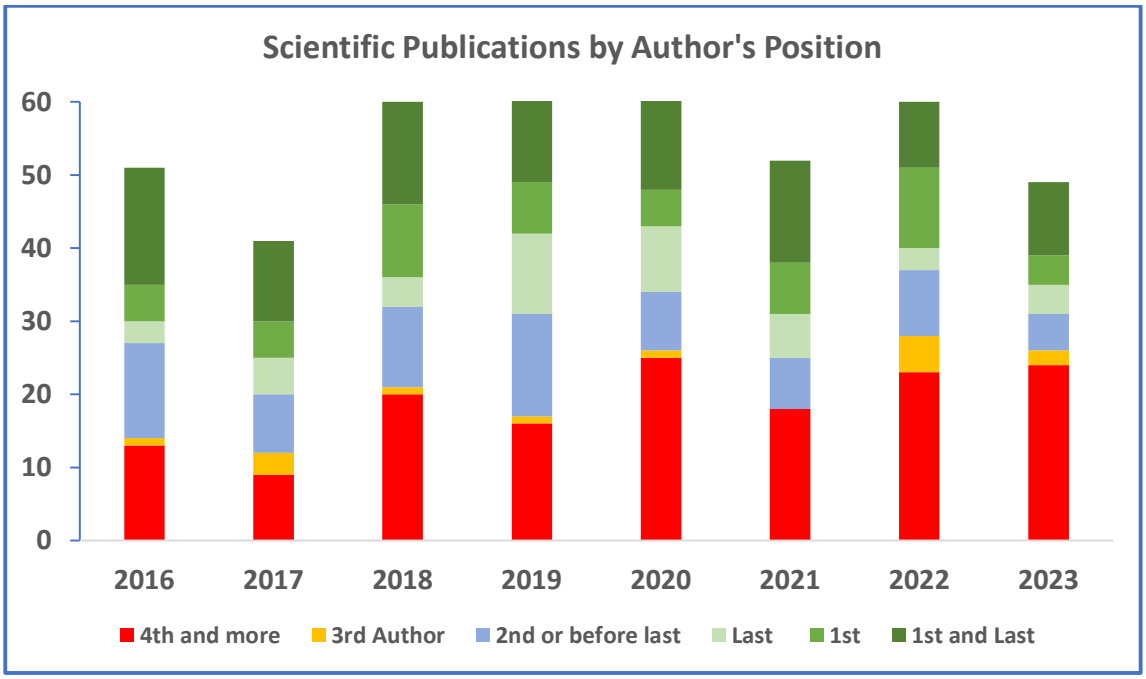


Figure 5: Scientific publications by author's position (2016-2023)

3.7 Training and Internships in 2023

The 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.

Student Internships

Throughout 2023, 131 students interned at the IPC. This is higher than the 77 welcomed in 2022, a year still marked by the COVID-19 epidemic. Among the 131 students, 95 were Cambodian nationals, while the others were French, American, Bangladeshi, Colombian, Cameroonian, Dutch, Pakistani, Kenyan, Italian, and Ugandan. Their university affiliations are as follows: University of Health Sciences (53), University of Puthisastra (18), the Institute of Technology of Cambodia (3), the Royal University of Phnom Penh (2) while the remainder (59) were from various French and Belgian universities. 16 were PhD students (8 Cambodian), 33 were in master's level studies, 56 were bachelor-level students, and 14 were working toward associates' degrees.

International Master's in Infectious Disease

Since 2019, the IPC provides important and substantive support to the International Master's Program in Infectious Diseases, a two-year program jointly offered by the University of Health Sciences (UHS – Cambodia) and the Université Paris-Saclay (UPS – France).

Throughout the year, IPC is involved in the curriculum's delivery through lectures, practical teaching, and internship supervision. Each year, IPC scientists contribute more than 200 hours of training in total. The IPC appointed a senior scientist, Dr. Jean Popovici (head of the Malaria Research Unit) and a project manager, Emilie Carlot, as focal points to facilitate the coordination of this program with the UHS and UPS. Moreover, the IPC also supports this program by granting IPC scholarships to cover university fees and provide stipends for Cambodian students enrolled in this master's program. Two scholarships are awarded per program year to promising candidates. The IPC also accommodates students within its premises for a moderate fee, when vacancy allows.

The 2023–2024 academic year saw the IPC welcome 19 students of 8 nationalities (including 4 Cambodians) for the M2 program. Regrettably, the M1 program for this academic year was unable to proceed due to insufficient student registration.

As of 2024, a total of 52 students of 16 different nationalities were admitted to this International Master's Program.

	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 Cambodians)	**P2:** 9 students (4 Cambodian) + 4 new students
2022-2023	**P4**: 14 students (4 Cambodians)	**P3**: 9 students (3 Cambodians) + 4 new students
2023-2024	*Class was not open due to insufficient students' registrations*	**P4:** 12 students (4 Cambodians) + 7 new students

Table 2: Number of students enrolled in the International Master's Program since its creation (P = promotion)

3.8 Scientific Seminars

The IPC held scientific seminars every other week. Twenty-four were held in 2023 (table 3)

Date	Name, Surname	Grade and Unit	Title of seminars
26-Jan	Jimmy CADENES	M1* student Virology Unit, IPC	Characterization of infectious bronchitis virus in Cambodia and possible applications for poultry vaccination.
31-Jan	Pr Gerald SPAETH	Institut Pasteur, Université Paris Cité, INSERM U1201	Darwin in a dish: How Leishmania experimental evolution informs on novel bio-markers relevant for natural infection
3-Feb	Pr Christian DOERIG	School of Health and Biomedical Sciences, RMIT University	Host-directed therapy for infectious diseases: phosphosignalling as a target
10-Feb	Pr Felix REY	Head of Structural Virology Unit, Institut Pasteur	Structural studies of dengue virus antibody/antigen complexes
15-Mar	Pr Martin PFEFFER	Institute of Animal Hygiene and Veterinary Public Health, Leipzig University	Ecology of tick-borne pathogens in Germany
29-Mar	Dr Constanza TACOLI	Postdoc, Malaria Research Unit, IPC	P. vivax SeroTAT approach in Cambodia
19-Apr	Dr Lien DE CALUWE	Postdoc, Immunology Unit, IPC	Virus-host interactions in the chikungunya virus replication cycle
26-Apr	Eamkim EAR	Bachelor student, Epidemiology and Public Health Unit, IPC	HEPEDIAC study (Pilot therapeutic study of DAA treatment for children and adolescents with active HCV infection in Cambodia: The ANRS 12420 HEPEDIAC study)
17-May	Matilin LE BEUX	M1 student, Medical and Veterinary Entomology Unit, IPC	Development of an artificial intelligence technique for the recognition of mass spectra of mosquito vectors of Japanese encephalitis virus.
31-May	Valentin BROCHET	M1 student, Medical and Veterinary Entomology Unit, IPC	Mosquitoes in the classroom: children's perception of vector and vector-borne diseases in Cambodia.

7-Jun	Antoine PELRAS	M1 student, Medical and Veterinary Entomology Unit, IPC	Modeling the impact of environmental factors on the distribution of mosquito species in Cambodia
14-Jun	Lea BALDOR	M2* student, Malaria Research Unit, IPC	Evaluation of the in-vitro response of Plasmodium vivax clinical isolates to antibodies targeting PvRBP2B.
20-Jun	Dr Alexis DZIEDZIECH	Postdoc, Institut Pasteur	Identification of biomarkers for exposure to tick bites in both humans and animals
28-Jun	Dr Marine COMBE	Researcher, IRD, Montpellier, France.	Water-based eco-epidemiology as sentinel of the emergence of zoonotic diseases
19-Jul	Dynan SENG	PhD student, Malaria Research Unit, IPC	Study of the biology of hypnozoites and relapses in Plasmodium vivax
13-Sep	Dr Anthony RUBERTO	Postdoc, University of Georgia	Single-cell transcriptomics in malaria research
27-Sep	Dr Nadeem MUHAMMAD	*M1 student, Malaria Research Unit, IPC	Implementation of a long-amplicon sequencing-based G6PD genotype assay for MinION mobile sequencer.
9-Oct	Peter DAZAKS	President, EcoHealth Alliance	Analyzing emerging disease origins to help prevent future pandemics
11-Oct	Alexander TENDU	PhD student, Virology Unit	Bat Ectoparasites: Towards an understanding of their natural viromes.
18-Oct	Nisa YA	*M2 student, Immunology Unit, IPC	The Evaluation of T-cell Responses to Rabies Post-Exposure Prophylaxis (PEP).
25-Oct	Dr Nolwenn DHEILLY, Sarah TEMMAN, Beatrice REGNAULT	Research Director, And Research Engineers Institut Pasteur	Metagenomics to identify novel and unexpected pathogens for improved diagnostic and prevention.
8-Nov	Julien DOWDING	*M2 student, Epidemiology and Public Health Unit, IPC	Dengue forecasting in Cambodia using an ensemble model
29-Nov	Betty NALIKKA	PhD student, Virology Unit, IPC	Integrating molecular studies with existing ecological knowledge to study viruses in host populations
12-Dec	Cherie YU	PhD student, CIRAD	Optimizing disease surveillance in Pteropus Lylei using a mechanistic model

*M1 = Master 1, *M2 = Master 2

Table 3: List of the 24 seminars held in 2023

3.9 Visits and missions at IPC in 2023

The main visits, missions and delegations that have been received at the IPC are listed in Table 4.

Date	Visits and missions
18-Jan	KOICA experts led by Mr. SAO Dona, a Program Officer
27-Jan	H.E. Dominic WILLIAMS, British Ambassador to Cambodia
9/10-Feb	Professor Felix REY, Head of the Structural Virology Unit, Institut Pasteur Paris
21-Feb	Delegation from the AFD led by M. Remy RIOUX, General Director; Ms. Barbara POMPILI, Deputy of the Somme's 2nd constituency; Ms. Anne GENETET, Deputy of the 11th constituency for French residents overseas; Mr. Jean-Pierre MARCELLI, Regional Director of the AFD (Southeast Asia); Mr. Philippe BAUMEL, Parliament Relations Advisor at the AFD; Ms. Sandrine BOUCHER, Country Director, AFD Phnom Penh; Mr. Emmanuel DOLLFUS, Deputy Country Director, AFD Phnom Penh, and Ms. OK Malika, Program Officer, AFD Phnom Penh
3-Mar	Mr. Nakarin AUD-AI, Lab Supervisor, NHealth Cambodia
7-Mar	Delegation from Global Fund led by Olivier CAVEY, Senior Fund Portfolio Manager, High Impact Asia Department; Gloria PESARIN, Senior Program Officer, High Impact Asia Department and Brian KANYIKA, Expert in Public Health for Cambodia
9-Mar	Ms. Agnès SOUCAT, Head of the Health Division at AFD-HQ
15-Mar	Delegation from the European Union (EU) and the National Authority of Chemical Weapons (Cambodian Ministry of Defense)
15-May	Dr. Didier LAUREILLARD & Ms. Barbara BICHE, DATURA project PI
22-24-May	Prof. Fernando ARENZANA SEISDEDOS, Director of International Affairs of Institut Pasteur (Paris)
25-May	Dr. Francois-Xavier BABIN and Mr. SEK Maredy – Fondation Mérieux
7-Jun	Mr. François ROGER, CIRAD Director for Continental SE Asia
16-Jun	Dr. Solène PAUL, Intermedical Care
19-Jun	Mr. Jean-Pierre MARCELLI, Regional Director, AFD
22-Jun	Mr. Marc THAYRE, Deputy Head of Mission, British Embassy in Phnom Penh
27-Jun	Delegation of the European Union to The Kingdom of Cambodia, led by Mr. Bryan FORNARI, Head of Cooperation at the EUD
4-Jul	Ms. Marine COMBE & Rodolphe GOZLAN, Researcher, IRD
30-Aug	Delegation of the Malaria Consortium, led by Tom HESLOP, Director of global operations and Asia (U.K.-based); Mousumi RAHMAN, Country Director, Cambodia; Lieven VERNAEVE, Program Manager, Cambodia
30-Aug	Dr. Lindsey SHIELDS, team lead for One Health 4 Cambodia (OH4CAM), Dr Nguyen NGUYEN, and her colleague, Dr SOY Ty
31-Aug	Dr. Y Lima (Vice President and Director of the Academic Board of Chenla University), and his partnering professors from Taiwan
8-Sep	Mr. Matthieu SCHOENHALS, Head of the Immunology of Infectious Diseases Unit, Institut Pasteur de Madagascar
20-Sep	Mid-term evaluation visit from WHO, funders, consultants for the TB prevalence survey

21-Sep	Mr. TRAN Thi Anh-Dao, Attaché de Coopération Scientifique et universitaire, French Embassy
5-Oct	Mr. Thomas GONNET, Project team manager – Health and Social Protection, Agence Française de Développement
20-Oct	H. E. Ambassador Justin WHYATT, Australian Ambassador, and Mr. Naisim SUM, Senior Program Manager–Health
8-Nov	Karma RINZIN and Stephane RENAUDIN, delegates from the World Organization for Animal Health (WOAH)
10-Nov	Dr. Nicolas ZELLER, Medical Director of Fondation Mérieux based in Lyon (France), Mr. Louis DELORME, Deputy Director of International Operations of Fondation Mérieux based in Lyon (France) and Dr NAY Thi Ha, Regional Representative for Southeast Asia, Fondation Mérieux (based in Vientiane, Laos)

Table 4: Visits, missions and delegations received at the IPC in 2023

3.10 Retrospective of 2023 events linked to the 70th anniversary of the IPC

The year 2023 was marked by the grandiose celebration of the 70th anniversary of the *Institut Pasteur du Cambodge*, created at the request of the Kingdom's authorities in 1953, the year of the country's independence. Several events were organized throughout the year, culminating in a cocktail party and photographic exhibition at the French Embassy, conferences at the Institut Français du Cambodge and a Gala dinner for all employees in a Phnom Penh hotel.

On Tuesday 23 May 2023, the Institut Pasteur du Cambodge celebrated its 70th anniversary in Cambodia in the magnificent garden of the French Embassy. His Excellency the Minister of Health, Professor Mam Bunheng, His Excellency Mr. Jacques Pellet, French Ambassador to Cambodia, Mr. Fernando Arenzana-Seisdedo, Director of International Affairs at the Institut Pasteur (Paris), Professor André Spiegel, Director of the IPC, and other local authorities and institutions honored us with their presence. The event provided an opportunity to recall the importance of the IPC's work in the fields of research and public health, and a tribute was paid to all of its staff who worked at the Institute for these 70 years.

From left to right: His Excellency the Minister of Health, Professor Mam Bunheng, Professor André Spiegel, Director of the Institut Pasteur du Cambodge and His Excellency Mr. Jacques Pellet, French Ambassador to Cambodia during the cocktail party at the French Embassy.

A photographic exhibition, retracing some of the highlights of the Institute's history and presenting some of its emblematic activities, was also installed on the walls of the French Embassy in mid-May and on display until October 2023.

Some photos of the exhibition on the wall of the French Embassy in Cambodia

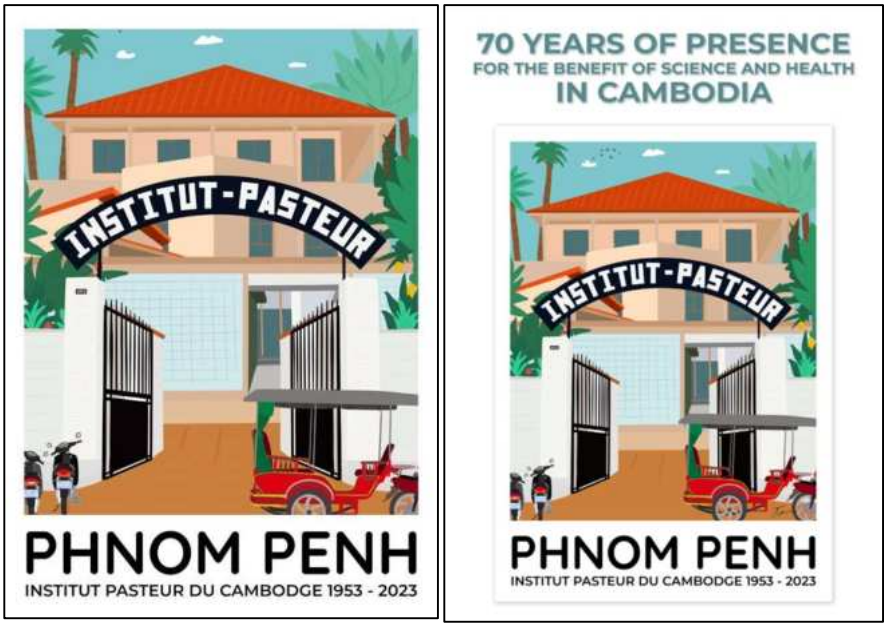
A first conference entitled "The three lives of Louis Pasteur", hosted by Professor Patrice Debré, member of the Académie Nationale de Médecine, was held at the Institut Français du Cambodge on Thursday 8 June 2023. Around a hundred people attended, including the French Ambassador to Cambodia, His Excellency Mr. Jacques Pellet, and Professor Yves Buisson, member of the Académie Nationale de Médecine and former director of the Institut Pasteur du Cambodge (1999-2001). The conference was arranged to take place during the Académie Nationale de Médecine's mission to Phnom Penh, as part of its long-standing collaboration with the University of Health Sciences.

Conference entitled "The three lives of Louis Pasteur" hosted by Professor Patrice Debré

A second conference entitled Institut Pasteur du Cambodge: 70 years of presence for the benefit of Science and Health in Cambodia, led by Professor André Spiegel, director of the Institut Pasteur du Cambodge, was held on Saturday, 16 September 2023 at the Institut Français du Cambodge. Around a hundred people attended, including the deputy director general of Calmette Hospital, His Excellency Professor Koy Vanny, and the French ambassador to Cambodia, His Excellency Mr. Jacques Pellet.


Conference entitled "L'Institut Pasteur du Cambodge: 70 years of presence for the benefit of Science and Health in Cambodia" led by Professor André Spiegel

A film, posters, a booklet — which presents a series of historical images generated through artificial intelligence by French artist and photographer Jérémie Montessuis — a book entitled "Insects in Cambodia", edited by SIPAR, and souvenirs such as tote bags, notebooks, pens, mugs, magnets and medals were distributed to commemorate this 70th anniversary.



The 70th anniversary poster and the cover of the historical booklet

Finally, on the occasion of this anniversary, a beautiful gala dinner was organised for all the employees of the Institute on Friday 8 December 2023, in the restaurant located on the 20th floor of the Sokha Hotel & Residence. Around 270 guests enjoyed a sit-down dinner with a breathtaking view of Phnom Penh. A number of events punctuated the anniversary dinner, including speeches, a video, an interactive quiz on the history of the IPC, and a singing competition. This unique event ended with a dance party to bring all the guests together and enjoy a festive and joyful moment.

Gala dinner for all IPC employees at the Sokha Hotel & Residence

3.11 Institutional Issues, Objectives and Outlook (2024–2028)

The general objectives remain the same as those of previous years, 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.

3.11.1 Maintaining the IPC in its National and Regional Scientific and Medical Context

The IPC maintains regular, frequent communication and interactions with the Ministry of Health and with the 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 maintain its efforts to mobilize and retain support from its different partners, whether Cambodian or otherwise, so that it can achieve its mission with maximum impact.

3.11.2 Developing the IPC's Partnerships with Other Scientific Institutions

Developing Partnerships with National Institutions

Partnerships with Cambodian ministries (Agriculture, Education, Environment, and others beyond the MoH) and their affiliated institutions, including but not limited to research centres, hospitals, universities, as well as with the private sector, are important to achieve the IPC's goals and objectives. Reinforcing and expanding these partnerships and collaborations would be beneficial for the IPC.

In light of the similarity and complementary scopes of the IPC and the 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 working jointly on ANRS sites in Cambodia.

Maintain and Strengthening the Hosting of Scientists from Other Institutions, Most Notably from CIRAD and IRD

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

3.11.3 Defining our Scientific Strategy

Organizing the 8th Meeting of the SAB

The IPC has not organized a Scientific Advisory Board meeting since February 2021, due to a sizeable turnover of IPC scientists and to the appointment of a new Minister of Health, who was tasked with nominating three of the board's members.

After considering the matter with the SAB's members, it was deemed impossible to arrange a meeting in 2024, due to calendar conflicts. Our current objective is to hold the meeting in the first quarter of 2025.

Developing a 5-year Strategic Plan

The last two SAB meetings recommended the development of a 5-year strategic plan. However, it did not seem possible to the new Management to begin such work without first having a thorough knowledge of the IPC and of the context in which it evolves.

This plan should be developed hand-in-hand with all IPC scientists as well as with key partners. The plan should also be coordinated with the MoH (hospitals, specialized Cambodian centres), the Ministry of Agriculture, Forestry and Fisheries, the Ministry of Environment, and the main universities in Cambodia.

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

Developing and Expanding Capacity in Bioinformatics

A working group was created in April 2021, bringing together different skills existing in this field at the IPC. Through our partnership with the Institut Pasteur and the Doherty Institute (University of Melbourne, Australia), we were able to sign a 2-year contract extension for Mr. Koen Vandelannoote (a bioinformatician), and to create the "Bacterial Phylogenomics Group" in early 2023, for a period of two years.

A senior bioinformatician was recruited in 2023 and is now working for the Immunology and Virology units. Efforts should be made toward the creation of a bioinformatics unit to train young Cambodian scientists. In this regard, dialogue has been initiated with the Cambodian Academy of Digital Technology (CADT) to develop collaborations (hosting bachelor's and master's students, joint projects) and possibly to recruit graduate students from this school who would work within this unit alongside scientific personnel from the MBL.

Supporting PhD Students

The IPC shall continue to support the training of young Cambodian scientists by covering the costs related to their PhD. In 2023, 8 PhD Students were supported, for a total of around €100,000.

3.11.4 Reducing Administrative Tasks for Scientists

In recent years, the IPC's development has been accompanied by an increasing number of scientists and of grant requests with conditional donors (NIH, Wellcome Trust, Bill & Melinda Gates Foundation, etc.). The time and effort required to monitor calls for projects, to submit, implement and coordinate projects, both administratively and financially, increasingly impedes our scientists' work, often reducing the amount of time they can devote to research. Hence, it appears necessary to support

them in these tasks by creating a new structure adapted to this mission within the IPC, namely the "Grants Office" (GO).

This lightening of the administrative burden is necessary to free more time for 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. This objective is in line with recommendations 4 and 5 from the last SAB. An experienced person was recruited in January 2024. This year, it will be necessary to define an effective method of organisation and to develop this GO within 3 to 5 years by relying on recruitment favouring local personnel.

A new application was developed (Notic) in 2023 and implemented at the beginning of 2024. This system should make it possible to decentralize part of the purchases to the unit level, and thus make it possible to improve fluidity and responsiveness.

3.11.5 Continuing the Development of Quality Standards

The IPC currently has three accredited laboratories (MBL, LEFS, and the Metrology Laboratory).

The Virology Unit, due to its size and to the critical activities it carries out (diagnoses critical for public health), has begun developing a quality approach in 2023.

Finally, a certification process (ISO 35001:2019) was initiated in 2023 for our BSL-3 laboratory as part of our commitment to continuous improvement in biosafety. Achieving this ISO 35001:2019 certification is anticipated for the second half of 2024.

3.11.6 Promoting our Staff's Career Development and Making the IPC a More Attractive Workplace

Strengthening the General Capacity of IPC Scientists

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

A course on scientific writing by EPICONCEPT is planned for May 2024.

Strengthening Management Skills

Current efforts to build up professionalism and skills in management, including supervision, mentorship, and structure-wide orientation, should be maintained.

Professional Development and Career Attractiveness

In order to identify "young talents" with high potential within the Institute and to plan career paths, the IPC organizes meetings with each head of unit, together with the Human Resources department and management (director, deputy director, CFO). These "Young Talents" meetings began in 2023 and will continue. The process makes it possible to identify young talents (promising laboratory technicians, young PhD students, etc.) and to determine paths allowing their personal development in line with the Institute's strategy).

Improve Workplace Safety and Security

Occupational Health Medicine will be strengthened in 2024, with the recruitment of an additional physician. in 2023, the procedures regarding the IPC's staff vaccination schedule and medical monitoring adapted to professional risks were reworked by a group of doctors from the IPC and by Human Resources service, and the new procedures will be formalized in 2024. The consultative committee on safety and security will be relaunched in early 2024.

Improving the IPC's Infrastructure: Project IPC 2030.

The "Project IPC 2030" (cf section 3.5) must be presented in more detail to the IPP and the MoH. After obtaining their authorisation, an active approach must be taken toward the donors (AFD, World Bank, European Union, etc.).

3.11.7 Fine-tuning the IPC's Economic Model

The IPC is a not-for-profit organization. Its economic model relies on the successful pursuit of research and institutional grants, and on the earning of revenue through services such as vaccinations and laboratory analyses.

The Medical Biology Laboratory's activities need to be expanded to generate additional revenue. This is possible, given the technical excellence and reputation it possesses. The laboratory's activity level, measured in "B" according to the French nomenclature of biological acts, which quantifies the activity, is too modest in view of the excellence of its technical platform and its potential to allow even more attractive prices.

The IPC's laboratory is facing increasing competition from non-accredited laboratories and suffers from accessibility constraints due to a lack of parking space for patients. The establishment of an off-site sampling centre warrants prompt consideration, as does an overall review of the MBL's general positioning in terms of price, value, and access. This project has been relaunched with the arrival of the new MBL's manager in January 2023. An extension of the opening hours should take place in 2024. In 2023, significant investment was made to increase the LEFS' capabilities to (personnel, new equipment for weighing heavy metals). Discussions are ongoing with CAPRED (the Cambodia–Australia Partnership for Resilient Economic Development) to set up testing equipment and procedures for (i) chemical analyses of water samples using GF-AAS, and (ii) pesticide and antibiotic residue analysis capabilities.

4 Activities at Institut Pasteur du Cambodge

4.1 Malaria Research Unit

4.1.1 Functional Structure

The Malaria Research Unit (MRU) is led by Jean Popovici since September 2022. The Unit encompasses five thematic areas: clinical studies, molecular biology & bioinformatics, liver-stage biology, blood-stage biology and developmental biology/host-parasite interactions.

As of January 2024, The Unit is composed of one Head of Unit (J. Popovici – IP permanent researcher), one Deputy Head (Khim Nimol – IPC permanent researcher), three postdoc researchers (Brice Feufack, Agnes Orban and Jeremy Salvador), one medical supervisor (Eng Virak), two PhD students (Seng Dynang and Lea Baldor), one field studies and biobank supervisor (Thin Sopheany) and seventeen technical & administrative staff.

The MRU is the WHO's main Southeast Asian laboratory for molecular surveillance of malaria drug resistance and, since January 2023, is a referral laboratory for the WHO Malaria NAAT EQA (Nucleic Acid Amplification Testing External Quality Assurance) scheme.

In 2023, we have created a Pasteur International Research Unit (PIU) associating our MRU at IPC with the Plasmodium Genetic Unit at Institut Pasteur de Madagascar (Benoit Witkowski) and the Infectious Disease Epidemiology and Analytics G5 group at Institut Pasteur Paris (Michael White). This PIU will enable us to further understand the challenges revolving elimination of *P. vivax*. Our MRU is also member of the Asia-Pacific International Center of Excellence in Malaria Research (ICEMR) aiming at understanding the epidemiology of malaria in Cambodia and in Papua-New Guinea.

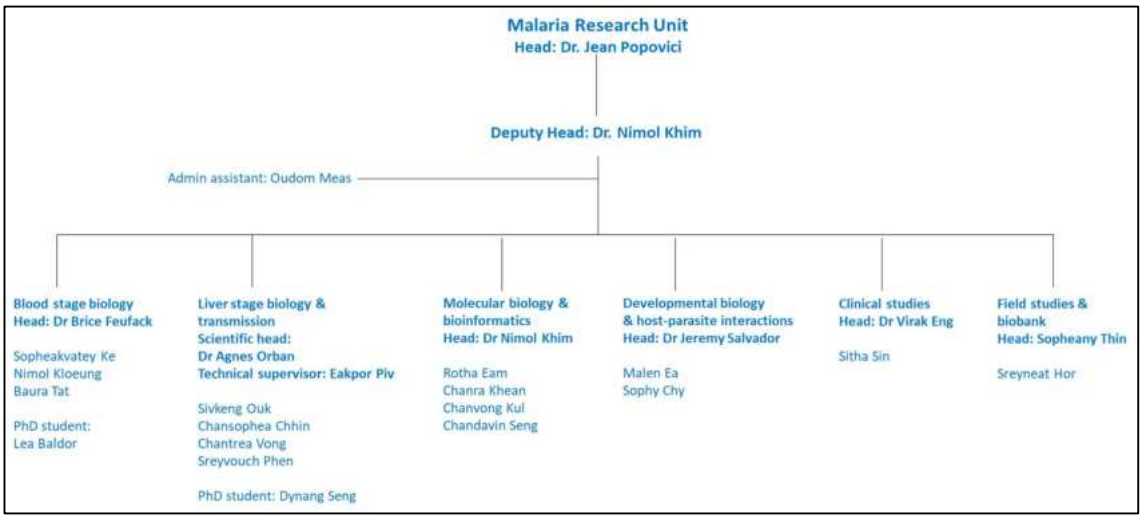


Figure 6: Malaria Research Unit Organigram

4.1.2 Research Programs – Major Achievements in 2023

Our unit's research activities are designed to provide insights useful for malaria elimination. These projects are mainly focused on Cambodia's key public health challenges but have implications beyond the country. Our activities are divided into four main axes: anti-malarial chemotherapy, pre-clinical vaccine development for *Plasmodium vivax*, host–parasite interactions and operational research for malaria elimination through improved diagnostics and innovative approaches. In the past years, our research has shifted from a main focus on *P. falciparum* (now on the verge of eradication in Cambodia) to an emphasis on *P. vivax*, a parasite far less studied and resilient to current elimination strategies.

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 WHO and the Global Fund. We also extend this surveillance to Papua New Guinea (PNG). In the past years, samples of *P. falciparum* were collected during therapeutic efficacy studies (TES) performed by Malaria Control Programs in each country of the region. However, with the numbers of cases decreasing, Cambodia has replaced traditional TES by integrated drug efficacy surveillance (iDES) where follow-up of infected patients is done continuously.

We are the main laboratory for characterizing resistance profiles of *P. falciparum* collected in these TES or iDES. We have confirmed in 2023 the trend observed in 2022 in Cambodia: *P. falciparum* are fully susceptible to currently used antimalarials and elimination of *P. falciparum* has massively progressed and we expect that Cambodia will have now nearly completely interrupted transmission. For Laos samples, we investigated TES data with suspected resistance emergence to lumefantrine but our data so far have not been able to confirm this. Similarly, for PNG samples, the few samples analysed did not allow to identify worrying signs of antimalarial resistance spread. Analyses of Vietnam samples are underway.

Research Project Name	RAI3 Molecular Markers
Funding	Global Fund, through WHO
Project duration	2021-2023
External collaborator	National Malaria Control Programs (NMCPs): Cambodia (LEANG Rithea), Vietnam, Laos and PNG, WHO

Therapeutic options against *P. vivax* liver stages.

P. vivax is characterized by the formation of dormant 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—mainly primaquine (PQ)—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 dehydrogenase (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 drug class that would be able to target hypnozoites in a safer manner. We have developed a method capable of medium throughput screening for drugs targeting this stage, making our unit one of the few laboratories able to perform this research at a global level. To date, more than 10,000 molecules have been screened using our platform with the identification of promising hits. This activity is financed by MMV. In parallel, we have developed a research program—financed by the National Institute of Health (NIH)—with the University of Georgia to guide structure-activity relationships. Thanks to this effort, insights in this elusive parasite biology stage are being acquired.

Name	LS drug discovery
Funding	Medicine for Malaria Venture (MMV, Switzerland)/National Institutes of Health (NIH, USA)
Project duration	2019-2024 (NIH); 2021-2022 (MMV)
External collaborator	University of Georgia USA (UGA, Dennis Kyle, Steven Maher, Anthony Ruberto)

Optimal drug regimen for *P. vivax* radical cure.

PQ represents the gold-standard molecule used for the *P. vivax* radical cure. Beside the hepatotoxicity of this drug in G6PD-subjects, there is no consensus on the posology to adopt. Moreover, the individual drug efficacy is poorly characterized, notably because of the difficulty in proposing a 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 the University of Maryland (UMD) which aims to determine the most effective PQ regimen. To avoid bias due to reinfection, we relocate enrolled patients in a no-transmission area during their entire follow-up. The first patients were recruited in November 2021, and 164 patients have been enrolled so far. The study is still ongoing, but given the conclusive results we obtained, we expect to have it completed soon. In addition, as part of this trial, we now evaluate the efficacy, safety and tolerability of weekly primaquine administration to G6PD deficient patients, as this regimen is now part of Cambodian National Treatment Guidelines.

Name	PQRC
Funding	NIH
Project duration	2020-2025
External collaborator	Université of Maryland USA (UMB, David Serre), National Center for Parasitology, Entomology, and Malaria Control Cambodia (CNM, Dysoley Lek)

Axis 2: Pre-clinical Vaccine Development for Plasmodium Vivax

Blood-stage vaccine development.

Thanks to the development of in vitro short-term cultures in the MRU in the past years, we are now able to evaluate blood-stage vaccine candidates to determine how they block parasite invasions in erythrocytes. We are currently focusing on PvDBP, PvRBP2b, PvAMA1 and PvRBP2a. We have expanded our analyses to PvAMA1 and the results obtained so far are very promising. Our work on PvRBP2a was initiated recently and is ongoing.

Name	RBC Invasion Blocking
Funding	NIH
Project duration	2020-2025
External collaborator	Case Western Reserve University USA (CWRU, Chris L. King), Walter and Eliza Hall Institute Australia (WEHI, Wai-Hong Tham), Burnet Institute (James Beeson), Mahidol University Thailand (Jetsumon Sattabongkot Prachumsri), CNM Cambodia (Dysoley Lek)

Name	Evaluation of PvRBP2a
Funding	A*STAR Singapore (Agency for Science, Technology and Research) and NTU Singapore (Nanyang Technological University)
Project duration	2023-2025
External collaborator	A*STAR Singapore (Laurent Renia)

Axis 3: Host-Parasite Interactions

Factors involved in *P. vivax* growth within its host.

We know very little about the factors influencing the growth of *P. vivax* in its human hosts. We started a project that aims at deciphering the contribution of human factors (specifically G6PD deficiency and HbE hemoglobinopathy) and of parasite genotypes on erythrocyte invasion and on the 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. The first scRNA-seq libraries were sequenced in 2023 showing that the pipeline is fully functional, and the project will continue in the coming years.

Name	Pv Growth
Funding	NIH
Project duration	2022-2027
External collaborator	UMB USA (David Serre), CNM Cambodia (Dysoley Lek)

Receptor-Ligand Interactions Involved in Duffy-Negative Red Blood Cell Invasion

The molecular mechanisms involved in the invasion of *P. vivax* into Duffy-negative erythrocytes are still unknown. Thanks to our ability to perform in-vitro short-term cultures of *P. vivax*, in collaboration with colleagues from Drexel University and Jimma University, we are aiming to identify the mechanisms that enable *P. vivax* to invade Duffy-negative red blood cells, an erythrocyte phenotype common in Africa, where increasing evidence of P. vivax infections is reported. Collection of parasites have been conducted in Ethiopia and samples will be shipped shortly to IPC to perform in vitro analyses.

Name	Pv Duffy neg
Funding	NIH
Project duration	2022-2027
External collaborator	Drexel University USA (Eugenia Lo), Jimma University Ethiopia (Delenesaw Yewhalaw)

Axis 4: Operational Research for Malaria Eradication

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, not only 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 eradication strategies.

Deciphering malaria epidemiology in Cambodia.

Our unit is part of the NIH Asia-Pacific ICEMR (Program Director: I. Mueller/ L. Robinson), which aims to address the key challenges to malaria elimination in the Asia-Pacific. We have applied serological investigations to evaluate the transmission of *P. vivax* in Eastern Cambodia and our results show that

this tool provides great opportunity to understand fine-scale epidemiology. We have performed a final cross-sectional survey in 2023 to determine how much epidemiology has changed over time since the start of this study. Results are currently being analysed and will be presented in future reports.

Name	ICEMR Asia-Pacific
Funding	NIH
Project duration	2017-2024
External collaborator	WEHI Australia (Ivo Mueller), Burnett Institute Australia (Leanne Robinson), IPP France (Michael White), CNM Cambodia (Dysoley Lek)

Targeting asymptomatic reservoirs of *P. vivax.*

The results of epidemiology analyses performed in the past years have shown that, while *P. falciparum* is on the verge of elimination, a reservoir of *P. vivax* remains. This reservoir consists of populations at risk of exposure, who are immune, and who rarely present a sufficient parasite density to show a positive result on a rapid diagnostic test (RDT). Therefore, a rational approach is to propose a test and a treatment strategy focused on the populations most at risk of having been recently in contact with the parasite. Because of its inconstant presence in the blood, the methods aiming at a direct detection (PCR) of the parasite are not sufficiently conclusive. Instead, we propose a serology-based methodology aiming at the characterization of recently exposed individuals, followed by a radical cure treatment (seroTAT). Our objective is to provide evidence about the feasibility and the acceptability of this strategy in rural Cambodia. We have implemented a pilot seroTAT study to evaluate the feasibility and acceptability of such approach in communities. Our data show very promising results and suggest that these should be scaled-up for evaluation of impact on *P. vivax* prevalence.

Name	RAI3 SEROTAT
Funding	The Global Fund
Project duration	2020-2023
External collaborator	WEHI Australia (Ivo Mueller), CNM Cambodia (Dysoley Lek)

Developing a minIon approach for G6PD sequencing.

As G6PD deficiency is a major host polymorphism involved in primaquine safety, understanding the epidemiology of this human polymorphism and better associating response to primaquine with human genotypes requires having a field-compatible tool for generating sequences of this gene. We have developped a MinIon pipeline to perform this sequencing in malaria-endemic areas. Our data show very good genotyping obtained.

Name	G6PD seq
Funding	Australian Centre of Research Excellence in Malaria Elimination (ACREME)
Project duration	2022-2023
External collaborator	WEHI Australia (Ivo Mueller), Menzies University Australia (Ric Price)

Surveillance of malaria infections among febrile illness in endemic areas.

We have conducted in 2023 a surveillance study to determine the true burden of malaria infections among febrile treatment seeking individuals. The rationale of this work is that a significant number of

infections might not be detected by current diagnostic tools (RDTs). We have enrolled nearly 1000 individuals from 6 Cambodian provinces. The study is complete and we are finalizing analyzes.

Name	Surveillance
Funding	IPC
Project duration	2023
External collaborator	CNM Cambodia (Dysoley Lek)

4.1.3 Research Programs – Outlooks for 2024

Are listed here projects that have started in 2023. These will be in addition to the ones listed above that are still ongoing in 2024.

Transmission-blocking vaccine development. We have partnered with the University of Oxford to evaluate transmission-blocking candidates against *P. vivax* using our standard membrane feeing assay.

Name	TB candidates
Funding	Oxford University UK
Project duration	2023-2024
External collaborator	University of Oxford UK (Sumi Biswas), IRD France (Anna Cohuet), CNM Cambodia (Dysoley Lek)

Pre-erythrocytic vaccine development. We have established a collaboration with the University of South Florida to evaluate pre-erythrocytic vaccine candidates to block sporozoites infection of hepatocytes.

Name	Sporo Pv
Funding	NIH
Project duration	2023-2025
External collaborator	University of South Florida USA (USF, John Adams)

Immune evasion of *P. vivax*. Following the identification of an in vitro immune evasion mechanism of *P. vivax* through a gene amplification (*pvdbp*), we have established a project started in 2023 to further understand the extent, dynamics and mechanisms associated to this phenotype. This project led by Jean Popovici and Eugenia Lo (Drexel University) will rely on two field sites, Cambodia and Ethiopia. Project is ongoing and results will be presented in future reports.

Name	PvDBP Immune Evasion
Funding	NIH
Project duration	2023-2028
External collaborator	Drexel University USA (Eugenia Lo), Ethiopian Public Health Institute Ethiopia (EPHI, Sindew Feleke & Abnet Assefa), CWRU USA (Chris L. King), UMB USA (David Serre), London School of Hygiene and Tropical Medicine UK (LSHTM, Rob Moon)

Clinical protection against P. vivax. We and others have shown that some individuals can display clinical protection against *P. vivax* enabling them to be asymptomatic upon infection. We have started a project to determine the factors involved in this protection. Led by Jean Popovici and Tineke Cantaert

at IPC, this project will study the genetic and immunologic determinants involved in this protection. Project is ongoing and results will be presented in future reports.

Name	Pv Immunology
Funding	NIH
Project duration	2023-2028
External collaborator	UMB USA (David Serre), CNM Cambodia (Dysoley Lek)

Mechanisms of Parasite Dormancy and Biology of Relapses. The mechanisms driving the biology of hypnozoites are completely unknown. We are combining analyses of finely characterized in-vivo relapses with in-vitro analyses of liver-stage infections to decipher the biology of these elusive parasites. Our first libraries for scRNA-seq were prepared and results are being analysed to make sure the pipeline is optimal for these studies.

Name	Pv dormancy
Funding	NIH
Project duration	2023-2028
External collaborator	UMB USA (David Serre), CNM Cambodia (Dysoley Lek)

4.1.4 Support to National Authorities

The IPC Malaria Unit supports the Cambodian Ministry of Health. Specifically, our unit is a main collaborator and a technical partner of the Cambodia National Malaria Control Program (NMCP), managed by the National Center for Parasitology, Entomology and Malaria Control (CNM). Our unit offers its expertise for malaria diagnostic, surveillance of drug resistance, evaluation of recrudescence.

4.1.5 Teaching and Training

PhD students

- Seng Dynang: Study of the biology of hypnozoites and relapses in *Plasmodium vivax* (started in October 2022).
- Lea Baldor: Extent, dynamics and mechanisms of *Plasmodium vivax* immune evasion caused by PvDBP gene amplification (started October 2023)

Master & Engineer students

- Sivkeng Ouk
- Rominea Yeat
- Baura Tat
- Mary Chim
- Muhammad Nadeem
- Justine Escard
- Laurine Moenne-Loccoz
- Raphael Genin

Teaching

- Jean Popovici coordinated the TU Host-Pathogen Interactions of the Joint Master's offered by the UHS and the *Université de Paris-Saclay*. He provided 3 hours on antigenic variation in Plasmodium parasites and 3 hours on multipartite interactions for second-year master's students. He is jury member and one of the coordinators for IPC of this Master degree.

- Brice Feufack, Seng Dynang, Khim Nimol and Agnes Orban: supervised a 24h practical class on the techniques used to study malaria parasites.

4.1.6 Outlook for upcoming years

A number of ongoing projects listed above will continue for the next 4 to 5 years.

Our research will keep focusing on the challenges related to malaria in the GMS with a specific attention on *P. vivax*. These will keep combining basic laboratory research with applied, operational questions for elimination. We are expanding our network of collaborators to leverage our knowledge and expertise (ie. in vitro culture of *P. vivax*) and use it at the service of other epidemiological contexts where malaria elimination is far from being achieved (ie. Ethiopia, Papua New Guinea). In addition, we have to anticipate the complete elimination of *P. falciparum* in the region for the very near future and, though less likely, of *P. vivax*. In that regard, we expect to expand our research to other pathogens than strictly *Plasmodium* parasites. Our immediate attention for this matter is to identify public health needs in Cambodia (and the region) with significant research gaps currently unaddressed at IPC to determine the most strategic decisions to be made for future research to be done in our unit. This could obviously include other parasitic infections, but also perhaps fungal infections or even bacterial pathogens.

4.1.7 Scientific Publications 2023

1. **Antimalarial drug efficacy and resistance in malaria-endemic countries in HANMAT-PIAM_net countries of the Eastern Mediterranean Region 2016-2020: Clinical and genetic studies.**
   Adam M, Nahzat S, Kakar Q, Assada M, Witkowski B, Tag Eldin Elshafie A, Abuobaida D, Safi N, Khan MA, Nagi M, Mustafa SA, Kohestani K, Muhammad J, Khim N, Al-Hadi M, Elfaki TM, Habib MN, Khairy AKA, Hamid H, Uddin Z, Amer Y, Hassan AH, Elhag MS, Sediqi AW, Kakar I, Abdul-Ghani R, Amran JGH, Abdallrahim TA, Tamim MS, Aljasari A, Rasmussen C, Azkoul L, Warsame M.
   Trop Med Int Health. 2023 Oct;28(10):817-829. doi: 10.1111/tmi.13929. Epub 2023 Sep 13. PMID: 37705047

2. **Hemisynthetic alkaloids derived from trilobine are antimalarials with sustained activity in multidrug-resistant Plasmodium falciparum.**
   Nardella F, Dobrescu I, Hassan H, Rodrigues F, Thiberge S, Mancio-Silva L, Tafit A, Jallet C, Cadet-Daniel V, Goussin S, Lorthiois A, Menon Y, Molinier N, Pechalrieu D, Long C, Sautel F, Matondo M, Duchateau M, Médard G, Witkowski B, Scherf A, Halby L, Arimondo PB.
   iScience. 2023 Jan 11;26(2):105940. doi: 10.1016/j.isci.2023.105940. eCollection 2023 Feb 17. PMID: 36718363

3. **Human monoclonal antibodies inhibit invasion of transgenic Plasmodium knowlesi expressing Plasmodium vivax Duffy binding protein.**
   Quentin D. Watson, Lenore L. Carias, Alyssa Malachin, Karli R. Redinger, Jürgen Bosch, Martino Bardelli, Lea Baldor, Lionel Brice Feufack-Donfack, Jean Popovici, Robert W. Moon, Simon J. Draper, Peter A. Zimmerman & Christopher L. King
   Malaria Journal 2023 Dec 4;22(1):369. doi: 10.1186/s12936-023-04766-1.

4. **Immunogenicity of a Plasmodium vivax vaccine based on the duffy binding protein formulated using adjuvants compatible for use in humans.**
   Francisco J Martinez, Micheline Guillotte-Blisnick, Christèle Huon, Patrick England, Jean Popovici, Hélène Laude, Laurence Arowas, Marie-Noëlle Ungeheuer, Jenny M Reimer, Darrick Carter, Steve Reed, Paushali Mukherjee, Virander S Chauhan, Chetan E Chitnis
   Scientific Reports 2023 Aug 25;13(1):13904. doi: 10.1038/s41598-023-40043-6.

5. **Naturally acquired antibodies to gametocyte antigens are associated with reduced transmission of Plasmodium vivax gametocytes to Anopheles arabiensis mosquitoes.**
   Tebeje SK, Chali W, Hailemeskel E, Ramjith J, Gashaw A, Ashine T, Nebret D, Esayas E, Emiru T, Tsegaye T, Teelen K, Lanke K, Takashima E, Tsuboi T, Salinas ND, Tolia NH, Narum D, Drakeley C, Witkowski B, Vantaux A, Jore MM, Stone WJR, Hansen IS, Tadesse FG, Bousema T.
   Front Cell Infect Microbiol. 2023 Jan 16;12:1106369. doi: 10.3389/fcimb.2022.1106369. eCollection 2022. PMID: 36726645 Free PMC article.

6. **Plasmodium vivax Malaria in Duffy-Positive Patients in Rwanda.**
   Welmoed van Loon, Rafael Oliveira , Clara Bergmann, Felix Habarugira, Costanza Tacoli, Julia Jäger, Darius Savelsberg, Djibril Mbarushimana, Jules M Ndoli, Augustin Sendegeya, Claude Bayingana, Frank P Mockenhaupt
   American Journal of Tropical Medicine and Hygiene 2023 Aug 7:tpmd230143. doi: 10.4269/ajtmh.23-0143. Online ahead of print.

7. **Prevalence and characterization of piperaquine, mefloquine and artemisinin derivatives triple-resistant Plasmodium falciparum in Cambodia.**
   Mairet-Khedim M, Roesch C, Khim N, Srun S, Bouillon A, Kim S, Ke S, Kauy C, Kloeung N, Eam R, Khean C, Kul C, Chy S, Leang R, Ringwald P, Barale JC, Witkowski B. J Antimicrob Chemother. 2023 Feb 1;78(2):411-417. doi: 10.1093/jac/dkac403. PMID: 36508338

8. **The spatial signature of Plasmodium vivax and Plasmodium falciparum infections: quantifying the clustering of infections in cross-sectional surveys and cohort studies**
   Mirco Sandfort, Wuelton Monteiro, Marcus Lacerda, Wang Nguitragool, Jetsumon Sattabongkot, Andreea Waltmann, Henrik Salje, Amélie Vantaux, Benoit Witkowski, Leanne J Robinson, Ivo Mueller, Michael White
   Malaria Journal 2023 Mar 4;22(1):75. DOI: 10.1186/s12936-023-04515-4

4.2 Epidemiology & Public Health Unit

4.2.1 Functional Structure

The Epidemiology and Public health Unit (EPHU), conducts operational epidemiological research studies on major public health challenges in Cambodia. Our mission is to conduct epidemiological and clinical research studies based on a cross-sectional and multidisciplinary approach, aiming to inform interventions and evidence-based policy recommendations.

Our objective aims to enhance population health and well-being, and to mitigate health disparities. Our research activities draw on various disciplines, such as epidemiology related to infectious diseases and environmental health, clinical research, biostatistics and modelling, health geography, and the human and social sciences within the health domain.

Recent research themes have focused on specific public health issues in Cambodia and, more broadly, Southeast Asia. These include rabies, arboviruses, HIV, tuberculosis, hepatitis, malaria, antibiotic resistance, leptospirosis, influenza viruses, SARS-CoV-2, among others. Beyond projects initiated before 2023, activities conducted in 2023 have strengthened the unit's research strategy by emphasizing the development of research projects directly coordinated by the unit and launching new initiatives such as the national tuberculosis prevalence survey, the HEPEDIAC and CAP-RAGE studies. These efforts are part of a response to the complex epidemiological context in Cambodia, highlighting the need for improved disease surveillance and diagnostic capabilities. To address these challenges, the unit, comprising 52 members in 2023, was organized into five specific and complementary groups (Figure xx):

- **The Community Epidemiology Group**, led by Dr Ly Sowath, which has extensive experience in community epidemiology research projects on rabies, dengue, avian influenza and investigations of epidemics and health emergencies;
- **The Clinical Research Group**, led by Dr Nathalie de Rekeneire, which pilots' clinical trials and contributes 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 Hélène Guis, which arises from a collaboration between CIRAD and IPC to tackle the human, animal and environmental components of disease transmission and prevention in a constructive and coordinated One Health approach—its focus is on zoonotic and vector-borne diseases;
- **The Methodology and Data Analysis** group**,** coordinated by Dr Claude Flamand, which aims to develop and implement methodological tools and approaches adapted to the studies carried out including study designs, data management and analysis resulting from the research work carried out.
- **The GeoHealth Group**, led by Dr Vincent Herbreteau from the IRD, which is specialized in the study of the effects of environmental and geographical factors on human health, and brings a useful expertise for research activities conducted within the unit;

The research activities of the EPHU 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). 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, the Sihanouk Hospital Center of Hope (SHCH), the Jayavarman VII Hospital, the Kantha Bopha Children's Hospitals and the National Pediatric Hospital (NPH). Robust partnerships with the University of Health Sciences (UHS) 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 International Vaccine Initiative (IVI), the European Union, *Fondation Total*, *Institut National de la Santé et de la Recherche Médicale* (INSERM), *L'Agence Inter-Etablissements 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)*, the *Agence Française de Développement* (AFD) and the French Embassy in Cambodia and the French Ministry of Foreign Affairs.

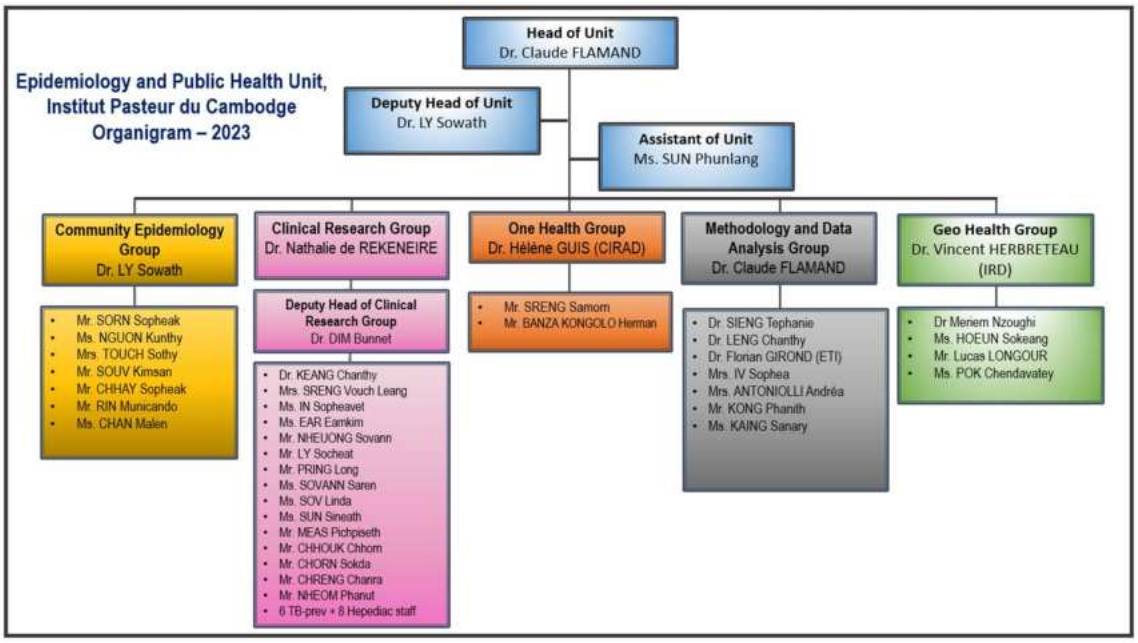


Figure 7: Epidemiology and Public Health Unit organigram, 2023

4.2.2 Research Programs - Major Achievements In 2023

Rabies

Follow-up of Patients Receiving the WHO 2018 - Recommended Rabies PEP Using Intradermal Vaccination Protocol

This study aims to evaluate the duration of the protective antibody response by following a cohort of approximately 170 patients at 14 days, 6 months, 1 year, and 3 years after receiving the first dose of the vaccine schedule. The cohort consists of patients who were bitten by dogs with a known rabies infection status (laboratory-confirmed as Negative or Positive) and who received a 3-session intradermal rabies post-exposure prophylaxis (PEP) at the Institut Pasteur du Cambodge (IPC). As of

January 2024, follow-up and blood sampling have been completed for years 1, 2, and 3 in 153, 76, and 50 participants, respectively. No PEP failures were encountered, and a protective level of immunity against rabies was observed among participants at one year. A manuscript detailing these findings is currently being prepared for submission.

Collaborations	Epidemiology and Public Health Unit (Touch Sothy, Ly Sowath), Immunology Unit (Tineke Cantaert), Virology Unit (Duong Veasna), Vaccination Unit (Peng Yiksing)
Funding	Institut Pasteur du Cambodge: (2019-2024)

KAP Rabies

In September 2023, the Institut Pasteur du Cambodge (IPC) and the French Agricultural Research Centre for International Development (Cirad), supported by the Ministry of Health and Ministry of Agriculture, Forestry and Fisheries, initiated a national online survey to evaluate rabies post-exposure prophylaxis (PEP) availability, and understand the knowledge, attitudes, and practices (KAP) of healthcare workers towards rabies. Including over 780 health facilities across 21 provinces in 2023, preliminary findings reveal that over 80% of facilities encountered animal bite victims, with a third providing rabies vaccination. The project, aiming to complete data collection by early 2024, will offer insights into national PEP coverage, estimate rabies burden, and highlight healthcare workers' training needs. Additionally, it encompasses developing rabies awareness materials and a targeted training program set for 2024 in Svay Rieng province, addressing crucial gaps in rabies prevention and management in Cambodia.

Collaborations	H. Guis (CIRAD), S. Ly, C. Flamand. In coll. with CDC MoH and GDAHP MAFF
Funding	French Ministry of Europe and Foreign Affairs through the Solidarity Funds for Innovative Projects (FSPI-R) :2023-2024

Arboviruses

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

We are evaluating the occurrence of dengue and dengue-like syndromes in Kampong Thom Province through the surveillance of children and adult inpatients at three referral hospitals in Kampong Thom Province, and at Jayavarman VII Pediatric Hospital in Siem Reap Province and through investigations in and around households if dengue Index Cases identified among positive cases found at hospitals. Sequential laboratory monitoring of cases will continue for up to three years. In hospital settings, 2,139 patients were screened, with 788 confirmed to have dengue infection through PCR testing. Community investigations surrounding the households of 91 Index Cases were conducted, screening 3,526 residents by PCR, of which 91 tested positives for dengue. During 2022-2023, DENV serotype 2 was the most prevalent, followed by DENV4. These detections enhance the surveillance data of the National Dengue Control Program, which primarily relies on syndromic passive surveillance in pediatric hospitals. Further analysis will explore the prevalence and determinants of dengue within the community.

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

One Health

AFRICAM

AFRICAM is a One Health project developed in the framework of the Preventing Zoonotic Disease Emergence (PREZODE) initiative, coordinated by CIRAD and IRD in four African countries and in Cambodia. The main objectives of the project are to 1) to study the zoonotic risk at different interfaces between humans, animals, and the environment, taking into account climatic and environmental dynamics; 2) to implement activities to reduce the risk of emergence, and 3) to further develop existing surveillance systems, working towards a One Health network and integrated surveillance.

Collaborations	Team Leader: Anne-Laure Banuls (IRD), CoPi: Flavie Goutard (Cirad), Country focal points: H. Guis and V. Herbreteau. Partners in Cambodia: 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 - Pilot survey on the potential role of domestic dogs as sentinels of human exposure to vector-borne and zoonotic diseases in Cambodia

The DogZooSea project aims to improve knowledge and management of two major public health problems in Cambodia and neighboring countries in SE Asia: rabies (for which dogs are the main reservoir) and arthropod-borne viruses (for which dogs could be sentinels for human risks of infection). The project activities included 1) documenting dog owners' perceptions and management practices in a rural and a peri-urban village, 2) assessing dog distribution and exposure to flaviviruses in 43 pairs of owners and their dogs sampled twice in the same villages, and 3) assessing the existence of non-lethal exposures to rabies in dogs in the rural village where no dogs had been vaccinated against rabies. Results showed that knowledge on dog diseases was higher in the peri-urban village and that the dogs were described as being more mobile in the rural village. Raw serological data from humans showed that DENV-2 seroprevalence was 98% and JEV seroprevalence was 79%. In dogs, the seroprevalence for DNV was 47% and JEV seroprevalence was 52%. WNV antibodies were detected in 3 humans and 5 dogs but no seroconversion was observed. Serological data will be combined with similar data collected in Thailand and Indonesia and analysed by owner-dog pairs. Rabies antibodies were detected in 3/107 unvaccinated dogs supporting growing evidence on the existence of non-lethal exposure to the virus.

Collaborations	Epidemiology and Public Health Unit: Ly Sowath, Helene Guis, Sorn Sopheak), Virology Unit (Heidi Auerswald), CIRAD-KU (Michel De Garine Wichatitsky), *Universitas Gadjah Mada* (Yogyakarta, Indonesia), Faculty of Veterinary Technology of the Kasetsart University (Bangkok, Thailand)
Funding	FSPI-One Health in Practice in Southeast Asia (OH SEA) Agence Universitaire de la Francophonie (AUF) (2022-2023)

HIV and/or Tuberculosis

DATURA-ANRS 12424 Clinical Trial: Determination of the Adequate Tuberculosis Regimen in Adults and Adolescents Hospitalized with HIV-Associated Severe Immune Suppression (CD4 ≤ 100 cells/µL)

Mortality in people entering into HIV care late and who have 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. At the end of December 2023, among 124 patients who were pre-enrolled, 89 were included in this study. Recruitment and follow-up of patients is ongoing.

Collaborations	Team leaders: Epidemiology and Public Health Unit: Nathalie de Rekeneire, Dim Bunnet (Clinical Research Group), Partners: NCHADS, Cambodia-China Friendship Preah Kossamak Hospital, Khmer-Soviet Friendship Hospital, Calmette Hospital, NCHADS Clinic and Laboratory
Funding	ANRS-MIE (2021-2024)

Third TB National Prevalence Survey in Cambodia

The overall goal of the third national tuberculosis prevalence survey is to gain a much better understanding of the burden and trend of the TB disease and to develop a plan to improve ways of TB control and care toward Ending TB along SDGs and the End TB Strategy targets. Dr Bunnet DIM is the co-PI of this project. Our group is involved in the data management part of this survey, the mycobacteriology laboratory of the Medical Biology Laboratory in the cultures and the IT unit in the host server maintenance. The implementation of this survey has been completed for 59 clusters; the next 25 clusters will be finished by May 2024.

Collaborations	Team leaders: Epidemiology and Public Health Unit: Nathalie de Rekeneire, Dim Bunnet (Clinical Research Group), Cheng Sokleaph (Deputy Head of Medical Biology Laboratory), Xavier Faure (Head of Informatics Technology) Partners: CENAT, RIT/JATA, HSD, CATA, USAID
Funding	DFAT (WHO), Global fund and USAID

The IPC team is responsible for the data management, performing sputum direct exam and cultures (MGIT and Solid) for this prevalence survey.

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 a 6-month daily isoniazid-based Tuberculosis Preventive Treatment (TPT) regimen (6H), on the TPT coverage among PLHIV-attending adult OI/ART clinics in Cambodia. The identification of barriers to TPT is completed. The follow-up of the study participants ended in August 2022. The year 2023 was dedicated to data cleaning, site visits, and statistical analysis. An external review of this project was performed in October 2023. The results of the study showed that an approach including introduction of 3HP, comprehensive health care workers training and PLHIV information based on previously identified barriers led to an increase in TPT coverage from 15% up 86% in adult PLHIV attending HIV clinics. No proven efficacy of the introduction of the intervention itself within this step wedge cluster randomized trials (continued increase).

Hepatitis

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 the sofosbuvir/daclatasvir combination for children who are at least 6 years old, and for adolescents with active HCV infection. The recruitment is this study started in August 2023. At the end of 2023, among 6066 children included in the screening phase, 7 children were included in the therapeutic phase. It is planned to add another recruitment site in Battambang.

Collaborations	Team Leaders: Nathalie de Rekeneire, Dim Bunnet (Clinical Research Group, IPC). Olivier SÉGÉRAL Partners: NCHADS and OI/ART sites, Jayavarman VII Hospital, Kantha Bopha 1 and 2 Hospitals, National Pediatric Hospital
Funding	ANRS-MIE (2022-2024)

Bacteriological Diseases and Antibiotic Resistance

RAMSES - Resistance to AntiMicrobials: Socio-Economic and regulatory factors influencing emergence and dissemination in the South

As a component of the AVIESAN "AMR South" network, this project investigates socio-economic and regulatory influences on antimicrobial resistance (AMR) emergence and spread, particularly in low-and-middle-income countries (LMICs). It aims to elucidate how socio-economic factors drive antimicrobial circulation and the availability of resources to mitigate AMR in humans, animals, and the environment. The study, comprising 75 interviews in three villages, interactions with 20 Cambodian health professionals, and consultations with 7 labs, shows that urban to rural communities self-medicate, guided by symptoms and deterred by the cost of microbiological tests, often pricier than antibiotics themselves. Antibiotics, considered vital by these communities, underscore the significant impact of socio-economic and cultural factors on AMR understanding and actions. Specifically, chicken farmers use antibiotics for market-bound chickens to boost growth and as a preventive measure, while those for personal consumption are raised without antibiotics. Following Covid19, renewed emphasis on hygiene has emerged, yet improper disposal of medicines by communities contributes to environmental pollution. The study aims for completion and publication within the year.

Collaborations	Epidemiology and Public Health Unit (Sieng Tephanie, Ly Sowath) Scientific coordinators: Alexandre Hobeika (CIRAD), Adèle Kacou N'Douba (University of Abidjan)
Funding	CIRAD - 2022 - 2024

Malaria

Blocking Malaria Transmission in Vulnerable Forest Populations Through Forest Malaria Workers: A Key for Malaria elimination in Cambodia

Cambodia is on a mission to eliminate P. falciparum malaria by 2023 and all human malaria species by 2025, aligning its efforts with the WHO's Mekong Malaria Elimination program. The focus of this project is to uncover the key factors contributing to malaria transmission within Cambodian forests and to evaluate the effectiveness of Intermittent Preventive Treatment for Forest Goers (IPTf) in curtailing malaria among vulnerable populations in these locales. Between March 11, 2019, and January 30, 2021, a total of 2,198 Forest Goers participated in 3,579 interviews, conducted over a span of two years. The year 2023 highlighted the analysis and utilization of project-related data, enriching

our approach and insight into eradicating malaria in Cambodia. Following the IPTf initiative, there was a marked reduction in PCR-confirmed malaria prevalence, from 2.9% to 0.5% for P. falciparum and from 21.0% to 4.7% for P. vivax. Recognizing the efficacy of this strategy, Cambodian National Malaria Program has deemed it an essential component of the country's malaria elimination efforts, advocating for customized strategies tailored to forest environments to meet the ambitious goal of eradicating all human malaria species by 2025.

Collaborations	Collaborations: Epidemiology and Public Health Unit: Patrice Piola, Iv Sophea, Claude Flamand, Malaria Unit (Benoit Witowski, Jean Popovici). 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 / French Embassy – 2019-2023

4.2.3 Research Programs - Outlook for 2024

The ELDORADO Trial: EvaLuation of DORAvirine in combination with tenofovir disoproxil fumarate plus lamivudine, versus DOlutegravir in combination with tenofovir disoproxil fumarate plus emtricitabine or lamivudine in treatment-naïve HIV-1 infected subjects.

The integrase strand transfer inhibitor (INSTI) dolutegravir (DTG) is now globally recommended by the World Health Organization (WHO), in combination with an NRTI backbone (TDF + 3TC), as first-line antiretroviral therapy in adults living with HIV. However, there are growing concerns and evidence on the toxicity of dolutegravir with massive and steady weight gains under treatment—notably among women in Africa—and an increase in diabetes mellitus. Doravirine is a potent and relatively novel non-nucleoside reverse transcriptase inhibitor (NNRTI) for the treatment of HIV-1 infections in antiretroviral-naïve HIV-infected subjects. This multicentre randomized trial will assess the non-inferiority of doravirine in association with tenofovir and lamivudine, as compared to dolutegravir in association with tenofovir and lamivudine or emtricitabine, in terms of virologic efficacy at week 4. It will also assess whether doravirine-based regimens less frequently lead to weight gain and adverse side effects than dolutegravir-based regimens. The study is under preparation. The protocol will be sent to the National ethics committee for review in April 2024.

Collaborations	Team leaders: Nathalie de Rekeneire, Dim Bunnet (Clinical Research Group, IPC) Partners: NCHADS, NCHADS Clinic and Laboratory, Social Health Science clinic (SHC) and AIDS Health Care Foundation (AHF)
Funding	ANRS-MIE (2024-2028)

Prevalence of invasive fungal infection – Histoplasma spp., Talaromyces marneffei, Cryptococcus spp.- in severe immunocompromised HIV-infected patients in Cambodia

Histoplasmosis, talaromycosis and cryptococcosis are serious invasive fungal infections in patients with advanced HIV disease. Even though these pathogens are probably endemic in Cambodia, little or no data is available, especially for histoplasmosis and talaromycosis. The lack of awareness of these infections and the insufficient availability of reliable diagnostic methods lead to delayed diagnosis. In the last years, antigenic and molecular methods have emerged as promising tools for the rapid diagnosis of invasive fungal infections. Our main objective is to assess, using these new methods, the prevalence of these invasive fungal infections in patients with advanced HIV disease in Cambodia. Our main secondary objective is to raise awareness and train local actors on these infections and new and simple diagnosis tools.

We will use the STATIS biobank, a study of the diagnostic management of tuberculosis in 199 severely immunocompromised HIV-positive patients in Cambodia. We will first perform specific antigenic tests for each pathogen. As the cross-reactivity of these tests between histoplasmosis and talaromycosis is unknown, we will confirm positive results by specific quantitative PCR. We will organize a laboratory workshop to train a team in the use of the test and to develop a training program, and a clinical workshop for clinicians in charge of patients at risk of Invasive fungal infections in Phnom Penh hospitals.

Collaborations	Team leaders: Nathalie de Rekeneire, Dim Bunnet (Clinical Research Group, IPC) Partners: Dr Sokleaph Cheng (PI Sud, IPC), Pr Aude Sturny-Leclère (PI Nord, IPP), Dr Emilie Mosnier (UHS), Pr Antoine Adenis (CIC1424 Inserm of the Cayenne hospital center)
Funding	ANRS-MIE (2024-2025)

National Population-Based Survey to Study Exposure and Transmission Dynamics of Priority Infectious Diseases from a One Health Perspective

Despite the increasing global impact of infectious diseases and the enhancement of surveillance systems, significant gaps remain in understanding the transmission mechanisms and maintenance of pathogens in exposed populations. Traditional epidemiological surveillance typically captures only symptomatic cases, neglecting asymptomatic infections or those not seeking medical attention, as well as the broader immune landscape at the population level. To address these limitations, serological surveys have long been utilized to detect immune responses in serum, offering insights into population-level infection rates for a variety of pathogens. However, accurately determining an individual's infection status and pinpointing the timing of infection involve complex challenges, influenced by a mix of human, virological, and environmental factors. Such information is vital for assessing transmission risks, but conducting in-depth studies on immunological status in affected populations demands extensive financial, human, and material resources, often scarce in the most impacted regions. With recent advancements in technology, sero-epidemiology has emerged as a potent tool that effectively integrates epidemiology, mathematical modelling, and public health to facilitate the development of risk reduction strategies. In 2024, we aim to launch a nationwide population-based serological survey to estimate the burden of multiple pathogens identified on the government's priority list of infectious diseases. This survey will enable us to reconstruct the spatiotemporal transmission dynamics and map infection risks with high precision. The upcoming year will focus on establishing a collaborative framework with project partners, identifying additional funding sources, developing the study protocol, and securing the necessary ethical and regulatory approvals.

Collaborations	Team leaders: Epidemiology and Public Health Unit (Claude Flamand, Hélène Guis, Sowath Ly, Tephanie Sieng), Virology Unit (Erik Karlsson, Veasna Duong, Heïdi Auerswald, Gary Huong), LBM (Sokleaph Cheng, Bertrand Guillard), Medical and Veterinary Entomology Unit (Sébastien Boyer)
Funding	AFD/Ecomore 3 Project: 2024-2027

4.2.4 Support to National Authorities

The following summarizes key support to Cambodian national authorities over the year 2023.

- N. de Rekeneire and Dim B. are members of the Cambodian Committee for TB Research (CCBR). They are also part of the technical working group (TWG) on HIV.

- Dim B. is involved with the CENAT for the TB prevalence survey that started in May 2023.
- Ly S. worked with the Emergency Operational Center (EOC) for Covid-19, coordinated by the Cambodian CDC-MoH.
- Ly S. and V. Herbreteau teams provided support for data management for Covid-19 lab testing at IPC and ensured the transfer of quality data to the CDC-MoH.
- C. Flamand participated to Dengue and Influenzae surveillance workshops organized by CDC-MoH and WHO.
- T. Sieng participated to the AMR Technical Working Group

4.2.5 Teaching and Training

Teaching and Training

- Ly Sowath, Lectures for foundation year students at the University of Health Science,
- Dim Bunnet, Teaching Clinical Research at the Master of Infectiology, University of Health Science & Paris-Saclay University
- Sovann Nheuong, Teaching Data management in clinical research at the Master of Infectiology, University of Health Science & Paris-Saclay University
- Socheat Ly, Teaching Data management in clinical research at the Master of Infectiology, University of Health Science & Paris-Saclay University
- Claude Flamand, Teaching in Biostatistics and Clinical Trials Methodology at the Master I & II of Infectiology, University of Health Science & Paris-Saclay University
- Claude Flamand, Teaching "Predicting Vector-Borne Diseases Emergences" and "One Health Approaches", Open Medical Institute Course "Vector Borne Diseases", November 2023
- Claude Flamand, Teaching Modeling Vector-Borne Diseases in Pasteur Network Entomology Course, New Caledonia, December 2023

PhD students

- Mrs Iv Sophea, Paul Sabatier-Toulouse III University, 2019-2023 – Thesis Title: Blocking Malaria Transmission in Vulnerable Populations of the Forest by the Forest Health Workers: Key to Malaria Elimination in Cambodia. (Co-director: C. Flamand)
- Ms Andréa Antoniolli; Paris Cité University, ED 393 from 2022 – * Thesis title: Transmission of leptospirosis in Cambodia. (Director: C. Flamand)
- Mr Herman Banza Kongolo, Paris Cité University, ED 393 from 2022 – * Thesis title: Estimation of the rabies load burden in Cambodia (Director: C. Flamand, Co-supervisor: H. Guis)
- Mr Sopheak SORN, Paris Cité University, ED 393 from 2023 – * Thesis title: Transmission dynamics and determinants of arboviruses in Cambodia (Director: C. Flamand, Co-supervisor: Dr Sowath Ly)

Master's students

- Mr Nheuong Sovann (UHS-Epidemiology, 2020–2023).
- Ms Sov Linda (Master of Science in Epidemiology at National Institute of Public Health, 2020–2023).
- Ms In Sopheavet (Master in Epidemiology, National Institute of Public Health, 2022-2024)
- Ms Sun Sineath (Master of Health and Community Development, National Institute of Public Health, 2022–2024)

- Mr Long Pring (Master in Epidemiology, National Institute of Public Health, 2022–2024)
- Ms San Kimsey (Master in Public Health, National Institute of Public Health, 2022–2024)
- Mr Lay Virak (Master in Epidemiology, National Institute of Public Health, 2022–2024)
- Mrs. Sreng Vouchleang (Master's in Epidemiology, National Institute of Public Health, 2023–2025)
- Ms Chan Malen (Master of Applied Epidemiology - MAE, ASEAN–Australia Health Security Fellowship Program, National University of Australia - ANU, 2022–2023)
- Mr Giacomini Anthony (Master II in Gestion Intégrée des Zoonoses et des Maladies Animales Tropicales (GIZMAT), Montpellier University, 2023)
- Ms Hoen Thavry (Master II in Epidemiology, National Institute of Public Health, 2023–2024)
- Ms Nathalie Passard (Master II in Public Health, SESSTIM, Aix-Marseille University, 2023-2024)
- Mr Julien Dowding, Specialized Master's in Public Health, Pasteur-CNAM School

Student internships

- Mr. Nheom Phanut (Bachelor in Geography and Land Management at RUPP; 2018–Ongoing).

4.2.6 Outlook

The research strategy of the Epidemiology and Public Health Unit for the upcoming years is focused on developing multidisciplinary and integrated research programs aligned with Cambodia's identified priorities. These programs aim to enhance our understanding of priority infectious diseases and inform targeted diagnosis, prevention, treatment, and public health strategies. Addressing the significant challenges associated with both existing projects slated for 2024 and potential new multicentric clinical research projects will require strengthening the clinical research group within the unit through the recruitment of high-level medical and clinical research scientific experts.

Additionally, we plan to embark on multi-scale collaborative One Health projects, leveraging multi-institutional and cross-sectional initiatives such as B-Coming, Prezode-Africam coordinated by IRD, and Ecomore 3 funded by AFD, after years of preparatory work. These projects are expected to not only bolster the unit's research strategy but also enhance team resources through the acquisition of additional funding essential for project challenges. Despite significant recent advancements in capacity building, highlighted by the enrollment of numerous unit staff members in academic Master's and PhD programs, we will continue to place special emphasis on training and capacity enhancement across various domains including clinical research, data management, epidemiology, and biostatistics. Both in-house and university training programs, which offer Master's and PhD degrees, are set to play a crucial role in sustaining and bolstering the unit's capabilities with long-term resources.

The departure of Vincent Herbreteau, who led the GeoHealth group within the unit at the end of 2023, underscores the need to strengthen the capacities and resources in spatial analysis and geomatics of the unit. It will be essential to reinforce these capabilities within the methodology and data analysis group and prioritize the recruitment of a senior data scientist in the coming years to address future challenges, particularly in the study of health-environment and climate relationships. Attracting talented Cambodian researchers and international postdoctoral scientists and expanding institutional and regional collaborations are essential to continue providing pertinent research findings and relevant support to IPC units and partners in the SEA region.

Furthermore, establishing a specialized social science team, facilitated by the recruitment of Téphanie Sieng as a post-doc in socio-anthropology, aims to integrate socio-anthropological approaches into the unit's projects.

Finally, given the critical public health issues in Cambodia, it is imperative to explore opportunities to broaden research themes to include non-communicable diseases (respiratory diseases, cardiovascular diseases, cancers, etc.).

4.2.7 Scientific Publications 2023

1. **A qualitative study of the experience of COVID-19 patients in Burkina Faso**
   B. Konaté, R. Médah, I. Traoré, S. Ouedraogo, N.F. Kaboré, A.K. Mamguem, O. Billa, D. Kania, H. Badolo, E. Ouédraogo, N. de Rekeneire, A. Poda, A.E. Diendéré, B.Ouédraogo, H. Tinto, T.S. Dabakuyo-Yonli.
   Am J Trop Med Hyg. 2023 Dec 18;110(1):170-178. doi: 10.4269/ajtmh.22-0351.

2. **Acceptability of decentralizing childhood tuberculosis diagnosis in low-income countries with high tuberculosis incidence: experiences and perceptions from health care workers in sub-Saharan Africa and South-East Asia.**
   J. Basant, Yara Voss De Lima, D. Mbang Massom, S. Kaing, MF. Banga, E. Tamba Kamara, S. Sesay, L. Borand, JV. Taguebue, R. Moh, C. Khosa, G. Breton, J. Mwanga-Amumpaire, M. Bonnet, E. Wobudeya, O. Marcy, J. Orne-Gliemann, the TB-Speed Decentralization study group.
   PLOS Global Public Health, 2023, 3(10): e0001525. https://doi.org/10.1371/journal.pgph.0001525

3. **Characterization of soluble TLR2 and CD14 levels during acute dengue virus infection.**
   V. Upasani, B.M. Ter Ellen, S. Sann, S. Lay, S. Heng, D. Laurent, S. Ly, V. Duong, P. Dussart, J.M. Smit, T. Cantaert, I.A. Rodenhuis-Zybert.
   Heliyon. 2023 Jun 21;9(6):e17265. doi: 10.1016/j.heliyon.2023.e17265. eCollection 2023 Jun.

4. **Forest malaria' in Myanmar? Tracking transmission landscapes in a diversity of environments.**
   E. Legendre, F. Girond, V. Herbreteau, S. Hoeun, S. Rebaudet, A.M. Thu, J.D. Rae, L. Lehot, S. Dieng, G. Delmas, F. Nosten, J. Gaudart & J. Landier.
   Parasit Vectors. 2023 Sep 12;16(1):324. doi: 10.1186/s13071-023-05915-w.

5. **High performance of systematic combined urine Liboarabinomannan test and Sputum Xpert MTB/RIF for Tuberculosis screening in severely immunosuppressed ambulatory adults with Human Immunodeficiency Virus.**
   M. Bonnet, D. Gabillard, S. Domoua, C. Muzoora, E. Messou, S. Samreth, D. B. Nguyen, A. Badje, S. Juchet, B. Dim, L. Borand, N. Natukunda, T. Hong Tran, X. Anglaret, D. Laureillard, F.-X. Blanc. STATIS ANRS 12290 Trial Team
   Clinical Infectious Diseases, 2023, https://doi.org/10.1093/cid/ciad125.

6. **Inappropriate antibiotic prescribing and its determinants among outpatient children in 3 low- and middle-income countries: A multicentric community-based cohort study.**
   A. Ardillon, L. Ramblière, E. Kermorvant-Duchemin, T. Sok, A. Zafitsara Zo, J.B. Diouf, P. Long, S. Lach, F. Diene Sarr, L. Borand, F. Cheysson, J.M. Collard, P. Herindrainy, A. de Lauzanne, M. Vray, E. Delarocque-Astagneau, D. Guillemot, B.T. Huynh; BIRDY study group.
   PLoS Med. 2023 Jun 6;20(6):e1004211. doi: 10.1371/journal.pmed.1004211. eCollection 2023 Jun

7. **Malaria temporal dynamic clustering for surveillance and intervention planning**
   E. Legendre , L. Lehot, S. Dieng , S. Rebaudet, A.M. Thu, J. D Rae, G. Delmas, F. Girond, V. Herbreteau, F. Nosten, J. Landier, J. Gaudart..
   Epidemics . 2023 Jun;43:100682. doi: 10.1016/j.epidem.2023.100682. Epub 2023 Mar 27.

8. **Management of depression in people living wit HIV/AIDS in Senegal: Acceptability, feasibility and benefits of group interpersonal therapy.**
   C. Bernard, H. Font, S. Ziadeh, J.M. Tine, A. Diaw, I. Ndiaye, O. Samba, T. Bottai, L. Jacquesy, H. Verdeli, N.F. Ngom, F. Dabis, M. Seydi, N. de Rekeneire; IeDEA West Africa Cohort Collaboration.
   Glob Ment Health (Camb). 2023 Jun 30;10:e36. doi:10.1017/gmh.2023.31. eCollection 2023.

9. **Mosquito diversity (Diptera: Culicidae) and medical importance in four Cambodian forests.**
   A. Rakotonirina, P.O. Maquart, C. Flamand, C. Sokha, S. Boyer.
   Parasites & Vectors 2023-03-21 DOI : 10.1186/s13071-023-05729-w.

10. **Natural killer repertoire restoration in TB/HIV co-infected individuals experienced an immune reconstitution syndrome**
    P. Pean, Y. Madec, E. Nerrienet, L. Borand, D. Laureillard, M. Fernandez, O. Marcy, D. Scott-Algara; CAMELIA Study Team.
    Pathogens. 2023 Oct 13;12(10):1241. doi:10.3390/pathogens12101241.

11. **One assay to test them all: Multiplex assays for expansion of respiratory virus surveillance.**
    N. Boukli, C. Flamand, K. L. Chea, L. Heng, S. Keo, K. Sour, S. In, P. Chhim, B. Chhor, L. Kruy, Jelena D. M. Feenstra, Manoj Gandhi, Obiageli Okafor, Camilla Ulekleiv, Heidi Auerswald, Viseth Srey Horm and Erik A. Karlsson.
    Front. Med., 24 April 2023 doi.org/10.3389/fmed.2023.1161268

12. **Plugging the leaks: antibiotic resistance at human–animal interfaces in low-resource settings.**
    M. L. Nadimpalli, M. Stegger, R. Viau, V. Yith, A. de Lauzanne, N. Sem, L. Borand, B-T. Huynh, S. Brisse, V. Passet, S. Overballe-Petersen, M. Aziz, M. Gouali, J. Jacobs, T. Phe, B. A. Hungate, V.O. Leshyk, A. J. Pickering, F. Gravey, C.M. Liu, T.J. Johnson, S. Le Hello, L.B. Price.
    Frontiers in Ecology and the Environment, 2023, 21(9): 428–434, doi: 10.1002/fee.2639.

13. **Rabies surveillance in Madagascar from 2011 to 2021: can we reach the target?**
    S.F. Andriamandimby, M.H. Volasoa, N.P. Razafindraibe, D.B. Ranoaritiana, M.H. Razafindramparany, T. Rafisandratantsoa, L.A. Nomenjanahary, N. Rakotondrabe, M.A. Andriamananjara, H. Guis, V. Lacoste, A. Dreyfus.
    Front Vet Sci. 2023 Oct 12;10:1270532. doi: 10.3389/fvets.2023.1270532. eCollection 2023.

14. **Side-by-side comparative study of the immunogenicity of the intramuscular and intradermal rabies post-exposure prophylaxis regimens in a cohort of suspected RABV exposed individuals.**
    H. Auerswald, A. Maestri, S. Touch, S. In, N. Ya, B. Heng, V. Bosch-Castells, C. Augard, C. Petit, P. Dussart, Y. Peng, T. Cantaert, S. Ly.
    Clin Infect Dis. 2023 Jun 20;ciad304. doi: 10.1093/cid/ciad304

15. **Stillbirths and neonatal mortality in LMICs: A community-based mother-infant cohort study**
    L. Rambliere, A. de Lauzanne, J.B. Diouf, A.Z. Zo, M. Landau, P. Herindrainy, D. Hivernaud, F.D. Sarr, T. Sok, M. Vray, J.M. Collard, L. Borand, E. Delarocque-Astagneau, D. Guillemot, E. Kermorvant-Duchemin, BT. Huynh; BIRDY study group..
    J Glob Health. 2023 Apr 14;13:04031. doi: 10.7189/jogh.13.04031

16. **Traditional medicine consumption in postpartum for HBV-infected women enrolled in the ANRS 12345 TA PROHM study in Cambodia.**
    S. Moeung, F. Chassagne, S. Goyet, S. Nhoeung, L Sun, D. Yang, S. Vilhem, B. Dim, S. Ly, L. Sov, V. Sreng, S. Chorn, S. Chhun, L. Borand, S. Kim, O. Segeral. PLoS One. 2023 Aug 10;18(8):e0288389. doi: 10.1371/journal.pone.0288389. eCollection 2023.

17. **Uncovering the endemic circulation of rabies in Cambodia.**
    M. Layan, L. Dacheux, P. Lemey, K. Brunker, L. Ma, C. Troupin, P. Dussart, V. Chevalier, J. L. N. Wood, S. Ly, V. Duong, H. Bourhy, S. Dellicour.
    Molecular Ecology 04 August 2023 DOI :**10.1111/mec.17087**

18. **Vaccination coverage and risk factors associated with incomplete vaccination among children in Cambodia, Madagascar and Senegal.**
F. Verrier, A. de Lauzanne, J.B. Niokhhor Diouf, A.Z. Zo, L. Ramblière, P. Herindrainy, F.D. Sarr, T. Sok, M. Vray, J.M. Collard, L. Borand, E. Kermorvant-Duchemin, E. Delarocque-Astagneau, D. Guillemot, B.-T. Huynh; Bacterial Infections and Antibiotic-Resistant Diseases Among Young Children in Low-Income Countries (BIRDY) Study Group.
Open Forum Infect Dis. 2023 Mar 10;10(4):ofad136. doi: 10.1093/ofid/ofad136. eCollection 2023 Apr.

4.2.8 Oral Communications and Posters

1. **25 years of rabies prophylaxis post-exposition in Cambodia (1998 - 2022).**
Banza Kongolo H, Guis H, Chan M, Thai P, Thap V, Yin H, Flamand C, Ly S, Peng Y.
"Social-ecological approach of dog-associated zoonotic diseases"5th Veterinary Technology & Nursing International Seminar: Joint Symposium 5th KU-Vet Tech / TICA-Innovative Animal Health / ANR-SEAdogSEA and Conference on One Health; Kasetsart University, Veterinary Technology, Nursing, Environmental Health, and Scientific Integration 2023, Bangkok, Thailand, 13th-14th December 2023.

2. **Descriptive analysis of rabies post-exposition prophylaxis in Cambodia from 1998 to 2022.**
Banza Kongolo H, Guis H, Chan M, Thai P, Thap V, Yin H, Girond F, Ly S, Peng Y, Flamand C.
Poster, Séminaire de l'école doctorale E393, Saint Malo, France 5-7 février 2024.

3. **Estimating rabies post-exposure prophylaxis delivery capacity and knowledge, attitudes and practices towards rabies among health staff in Cambodia – preliminary results.**
Guis H, Kaing S, Leng C, Kong P, Sendoeurn Y, Peng Y, Ly S, Flamand C.
"Social-ecological approach of dog-associated zoonotic diseases" 5th Veterinary Technology & Nursing International Seminar: Joint Symposium 5th KU-Vet Tech / TICA-Innovative Animal Health / ANR-SEAdogSEA and Conference on One Health; Kasetsart University, Veterinary Technology, Nursing, Environmental Health, and Scientific Integration 2023, Bangkok, Thailand, 13th-14th December 2023.

4. **Impact of tuberculosis and other factors on mortality in children admitted with severe pneumonia in sub-Saharan Africa and South-East Asia: TB-SPEED pneumonia study.**
E. Komena, M. Nguyet, m. itchy, M. Cardena, L. Borand, C. Chabala, C. Khosa, J.-V. Taguebue, E. Wobudeya, O. Marcy, F.D.A. Tanoh, R. Moh.
The Union Meeting, Paris, France, November 16-19, 2023.

5. **Introducing a social-ecological approach of dog-borne diseases in SE Asia.**
De Garine-Wichatitsky M, Artama WT, Ly S, Guis H, Karnchanabanthoeng A, Michaux J, Morand S.
"Social-ecological approach of dog-associated zoonotic diseases" 5th Veterinary Technology & Nursing International Seminar: Joint Symposium 5th KU-Vet Tech / TICA-Innovative Animal Health / ANR-SEAdogSEA and Conference on One Health; Kasetsart University, Veterinary Technology, Nursing, Environmental Health, and Scientific Integration 2023, Bangkok, Thailand, 13th-14th December 2023.

6. **Knowledge, attitudes and practices towards dogs and dog-related diseases in two Cambodian villages.**
Nicolas A, Sorn S, Van Leeuwen P, Chhay S, Auerswald H, Michaux J, Ly S, De Garine Wichatitski M, Guis H.
"Social-ecological approach of dog-associated zoonotic diseases" 5th Veterinary Technology & Nursing International Seminar: Joint Symposium 5th KU-Vet Tech / TICA-Innovative Animal Health / ANR-SEAdogSEA and Conference on One Health; Kasetsart University, Veterinary Technology, Nursing, Environmental Health, and Scientific Integration 2023, Bangkok, Thailand, 13th-14th December 2023.

7. **One Health characterization of leptospirosis transmission in humans, animals and the environment: a systematic review with meta-analysis.**
   Antoniolli A, Guis H, Goarant C, Flamand C.
   Poster, Séminaire de l'école doctorale E393, Saint Malo, France, 5-7 février 2024.

8. **Optimizing tuberculosis preventive treatment initiation among people with HIV in Cambodia: results of the OPTICAM cluster randomized trial.**
   L. Borand, V. Ouk, M. Huyen Ton Nu Nguyet, S. Chay, B. Dim, V. Sreng, S. Nhoeung, K. Seak, D. Laureillard, K.E. Khun, O. Marcy, C.Y.Huot.
   The Union Meeting, Paris, France, November 16-19, 2023.

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) Investigation of immunopathological mechanisms of mosquito-borne infections, (2) identification of new immune-related biomarkers for infectious diseases, and (3) development of new vaccination strategies for neglected tropical diseases. Moreover, we are hosting a transversal single cell analysis platform.

4.3.1 Functional Structure

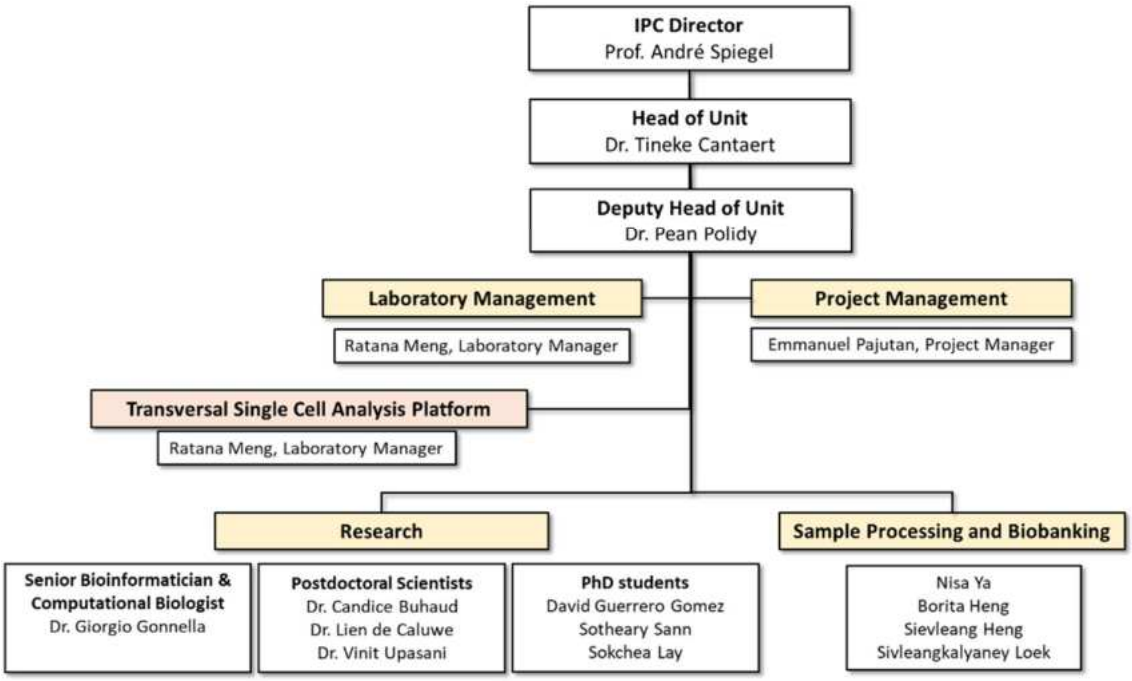


Figure 8: Immunology Unit Organizational Chart

4.3.2 Research Programs – Major Achievements in 2023

Axis 1: Investigation of immunopathological mechanisms of mosquito-borne infections.

1.1. Determining of disease mechanisms leading to severe dengue in secondary dengue-infected cases

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.

Assessment of the interaction between IgG Fc and FcgR during dengue infection.

Although antiviral antibodies generally confer protective functions, antibodies against dengue virus are associated with enhanced disease susceptibility. In previous work, we showed that neither antibody titers nor neutralizing activity correlated with disease severity in dengue-infected populations. Rather, dengue infection induced a specific increase in immunoglobulin G1 afucosylation, and the levels of afucosylated IgG1 were predictive of dengue disease severity. Moreover, increased IgG1 afucosylation levels correlated with increased hematocrit and decreased platelet counts, 2

hallmarks of dengue disease severity. (Bournazos et al, Science 2021). In the continued collaboration with Rockefeller University, USA, we now assessed the in vivo mechanisms of antibody-mediated dengue pathogenesis. we discovered that the pathogenic activity of anti-DENV antibodies is exclusively mediated through engagement of FcγRIIIa on splenic macrophages, resulting in inflammatory sequelae and mortality. These findings highlight the importance of IgG–FcγRIIIa interactions in dengue, with important implications for the design of safer vaccination approaches and effective therapeutic strategies (Yamin et al, Nature Microbiol 2023).

Start/End Year	2023-2028
Collaborations	Virology Unit, IP Cambodia (Duong V), Rockefeller University (Ravetch J, Bournazos S)
Funding	Calmette-Yersin Pasteur Network Postdoctoral Research Grant 2023-2024 R01

Evaluation of regulatory T cells in dengue

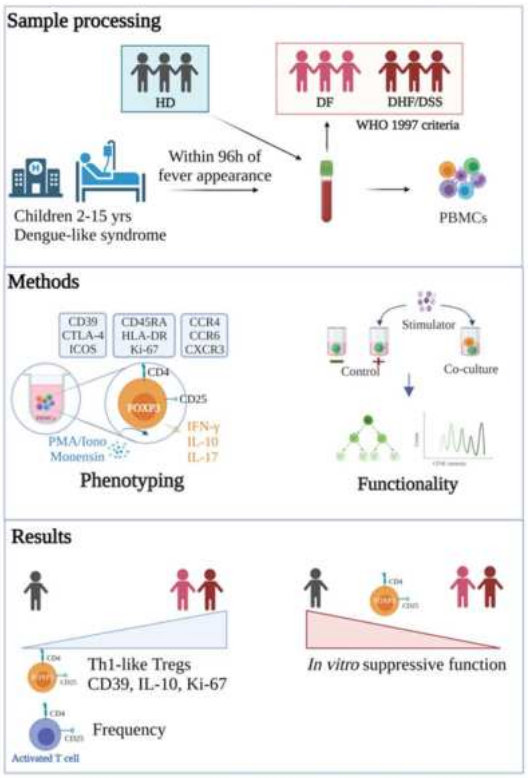


Figure 9: Graphical abstract. Phenotypical and functional analysis of regulatory T cells in dengue-infected patients uncovers alterations in Treg composition and suppressive capacity on dengue patients. (Sann et al, submitted)

Regulatory T cells (Tregs) are essential in maintaining immune homeostasis and peripheral tolerance. In the context of infectious diseases, Tregs have both beneficial and harmful effects: while they dampen excessive immune activation, they also can suppress beneficial antigen-specific immune responses. Given the current increased understanding of different Treg subsets and their functional properties, we aimed to define in detail the phenotype and function of different Treg subsets and their association with disease severity in a unique cohort of more than forty Cambodian children undergoing an acute secondary dengue infection with variable disease outcome. We show that Tregs are expanded and are highly proliferative during dengue infection compared to Tregs obtained from age-matched healthy controls. Tregs are skewed to a Th1-like Treg phenotype and are highly activated. In agreement, Tregs from dengue patients produce more pro-inflammatory cytokines compared to age-matched healthy donors. In accordance with these phenotypic characteristics, Tregs from dengue patients have less suppressive capacity as observed in an *in vitro* Treg suppression assay. Taken together these data suggest that Tregs fail to resolve ongoing inflammation during dengue infection and hence contribute to the immunopathology of severe dengue disease (Figure 9). Given the complexity of dengue immunopathology and the development of exacerbated immune responses in severe dengue, these data clarify the role of regulatory T cells in dengue immunopathogenesis (Sann et al, submitted).

Start/End Year	2019-2024
Collaborations	University of Hasselt, Belgium (Kleinewietfeld M)
Funding	Wellcome Trust/HHMI International Research Scholar

Centers for Research in Infectious Diseases (CREID) network of the Pasteur Institute (PICREID)

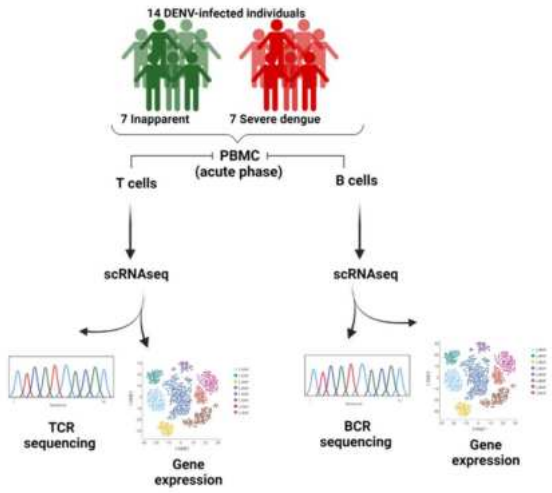


Figure 10: Summary of the experiments performed to uncover novel mechanisms of pathogenesis related to adaptive immune cells in dengue. Single cell RNAsequencing was performed on purified B and T cell subsets of dengue patients with different disease severity.

IP Cambodia is part of the NIH funded consortium PI-CREID. Here, the Immunology Unit is involved in the 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. For this study, we performed single cell RNA sequencing (scRNAseq) of isolated B and T cells from dengue patients with either hospitalized dengue or inapparent dengue disease (Figure 10). We coupled this to B cell receptor (BCR) and T cell receptor (TCR) analysis. We identified a subset of metabolically active and proliferating CD8 T cells, which we poise to be antigen-specific cells. These antigen-experienced, CD8 T cells have a T effector memory phenotype with decreased cytotoxic potential in hospitalized patients compared to inapparent cases. In addition, hospitalized dengue patients are characterized by decreased frequencies of CD4+ T effector memory T cells showing decreased activation, a decreased interferon signature, and decreased expression of anti-inflammatory regulators. The analysis of TCR and BCR gene usage is ongoing.

Start/End Year	2020-2025
Collaborations	Virology Unit, IP Cambodia (Duong V), Epidemiology and Public Health Unit, IP Cambodia (Ly S), Medical and Veterinary Entomology Unit, IP Cambodia (Boyer S), Institut Pasteur Paris (Sakuntabhai A, Simon-Loriere E, Han M)
Funding	NIH PICREID (1U01AI151758 – 01): 2020-2025

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. In 2022, we assessed the cutaneous innate and adaptive immune responses via controlled *Aedes aegypti* feedings in humans living in an Aedes-endemic country (Guerrero, Vo et al, Nat Comm 2022). In a follow up study of this work, our objective was to gain a deeper insight into the impact of different strains of *Aedes* mosquitoes and DENV on the local human skin immune response, particularly in individuals residing in regions where *Aedes* mosquitoes and dengue are prevalent. To accomplish this, leftover cutaneous tegument specimens

from eye-lid aesthetical surgery were used to isolate primary human skin cells. We examined changes in the activation and differentiation markers of these skin immune cells when exposed to mosquito salivary gland (SGE) extract in presence or absence of dengue. Our findings revealed that exposure to *Aedes* mosquito saliva led to significant changes in the characteristics of immune skin cells. These changes included a notable increase in the number of monocytes expressing the CCR2 marker, a higher proportion of neutrophils expressing CD69, and a substantial increase in the proportion of infected monocytes isolated from the human skin biopsies (Guerrero et al, submitted).

Start/End Year	2020-2024
Collaborations	Medical and Veterinary Entomology Unit, IP Cambodia (Boyer S), Laboratory of Malaria and Vector Research, NIAID, National Institutes of Health (NIH) (Manning J, Oliveira F), MiVEGEC Unit, IRD, UR224 (MISSE D)
Funding	Calmette-Yersin Pasteur Network PhD grant. 2020-2023

1.3. Understanding of immune correlates of protection to Plasmodium vivax malaria

Plasmodium vivax (Pv) is the most widespread human malaria parasite and is particularly resilient to current elimination efforts. The development of a blood-stage vaccine would be a game-changer for Pv control. Naturally acquired immunity to Pv has been observed, where exposed subjects can develop partial immunity after multiple exposures to Pv enabling the control of parasite densities. However, life-long exposure rarely seems to confer sterile immunity and individuals can carry low-level parasitemia. Increased understanding of the immunological mechanisms, potential antigenic targets and parasite factors that confer protection to clinical Pv malaria is urgently needed for future vaccine development. Leveraging a longitudinal cohort, we have constituted in endemic area of Cambodia in collaboration with the Malaria Research Unit, we have identified individuals displaying remarkable clinical protection against Pv. In this cohort, we aim to correlate protection to phenotypic and functional alterations of the immune response. We are currently performing single cell RNA sequencing to uncover changes in cell populations, in depth flow cytometry analysis and in vitro functional assays with short term cultures of Pv parasite-infected reticulocytes.

Start/End Year	2023-2028
Collaborations	Malaria Research Unit (Popovici J)
Funding	NIH R01

1.4. Pasteur International Unit

Understanding the detailed interactions between pathogens and the immune system and determining the correlates of protection versus pathology are critical for understanding and controlling infectious diseases and pave the way for the development of new diagnostics, vaccines, therapeutics, or innovative infection-reduction strategies. To address current public health challenges and anticipate those that may arise in the future (Disease X), the global objective of this proposal is to build a sustainable immunology research ecosystem in the African (Senegal and Madagascar) and South-East Asian (Cambodia) Pasteur Institutes around COVID-19 and dengue research projects. The creation of the PIU was approved in 2023 and the PIU agreement will be signed in 2024.

Start/End Year	2023-2029
Collaborations	Immunopathophysiology and infectious diseases dpt, Institut Pasteur de Dakar (VIGAN-WOMAS I) Immunology and Infectious Disease Unit, Institut Pasteur de Madagascar (Schoenhals M)
Funding	Institut Pasteur du Cambodge/Pasteur Network

Axis 2: Biomarkers of Infectious Diseases

2.1. Agent National de Recherche sur le SIDA/les hépatites virales et les maladies infectieuses d'émergence (ANRS) No 12358 : "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 (has-mir-146a-5p, has-mir-29c-3p and has-mir-29a-3p) can be used as the biomarker to differentiate IRIS from non-IRIS before any treatment initiation and predict the occurrence of IRIS. Patent No: NT/ NG/IDM-22-0055.

Start/End Year	2017-2019
Collaborations	IP Paris (Scott D, Madec Y), IP Cambodia (Borand L), Center of Hope, Cambodia (Pichsovannary S)
Funding	ANRS-MIE/INSERM

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 manuscript is submitted in December 2023.

Start/End Year	2019-2021
Collaborations	Univ. Paris 7 Diderot, Paris, France (Weiss Laurence), PACCI, Ivory Coast (Moh R), IPC Cambodia (Borand L), Center of Hope, Cambodia (Pichsovannary S)
Funding	ANRS-MIE/INSERM

2.3 Characterization of soluble TLR2 and CD14 levels during acute dengue virus infection

Dengue virus infection results in a broad spectrum of diseases ranging from mild dengue fever (DF) to severe dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Hitherto, there is no consensus biomarker for the prediction of severe dengue disease in patients. Yet, early identification of patients who progress to severe dengue is pivotal for better clinical management. We have recently reported that an increased frequency of classical (CD14 ++CD16-) monocytes with sustained high TLR2

expression in acutely infected dengue patients correlates with severe dengue development. Here, we hypothesized that the relatively lower TLR2 and CD14 expression in mild dengue patients is due to the shedding of their soluble forms (sTLR2 and sCD14) and that these could be used as indicators of disease progression. Therefore, using commercial sandwich ELISAs, we evaluated the release of sTLR2 and sCD14 by peripheral blood mononuclear cells (PBMCs) in response to in vitro dengue virus (DENV) infection and assessed their levels in acute-phase plasma of 109 dengue patients. We show that while both sTLR2 and sCD14 are released by PBMCs in response to DENV infection in vitro, their co-circulation in an acute phase of the disease is not always apparent. In fact, sTLR2 was found only in 20% of patients irrespective of disease status. In contrast, sCD14 levels were detected in all patients and were significantly elevated in DF patients when compared to DHF patients and age-matched healthy donors. Altogether, our results suggest that sCD14 may help in identifying patients at risk of severe dengue at hospital admittance. (Upasani et al, Heylion, 2023)

Start/End Year	2020-2023
Collaborations	University of Groningen (Rodenhuis-Zybert I)
Funding	Calmette Yersin PhD grant

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.

In a follow up study, we compared the development of protective neutralizing antibodies and the development of RABV-specific CD4+ and CD8+ T cells in this shortened IPC protocol compared to the intramuscular (IM) 1-site, 4-visit vaccination protocol (4-dose Essen regimen). We analyzed the development of neutralizing antibodies (nAbs) by fluorescent antibody virus neutralization test (FAVNT) and rapid fluorescent focus inhibition test (RFFIT), and the T cell response to a glycoprotein-derived peptide pool at baseline and at 14 and 28 days after post-exposure prophylaxis (PEP) in 210 patients with a category II or III animal exposure in a rabies-endemic country receiving either ID (n=112) or IM (n=98) PEP regimen. At day 28, all study participants seroconverted for rabies nAbs (≥0.5 IU/mL), irrespective of PEP scheme, age, or administration of rabies immunoglobulin (RIG) and nAb titers did not differ for the two PEP schemes. Both PEP schemes induced a RABV-specific T cell response (measured 28 days after the first dose), which was polyfunctional and did not differ by PEP scheme. In conclusion, we demonstrated under real-life PEP conditions in a direct comparative trial that the 1-week ID IPC regimen course is as sufficient in inducing an anti-rabies immune response as a 2-week IM 4-dose Essen regimen (Maestri, Auerswald et al, CID, 2023)

Start/End Year	2019-2023
Collaborations	Virology Unit, IP Cambodia (Duong V), Epidemiology and public Health Unit, IP Cambodia (Ly S), International Vaccination Center, IP Cambodia (Peng Y)
Funding	IPC internal project

4.3.3 Research Programs – Outlook 2024

Axis 1: Investigation of immunopathological mechanisms of mosquito-borne infections.

1.1. Determining of disease mechanisms leading to severe dengue in secondary dengue-infected cases

Humoral immune responses to dengue virus infection.

The Envelope (E) protein of DENV is known as the major target of antibody (Ab) response. Anti-Envelope domain III Abs (anti-EDIII Ab) are mainly serotype-specific, whereas anti-fusion loop (FL) Abs can induce antibody dependent enhancement (ADE) in vitro. Abs targeting the E-dimer epitope (EDE) are potent and broadly neutralizing against all four dengue serotypes. Resolving the complex polyclonal response to E protein might uncover novel correlates of protection after infection. Therefore, we aim to set up a new assay to detect and quantify E protein-epitope specific Abs in plasma obtained from DENV infected humans by multiplex immunoassay (MIA). Next, we aim to correlate levels of anti-EDIII, anti-E dimer and anti-fusion loop antibodies with disease outcome and follow the development of these antibodies over time.

Start/End Year	2017-2025
Collaborations	Virology Unit, IP Cambodia (Duong V), Institut Pasteur Paris (Barba-Spaeth G, Rey F), Antwerp University (Arien K)
Funding	NIH PICREID (1U01AI151758 – 01): 2020-2025 Calmette-Yersin Pasteur Network Postdoctoral Research Grant 2023-2024

Assessment of the interaction between IgG Fc and FcgR during dengue 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. We have recently shown that DENV infection causes a specific increase in afucosylated IgG glycoforms, which correlates with disease severity and has prognostic potential (Bournazos et al, Science 2021). Hence, we identified a key role for the Fc glycan structure in dengue pathogenesis, but the mechanism underlying this observation remains to be determined. Different IgG Fc glycoforms have different affinities for Fc gamma receptors, which activate and initiate downstream effector functions. One of these mechanisms crucially dependent on IgG Fc-Fc gamma receptor interactions is antibody-dependent enhancement (ADE). Therefore, we seek to investigate how DENV infection modulates the glycosylation profile of IgG Fc and how IgG Fc-Fc gamma receptor interactions contribute to disease outcome after dengue infection. DENV could modulate the IgG1 glycosylation profile either by eliciting distinctive inflammatory cues to B cells or through direct infection of B cells. Dynamic changes in glycoenzyme expression over time will be assessed and in vitro conditions and pathways that lead to changes in glycoenzyme expression will be identified. 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.

Start/End Year	2023-2028
Collaborations	Virology Unit, IP Cambodia (Duong V), Rockefeller University (Ravetch J, Bournazos S)
Funding	Calmette-Yersin Pasteur Network Postdoctoral Research Grant 2023-2024 NIH R01

Centers for Research in Infectious Diseases (CREID) network of the Pasteur Institute (PICREID).

In the last year of the grant, we aim to discover new anti-dengue antibodies with high cross-neutralization potential for dengue. We have established 2 pipelines for the discovery of novel potent anti-dengue antibodies (Figure 11).

Given the low frequency of antigen-specific B cells and the high cost of the scRNAseq, we have optimized a patient-barcoded approach, which allows to pool multiple individuals before the 10x single cell analysis pipeline. B-cell receptor sequences showing a high clonal expansion profile will be selected for small-scale in-house mAb production to dengue E-dimer antigens. mAbs will be further characterized for breath, potency and effector function capacity. Secondly, we have established an additional mAb production pipeline involving single B cell cultures to enhance the efficient down-selection of highly functional mAbs. Here, single E-dimer-specific B cells are sorted and cultured. Supernatants will be screened for IgG production, E-dimer binding and functionality. Strong serotype cross-neutralizers and/or strong inducers of antibody effector functions will be selected for in house recombinant mAb production and further characterization. Stabilized E-dimer antigens are obtained from F.Rey and G. Barba-Spaeth from Institut Pasteur Paris.

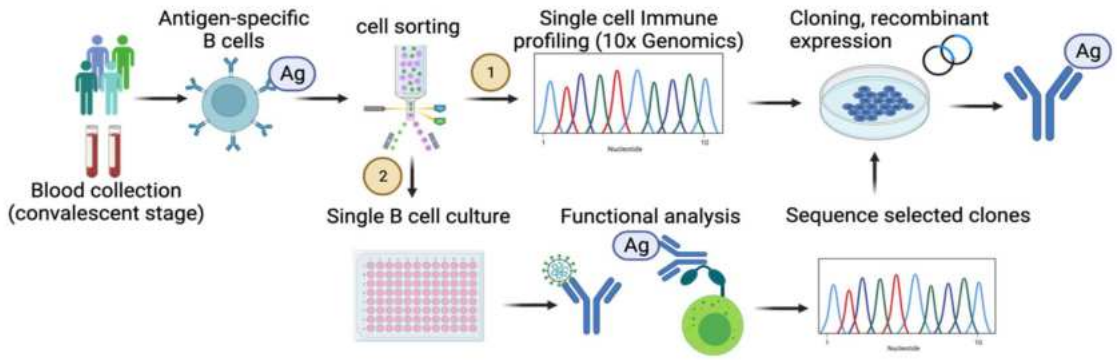


Figure 11: Experimental pipeline for the production of mAbs.

Start/End Year	2020-2025
Collaborations	Virology Unit, IP Cambodia (Duong V), Epidemiology and Public Health Unit, IP Cambodia (Ly S), Medical and Veterinary Entomology Unit, IP Cambodia (Boyer S), Institut Pasteur Paris (Sakuntabhai A, Simon-Loriere E)
Funding	NIH PICREID (1U01AI151758 – 01): 2020-2025

1.2. 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 continue our collaboration with the LMVR, NIAID, NIH to further assess the immune responses to mosquito saliva in the skin of Cambodian individuals with a new clinical trial design.

Start/End Year	2023-2026
Collaborations	Medical and Veterinary Entomology Unit, IP Cambodia (Boyer S), Laboratory of Malaria and Vector Research, NIAID, NIH (Oliveira F)
Funding	NIH

1.3. Understanding of immune correlates of protection to *Plasmodium vivax* malaria

Plasmodium vivax (Pv) is the most widespread human malaria parasite and is particularly resilient to current elimination effortsonly a minority of infected individuals develop naturally-acquired antibodies inhibiting the binding of Pv Duffy binding protein (DBP) to the Duffy receptor (binding inhibitory antibodies, BIabs). The mechanisms underlying the acquisition of BIabs are unknown. Individuals not producing BIabs can still be protected against Pv clinical malaria indicating that additional immunological and/or genetic factors can confer protection. Increased understanding of the immunological mechanisms and potential antigenic targets that confer protection to clinical Pv malaria is urgently needed for future vaccine development. We aim to understand the mechanisms driving the development of BIabs against PvDBP in humans. Phenotype, gene expression profile and B cell receptor (BCR) characteristics of DBP-specific memory B cell subsets will be assessed by flow cytometry and combining single-cell RNA and BCR sequencing in naturally infected participants with characterized amounts of BIabs. DBP-specific CD4+ T subsets will be interrogated for function and phenotyped by flow cytometry. In addition, the function of anti-DBP antibodies will be assessed by various cell-based effector function assays using merozoites as target. In addition, we aim to identify host immune factors associated to protection from clinical P vivax malaria independent of Blab development. To have a better understanding of the immunological factors associated to protection from Pv, we will study the single cell landscape of the immune response in individuals displaying various levels of protection against Pv malaria. Moreover, functional *in vitro* studies using sorted primary cell populations from these individuals will be employed to assess the function of monocytes, T and B cell subsets in Pv infection.

Start/End Year	2023-2028
Collaborations	Malaria Research Unit, IP Cambodia (Popovici J) University of Maryland (Serre G)
Funding	NIH – R01

1.4. Development of monoclonal antibodies to Crimean-Congo hemorrhagic fever virus (CCHFV)

The CCHFVACIM project is an ambitious collaborative effort aimed at developing both prophylactic and therapeutic effective countermeasures against Crimean Congo Haemorrhagic Fever Virus (CCHFV), one of the most threatening vector-borne pathogens, widely distributed. Deep structural biology studies on viral glycoproteins and investigation of the immunogenicity of the viral antigens will be combined with optimization of an mRNA vaccine candidate against the virus and characterisation of the resulting protective immunity, as well as with the development of immunotherapeutic monoclonal antibodies (mAbs) based on CCHFV's antigenic targets. The role of the Immunology Unit in this project is to produce highly neutralizing antibodies directed to the recombinant glycoprotein of CCHFV using our single-cell culture approach. At the same time, the B cell receptor (BCR) repertoire of Gn/Gc-specific B cells will be interrogated and monoclonal antibodies will be produced for functional and structural analysis.

Start/End Year	2024-2028
Collaborations	Institut Pasteur (Rey F) Karolinska Institute (Mirazimi A)
Funding	EU HORIZON-HLTH-2023-DISEASE-03 -CCHFVACIM

1.5. Pasteur International Unit

The PIU-Immuno will be leading multidisciplinary studies in these three uneven contexts (different genetic background, population immunity and demographic structure) to understand in detail the human T and B cell responses to SARS-CoV-2 Variants of Concern (VOCs) and the different dengue serotypes. The PIU aims to describe population immunity status against SARS-CoV-2 VOC and flaviviruses, discover immune response signatures that can predict clinical outcome of dengue or SARS-CoV-2 infection, identify host factors that drive immunopathology and aims to develop cross-neutralizing antibodies. We will capitalize on our access to unique collections of well documented human clinical samples and cutting-edge technologies (flow cytometry, single cell sorting, single cell RNA sequencing) already in place. The long-term vision of our PIU is to exploit immunological investigations to improve health outcomes in Africa, Indian Ocean and SEA and to leverage the critical mass of the next generation of internationally competitive immunologists. Standardised protocols will be shared between the different study sites and an intercountry training program will be set up to enable local and sustainable capacity building.

Start/End Year	2023-2029
Collaborations	Immunopathophysiology and Infectious Diseases Dpt, Institut Pasteur de Dakar (Vigan-Womas I) Immunology and Infectious Disease Unit, Institut Pasteur de Madagascar (Schoenhals M)
Funding	Institut Pasteur du Cambodge/Pasteur Network

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). The intermediate results submitted for evaluation to DARRI and Institut Carnot Pasteur MS.

Start/End Year	2022-2023
Collaborations	IP Paris (Scott D), IP Cambodia (Pean P), IP Cambodia (Borand L)
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 2024 are (1) a grant proposal to *Programme Transversal de Recherche* 2023 (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 (a nested sub-study in DATURA 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). Preliminary, 18 serum PCR positive of respiratory viral (n=12) and respiratory bacterial infection (n=8) were tested with 17 circulating microRNA related to innate immune responses. We found a higher expression of serum microRNAs in respiratory viral infected children versus respiratory bacterial infected (figure). Collaboration with Dr. Karlsson (IPC virology unit). Next, proposal to internal fund or AMR consortia fund in 2024) (5) biomarkers of Schistosomiasis infection,

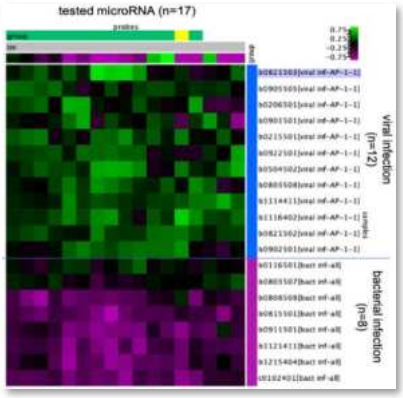


Figure 12: Heatmap displays the expression levels of serum microRNA (fold change MFI) in viral

Start/End Year	2023-2025
Collaborations	IP Rome (Scagnolari C), Virology Unit, IP Cambodia (Karlsson E), CNM/MoH (Virak K)
Funding	To be identified

Axis 3: Vaccine Responses to Rabies Virus Vaccination

We aim to continue our long-term follow up of individuals bitten by rabid suspected dogs receiving the IPC PEP regimen. Individuals reach the 3 years follow up period in 2024. Rabies virus neutralizing antibodies and rabies-specific T cells will be determined.

Start/End Year	2020-2024
Collaborations	Virology Unit, IP Cambodia (Duong V), Epidemiology and Public Health Unit, IP Cambodia (Ly S), International Vaccination Center, IP Cambodia (Peng Y)
Funding	Internal IPC project: 2020-2024

Axis 4: Implementation of flow cytometry immune-phenotyping for acute leukemia and chronic lymphoproliferative disorder (CLPD).

The aim of the study is to improve diagnosis of acute leukemia and CLPD in Cambodia. Therefore, we will demonstrate the feasibility and standardization of flow cytometry technique for cell lineage identification in acute leukemia and chronic lymphoproliferative disorders (CLPD) pediatric patients (n=100). We use IVD BD™ ALOT and LST commercial kits for cell lineage assessment and clonotype determination, respectively. Data acquisition and analysis perform using BD FACS Canto II flow cytometer. The specimen are bone marrow aspirate (BMA) and peripheral venous blood (PB) of sick

children admitted in Kantha Bopha hospital. From March to December 2023, BMA and PB specimens of 32 children clinically suspected acute leukemia are analyzed. The majority of suspected acute leukemia specimen are associated with bi-phenotypic of B cells with myeloid cells ALL (n=18); early precursor T cells with myeloid cells lineages ALL (n=7); B cell lineage ALL (n=3) and acute myeloid leukemia (n=4).

Start/End Year	2022-2023
Collaborations	Medical Biology Laboratory, IP Cambodia (Guillard B), Kantha Bopha Children Hospital (Laurent D)
Funding	Internal IPC project: 2022-2023

4.3.4 Support to National Authorities

Tineke Cantaert, PhD and Polidy 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 Université Paris Saclay, Paris, France).

4.3.5 Teaching and Training

PhD students

- Sann Sotheary: University of Hasselt, Belgium (2019-2024). Visits to U Hasselt are covered by a BOF/BILA grant of the Flemish Government
- Guerrero-Gomez David: University of Montpellier, France (2020-2023)
- Lay Sokchea: Antwerp University, Belgium (2023-2025)

Internship/Master students

- Chyvorn Nob: Royal University of Phnom Penh (2023), Bachelor thesis, Characterization of the Anti-Envelope Protein Antibody Response in Dengue Virus Infection
- Chany Kong: Royal University of Phnom Penh (2023), Bachelor thesis, On The Effects of Mosquito Salivary Gland Extract in DENV Infected Cells' Activation Phenotype and Virus Replication
- Laurie Euvrard: Institut Sup Biotech, Paris Master 1 Internship, Detection of antigen-specific B lymphocytes: application to dengue virus. Supported by "Fondation Pierre-Ledoux pour la jeunesse international"
- Wajed Shah: University Paris-Saclay (2023), Master 1 Internship, Optimization of monoclonal antibody production capcity: applications to dengue virus
- Clementine Roux: University Paris-Saclay (2023), Master 1 Internship, Identification of the stimuli altering IgG glycosylation during dengue virus infection
- Nisa YA: University Paris-Saclay, Phnom Penh (2023), Master 2 Internship, The Evaluation of T cell Responses to Rabies Vaccination in Patients Receiving the WHO 2018 Recommended Rabies Post-Exposure Prophylaxis (PEP)

Teaching

- Tineke Cantaert, PhD and Polidy Pean, MD, PhD: Master Infectious Diseases, University Paris-Saclay-University of Health Sciences, 10 hours (M1 and M2)
- Tineke Cantaert, PhD and Polidy Pean, PD, PhD: Member of the Steering Committee International Master Infectious Diseases and coordinator of the Immunology Module (M1).

- Tineke Cantaert, PhD: Master Immunology-ImmunoPathology, Sorbonne University, 2 hours (M2)

Course on flow cytometry and applications

In September 2023, the Immunology Unit held its first 6-day course on "Flow cytometry and applications". The course was funded by Wellcome Trust, the Pasteur Network and the PICREID program. The course on Flow Cytometry and Applications was a hands-on practical course with small groups of participants. The course emphasized on the principles of flow cytometry such as standardization and calibration of the machine, routine daily use and maintenance, parameter setting and compensations for measurements. In addition, it will discuss optimal panel design, data analysis and both research and clinical analysis applications. Out of forty-six (46) applicants, sixteen (16) were selected to attend the course. There were four (4) Cambodian students and twelve (12) international students coming from Centre Pasteur de Cameroon, St. Luke's Medical Center in the Philippines, Pasteur Institute of Cote d'Ivoire, Institute Pasteur de Dakar, Chulalongkorn University in Thailand, Institute Pasteur Korea, Pasteur Institute of Iran, Institut Pasteur de Tunis, Institut Pasteur de Madagascar, Oxford University Clinical Research Ho Chi Minh, and UKM Medical Molecular Biology Institute in Malaysia. Two faculties were invited from Institut Pasteur de Tunis and Institut Pasteur de Madagascar.

4.3.6 Outlook

The strengths of the immunology unit in infectious diseases research are 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. 2023 is especially marked by the organization of a course "Flow cytometry and applications", and by the training of 6 students, of which 50% were of Cambodian nationality. We continue to emphasize collaborative work in an environment of cultural diversity. We aim to create an educational learning environment for students, PhD students and postdoctoral researchers with weekly laboratory meetings and monthly journal clubs. A skilled bioinformatician has been hired who is increasing our capacity for -omics and big dataset analysis, and who is committed to transfer his knowledge to Cambodian scientists.

Within the Pasteur network, we have formed a Pasteur International Unit with the Immunology Units of IP Madagascar and IP Dakar, respectively, to increase the visibility of Immunology in the Pasteur Network. In 2023, two innovative grant proposals have been awarded (NIH R01) to continue our work on the role of FcgR in dengue disease and to initiate a new project on *Plasmodium vivax* malaria. Moreover, multiple proposals were submitted to broaden the scope of the Unit to additional arbovirus infections (eg. Crimean Congo hemorrhagic fever) or other viral infections of high prevalence in Cambodia (eg. EV71). In the upcoming years, we aim to create a platform within the Unit for monoclonal antibody production and immunomonitoring.

4.3.7 Scientific Publications 2023

1. **Characterization of soluble TLR2 and CD14 levels during acute dengue virus infection.**
   Upasani V, Ter Ellen BM, Sann S, Lay S, Heng S, Laurent D, Ly S, Duong V, Dussart P, Smit JM, Cantaert T, Rodenhuis-Zybert IA.
   Heliyon. 2023 Jun 21;9(6):e17265. doi: 10.1016/j.heliyon.2023.e17265. eCollection 2023 Jun. PMID: 37416678

2. **Human FcγRIIIa activation on splenic macrophages drives dengue pathogenesis in mice.**
   Yamin R, Kao KS, MacDonald MR, Cantaert T, Rice CM, Ravetch JV, Bournazos S.
   Nat Microbiol. 2023 Aug;8(8):1468-1479. doi: 10.1038/s41564-023-01421-y. Epub 2023 Jul 10. PMID: 37429907

3. **Side-by-side Comparative Study of the Immunogenicity of the Intramuscular and Intradermal Rabies Post-exposure Prophylaxis Regimens in a Cohort of Suspected Rabies Virus Exposed Individuals.**
   Auerswald H, Maestri A, Touch S, In S, Ya N, Heng B, Bosch-Castells V, Augard C, Petit C, Dussart P, Peng Y, Cantaert T, Ly S.
   Clin Infect Dis. 2023 Sep 18;77(6):910-916. doi: 10.1093/cid/ciad304. PMID: 37337899

4.4 Virology Unit

4.4.1 Functional Structure of the Unit

For over 25 years, the Virology Unit (the Unit) is committed to conduct biomedical research and surveillance, and contributes to the prevention and control in infectious diseases in Cambodia, in Southeast Asia, and around the globe. These activities comprise four main components, 1) arboviruses (e.g. dengue, Zika, chikungunya and Japanese encephalitis viruses), 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) Rabies and other viruses (enteroviruses, hepatitis viruses, 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 structured in three components: 1. Laboratory Management; 2. Technical Groups and 3. Research and Surveillance Teams (Figure 13).

In 2023, the unit comprises 48 staff including 2 Post-Doc, 7 PhDs, 5 PhD candidates, 5 master's degree holders, 4 master students, 2 medical doctors, 1 pharmacist and 22 technical staff. The ratio female/male was 1.53 and there are 10 nationalities including 37 Cambodian nationality.

In 2023, the Unit welcomed three scientists from IP Shanghai, Dr. Chung Kei Wong, Dr. Kathrina Mae Bienes Ulilang and Mr. Victor Omondi. They brought some expertise in CRISPR-Cas molecular tools, vaccine development, and other skills in metagenomic sequencing and field work experiences to broaden to research scope to the Unit. In addition, Dr. Anna Signe Fomsgaard joined the unit to work with Dr. Erik Karlsson to assist in developing and implementing novel, innovative methods for field-based diagnostics, identification and characterization of infectious diseases to improve emerging infectious diseases research and surveillance in Cambodia.

Within the research and surveillance groups, the Virology Unit has developed numerous research programs conducted in collaboration with other units at IPC—the Epidemiology and Public Health Unit (EPH), the Entomology Unit, and the Immunology Unit—as well as with governmental partners from the Ministry of Health (Cambodian CDC-MoH, National Center for Parasitology, Entomology and Malaria Control, National Institute of Public Health), the Ministry of Agriculture, Forestry and Fisheries (National Animal Health and Production Research Institute, General Directorate of Animal Health, Production and Forestry Administration), the Ministry of Environment (Department of Wildlife) and other collaborators across the globe. These programs focus on infectious diseases of concern to the Cambodian population.

The unit provide diagnosis of arboviruses and rabies support to the national control programs under the MoH and host 3 national and international reference laboratories, including the National Influenza Center, the WHO's H5 Reference Laboratory, and a WHO Global COVID-19 Reference Laboratory.

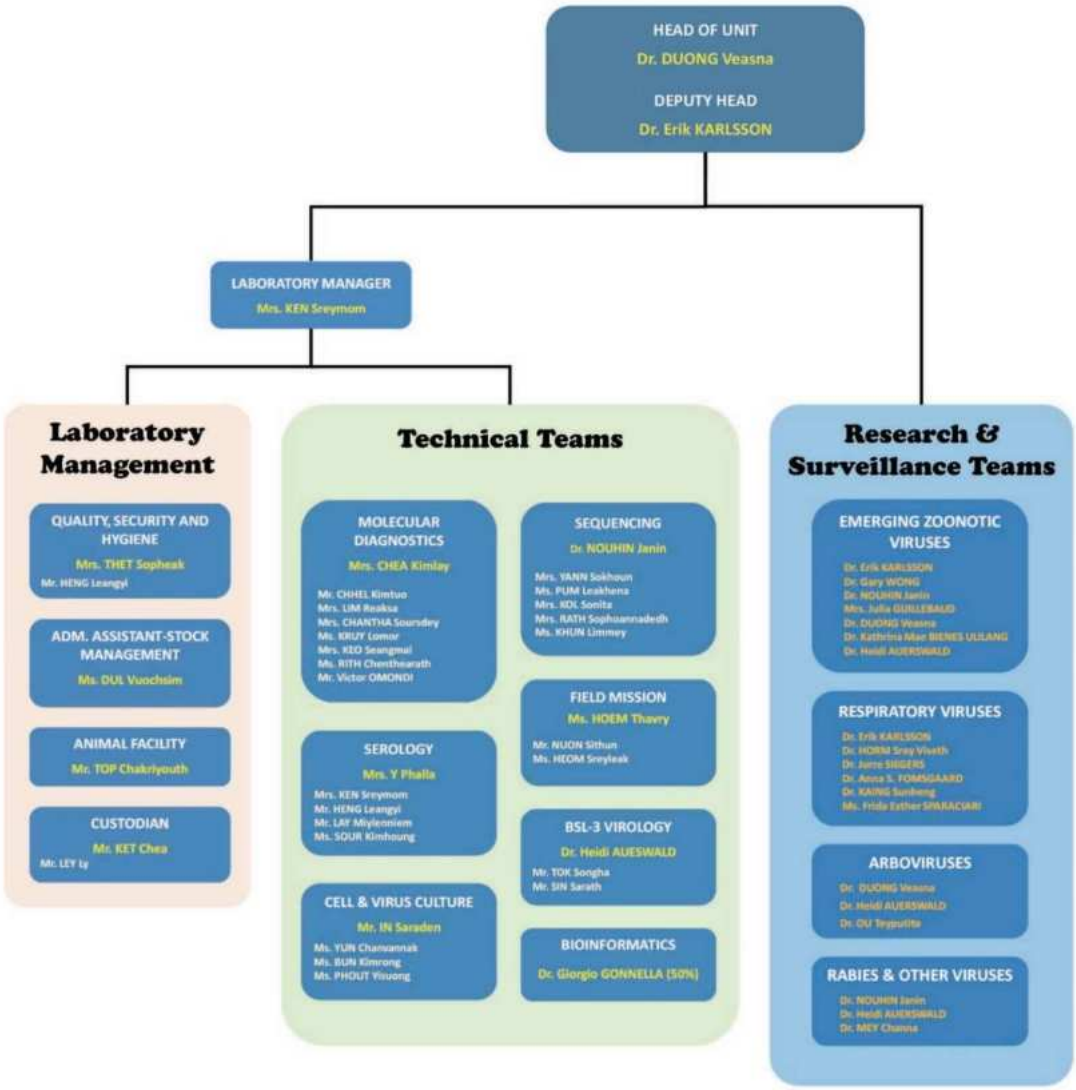


Figure 13: Virology Unit organigram, 2023

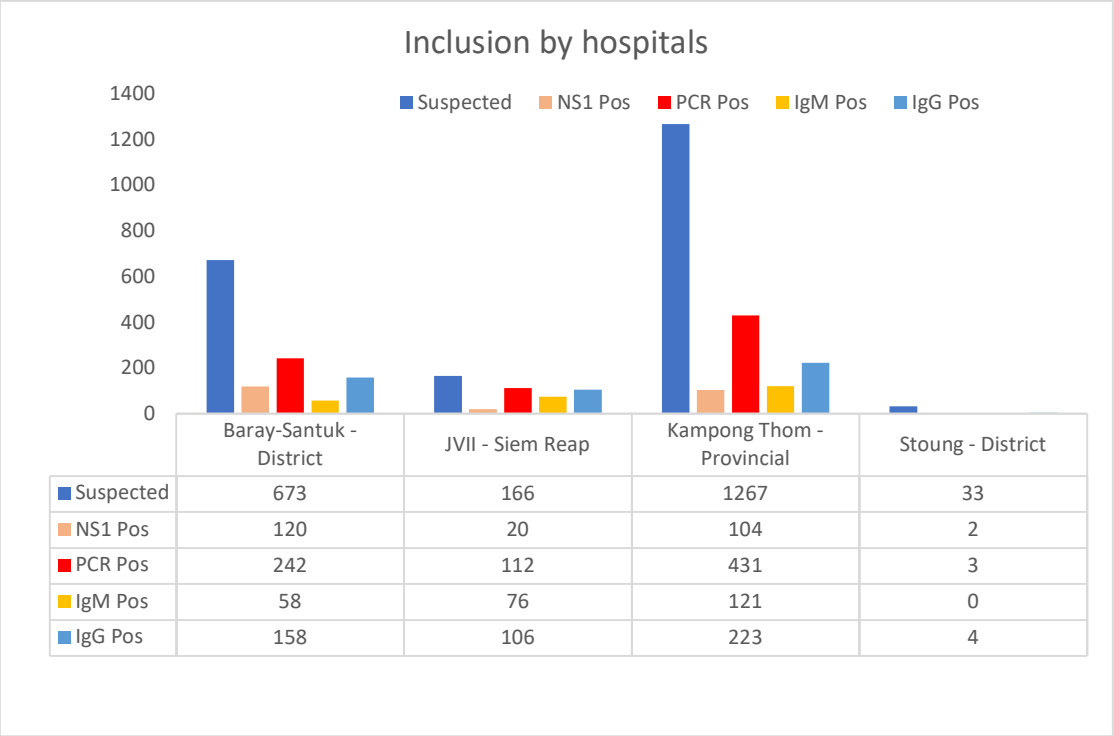
4.4.2 Research Programs – Major Achievements in 2023

Axis 1: Arboviral Disease

DenThom: Study of dengue-like illnesses 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 illnesses 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 so far. The full description of this project is detailed in the Epidemiology and Public Health Unit, the Immunology Unit and Medical & Veterinary Entomology Unit sections (see p.42, 56 & 106). The Virology Unit is involved in the project's implementation in the field, in collaboration with the Epidemiology and Public Health unit as well as in the diagnosis of arboviruses in humans and mosquitoes using serological and molecular tools. As of first February 2024, a total of 2139 suspected dengue cases from the four hospitals were included, and 788 were positive for dengue virus (DENV) infection (RT-PCR confirmation). The majority of the samples came from Kampong Thom and Baray-Santuk hospitals (Figure 14). Following the household

investigation around the index cases that started on 1 November 2022, we included 3526 participants and 91 were PCR confirmed DENV positive. In addition, we have established the Multiplex Immunoassay (MIA) for arboviruses IgG antibodies detection using Ag (EDIII or E) coupled beads from our collaborator at IP Paris, Dr. Jessica Vanhomwegan. At the same time, we are exploring other antigens including NS1 from commercial company to complement the panel. This MIA will be used to screen participants from the household investigation for the seroprevalence study.



	Baray-Santuk - District	JVII - Siem Reap	Kampong Thom - Provincial	Stoung - District
Suspected	673	166	1267	33
NS1 Pos	120	20	104	2
PCR Pos	242	112	431	3
IgM Pos	58	76	121	0
IgG Pos	158	106	223	4

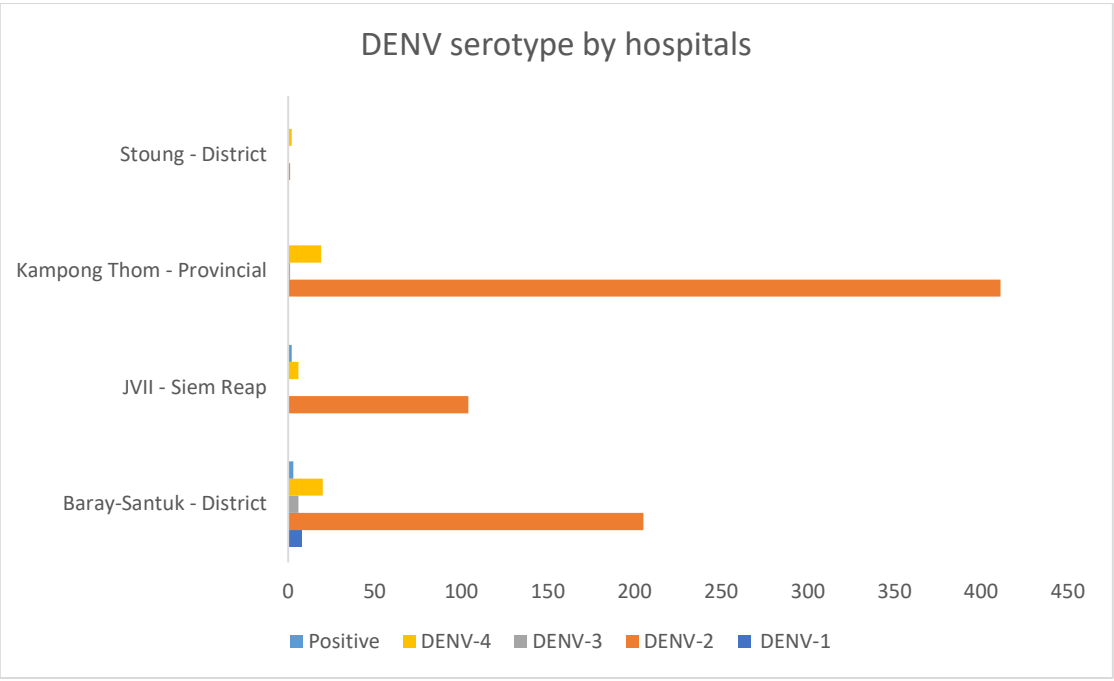


Figure 14: Total cases of suspected and confirmed dengue infection from the four hospitals

Research Project Name	DenThom
Funding	NIH collaborative agreement (1-IPC-NIH-U01-AS-2020)
Project duration	August 2020 to May 2025
Collaboration	Institut Pasteur du Cambodge: Virology unit (Duong Veasna, Ou Tey Putita, Ken Sreymom), Epidemiology and Public Health Unit (Ly Sowath), Immunology Unit (Tineke Cantaert), and Medical and Veterinary Entomology Unit (Sebastien Boyer) IP, Paris (Anavaj Sakuntabhai) Jayavarman VII hospital, Siem Reap, Cambodia Kampong Thom, Stoung and Baray-Santuk hospitals, Kampong Thom

Investigation of Dog-Associated Diseases in Southeast Asia (DogZooSEA and SEAdogSEA).

An investigation was conducted into dog-borne diseases in Southeast Asia, focusing on the perceptions and practices of villagers regarding dog ecology and epidemiology, as well as dog distribution and population dynamics. The project, which included sample collection from Cambodia, Indonesia, and Thailand, involved serological investigations at the IPC's Virology Unit. Paired serum samples from dogs and their owners were collected during a follow-up survey were sent to IPC and used to assess the prevalence of arboviruses (dengue, Japanese encephalitis, West Nile viruses) using virus-specific neutralization tests. This investigation also aimed to evaluate the potential role of dogs as sentinels for arboviruses. Additionally, the dog samples were tested for rabies antibodies to study non-lethal exposure to canine rabies. Laboratory analysis of the Cambodian samples was completed in 2023, with samples from Thailand and Bali scheduled for testing in the first quarter of 2024. Data analysis is pending until results from other countries will be obtained.

Research Project Name	DogZooSEA and SEAdogSEA
Funding	FSPI-One Health in Practice in Southeast Asia (OH SEA):
Project duration	February 2022 – March 2023
Collaboration	Institut Pasteur du Cambodge: Virology Unit (Duong Veasna and Heidi Auerswald), and Epidemiology and Public Health Unit (Ly Sowath, Helene Guis and Sorn Sopheak) CIRAD-KU (Michel De Garine Wichatitsky) Kasetsart University Bangkok (Anamika Kritiyakan) Gadjah Mada University Yogyakarta (Wayan T. Artama)

Current knowledge of exposure to ticks and tick-borne diseases among rural population in Cambodia

In Cambodia, ticks and tick-borne diseases are neglected and data are still lacking. The objectives of the study are (i) to identify tick species and associated pathogens including viruses and bacteria circulating in a rural community of Cambodia and (ii) to investigate transmission risk of the identified tick-borne pathogens in human by assessing seroprevalence (IgG) and tick bite biomarkers in people living in the communities where ticks are captured. Our study has been nestled in a study, namely "Study of dengue-like illness in Kampong Thom Province, Cambodia" (DENTHOM) which is part of a NIH/NIAID-funded project, namely "Inter-regional study of transmission, adaptation and pathogenesis of viruses with pandemic potential in Southeast Asia and West/Central Africa" (U01 AI151758-01).
In collaboration with Entomology Unit, a total of 1,433 ticks were collected from 24 clusters containing 82 households, with dogs as the primary host for 1,272 (97.3%) of the collected ticks. Among the 1,433 ticks, 1,307 (91%) were in the adult stage, 118 (8%) in the nymph stage, and 8 (1%) in the larval stage.

Within the 1,307 adult ticks, 1,272 (97.3%) belonged to *Rhipicephalus sanguineus*, 31 (2.4%) to *Rhipicephalus microplus*, and 4 (0.3%) to *Rhipicephalus haemaphysaloides*. None of targeted viruses (SFTSV, TBEV, and CCHFV) were detected with PCR approach. Bacterial detections (*Rickettsia, Borrelia, Ehrlichia, and Coxiella)* are underway. To enhance the detection of tick-borne pathogens, 91 pools (15 – 16 ticks/pool) underwent metagenomics sequencing. Data analysis is currently in progress. To assess the transmission risk of targeted pathogens in human, we analyzed a subset of 200 serum samples collected from individuals residing in area where ticks were collected using commercial ELISA kits for the detection of IgG antibodies. Among these samples, 56% tested positive for anti-TBEV IgG, 6.7% for anti-SFTV IgG, 2.5% for anti-CCHFV IgG, 10.5% for anti-scrub typhus IgG, 5% for anti-Borellia, and 0.6% for anti-Coxiella IgG. Samples tested positive for anti-SFTSV, TBEV, and CCHFV IgG will be sent to Hokkaido University for sero-neutralization assay scheduled in 2024 to confirm the ELISA results. To investigate transmission risk from tick bites, we plan to apply ELISA technique to screen IgG antibodies response to different tick bite biomarkers. Through use of an exploratory mircroarray assay (PepperPrint™), we identified novel biomarker candidates from the infested sheep sera. Among the candidates tested, we found that there was a more specific response against the IgG antibody and that certain peptides had very strong binding affinity. Additionally, peptide candidates had differential binding affinity based on the individual's exposure to ticks. Further cross-validation is being tested against sera from clinical and field samples.

Research Project Name	Current knowledge of exposure to ticks and tick-borne diseases among rural population in Cambodia
Funding	NIH (Award Number 3U01AI151758-03S1)
Project duration	August 2022 – October 2023
Collaboration	Institut Pasteur du Cambodge: Virology Unit (J. Nouhin, L. Khun, L. Heng, P. Y, S. Ken, and V. Duong); Medical and Veterinary Entomology Unit (P.-O. Maquart, S. Yean, K. Heng, S. Leng and S. Boyer); Epidemiology and Public Health Unit (S. Sorn and S. Ly) Institut Pasteur Paris (A. Dziedziech, S. Bonnet, R. Paul, S. Mohamed Ali, M. Eloit, and A. Sakuntabhai)

Axis 2: Respiratory Viruses

Avian influenza surveillance in key Live Bird Markets (LBMs).

In 2017, in collaboration with the Food and Agriculture Organization of the United Nations (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) of the bird sample. Surveillance resumed in fall 2021 (after some delays due to COVID-19). In 2023, this collection continued in the beginning and late parts of the year, with 3,600 samples collected from 1,800 birds, and 425 environmental samples (see below).

The collection, analysis, and sequencing are complete for the 2017–2022 sessions, 2023 is ongoing, and several manuscripts are in preparation (see below). This collaboration is planned to continue for the 2024 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 2023 are being sequenced and phylogenetic, antigenic, and molecular risk assessments are underway in collaboration with the WHOCC in Melbourne, the University of Hong Kong, and other partners. Since 2021, IPC has worked with partners at the Johns Hopkins University's Applied Physics Laboratory to use a novel multi-segment, barcoded PCR for sequencing using 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.

Between 2014 and 2018, the detections of circulating HPAI A/H5N1 in Cambodia were clade 2.3.2.1c viruses in poultry. Through longitudinal, active surveillance between IPC and the National Animal Health and Production Institute (NAHPRI) with support from the Food and Agriculture Organization of the United Nations (FAO), it is known that HPAI A/H5N1 clade 2.3.2.1c viruses were still detected regularly into 2023. However, since 2014, HPAI viruses with a H5 HA gene that group into the genetic clade designated as 2.3.4.4, have spread globally causing severe outbreaks in Africa, Europe, Asia, and most recently in North and South America, with current recirculation of A/H5N1 clade 2.3.4.4b viruses into Asian bird populations. These viruses have caused devastating outbreaks in poultry, rapidly evolve, and continuously reassort with other AIVs, posing not only a threat to food security in many parts of the world but also a significant human zoonotic risk. HPAI A/H5N6 clade 2.3.4.4g viruses were already found in Takeo and Orussey markets in chickens in 2019 and ducks in 2020, and HPAI A/H5N6 clade 2.3.4.4h viruses were detected sporadically in Kampot province in late-2018, Takeo province in 2019-2020, and Phnom Penh in 2020.

Recently, HPAI A/H5N1 viruses of clade 2.3.4.4b were detected in live bird market surveillance during Lunar New Year, 2023 with analysis ongoing. Concerningly, A/H5N1 clade 2.3.4.4b viruses containing HA genes closely related to A/Astrakhan/3212/2020 have caused major devastation in poultry and wild birds globally, and numerous spillovers into mammalian hosts such as mink and marine mammals, and human infections in the UK, the USA, and Ecuador. In addition, HPAI A/H5N1 clade 2.3.2.1c viruses were still detected regularly into 2023 alongside these 2.3.4.4b viruses.

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. Its detection frequency increased in March and April of the same year, and it has continued to be detected sporadically in 2019 in the country. A paper detailing these initial findings was published in 2019.

Throughout the 2020–2023 seasons, A/H7Nx viruses continued to be detected in waves. These samples have been sequenced and a detailed report of A/H7Nx circulation and evolution within Cambodian LBMs is expected in mid- to late 2024.

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 and 2019. The majority of Cambodian A/H9N2 viruses detected after 2015 belong to two genotypes, P and V. Human infection with A/H9 is rare, but not unheard of. The first human infection with A(H9) virus was reported in Hong Kong in 1998. Infections have occurred mainly in China and Hong Kong; however, some cases have been reported in Bangladesh, Pakistan, Oman, Senegal, and Egypt. Since 1998, >80 A(H9) human infections have been documented. The majority of cases were children. In 2022, 6 cases of avian influenza A(H9N2) were reported to WHO from China. In the Western Pacific region, 71 cases of human infection with avian influenza A(H9N2) including two deaths (both with underlying conditions) have been reported to WHO since December 2015. Cambodia detected 2 cases of A/H9N2, one in 2021 (published) and one in 2022 (paper expected mid-2024). A detailed report of A/H9N2 circulation and evolution within Cambodian LBMs is expected in mid- to late 2024.

Detection of rare avian influenza subtypes in Cambodian poultry.

AIV in Asia is a complex system of many subtypes and a highly porous wild bird-poultry interface. Certain AIV subtypes, such as H14, are underrepresented in current surveillance efforts, and remain poorly understood in terms of its ecology and evolution. In 2022, we detected of rare subtype H14 in Southeast Asia, a geographic location and domestic bird system not previously known to harbor this subtype. These H14 viruses have a complex evolutionary history across gene segments including reassortment events, with sequences similar to endemic AIVs in Cambodian ducks, Eurasian low pathogenic AIVs and Eurasian highly pathogenic H5Nx AIVs (Figure 15). The detection of H14 in Southeast Asia further advances our knowledge of the ecology and evolution of this subtype and reinforces the need for future vigilance in longitudinal, active surveillance in domestic poultry and wild birds as well as *in vivo* and *in vitro* risk assessment to include rare AIV subtypes that could become endemic in poultry systems. The manuscript was published in Emerging Microbes & Infections.

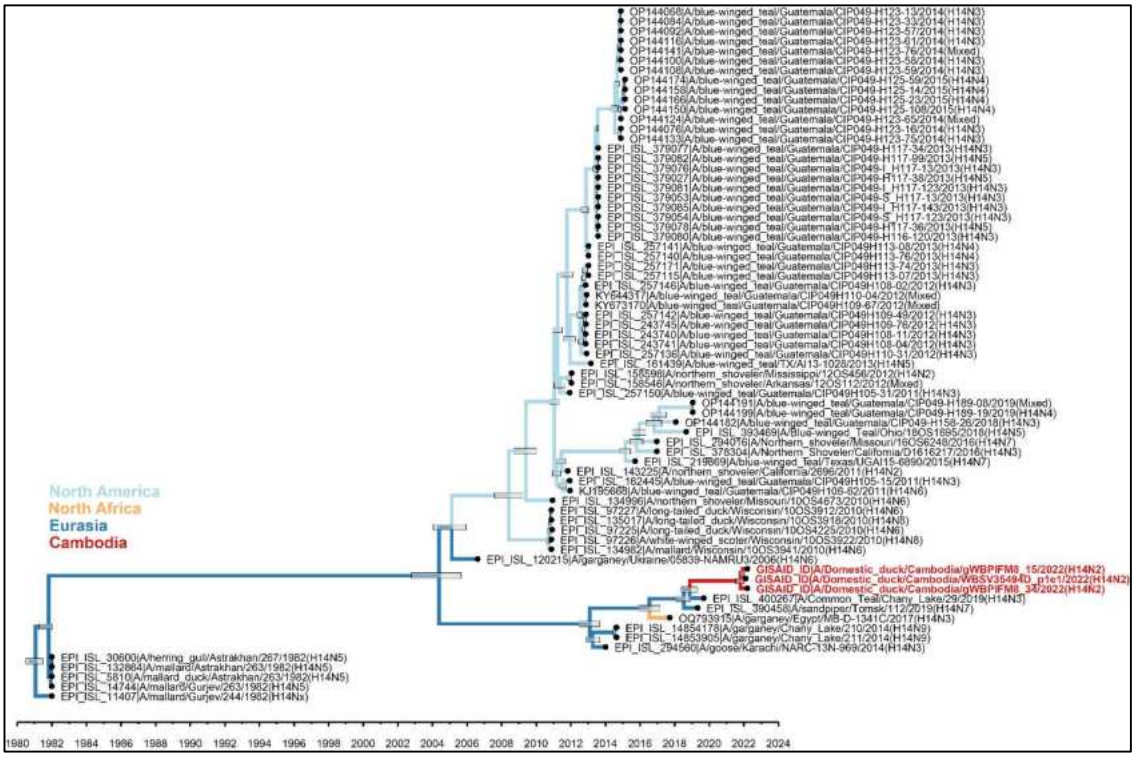


Figure 15: Evolutionary relationships and divergence of HA gene of H14Nx viruses detected in Cambodia. Phylogeny includes all H14 HA sequences available in public influenza databases (GISAID, NCBI, and BV-BRC) with sequences generated in this study shown in red. Branches are colored by geographic location. Tree is scaled to time with node bars corresponding to the 95% HPD.

Research Project Name	Avian influenza surveillance in key Live Bird Markets
Funding	Food and Agriculture Organization of the United Nations
Project duration	2023
Collaboration	Institut Pasteur du Cambodge: Virology Unit (E. Karlsson), EPH Unit (S. Ly, M. Chan) Ministry of Agriculture, Forestry and Fisheries: National Animal Health and Production Research Institute, Cambodia

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 and the National

University of Singapore (NUS) for attempts at full genome sequencing to understand the viruses detected in these animals. These analyses have been completed.

Further swine-related work is funded through 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's CEIRR/Royal Veterinary College (RVC), established swine and human surveillance, and began ethical clearance for the studies. Sampling and analysis commenced in 2022, with collection continuing into 2023. No influenza viruses have been detected in swine samples in 2023.

Research Project Name	Swine influenza viruses
Funding	US-DHHS, FAO, NIH/NIAID/CEIRR
Project Duration	2021-2028
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, Australia, Duke-NUS, WHO, RVC/UPenn, AAHL/CSIRO, London School of Tropical Medicine and Hygiene, UHS, CEIRRA

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

In collaboration with Kantha Bopha Hospital (KBH), IPC has been conducting surveillance of respiratory infections in Cambodian children for over 10 years as a first line strategy for human A/H5N1 detection. While Cambodia has not experienced a human infection with A/H5N1 since 2014, this surveillance program includes a number of severe seasonal influenza infections, especially in children.

Attempts are being made to classify viruses of interest, especially the Respiratory Syncytial Virus (RSV), coronaviruses, and paramyxoviruses. Some samples were shipped to collaborators at Duke-NUS in Singapore for next-generation sequencing in late 2020. Phylogenetic analysis and sequencing of the 2021-2023 samples are ongoing. Analysis of the etiology database was completed in 2023 and we are actively collaborating with epidemiologists and biostatisticians for the best way to analyze and present the data. A manuscript describing the 2014–2023 comparative data between Severe Acute Respiratory Illness (SARI) cases and healthy children, as well as reasons for increased mortality from influenza between 2016 and 2017, as compared to 2014–2015 is in preparation and is projected to be submitted mid-2024.

Research Project Name	Investigation of etiology and risks for morbidity and mortality from influenza-associated SARI in Cambodian children
Funding	US-DHHS, USAID, FAO, WHO, Virology internal funding
Project Duration	2014-2022
Collaborations	Institut Pasteur du Cambodge : Virology unit (E. Karlsson) MoH/CCDC, WHO CC Melbourne/Peter Doherty Institute, Duke-NUS, WHO, US-CDC, NIPH; Kantha Bopha Hospital; NAMRU-2

Surveillance activities in Cambodia and using novel collection and sequencing techniques.

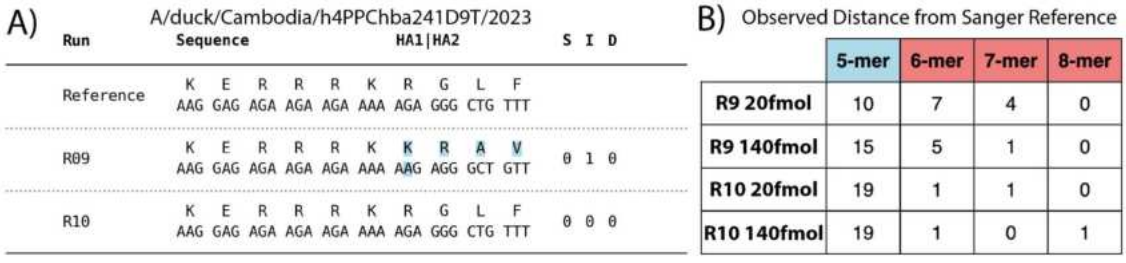
In Cambodia, current pathogen surveillance systems rely primarily on sampling and testing individual animals – a practice that is both costly and time-consuming, and prevents widespread coverage of all high-risk areas. One way to address this issue is the incorporation of environmental sampling (ES) into surveillance programs. ES includes samples or swabs taken from soil, water sources (drinking, carcass wash, lakes and ponds), feeding sources, feathers, air, and surfaces such as cages, chopping boards,

and defeathering machines. As such, environmental pathogen surveillance casts a wide net at high-risk interfaces, potentially improving surveillance coverage and supporting expanded sampling on a longitudinal basis. We hypothesize that utilizing ES can: 1) improve, expand and simplify existing methods of pathogen surveillance; 2) reduce the cost of pathogen surveillance programs; 3) reduce direct contact between people and large numbers of animals, thereby improving biosafety, animal welfare, and reducing occupational exposure risks; and 4) set a precedent for lower-middle income countries (LMICs) to conduct broad pathogen surveillance cost-effectively. In 2023, we were able to collect 425 ES for comparison to individual bird samples. Sampling continues into 2024 and analysis is ongoing.

Research Project Name	Surveillance activities in Cambodia and using novel collection and sequencing techniques
Funding	Food and Agriculture Organization of the United Nations
Project duration	2022-2023
Collaboration	Institut Pasteur du Cambodge : Virology unit (E. Karlsson) Ministry of Agriculture, Forestry and Fisheries: National Animal Health and Production Research Institute

Improving Resolution of Highly Pathogenic Avian Influenza Virus Haemagglutinin Cleavage Site Using Oxford Nanopore R10 Sequencing Chemistry.

Rapid and accurate genomic surveillance is critical for monitoring viral mutations, tracing transmission, and guiding interventions in near real-time. Oxford Nanopore sequencing holds promise for real-time influenza genotyping, but data quality from R9 chemistry has limited its adoption due to challenges resolving low-complexity regions such as the biologically critical hemagglutinin cleavage site, a homopolymer of basic amino acids that distinguish highly pathogenic strains. In this study, human and avian influenza isolates (n=45) from Cambodia were sequenced using both R9.4.1 and R10.4.1 flow cells and chemistries to evaluate performance between approaches. Overall, R10.4.1 yielded increased data output with higher average quality compared to R9.4.1, producing improved consensus sequences using a reference-based bioinformatics approach. R10.4.1 had significantly lower minor population insertion and deletion frequencies, driven by improved performance in low sequence complexity regions prone to insertion and deletion errors, such as homopolymers. Within the hemagglutinin cleavage site, R10.4.1 resolved the correct motif in 90% of genomes compared to only 60% with R9.4.1. Further examination showed reduced frameshift mutations in consensus sequences generated with R10.4.1 that could result in incorrectly classified virulence (Figure 16). Improved consensus genome quality from nanopore sequencing approaches, especially across biologically important low-complexity regions, is critical to reduce subjective hand-curation and will improve local and global genomic surveillance responses. Paper is under revision at *npj Viruses*.



	5-mer	6-mer	7-mer	8-mer
R9 20fmol	10	7	4	0
R9 140fmol	15	5	1	0
R10 20fmol	19	1	1	0
R10 140fmol	19	1	0	1

Figure 16: Improved Resolution of the H5 Multi-Basic Cleavage Site. (A) Representative multiple sequence alignment for HA segment inclusive of the multibasic cleavage motif, composed of Sanger (Reference), R9, and R10 consensus outputs. 20fmol and 140fmol consensus outputs were identical for both R9 and R10 chemistries

for this sample. R9 output includes a single base insertion at the 3' end of the adenine homopolymer upstream of the RGLF motif, resulting in an apparent frameshift mutation (highlighted in blue). S = # of substitutions / I = # of insertions / D = # of deletions. (B) Length of adenine homopolymer in consensus outputs for both chemistries and loading concentrations for all high pathogenicity avian influenza samples (n = 21). Lengths greater than five bases are assumed to be artifactual insertions.

Research Project Name	Improving Resolution of Highly Pathogenic Avian Influenza Virus Haemagglutinin Cleavage Site Using Oxford Nanopore R10 Sequencing Chemistry
Funding	WHO, Internal Funding
Project duration	2023
Collaboration	John's Hopkins Applied Physics Laboratory

Axis 3: Zoonoses

Biodiversity conservation to mitigate the risks of emerging infectious diseases (BCOMING).

The BCOMING project main objective is to co-construct innovations with all stakeholders in biodiversity hotspots to reduce the risks of infectious disease emergence through biodiversity conservation and zoonotic disease surveillance. The activities of the project will be implemented in Europe and three tropical biodiversity hotspots in Southeast Asia, West Africa and the Caribbean. In Cambodia, the BCOMING project will help understanding and preventing the risk of emergence of coronaviruses (CoV) and paramyxoviruses. It will be implemented in four Provinces with different levels of anthropisation and biodiversity protection measures: Phnom Penh (urban environment), Battambang (intensive agriculture), Steung Treng (fragmented forest and rural agriculture), and Mondulkiri (pristine forest).

Biological sample collection was initiated with the longitudinal follow-up of bats in three selected karst hills in Stung Treng province with four field missions implemented in March, May, June and December 2023. Over the four sessions, concurrent sampling of bats individuals, ectoparasites, vectors (mosquitoes, sand flies) and environment specimens (bat guano, air, feces, soil, hair) was deployed. A total of 686 bats were captured over 5 sessions between Marc 2023 and March 2024. Eight confirmed genera were identified (2 additional are suspected). *Rhinolophus* species represented 94.4% (648/686) of the sample size, and *R. shameli* accounted for 83.3% (572/686).

Laboratory analysis and further characterization are ongoing but already highlighted a co-circulation of SARS-CoV-2-related viruses in different *Rhinolophus* bat species.

Research Project Name	BCOMING
Funding	European Union HORIZON-CL6-2021-BIODIV-01
Project duration	August 2022 - July 2026
Collaboration	Institut Pasteur du Cambodge: Virology Unit (Duong Veasna and Julia Guillebaud), Epidemiology and Public Health Unit (Ly Sowath, Medical and Veterinary Entomology Unit (Sébastien Boyer) CIRAD-ASTRE (Julien Cappelle) IRD-TransVIHMI (Martine Peeters and Ahidjo Ayouba) IRD-ISEM (Rodolphe Gozlan and Marine Combe) MERFI (Alex Smajgl) Université de Liège (Johan Michaux and Pauline Van Leeuwen) Avia-GIS (Guy Hendrickx and Cedric Marsboom) INRAE University of Antwerp (Vincent Sluydts) Helmoltz Centre for Infection Research (Sébastien Calvignac-Spencer and Lorenzo Lagostina) CERFIG (Alpha Kabinet Keita) iDE (Moung Vandy) Nature Metrics (Tiffany Jedrecka and Kate Denton) Flora and Fauna International (Pablo Sinovas and Thi Sothearen) Europa Media (Gabriella Lovasz and Zsuzsanna Selmeczyk)

Benchmarking novel environmental surveillance in live bird markets, abattoirs, caves and other high-risk areas for expanded early-warning surveillance.

In Cambodia, current pathogen surveillance systems rely primarily on sampling and testing individual animals—a practice that is both costly and time consuming and prevents widespread coverage of all high-risk areas. One way to address this issue is the incorporation of environmental sampling (ES) into surveillance programs. ES includes samples or swabs taken from soil, water sources, feeding sources, feathers, and even the air. As such, environmental pathogen surveillance casts a wide net at high-risk interfaces, potentially improving surveillance coverage and supporting expanded sampling on a longitudinal basis. In addition, after collection, samples are currently with molecular diagnostics tested after transport back to a laboratory, another time-consuming process. Therefore, the incorporation of field-based detection methodology can greatly reduce the time requirements for pathogen detection and give greater ability to respond to zoonotic pathogens of concern. Comparison of air, water, and surface sampling, PCR detection techniques, and metagenomics for these samples is ongoing, and a publication is expected by mid-2024.

Research Project Name	Benchmarking novel environmental surveillance in live bird markets, abattoirs, caves and other high-risk areas for expanded early-warning surveillance
Funding	Food and Agriculture Organization of the United Nations Office of Innovation, Food and Agriculture Organization of the United Nations Bill and Melinda Gates Foundation
Project duration	2022-2024
Collaboration	Institut Pasteur du Cambodge : Virology unit (Erik Karlsson); Ministry of Agriculture, Forestry and Fisheries: National Animal Health and Production Research Institute; Forestry Administration

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

In order to further expand zoonotic disease detection at high-risk interfaces, enriched viral metagenomic sequencing (TEMVS) was performed on air samples versus pools of individual animal samples using Twist Bioscience's Comprehensive Viral Research Panel (CVRP) on an Illumina MiSeq platform, and the results were analysed using several commercial software packages such One Codex, Genome Detective and Chan Zuckerberg ID. In LBMs, ES reflected avian influenza viral (AIV) diversity found in individual animal samples. In some cases, TEMVS significantly improved the sensitivity and genome coverage of AIV whole genome sequencing compared to in-house developed Oxford nanopore sequencing. In bat caves/roosts ES, paramyxoviruses, coronaviruses and astroviruses were detected in faeces, air and urine. Individual animal and ES samples from pig slaughterhouses revealed the presence of coronaviruses, astroviruses and occasional orthomyxoviruses. Generally, conventional PCR screening and viral metagenomics agrees for LBMs and pig slaughterhouses but contains discrepancies for bat samples. In addition, viral metagenomic simultaneously identified numerous animals and occasionally human pathogens in full genome resolution that are understudied in Cambodia. Taken together, ES coupled with TEMVS is a powerful tool to improve/expand surveillance capacity.

However, current (commercial) metagenomic analysis software requires more exhaustive reference databases to be able to detect emerging (divergent) pathogens at the human–animal interface. Overall, coupling TEMVS with ES can: 1) improve pathogen surveillance, 2) reduce costs, 3) improve biosafety and animal welfare, and reduce occupational exposure risks; and 4) act as a first line of detection for high-risk human–animal interfaces. The preliminary data is showed in Figure 17.

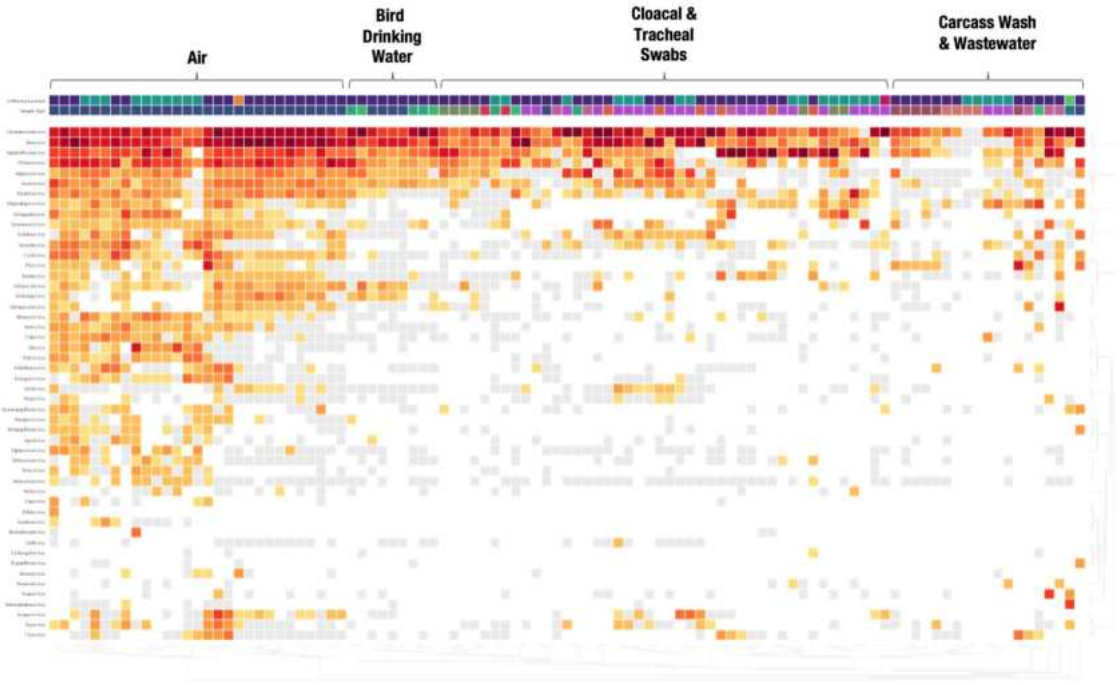


Figure 17: Comparison of air samples in LBM with other ES and individual duck and chicken samples

Research Project Name	In the Air Tonight: Metagenomic Pathogen Discovery as Tools in Pathogen Surveillance
Funding	NIAID Centers for Research in Emerging Infectious Diseases – EID-SEARCH:
Project duration	August 2022 – April 2023
Collaboration	Institut Pasteur du Cambodge: Virology unit (Erik Karlsson, Jurre Siegers); Sequencing Platform (Heang Vireak) Ministry of Agriculture, Forestry and Fisheries: National Animal Health and Production Research Institute; Forestry Administration EcoHealth Alliance

Improving multiplex, pan-viral PCR and Nanopore sequencing for real-time, in-field detection of zoonotic pathogens

Backyard livestock farms, LBMs, and wildlife farms often have limited biosafety measures in place despite representing potentially risky human-livestock-wildlife interfaces, and as a result, have repeatedly been associated with the (re-)emergence of zoonotic diseases. Therefore, developing early warning systems at these high-risk locations will be critical for monitoring and preventing zoonotic diseases from generating the next pandemic. Previous pathogen surveillance and virus diversity studies at human-livestock-wildlife interfaces in rely heavily on consensus time consuming and costly methodology mostly focused on a single pathogen and/or species, and may result in delayed disease detection and reporting. Every second counts between first detection and response. Indeed, the total cost of an outbreak grows exponentially as time from detection increases, making identification critical at emergence or early stages of spread. It is critical to get "left of sneeze." To this end, the proposed project aims to evaluate family level consensus-based approaches for multiple viral pathogens on broad coverage samples coupled with real-time, in-field sequencing as a modern alternative to pathogen surveillance at high-risk interfaces. Development of these novel primers and sequencing pipelines is ongoing into 2024.

Research Project Name	Improving multiplex, pan-viral PCR and Nanopore sequencing for real-time, in-field detection of zoonotic pathogens
Funding	International Atomic Energy Agency (IAEA) ZODIAC Project
Project duration	2023-2025
Collaboration	Institut Pasteur du Cambodge : Virology unit (E. Karlsson) John's Hopkins Applied Physics Laboratory International Atomic Energy Agency (IAEA) Food and Agriculture Organization of the United Nations (FAO)

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 on 12-14 June 2019 in Phnom Penh, Cambodia. The inaugural meeting, hosted by IPC with sponsorship from the Defense Threat Reduction Agency, the Cooperative Threat Reduction, the 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 organisations, connecting both formal and informal human and animal influenza surveillance networks at a global level. Its purpose is to apply a multisectoral, multi-level approach to integrating programs, policies, legislation, and research, thus allowing better One Health outcomes.

CANARIES continued in 2023, 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, awarding grants, and leading other collaborative efforts. A second consortium meeting is being planned for 2024.

Research Project Name	Continuation of the CANARIES Network
Funding	DTRA–UKGCRF DTRA through FAO
Project duration	2019-2022 2023-2028
Collaboration	Institut Pasteur du Cambodge : Virology unit (E. Karlsson)

COVID-19

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. In 2023, further work was extended to other bat cell lines (e.g., TB1 Lu purchased from ECACC) and primary bat cells. A paper was published in Microbiol Spectrum.

Research Project Name	Investigation of in vitro host-pathogen interaction between coronaviruses and bat cells
Funding	Virology Unit's internal funding
Project duration	2021-2024
Collaboration	Institut Pasteur du Cambodge: Virology Unit (Erik Karlsson, Heidi Auerswald and Duong Veasna) Duke-NUS Medical School Singapore (Gavin J.D. SMITH)

Axis 4: Rabies and other viruses

Serological long-term follow-up of patients who received the IPC's PEP regimen

In a comprehensive long-term follow-up study, patients who received rabies Post-Exposure Prophylaxis (PEP) at the Rabies Prevention Center in Phnom Penh were monitored to investigate the development and persistence of rabies neutralizing antibodies (nAb). This study involved both the Virology and Immunology Units, focusing on assessing the humoral and cellular immune responses of these individuals up to 2-3 years post-treatment. The Virology Unit conducted analyses using the Fluorescent Antibody Virus Neutralization Test (FAVNT) on serum samples from PEP recipients. These samples were collected before vaccination (Day 0), shortly after vaccination (Days 7 and 14), and subsequently at six months and one year following PEP treatment, to track the persistence of rabies neutralizing antibodies.

Simultaneously, the Immunology Unit examined the cellular immune response, particularly the long-term activation of T-cells. This dual approach was crucial in providing insights into the long-term efficacy and immune memory following rabies PEP, thereby informing future improvements in treatment protocols and understanding the overall immune dynamics post-PEP.

Research Project Name	Evaluation of long-term immunity following the IPC PEP protocol
Funding	IPC internal project, 2019-2023
Project duration	January 2021 –December 2023
Collaboration	Epidemiology and Public Health unit (Ly Sowath) Immunology unit (Tineke Cantaert) Virology Unit (Heidi Auerswald)

Improved monitoring of rabies activities with a Laboratory Information Management System (LIMS)

Starting from 2023, the Virology Unit's routine rabies diagnostic activities began to be documented in a FAO-supported veterinary public health Laboratory Information Management System (LIMS) known as SILAB. This system oversees all samples received for direct diagnosis by Direct Fluorescent Antibody test (DFAT), and for serological diagnosis by FAVNT, which is offered as a paid service. The implementation of SILAB facilitates faster result reporting to both customers and authorities. Following an assessment of the laboratory's needs for rabies diagnostic operations, two LIMS administrators were trained to manage rabies-related activities using SILAB. Additionally, four technicians were trained in sample and result entry. The adoption of the SILAB LIMS enhances sample tracking, testing, and reporting, and improves the overall management of the Virology Unit's diagnostic capabilities.

Research Project Name	SILAB-IPC
Funding	Food and Agriculture Organization of the United Nations (FAO)
Project duration	September 2023 –December 2023
Collaboration	Institut Pasteur du Cambodge: Virology Unit (Erik Karlsson, Heidi Auerswald); Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise (IZSAM, Ecole DEL NEGRO)

Human papillomavirus E6 and E7 coding gene variations and their possible association with the occurrence of cervical intraepithelial neoplasia.

Cervical cancers remain a public health concern in most countries. It is caused by sexually acquired infection with human papillomavirus (HPV), notably genotypes 16 and 18. Several 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 the study is to describe and characterize HPV diversity circulating in HIV-infected women in Cambodia using NGS approach. Secondarily, we will assess the associations between variants in HPV E6 and E7 coding genes and cervical intraepithelial neoplasia (CIN) status. A total of 60 women co-infected with HIV and HPV were included in the study. Rolling-circle amplification metagenomics approach and Illumina MiSeq technology have been used to obtain HPV whole genome. The sequencing activities were completed for all included samples (20 samples in 2022 and 40 samples in 2023). Data analysis is ongoing and a publication is expected by end of 2024.

Research Project Name	Human papillomavirus E6 and E7 coding gene variations and their possible association with the occurrence of cervical intraepithelial neoplasia.
Funding	IPC Internal Funding
Project duration	2022 – 2023
Collaboration	Institut Pasteur du Cambodge: Virology Unit (J. Nouhin, N. Boukli, L. Khun, J. Guillebaud, and G. Gonnella), Sequencing Mini-Platform (J. Nouhin, N. Khim and V. Heang). Calmette Hospital (S. Limsreng, A. Korn). University of Health Sciences (S. Kim, S. Moeung). ANRS (O. Segeral). Institut de Recherche pour le Développement (P. De Beaudrap).

4.4.3 Research Programs – Outlooks for 2024

Axis 1: Arboviral Disease

ECOMORE 3

In the lines of the strengthening of health security in the Indo-Pacific region, the ECOMORE 3 will aim to study the circulation of priority zoonotic and vector-borne diseases in Southeast Asia using innovative laboratory diagnostic approaches, associating them with the measurement of environmental and climatic factors, and characterizing vectors and reservoirs. The ECOMORE consortium, transversally coordinated by Institut Pasteur, will reunite five partners located in 4 countries: the Institut Pasteur du Cambodge (IPC) in Phnom Penh, the Institut Pasteur du Laos (IPL) in Vientiane, and the National Institute of Hygiene and Epidemiology (NIHE) in Hanoï, Vietnam, the Research Institute of Tropical Medicine (RITM) in Manila. All these institutes are national institute affiliated to their respective Ministries of Health and play an active role in the health system of their countries. In addition, the French National Research Institute for Sustainable Development (IRD) will dedicate its effort to the climate component of the program.

In Cambodia, the multidisciplinary team (virologists, entomologists, epidemiologists, climatologists…) aims to conduct, with a One-Health approach, a nationally-representative longitudinal survey on zoonotic and endemic diseases, as well as to study the impact of human activities, land use and climate changes on the distribution of the main vectors in the country. In parallel, innovative diagnostic tools will be developed to support these studies all while enhancing.

The project will specifically aim to: strengthen integrated approaches to human, animal, and environmental health; and establish a framework for inter-network cooperation on epidemic preparedness and response (PPR). These two objectives will be structured around four areas of work: Laboratory Capacities, Climate and Health, Surveillance and Response.

Research Project Name	ECOMORE 3
Funding	AFD
Project duration	2024-2027
Collaboration	Institut Pasteur du Cambodge: Virology Unit (Veasna Duong, Chung Kei Wong, and Kathrina Mae Bienes Ulilang) Institut Pasteur du Cambodge: Epidemiology and Public Health Unit (Claude Flamand and Sowath Ly) Institut Pasteur du Cambodge : Medical and Veterinary Entomology Unit (Sébastien Boyer) Institut Pasteur du Laos, Lao RDP National Institute of Hygiene and Epidemiology (NIHE), Vietnam Research Institute of Tropical Medicine (RITM), the Philippines

Axis 2: Respiratory Viruses

Aetiologic Agents of Community-Acquired Pneumonia in Cambodia - CAP study

The primary objectives of the study are to: 1. determine the aetiological agent of the CAP and 2. detect novel viruses or pathogens causing CAP.

The project will be implemented at Calmette hospital for adult patients and at Kuntha Bopha hospital for pediatric patients. The respiratory samples will be screened using multiplex RT-PCR for both viral and bacterial pathogens and a subset of negative samples will be used for novel pathogens detection using metagenomic sequencing. The serum samples will be screened for IgG antibodies against emerging pathogens using multiplex serological assay developed by Singaporean team at National Center for Infectious Disease.

The project protocol was submitted to the National Ethic Committee for Health Research in Cambodia for approval.

Research Project Name	CAP-Study
Funding	PREPARE Programme, National Centre for Infectious Diseases, Singapore
Project duration	2024-2025
Collaboration	Institut Pasteur du Cambodge: Virology Unit (Veasna Duong, Erik Karlsson, and Kang Sunheng) Calmette Hospital (Dr. Sotharith Bory) Kuntha Bopha Hospital (Dr. Nguon Yaneth), Cambodoa National Centre for Infectious Diseases, Singapore (Dr. Yeo Tsin Wen)

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

The objective of the project is to build capacity 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.

Research Project Name	CEIRR Pilot Project: Capacity building for in vitro influenza diagnostics.
Funding	CEIRR/NIAID SUBMITTED IN 2024
Project duration	2024–2026
Collaboration	Institut Pasteur du Cambodge: Virology Unit (Erik Karlsson and Heidi Auerswald), St. Jude Children's Research Hospital (Stacey Schultz)

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

The objective is to assess the immune responses, transmission, and infectivity of AIV in native chicken and duck breeds in the Greater Mekong Region to understand circulation, reassortment, zoonotic risk, and preventive mechanisms.

Research Project Name	Assessing the transmission, carrying capacity, and pathogenesis of the avian influenza virus associated with native chicken and duck breeds in the Greater Mekong Subregion
Funding	Wellcome Trust; PENDING – To be submitted July 2024 CEIRR/NIAID - SUBMITTED IN 2024 (2024–2026) Food and Agriculture Organization of the United Nations/USAID – PENDING 2024
Project duration	2024–2026
Collaboration	Institut Pasteur du Cambodge: Virology Unit (Erik Karlsson) Cambridge (Jim Kaufman) Ben-Gurion University of the Negev (Tomer Hertz)

Pilot implementation of the SILAB LIMS system in Cambodia for use in AIV surveillance.

The aim of this pilot program is to implement an ISO-accredited LIMS system in the AIV laboratory to investigate its utility, data sharing possibilities, and to facilitate accreditation.

Research Project Name	Pilot implementation of the SILAB LIMS system in Cambodia for use in AIV surveillance.
Funding	FAO
Project duration	Started March 2022 and continued in 2024
Collaboration	Institut Pasteur du Cambodge: Virology Unit (Erik Karlsson, Heidi Auerswald) Theramo

Expanded Environmental Surveillance and Metagenomics for Zoonotic Risk/Prevention in Cambodian Live Animal Markets

This project was funded by the Bill and Melinda Gates Foundation with the objective of assessing the highly multiplexed PCR and metagenomics against standard detection techniques to improve environmental surveillance for early warnings. The expansion of this project intends to scale up efforts in Cambodia, further develop the novel technologies employed, understand how the data is used for action and reporting.

Research Project Name	Expanded Environmental Surveillance and Metagenomics for Zoonotic Risk/Prevention in Cambodian Live Animal Markets
Funding	Bill and Melinda Gates Foundation
Project duration	PENDING Expanded Funding 2024-2026
Collaboration	Institut Pasteur du Cambodge : Virology unit (Erik Karlsson) Duke-NUS (Gavin SMITH), Singapore Asia Pacific Genomics Institute, Singapore

Scaling up early warning surveillance using novel collection and sequencing techniques.

The objective of this proposal is to expand air-based surveillance in live animal markets employing and cutting-edge detection and sequencing methods and to further benchmark their performance and utility against standard surveillance techniques. Scale up focuses on using techniques piloted in Cambodia to expand to surveillance in Indonesia, Vietnam, Laos, and the Philippines.

Research Project Name	Scaling up early warning surveillance using novel collection and sequencing techniques.
Funding	UN Global Pulse Scale Accelerator Award, PENDING 2024 Hong Kong Jockey Club, PENDING 2024 WHO International Pathogen Surveillance Network Country Scale-Up *Accelerator – SUBMITTED in 2024*
Project duration	2024-2025
Collaboration	Institut Pasteur du Cambodge: Virology Unit (Erik Karlsson) Food and Agriculture Organization of the United Nations (FAO) Hong Kong University IP Laos Other ministries, stakholders in partner countries

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

The proposal focuses on capacity building for sequencing bat genomes, immune responses, and on the generation of primary bat cell lines linked to a scientific exploration of what may make Rhinolophilus species adept at carrying SARS-CoV-2-related viruses and the potential impacts of reverse zoonoses.

Research Project Name	Understanding the Original Variant of Concern: In vitro Studies of SARS-CoV-2 in Bats and the Consequences of Reverse Spillover
Funding	Wellcome Trust; PENDING – to be submitted July 2024
Project duration	2024-2027
Collaboration	Institut Pasteur du Cambodge: Virology Unit (Erik Karlsson) Cambridge University (Jim Kaufman), UK Duke-NUS (Gavin Smith), Singapore

TrackFlu - Tracking the spread of avian influenza viruses in live bird market networks

This project aims at investigating the transmission mechanisms of avian influenza viruses in live bird market networks in Cambodia using advanced snowball sampling, modeling, and phylogeography and phylodynamics.

Research Project Name	TrackFLu
Funding	European Research Council
Project duration	2024-2029
Collaboration	Institut Pasteur du Cambodge: Virology Unit (Erik Karlsson) INRAE (Claire Guinat)

ACIP: AVERT-Cam: An Early Warning System for AIV Transmission and Gene Flow in Cambodia

Avian influenza viruses (AIV) pose a serious public health threat in Cambodia, with highly pathogenic avian influenza (HPAI) H5N1 viruses and multiple subtypes of low pathogenic avian influenza (LPAI) viruses circulating. Despite surveillance efforts, knowledge gaps remain concerning the factors influencing its transmission and maintenance, and the effectiveness of current control measures. To address these gaps, our consortium, comprising Institut Pasteur Cambodia, Hong Kong and Paris, aims to address these gaps by developing an early warning system for AIV in Cambodia. Main objectives include:

1. Identify factors that influence the maintenance and transmission of AIV genetic and antigenic diversity.
2. Identify potential sources and routes of AIV transmission.
3. Evaluate the effectiveness of ongoing AIV control measures, including non-pharmaceutical interventions (NPIs) and vaccination strategies, in mitigating AIV transmission and evolution in poultry populations.

Research Project Name	ACIP: AVERT-Cam
Funding	IP Paris
Project duration	2024-2025
Collaboration	Institut Pasteur du Cambodge: Virology Unit (Erik Karlsson) Hong Kong University-Pasteur Research Centre (Dhanasekaran Sijaykrishna) Institut Pasteur Paris (Duchene Sébastian)

Axis 3: Zoonoses

PREZODE-AFRICAM

This project was funded by the PREZODE initiative and led by IRD and CIRAD. The main aim is to study the impacts of hydrological dynamics and climatic and environmental factors on the risks of emergence of zoonotic diseases in diversified ecosystems representing key animal/human/environment interfaces. It also aims to implement activities to reduce the emergence of zoonotic risks and strengthen, in coordination with local and national partners, the existing surveillance systems towards an integrated One-Health surveillance.

The planning of research activities is ongoing. Field activities have not yet begun.

Research Project Name	PREZODE-AFRICAM
Funding	French Development Agency (AFD)
Project duration	2024-2026
Collaboration	Institut Pasteur du Cambodge: Virology Unit (Duong Veasna, Julia Guillebaud, Hoem Thavry, and Hul Vibol), Medical and Veterinary Entomology Unit (Sébastien Boyer); Epidemiology and Public Health Unit (Sorn Sopheak and Ly Sowath) IRD (Vincent Herbreteau and Anne-Laure Banuls) CIRAD (Hélène Guis)

Axis 4: Rabies and other viruses

ACIP: CLIMPATHIC – Strategy for Genomic Surveillance of Pathogens in Wastewater

The goal of this project is to develop tools for genomic surveillance of pathogens (endemic, (re)-emerging) in the context of climate change. The full strategy will be developed for dengue and Leptospira which are mentioned as important to follow in the context of climate change and can also be found in wastewater. However, this research proposal has to be considered as a pilot study using a pathogenic virus and bacteria to develop strategies applicable to global surveillance by monitoring pathogens in wastewater. In this proposal three types of strategy will be developed for genomic surveillance with a global increase in complexity: First, real-time PCR assays targeting these two pathogens will be adapted and implemented which will allow the monitoring of the circulation of a target pathogen and its presence and frequency in the population. Secondly, targeted sequencing allows getting precious information on the type of strain, pathogenicity, and vaccine escape of the circulating strains, including an approach to evaluate those present at lower frequencies will be developed. Third, metagenomics as an open approach that potentially allows the detection of any pathogens will be developed. These methods will be applied to wastewater samples, taking advantage of the national wastewater surveillance of SARS-CoV-2 already in place for each country of the consortium.

Research Project Name	CLIMPATHIC
Funding	ACIP - FUNDED 2024, IP Paris
Project duration	2024-2025
Collaboration	Institut Pasteur du Cambodge : Virology Unit (Erik Karlsson), Sciencano (Laura Van Poelvoorde) Institut Pasteur New Caledonia (IPNC)

4.4.4 Support to National Authorities

National Dengue Control Program (NDCP) in Cambodia

As part of a collaboration with WHO and NDCP, the Virology Unit receives samples of suspected dengue cases from six provincial hospitals, from the National Pediatric Hospital in Phnom Penh, and from IPC's Medial Biological Laboratory (MBL). Results from the virological monitoring of samples from patients with hemorrhagic syndromes are reported weekly or monthly to the various monitoring program participants (Director of the NDCP, hospital physicians, etc.). This long-term ongoing investigation shows that all four DENV serotypes are co-circulating in Cambodia with changing dominant serotypes (Figure 18). The surveillance data between 2000 and 2023 reveals that there were four big dengue outbreaks observed in 2007 by DENV-3, in 2012 by DENV-1, in 2019 by DENV-1 and in 2023 by DENV-2. DENV-4 circulates at background level. The genomic data showed that there were constant replacement of serotypes/genotypes/lineages and these replacements were often associated with big dengue outbreaks.

In 2023, the Virology Unit received 1244 samples from suspected dengue patients admitted to the sentinel hospitals and 1036 were confirmed positive by RT-PCR (83.3%). DENV-2 (n=756; 73.0%)) was the major serotype detected followed by DENV-4 (n=81; 7.8%), DENV-1 (n=10; 1%) and DENV-3 (0.1%). Besides, DENV, Zika virus (ZIKV) is reported to be endemic in Cambodia but in low prevalence (Figure 18). Chikungunya virus (CHIKV) was introduced in Cambodia in 2011 and in 2020. The virus was not able to become endemic and disappeared respectively in 2013 and 2022.

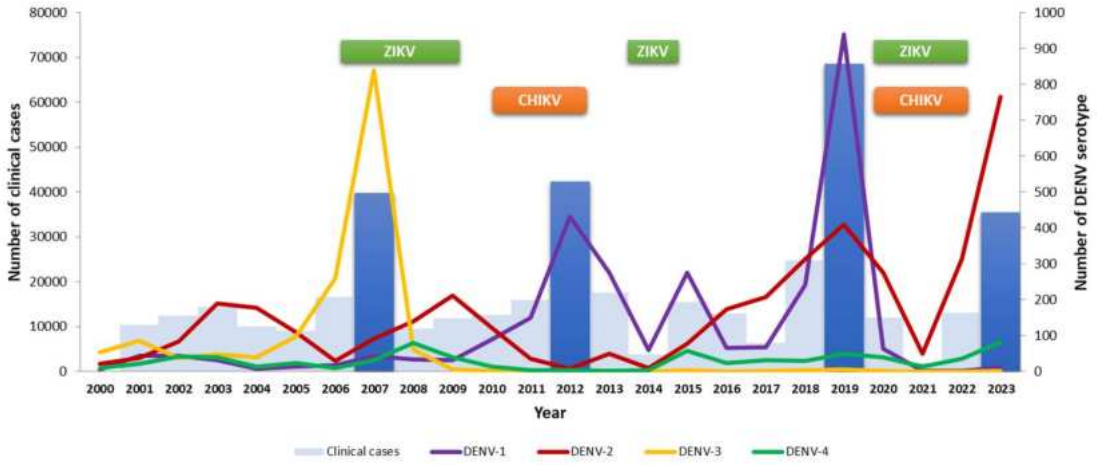


Figure 18: Dengue and other arboviruses circulation in Cambodia.

Cambodian National Influenza Center

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

IPC's Virology Unit has been Cambodia's National Influenza Center (NIC) since 2006 and in the same year, the influenza-like illness (ILI) surveillance was established, in collaboration with the MoH and WHO, and allows for the collection of influenza strains and data on seasonality. Currently, seven hospitals contribute to ILI surveillance: Kampot, Battambang, Kampong Cham, Mondulkiri, Svay Rieng, Angkor Children's Hospital (Siem Reap) and the National Pediatric Hospital (Phnom Penh). Each hospital randomly collects clinical samples from a maximum of 5 ILI patients per week. Samples are first 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; pending defunct).

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 diseases worldwide. Indeed, the 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 infection rates of many respiratory diseases despite continued, or even increased, testing for influenza in some countries.

Once international border restrictions were eased on 11 June 2020, Cambodia experienced an outbreak of influenza A(H3N2), that circulated in several provinces from July through to November 2020 including clustered detections in closed/semi-closed systems (prisons/pagodas), and also spreading in the general community. 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 Cambodia's inclusion in the vaccine's development represents a first for the country. A paper detailing the initial A(H3N2) outbreaks in mid- to late-2020 was published, and a second one which details the genetic and antigenic findings from the entire outbreak was also published in 2021.

Following the 2020 outbreak, IPC began to follow recommendations from the WHO's guidelines to maintain influenza surveillance, especially with the exceptionally low numbers of influenza infections detected worldwide in 2020 and 2021. Out of 4421 samples from symptomatic patients presenting an influenza-like illness or SARI during COVID-19 screenings in 2021, no human seasonal influenza was detected in Cambodia. In 2022, similar to the rest of the world, influenza began to rise again in Cambodia with an initial wave of A(H3N2) followed by Influenza B. In 2023, IPC tested 427 samples from ILI/SARI systems showing Cambodia experienced two waves of influenza, the first dominated by A/H1N1pdm and the second a mixture of A/H1N1pdm, A/H3N2, and B/Victoria (Figure 19)

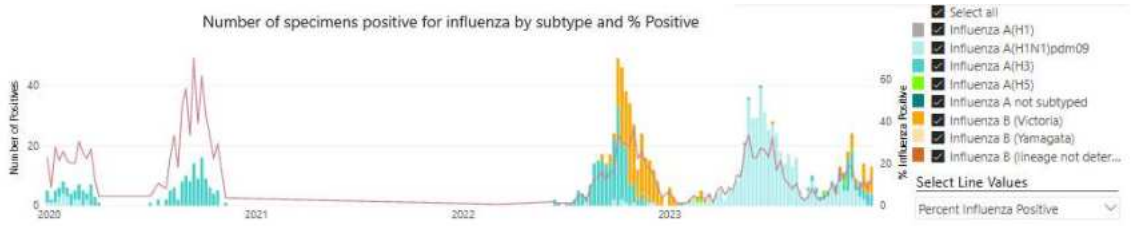


Figure 19: Number of influenza cases detected in sentinel and outbreak samples 2020-2023. No influenza cases were detected in Cambodia in 2021. Influenza returned to Cambodia in 2022. Data source: FluNet (https://www.who.int/tools/flunet)

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. RSV-B and PIV-3 were also detected sporadically. RSV and PIV were continued to be monitored in 2023 with 110 samples tested, detected RSV-A, RSV-B, PIV-1, and PIV-3 and sequencing, analysis, and publication are underway to describe these viruses in Cambodian children expected mid- to late 2023.

WHO H5 Reference Laboratory

Detection and response to A/H5N1 infections in humans in 2023.

Highly pathogenic avian influenza (HPAI; subtype A/H5N1) has been endemic in Cambodia since 2004 with 67 reported human cases (including 42 deaths; CFR 62.7%) and 65 reported poultry outbreaks up to February 2024. During 2013, a major increase in number of human HPAI A/H5N1 cases corresponded with the emergence of a reassortant Clade 1.1.2 virus that contained mutations linked with adaptation of the HPAI A/H5N1 virus to mammals. However, no inter-human transmission was identified following these changes, and no human cases of HPAI A/H5N1 had been reported since 2014. Between 2014 and 2018, the only circulating HPAI A/H5N1 viruses detected in Cambodia were of clade 2.3.2.1c. However, 2.3.4.4 viruses have been detected in Cambodian LBMs since 2019 (see below). In 2023, Cambodia detected 6 cases of human A/H5N1 in humans, 2 in February 2023 in Prey Veng, 1 in October in Svay Rieng, 1 in October in Prey Veng, and 2 in Kampot in November. All samples were sequenced within 24 hours of receiving sample using iMS-PCR and real-time sequencing on Oxford Nanopore GridION technology (see above). Coverage of the HA genome was 100x+ across the segment. Genetic analysis all cases indicate they are clade 2.3.2.1c (Figure 20) according to the hemagluttinin (HA) gene, similar to strains that have been circulating in Cambodia and Southeast Asia since 2013-2014. Sequences cluster closest to samples from sample cluster or poultry samples taken from vicinity of cases**.** Pattern of genomic epidemiology from the phylogenetic tree suggests all have exposure to dead or dying birds, which matches epidemiological data. No human-to-human transmission is suspected from phylogenetic analysis. Further work is ongoing and critical to perform deeper phylogenetic and molecular analysis of the genomic data, including full genomic assessment of other segments and relation to other recent samples in poultry.

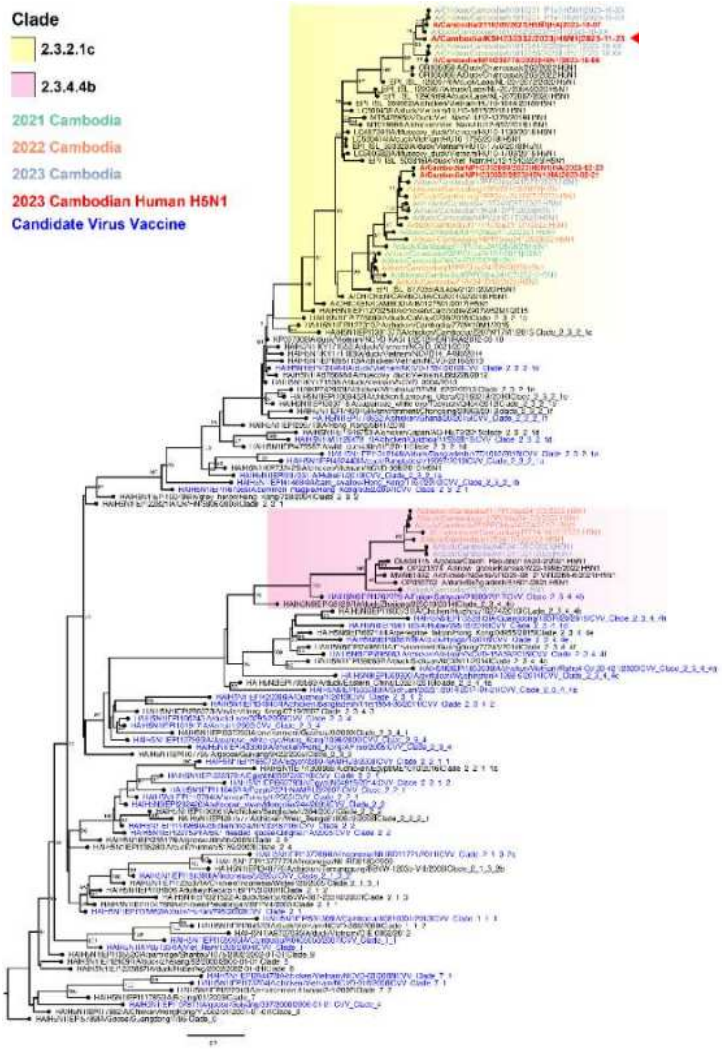


Figure 20: Phylogenetic subtree of A/H5Nx 2.3.2.1c hemagglutinin (HA) gene for typing of human A/H5N1 cases detected in Cambodia in 2023. Sequences were aligned using MAFFT v.7.490 (PMID: 23329690), trimmed using trimAL (PMID: 19505945) and phylogenetic trees were constructed using IQ-TREE v.2.0.3 (PMID: 32011700) with the best-fit nucleotide substitution model (GTR+F+I+G4 chosen according to Bayesian Information Criterion (BIC)). Trees were visualized and annotated using FigTree v.1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/) and Adobe Illustrator 27.3. Human A/H5N1 cases are highlighted in bold red. Bird market samples available from Cambodia are indicated in light green (2021), orange (2022), and light blue (2023). Candidate vaccine viruses are indicated in dark blue.

WHO Global COVID-19 Reference Laboratory

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, on 27 January, IPC confirmed the first COVID-19 case (a traveller from Wuhan) in Cambodia. In April 2020, the work done at IPC in response to the global COVID outbreak was recognized by designating the Virology Unit as a WHO COVID-19 global reference centre. 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. While testing significantly decreased since 2022, **by 31 December 2023, IPC had tested over 944.543 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.

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 as a reference to validate and verify all novel and incoming assays for SARS-CoV-2 before their use in the Kingdom. 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 that testing for all VoCs is widely available in the country. A publication detailing this work is expected by mid-2024.

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 can test 92 samples/day (644/week) in routine testing of new cases in Cambodia. These samples are chosen based on the availability of what was tested the previous day and selected from as many 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 2023, IPC had tested over 21,003 samples for VoC by RT-PCR to monitor Variants of Concern (VoC) circulating in and entering Cambodia (Figure 21).

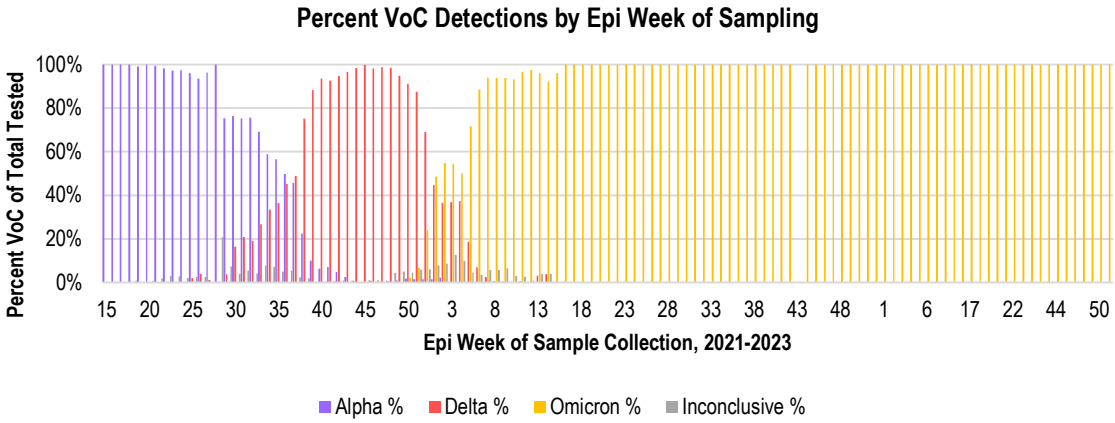


Figure 21: Detection of VOC in community samples in Cambodia by epidemiological week from April, 2021 to Dec 31st, 2023. Due to declining sample numbers after week 50 of 2021, percentages may not reflect true prevalence in the community.

SARS-CoV-2 Sequencing

IPC has been able to establish a highly multiplexed PCR amplicon approach using the ARTIC Network multiplex PCR primers set v3, v4, and MIDNIGHT 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 used weekly to sequence a limited number of samples to help with the COVID-19 response efforts and for monitoring clusters and community spread.

Between January 2020 and the end of December 31st 2023, IPC was able to sequence 3,405 samples (2.44 % of the total reported cases at that time) and submitted results to GISAID (Figure 22). Further work continues to monitor mutation rates in vaccinated versus unvaccinated individuals, transmission

dynamics, and the phylogeography of the viral spread. Two manuscripts have been written regarding SARS-CoV-2 sequencing in Cambodia. The first detailing the results from 2020 has been published in early 2023, the second looking at details from 2021 is in revision at *Communications Medicine* (see below).

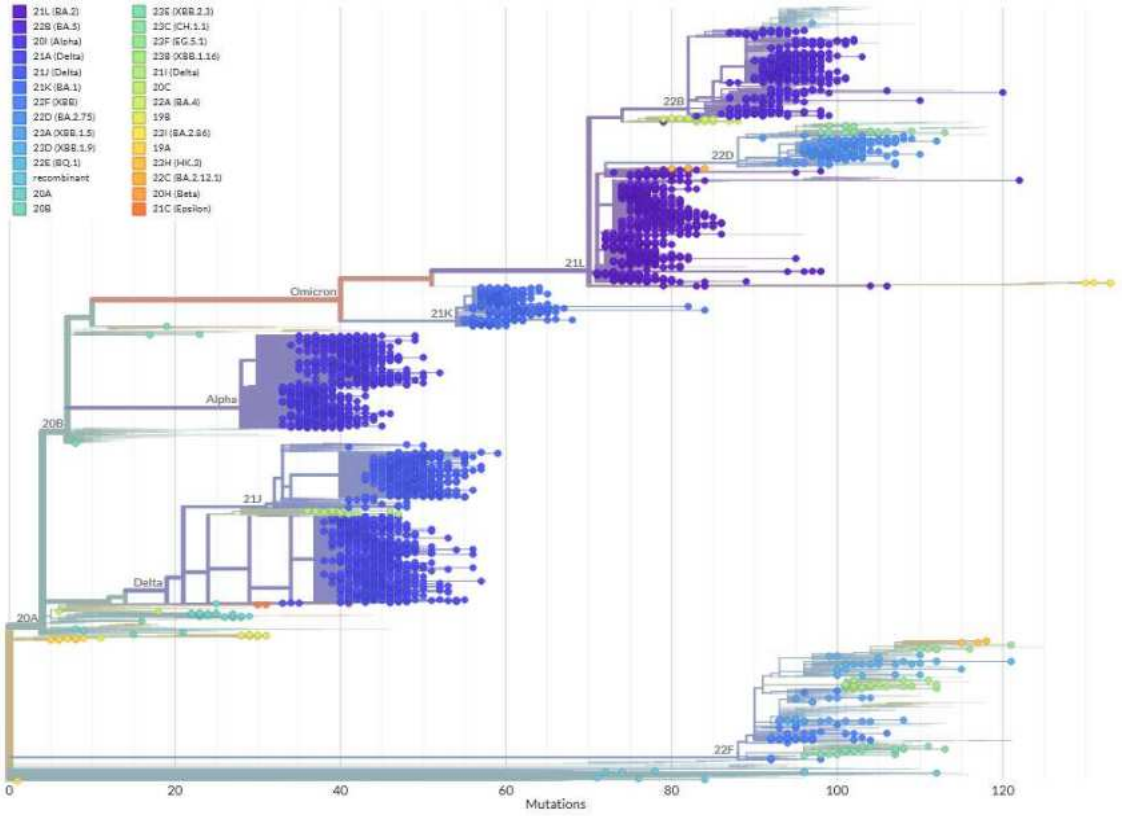


Figure 22: Phylogeny of SARS-CoV-2 sequences (n=4,039) between Jan 27th 2020 and Dec 31st 2023. Cambodian sequences are highlighted. Sequences from both IPC and NIPH are included in analysis as available from GISAID.

Monitoring Omicron and other VoC

Based on sequencing data at IPC and determined by PANGOLIN, Omicron subvariants groups BA.1, BA.2, BA.4, and BA.5 have been detected in Cambodia (Figure 23). Further breaking down the subvarinats based utilizing newly available tools on Nextclade and PANGOLIN, BA.1 and BA.2 can be further broken down into lineages of subvariants (Figure 22). Overall, BA.1 and BA.1.1 subvariants dominated until Week 4 of 2022, followed by a steep rise in BA.2 subvariants. BA.2 variants dominated in the beginning of 2022 with ~95% of Omicron sequences positive for this subvariant after Week 8, 2022. BA.2.12.1 was detected in one case in Week 21, 2022 and three more cases between week 23 and 25. BA.5 subsequently became the dominant variant in Cambodia from Week 23, 2022, mostly BA.5.2. BA.2.75 was first detected in one in Week 29. Other subvariants detected include BE, BF, BL, BM, BN, BQ, BW, CH, CP, GA, GE, GR XAZ, and XBB and have been increased in prevalence since Week 44, 2022. Subvariant BN (BN.1.2, BN.1.3, BN.1.4, BN.1.5) are now the dominant "other" dections since week 50, 2022. One sample of BQ.1.1 was detected in Week 48, 2022. XBB was first detected in one sample in Week 39, 2022 and has subsequently been detected 60 times with increasing frequency. XBB.1.5 was first detected in one sample in Week 3, 2023 AND XBB.1.16 began to be detected in May, 2023. XBB.1.16 and XBB.1.5 cocirculated with XBB.2 sublinages. *****VOI JN.1 was first detected in week 50, 2023 became the dominant variant detected.** However, the true prevlance may be unclear

due to numbers of samples being detected and sequenced, sample quality, and sequencing bias, which will affect percentages detected. Further work in 2024 will continue to monitor the introduction and spread of Omicron subvariants over time in different populations and expanded sequencing may need to occur to determine exact temporal and spatial spread.

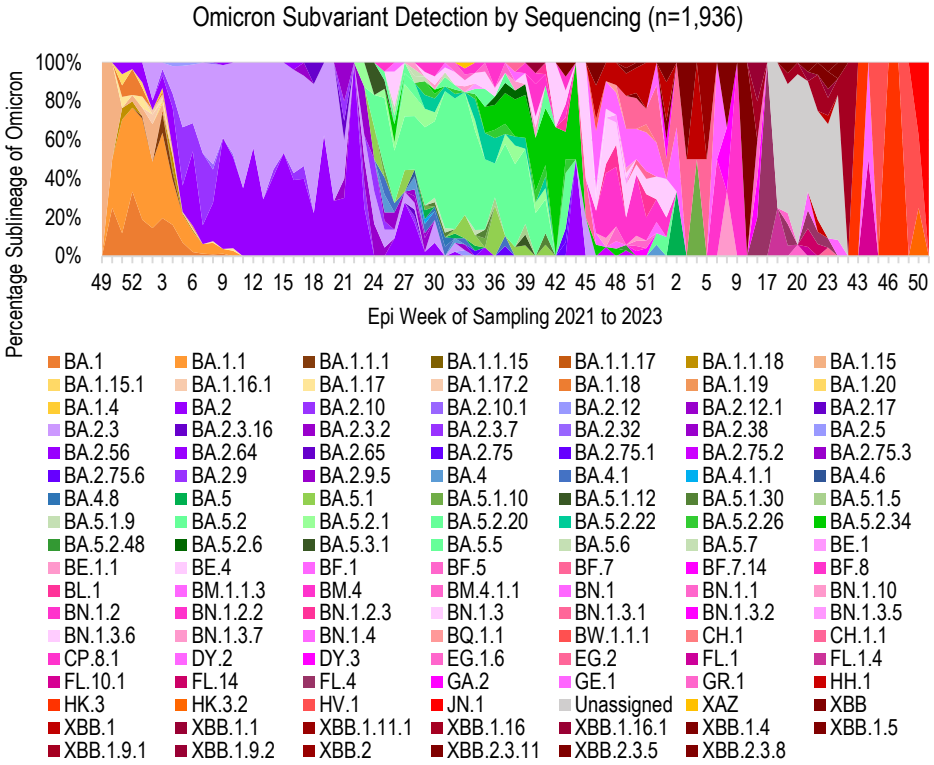


Figure 23: Detection of lineages of subvaraints of Omicron in fully sequenced samples (n=1,936) by epidemiological week of sampling from Dec. 14th, 2021 to Dec 31st, 2023. Percentage of each subvariant is represented as part of the entire sequencing for that individual week. BA.1 is represented in orange, BA.2 in purple, BA.4 in blue, BA.5 in green colors. "Other" subvariants such as BE, BF, BL. BM, BN, BQ, BW, CH, CP, XAZ, and XBB are in pink/red colors. XBB lineages are in dark red colors. JN.1 is in bright red. Sequences "unassigned" by GISAID/PANGOLIN are indicated in gray. Sequences from both IPC and NIPH are included in analysis as available from GISAID.

Analysis of genetic epidemiology of SARS-CoV-2 in Cambodia 2020-2021

The first case of COVID-19 in Cambodia was confirmed on 27 January 2020 in a traveller from Wuhan. Cambodia subsequently implemented strict travel restrictions, and although intermittent cases were reported during the first year of the COVID-19 pandemic no apparent widespread community transmission was detected. Investigating the routes of SARS-CoV-2 introduction into the country was critical for evaluating the implementation of public health interventions and assessing the effectiveness of social control measures. We detected 478 confirmed COVID-19 cases in Cambodia between 27 January 2020 and 14 February 2021, with 81.3 % being imported cases. Among them, 54 SARS-CoV-2 genomes were sequenced and analysed along with representative global lineages. Despite the low number of confirmed cases, we found a high diversity of Cambodian viruses that belonged to at least 17 distinct PANGO lineages. Phylogenetic inference of SARS-CoV-2 revealed that the genetic diversity of Cambodian viruses resulted from multiple independent introductions from diverse regions, predominantly Eastern Asia, Europe, and Southeast Asia. Most cases were quickly

isolated, limiting community spread, although there was an A.23.1 variant cluster in Phnom Penh in November 2020 that resulted in small-scale local transmission (Figure 24). The overall low incidence of COVID-19 infections suggests that Cambodia's early containment strategies, including travel restrictions, aggressive testing and strict quarantine measures, were effective in preventing large community outbreaks of COVID-19. These analyses have been published in early 2023.

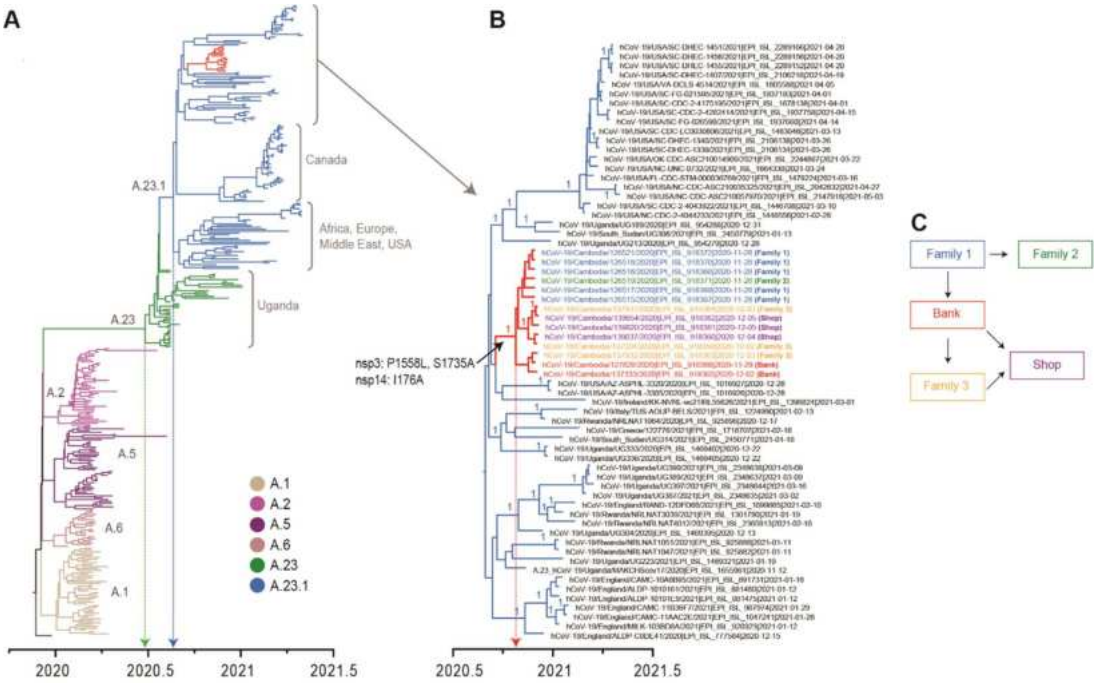


Figure 24: Temporal phylogeny of A.23 variants and related SARS-CoV-2 lineage A sequences (n=339). ***(A)*** *The maximum clade credibility (MCC) tree was reconstructed based on the whole genome of SARS-CoV-2 from Cambodia and globally. Colored branches represent various lineage A viruses. The green dotted line indicates the mean TMRCA estimate of A.23 lineage.* ***(B)*** *The estimated mean TMRCA of Cambodia A.23.1 viruses is indicated by the red dotted line. Three non-synonymous amino acid mutations unique to A.23.1 viruses from Cambodia are noted on the corresponding tree branch.* ***(C)*** *Relationships between A.23.1 virus infection clusters in Cambodia are determined through epidemiological investigation.*

Spatiotemporal evolution and transmission dynamics of Alpha and Delta SARS-CoV-2 variants contributing to sequential outbreaks in Cambodia during 2021

Tracking the emergence, introduction and spread of SARS-CoV-2 variants of concern are essential for informing public health strategies. In 2021, Cambodia faced significant challenges from two major epidemic waves caused by Alpha and Delta variants. Complete SARS-CoV-2 genomes from 1,163 COVID-19 patients in Cambodia from February–September 2021 were underwent evolutionary analyses to explore lineage diversification, and to infer virus population dynamics and transmission. The correlation between epidemic occurrence and public health control strategies were examined. Phylogeographic reconstruction was conducted to infer the spatiotemporal processes of Alpha and Delta variants. During the first wave, Cambodian Alpha variant diversified and rapidly expanded reaching an effective reproductive number exceeding 3.0 in March 2021. Subsequently, it acquired a spike E484K mutation, coinciding with the country's mass vaccination campaigns in April 2021. The Delta variant, with relatively stable growth, quickly displaced the Alpha variant, possibly due to key amino acid mutations associated with increased infectivity and transmissibility. Our findings suggest that the Alpha variant entered Cambodia through the capital city Phnom Penh, before spreading

nationwide. In contrast, the Delta variant was likely introduced via northern border provinces before disseminating to Phnom Penh and other provinces (Figure 25). Despite nationwide control measures and high vaccine coverage, the Delta variant rapidly replaced the Alpha variant, becoming predominant in Cambodia by August 2021. Genomic surveillance combined with public health strategies was vital for effectively tracking and responding to the emergence and dissemination of emerging variants.

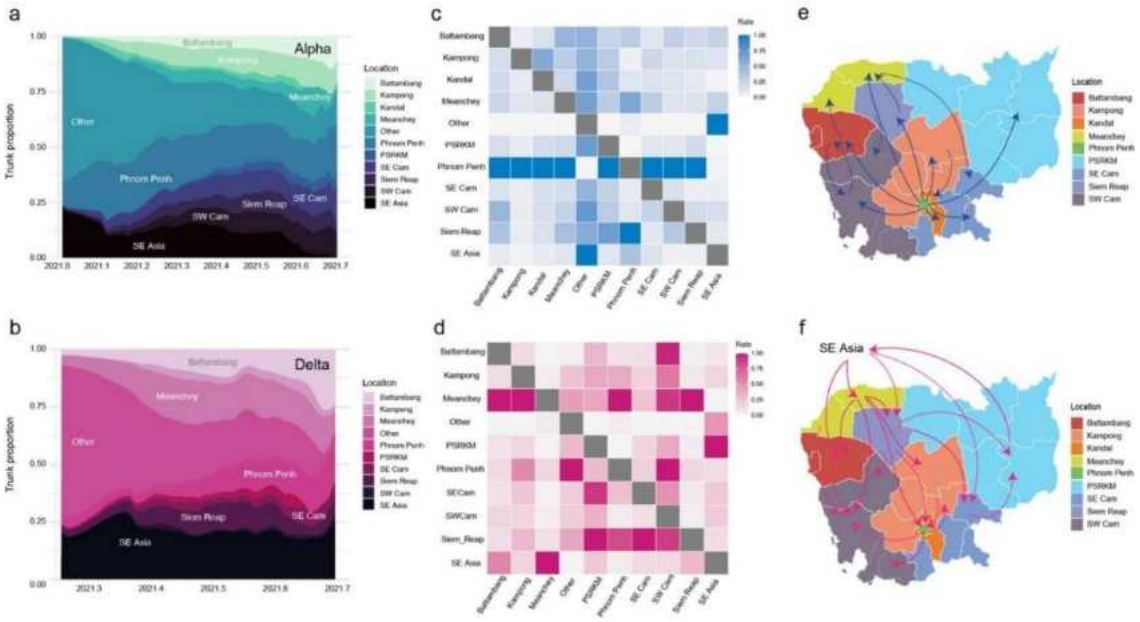


Figure 25: Spatiotemporal diffusion of the Alpha and Delta variants in Cambodia using discrete phylogeographic reconstruction. (a, b). Trunk reward proportion was estimated for each geographic location over time for the Alpha and Delta variant, respectively. (c, d). Asymmetric non-zero migration rates between pairwise location states were inferred using the Bayesian stochastic search variable selection (BSSVS) model. (e, f). Migration pathways with statistical support were inferred using SPREAD 3. Solid lines represent significant migration routes with strong support (Bayes factor of ≥100), and dotted lines represent transmission routes with moderate support (BF between 3 and 99). Abbreviations: Other, rest of the world; PSRKM include five provinces (Preah Vihear, Stung Treng, Ratanakiri, Kratie and Mondulkiri); SE Cam, Southeast Cambodia; SW Cam, Southwest Cambodia; and SE Asia, Southeast Asia.

Viral Isolation 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 the Delta VoC. This strain biobank is vital, not only for our continued validation and technical improvement work, but also for establishing serological assays for sero-epidemiological surveys and contact-tracing efforts. Further isolation attempts are made on all possibly viable samples available.

Diagnostic for rabies infection in animals

NAHPRI Training for Enhanced Rabies Diagnostic Capacity

To enhance the post-mortem rabies diagnostic capabilities at the National Animal Health and Production Research Institute (NAHPRI), a collaborative initiative was launched in partnership with the GIZ-funded support to the Royal Government of Cambodia. This is part of the GIZ global

programme for Pandemic Prevention and Response, One Health. The Virology Unit lends its technical expertise in rabies direct diagnostic through a twinning approach with NAHPRI. The focus is on diagnosing rabies virus in animal brain samples using the direct fluorescence antibody test (DFAT). This training was aimed at building their capacity in conducting DFAT for rabies virus. Throughout the project, quality assurance measures such as confirmatory testing and sample sharing upon request will be implemented to ensure the reliability and accuracy of the testing conducted at NAHPRI.

2023 Rabies Surveillance: Post-Mortem Diagnostic Outcomes at IPC Rabies Prevention Centers

The rabies surveillance activities conducted in 2023 encompassed free post-mortem diagnostics of animals involved in bite incidents, which were brought to one of the three IPC rabies prevention centers. This initiative was crucial in tracking and managing the incidence of rabies across different regions. In 2023, a total of 225 animals were tested for rabies (210 dogs and 15 cats). Out of these, 73% were found to be positive for rabies virus (160 dogs and 4 cats tested positive). The majority of these samples came from Prey Veng, indicating a higher incidence of rabies in this region compared to others.

PROVINCE	TESTED	POSITIVE	PREVALENCE
PREY VENG	44	38	86.4%
PHNOM PENH	30	14	46.7%
TAKEO	21	14	66.7%
KANDAL	20	14	70.0%
BATTAMBANG	20	14	70.0%
SVAY RIENG	16	14	87.5%
KAMPONG CHAM	14	12	85.7%
KAMPONG SPEU	14	9	64.3%
KAMPOT	13	8	61.5%
KAMPONG THOM	9	8	88.9%
TBONG KHMUM	4	4	100.0%
KAMPONG CHHNANG	4	4	100.0%
KRATIE	3	1	33.3%
BANTEAY MEANCHEY	2	2	100.0%
PREAH VIHEAR	2	2	100.0%
KEP	2	2	100.0%
OUDOR MEANCHEY	1	1	100.0%
SIHANOUKVILLE	1	0	0.0%
KOH KONG	1	1	100.0%
TOTAL	225	164	72.9%

The animal surveillance program not only aids in rabies monitoring but also serves as a valuable resource for global rabies research and control efforts. The biobank samples collected were utilized to support the FAO Reference Center on Rabies at the Istituto Zooprofilattico Sperimentale delle Venezie. These samples enabled the isolation of the virus, which was instrumental in creating a proficiency testing panel. This collaboration enhances the understanding of rabies virus characteristics and supports the development of more effective diagnostic and control strategies.

Diagnostic of Emerging Diseases

Confirmation and Sequencing of MPXV genomes from Monkey Pox cases from December 2023 in Phnom Penh

Full genome sequencing of MPXV was successful for 2 human cases (one from 11th December and one from 21st December, 2023) of mpox using AmpliSeq sequencing on Oxford Nanopore GridION technology. Phylogenetic analysis of the full genome of both cases indicates they are of the Clade IIb, lineage C.1 (Figure 26), similar to strains that have been circulating in Asia. Both samples phylogenetically cluster together, supporting potential for circulation within the community; however, further analysis is needed. Critically, these samples are genetically distant from the original case of mpox in Cambodia ex. Thailand which was of Clade IIb.A.2. IPC Virology Unit continues to support the mpox response in Cambodia into 2024.

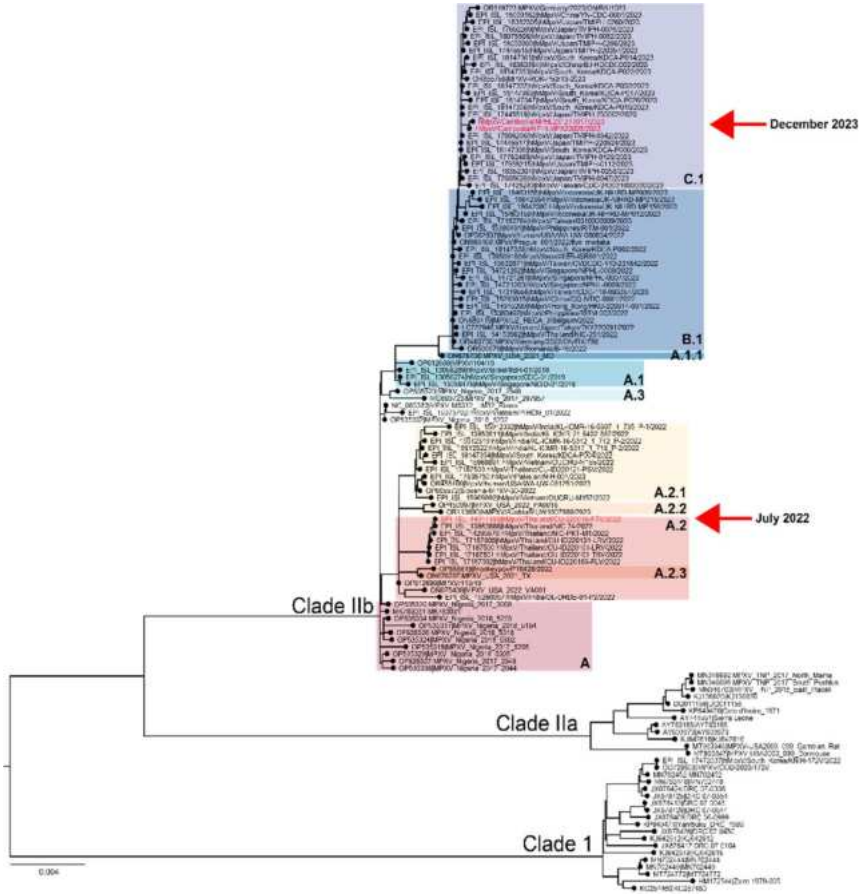


Figure 26: Phylogenetic tree of mPox sequences from cases detected in July 2022 and December 2023. Sequences were aligned using MAFFT v.7.490 (PMID: 23329690), trimmed using trimAL (PMID: 19505945) and phylogenetic trees were constructed using IQ-TREE v.2.0.3 (PMID: 32011700) with the best-fit nucleotide substitution model (GTR+F+I+G4 chosen according to Bayesian Information Criterion (BIC)). Trees were visualized and annotated using FigTree v.1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/) and Adobe Illustrator 27.3. mPox cases are highlighted in bold red. Detections and time are indicated by a red arrow. Lineages are indicated by color.

Implementation of Internal Quality Control for Respiratory Pathogen Testing at NIPH Laboratories

To establish a robust Internal Quality Control (IQC) system for the testing of Influenza A & B, SARS-CoV-2, and RSV A and B, an internal quality control was conducted for NIPH by providing control samples specifically for these pathogens. This initiative involved the use of GeneXpert cartridges and aimed to ensure the accuracy, reliability, and consistency of test results across 35 laboratories.

The IQC system was meticulously designed to regularly evaluate and maintain the performance of diagnostic testing. By using control samples of Influenza A & B, SARS-CoV-2, and RSV A & B, the system allows for the continuous monitoring of the testing process, identifying any deviations or inconsistencies in the results. This approach is vital for guaranteeing that the laboratories consistently produce reliable and accurate test outcomes, which is crucial in effective disease surveillance and patient care management. The implementation of this IQC system represents a significant step towards enhancing the quality of diagnostic services provided by NIPH across its network of laboratories.

4.4.5 Teaching and training

PhD students

- Ou Tey Putita, Université de Montpellier, France, 2021-2024, Diversity and characterization of coronaviruses circulating in bats in Cambodia
- Julia Guillebaud, University of Montpellier, France, 2022-2025, Assessment of the risk of emergence of coronaviruses in human population at the wildlife interface in Cambodia
- Frida Esther Sparaciari, James Cook University, Australia, 2023-2026, University, Development of a framework for determining zoonotic emerging infectious disease risk profiles at traditional food markets in Cambodia
- Alexander Tatuya Tendu, University of Chinese Academy of Sciences, 2022-2025. Characterization of viromes from mosquitoes collected in Kampong Thom, Cambodia, with a focus on flaviviruses.
- Betty Nalikka, University of Chinese Academy of Sciences, 2022-2025. Characterization of viromes from Scotophilus bats collected in Kampong Cham, Cambodia, with a focus on paramyxoviruses.

Master students

- Benoit Batallan, l'Université Paris Cité, France, November 2023 - June 2024, M2 Life Sciences & Health, International Track – Infectiology: Biology of Infectious Diseases – Cambodia, Etiology agents of severe community-acquired pneumonia in two hospitals in Phnom Penh
- Jule Spooren, Université Paris-Saclay, May - June 2023 M1 Life Sciences and health-International Track - Infectiology: Biology of infectious diseases – Cambodia, Current knowledge of tick-borne pathogens and potential spillover in human
- Lea Renouard, Sup'Biotech, May - August 2023, Pathogen detection using novel sampling techniques in Cambodia
- Nina Rault, Université de Montpellier, January - June 2023Exposition à différents coronavirus d'intérêts de populations humaines à l'interface avec la faune sauvage au Cambodge
- Thomas Martinez, Université de Montpellier, December 2023 – May 2024, Arboviruses in Cambodia : virological diagnostics and molecular epidemiology
- Thavry Hoem, Master of Science in Epidemiology program at National Institute of Public Health, 2022-2024, Knowledge, attitudes, and practices (KAP) towards zoonotic transmission from wildlife in the context of COVID-19 in two provinces, Cambodia

Bachelor students

- Mr Heng Leangyi, second-year bachelor's student in Medical Laboratory Technology at the University of Health Sciences, Cambodia

- Mr. Chel Kimtuo, second-year bachelor's student in Medical Laboratory Technology at the University of Health Sciences, Cambodia
- Mrs. Chantha Soursdey, second-year bachelor's student in Medical at University of Puthisastra, Cambodia

Teaching

- Veasna Duong, course "Arbovirus in Cambodia" (1.5h) for M2 Master Life Science and Health – Infectiology: Entomology: Vector-Borne Diseases at University of Health Sciences in Phnom Penh
- Veasna Duong, course "Zika virus" (1.5h) for M2 Master Life Science and Health – Infectiology: Advances in Virology at University of Health Sciences in Phnom Penh
- Veasna Duong, course "Arbovirus" (2h) in module Microbiology for Master Medical Biology at University of Health Sciences in Phnom Penh
- Veasna Duong, course "Picornaviridae" (2h) in module Microbiology for Master Medical Biology at University of Health Sciences in Phnom Penh
- Veasna Duong, course "Hemorragic Fever viruses" (2h) in module Microbiology for Master Medical Biology at University of Health Sciences in Phnom Penh
- Heidi Auerswald, course "Origin and Evolution of Rabies Virus" (1.5h) for M1 Master Life Science and Health – Infectiology: Biology of Infectious Diseases at University of Health Sciences in Phnom Penh
- Heidi Auerswald, course "Transmission Routes and Defenses" (1.5h) in module Host-pathogen Interactions for M2 Master Life Science and Health – Infectiology: Biology of Infectious Diseases at University of Health Sciences in Phnom Penh
- Heidi Auerswald, course "Local Adaptions sand Spatial Variation" (1.5h) in module Host-pathogen Interactions for M2 Master Life Science and Health – Infectiology: Biology of Infectious Diseases at University of Health Sciences in Phnom Penh
- Heidi Auerswald, course "Laboratory Techniques for the Diagnosis of Viral Infections" (1.5h) in module Microbiology for Master Medical Biology at University of Health Sciences in Phnom Penh
- Heidi Auerswald, course "Prophylaxis of Viral Diseases - Vaccination" (1.5h) in module Microbiology for Master Medical Biology at University of Health Sciences in Phnom Penh
- Heidi Auerswald, course "Host-pathogen Interactions " (1.5h) for Bachelor/Graduate in Veterinary Sciences at Pontificia Universidad Cattolica de Chile in Santiago
- Janin Nouhin, course "Viral hepatitis (A, B, C, D, and E)" (3h) in module Microbiology for Master Medical Biology at University of Health Sciences in Phnom Penh
- Janin Nouhin, course "HIV virology" (2h) in module Microbiology for Master Medical Biology at University of Health Sciences in Phnom Penh
- Channa Mey, course "Immunology" (6h) for year 3 medical students at University of Health Sciences in Phnom Penh

Received Training

- Heng Leangyi and Heidi Auerswald, along with staff from NAHPRI and the National Animal Health Laboratory from Laos, attended a 2-week training at the Istituto Zooprofilattico Sperimentale (IZS) in Teramo, Italy. This training was focused on deepening their knowledge and skills as administrators of the Laboratory Information Management System (LIMS) SILAB,

enhancing their ability to effectively manage and utilize this crucial laboratory information system.

- Heidi Auerswald participated in the PVS Sustainable Laboratories Expert Training at the WOAH Headquarters in Paris, France. This training aimed to enhance the capabilities of PVS Laboratory Experts, focusing on sustainable laboratory practices. It provided a platform for learning and networking, ensuring that participants are well-informed and equipped to implement sustainable practices in their laboratories.
- Janin Nouhin received a 5-week training on metagenomics for pathogen discovery in the Laboratory of Pathogen Discovery (LDP) at IP Paris, Paris, France.
- Janin Nouhin attended Grant Writing Workshop, Rockville, MD USA, June 16, 2023

4.4.6 Outlook for upcoming 3 – 5 years

In 2023, the Unit continues being restructured to delegate responsibilities to senior scientists and talented staff, and to meet the Unit's new research objectives. The arrival of three scientists from IP Shanghai and a Post-Doc from James Cook University with some expertise in molecular tools, vaccine development, and other skills in metagenomic sequencing and field work experiences have complemented the ongoing restructuration to broaden to research scope to the Unit.

To sustain and further build on this expansion, several critical short and medium-term plans to improve technical skills through training or technology transfer, to bring in senior researchers including post-docs or visiting scientists, to extend collaboration with complementary expertise including fundamental research, and to improve laboratory management are a priority for the Unit. It is also crucial for the Unit to continue to publish the huge amount of data generated.

We expect to accomplish and strengthen the following points:

- Capacity and capability for *in-vitro* work, including a greatly expanded biorepository of immortalized and primary cell lines, implementation of reverse genetic system for host-pathogen study such as virus-like particles and pseudoviruses;
- Skills in next generation sequencing (NGS) and metagenomics sequencing, full integration of sequencing capacity with our mini-Platform will continue to improve;
- To improve and strengthen bioinformatics activities, a dedicated junior bioinformatician will be recruited to work alongside with other scientists;
- Luminex assays were implemented on a number of viruses, including CoV and arboviruses, and will be expanded to other pathogens to support surveillance and research programs;
- Collaboration within IP-Paris, across the IPIN, and global partners will continue to strengthen in the aim of expanding the Unit's capability for fundamental research;
- Fostering the regional collaboration in the area of emerging infectious diseases to increase visibility and increase funding opportunities;
- Research programs in connection with basic science for mechanistic studies will continue to be developed;
- Through research programs, we continue to bring postdoctoral fellows and visiting scientists to complement the expanded activities;
- The Unit will enrol more students in Master's and PhD programs (both Cambodian students attending foreign universities and foreign students conducting research in the Virology Unit in collaboration with universities);
- The laboratory's database system will be improved using a more standardised laboratory information management system;

- The Unit is preparing for ISO17025 accreditation for its reference laboratory activities;
- Finalizing designation as a FAO Reference Center and WHO Collaborating Center, both for Innovation in Emerging and Endemic Virus Surveillance and response;
- We seek to strengthen and expand our activities as a reference laboratory, such as by obtaining the WOAH rabies reference laboratory status, by the expansion of our rabies serology service for testing non-domestic samples, by the development and updating of new assays including serology and molecular techniques, by a continued participation in proficiency testing for molecular, virological and serological diagnoses, and by becoming a reference laboratory for zoonotic research and emerging infectious diseases.

4.4.7 Scientific Publications 2023

1. **Arbovirus researchers unite: expanding genomic surveillance for an urgent global need**
   Gabriel Luz Wallau Author links open overlay panelNgu Njei Abanda, Adriano Abbud, Saro Abdella, Adugna Abera, Steve Ahuka-Mundeke, Francesca Falconi-Agapito, Kalichamy Alagarasu, Kevin K Ariën, Constancia Flávia Junqueira Ayres, Luisa Barzon, Joseph Humphrey Kofi Bonney, Sanaba Boumbaly, Philippe Buchy, Van-Mai Cao-Lormeau, Yu Kie Chem, Paul A Cardenas, Andres E Castillo, Adriana Delfraro, Gregor Devine, Veasna Duong, Myrielle Dupont-Rouzeyrol, Artem V Fadeev, Alvaro Fajardo, Luis Adrián Diaz, Lara Ferrero Gómez, Eduardo Samo Gudo, Gladys Gutierrez-Bugallo, Hapuarachchige Chanditha Hapuarachchi, Jean-Michel Heraud, Martin L Hibberd, Osvaldo Frederico Inlamea, Nik Jasmin, Kalysbek Kydyshov, Maria Ezekiely Kelly, Salim Khan, Andrey B Komissarov, Pornsawan Leaungwutiwong, Mariana Leguia, Yaniv Lustig, Rafael Maciel-de-Freitas, Gathsaurie Neelika Malavige, Alexander A Martinez, Maria L Mendoza, Luong T Mo, Brechla Moreno, Lydia Mwasi, Felipe Gomes Naveca, Lee Ching NG, Richard Njouom, Mauricio Lacerda Nogueira, Francine Ntoumi, Nehemie Nzoyikorera, Barbara A Parra, Mauricio Vázquez Pichardo, Kristine Joy Ragual Privaldos, Ricardo Rivero, Alejandra María Rojas, Richard Steiner Salvato, R Tedjo Sasmono, Jonas Schmidt-Chanasit, Etienne Simon-Loriere, Ava Kristy Dy Sy, Michael Talledo-Albujar, Daniel Thakuma Tizhe, Usenbaev Nurbolot Toloshovich, Vi Thuy Tran, Cécile Troupin, John Timothy Kayiwa, Andrew van den Hurk, Nikolaos Vasilakis, Atsbeha Gebreegziabxier Weldemariam, Sophie Yacoub, Zainun Zaini, Gabriel Luz Wallau
   The Lancet Global Health 2023-08-01 DOI : 10.1016/S2214-109X(23)00325-X

2. **Characterization of soluble TLR2 and CD14 levels during acute dengue virus infection.**
   Upasani V, Ter Ellen BM, Sann S, Lay S, Heng S, Laurent D, Ly S, Duong V, Dussart P, Smit JM, Cantaert T, Rodenhuis-Zybert IA.
   Heliyon 2023 Jun 21;9(6):e17265. doi: 10.1016/j.heliyon.2023.e17265. eCollection 2023 Jun.

3. **Construction and characterization of a new hepatitis C virus genotype 6a subgenomic replicon that is prone to render the sofosbuvir resistance.**
   Chaolun Liu, Mingzhe Guo, Lin Han, Jie Lu, Xiaogang Xiang, Qing Xie, Janin Nouhin, Veasna Duong, Yimin Tong, Jin Zhong
   Journal of Medical Virology 2023 Sep;95(9):e29103. doi: 10.1002/jmv.29103.

4. **Detection and Phylogenetic Analysis of Contemporary H14N2 Avian Influenza A Virus in Domestic Ducks in Southeast Asia (Cambodia)**
   Jurre Y. Siegers, Michelle Wille, Sokhoun Yann, Songha Tok, Sarath Sin, Sokha Chea, Alice Porco, Sreyem Sours, Vutha Chim, Samban Chea, Kimtuo Chhel, Sothyra Tum, Sorn San, Peter Thielen, Vijaykrishna Dhanasekaran, Erik A. Karlsson
   Emerging Microbes & Infections DOI: 10.1080/22221751.2023.2297552

5. **Detection and Phylogenetic Analysis of Highly Pathogenic A/H5N1 Avian Influenza Clade 2.3.4.4b Virus in Chile, 2022**
   Pedro Jimenez-Bluhm, Jurre Y. Siegers, Shaoyuan Tan, Bridgett Sharp, Pamela Freiden, Magdalena Johow, Katherinne Orozco, Soledad Ruiz, Cecilia Baumberger, Pablo Galdames, Maria Antonieta Gonzalez, Camila Rojas, Erik A. Karlsson, Christopher Hamilton-West, Stacey Schultz-Cherry
   Emerging Microbes & Infections **https://doi.org/10.1080/22221751.2023.2220569**

6. **Detection of Clade 2.3.4.4b Avian Influenza A(H5N8) Virus in Cambodia, 2021**
   Kimberly M Edwards, Jurre Y Siegers, Xiaoman Wei, Ammar Aziz, Yi-Mo Deng, Sokhoun Yann, Chan Bun, Seng Bunnary, Leonard Izzard, Makara Hak, Peter Thielen, Sothyra Tum, Frank Wong, Nicola S Lewis, Joe James, Filip Claes, Ian G Barr, Vijaykrishna Dhanasekaran, Erik A Karlsson
   Emerg Infect Dis. 2023 Jan;29(1):170-174. doi: 10.3201/eid2901.220934.

7. **Human immunodeficiency virus 1 5′-leader mutations in plasma viruses before and after the development of reverse transcriptase inhibitor-resistance mutations**
   Janin Nouhin, Philip Lei Tzou, Soo-Yon Rhee, Malaya K. Sahoo, Benjamin A. Pinsky, Miri Krupkin, Joseph D. Puglisi, Elisabetta V. Puglisi, Robert W. Shafer
   Journal of General Virology Published: 06 October 2023 https://doi.org/10.1099/jgv.0.001898

8. **Inundative, Dry-Powder, Inhaled Measles Vaccination to Prevent Deaths of Young Children in War-torn Regions**
   Benjamin L Sievers, Robert E Sievers, Eric L Sievers
   Open Forum Infectious Diseases 2023 Jun 5;10(6):ofad302. doi: 10.1093/ofid/ofad302. eCollection 2023 Jun.

9. **Longitudinal comparison of bacterial pathogen seropositivity among wet market vendors in the Lao People's Democratic Republic**
   Nilandone Senvanpan, Vilayouth Phimolsarnnousith, Sayaphet Rattanavong, Mayfong Mayxay, Daniel Reinharz, Amanda E Fine, Paul F Horwood, Philippe Dussart, Stuart D Blacksell, Mathieu Pruvot, Paul N Newton, Matthew T Robinson
   One Health. 2023 Aug 25:17:100618. doi: 10.1016/j.onehlt.2023.100618. eCollection 2023 Dec.

10. **National dengue surveillance, Cambodia 2002-2020**
    Christina Yek, Yimei Li, Andrea R Pacheco, Chanthap Lon, Veasna Duong, Philippe Dussart, Katherine I Young, Sophana Chea, Sreyngim Lay, Somnang Man, Souv Kimsan, Chea Huch, Rithea Leang, Rekol Huy, Cara E Brook, Jessica E Manning
    Bulletin of the World Health Organization 2023 Sep 1;101(9):605-616. doi: 10.2471/BLT.23.289713. Epub 2023 Jul 5.

11. **Neutralization of African enterovirus A71 genogroups by antibodies to canonical genogroups**
    Romain Volle, Lingjie Luo, Richter Razafindratsimandresy, Serge Alain Sadeuh-Mba, Ionela Gouandjika-Valisache, Paul Horwood, Veasna Duong, Philippe Buchy, Marie-Line Joffret, Zhong Huang, Erwin Duizer, Javier Martin, Lisa A Chakrabarti, Philippe Dussart, Nolwenn Jouvenet, Francis Delpeyroux, Maël Bessaud
    J Gen Virol . 2023 Nov;104(11). doi: 10.1099/jgv.0.001911

12. **Obesity Is Associated with an Impaired Baseline Repertoire of Anti-Influenza Virus Antibodies**
    Marwa Abd Alhadi, Lilach M. Friedman, Erik A. Karlsson, Liel Cohen-Lavi, Anat Burkovitz, Stacey Schultz-Cherry, Terry L. Noah, Samuel S. Weir, Lester M. Shulman, Melinda A. Beck, Tomer Hertz
    Microbiology Spectrum 26 April 2023 DOI**:** https://doi.org/10.1128/spectrum.00010-23

13. **One assay to test them all: Multiplex assays for expansion of respiratory virus surveillance**
Narjis Boukli, Claude Flamand, Kim Lay Chea, Leangyi Heng, Seangmai Keo, Kimhoung Sour, Sophea In, Panha Chhim, Bunthea Chhor, Lomor Kruy, Jelena D. M. Feenstra, Manoj Gandhi, Obiageli Okafor, Camilla Ulekleiv, Heidi Auerswald, Viseth Srey Horm and Erik A. Karlsson
Frontiers of Medicine, 24 April 2023 doi.org/10.3389/fmed.2023.1161268

14. **Ribosomal RNA (rRNA) sequences from 33 globally distributed mosquito species for improved metagenomics and species identification**
Cassandra Koh, Lionel Frangeul, Hervé Blanc, Carine Ngoagouni, Sébastien Boyer , Philippe Dussart, Nina Grau, Romain Girod, Jean-Bernard Duchemin, Maria-Carla Saleh
Elife. 2023 Jan 23;12:e82762. doi: 10.7554/eLife.82762.

15. **Side-by-side comparative study of the immunogenicity of the intramuscular and intradermal rabies post-exposure prophylaxis regimens in a cohort of suspected RABV exposed individuals**
Heidi Auerswald, Alvino Maestri, Sothy Touch, Saraden In, Nisa Ya, Borita Heng, Valérie Bosch-Castells, Christele Augard, Céline Petit, Philippe Dussart, Yiksing Peng, Tineke Cantaert, Sowath Ly
Clinical Infectious Diseases 2023 Jun 20;ciad304. doi: 10.1093/cid/ciad304

16. **Small mammals at the edge of deforestation in Cambodia: Transient community dynamics and potential pathways to pathogen emergence**
Mathieu Pruvot, Sokha Chea, Vibol Hul, Samat In, Vuthy Buor, Jill-Lea Ramassamy, Caroline Fillieux, Seng Sek, Ratha Sor, Sela Ros, Sithun Nuon, Sovannary San, Yaren Ty, Marany Chao, Srey emSours, Sreyleap Torng, Unthyda Choeurn, Udam Hun, Sophorn Ton, Samnang Y, Sonara Phon, Lina Kuy, Amanda Fine, Philippe Dussart, Veasna Duong, Paul F. Horwood, Sarah H. Olson
One Earth, Available online 29 November 2023. 10.1016/j.oneear.2023.11.003

17. **Uncovering the endemic circulation of rabies in Cambodia**
Maylis Layan, Laurent Dacheux, Philippe Lemey, Kirstyn Brunker, Laurence Ma, Cécile Troupin, Philippe Dussart, Véronique Chevalier, James L. N. Wood, Sowath Ly, Veasna Duong, Hervé Bourhy, Simon Dellicour
Molecular Ecology 04 August 2023 DOI : 10.1111/mec.17087

4.5 Medical & Veterinary Entomology Unit

4.5.1 Functional Structure

The Medical and Veterinary Entomology Unit was officially created on 1 October 2018 (our reference: N°413/IPC/DIR/2018), coinciding with the recruitment of Dr. Sebastien Boyer by Institut Pasteur in Paris for a permanent position. The unit's staff has 16 members. Two foreign scientists (Antsa Rakotonirina and Sébastien Boyer) lead the research. Ms. Yean Sony, a Cambodian national, began her PhD on ticks and tick-borne diseases in October 2022, in Cambodia. Mr. Bros Doeurk begun his PhD in October 2023.

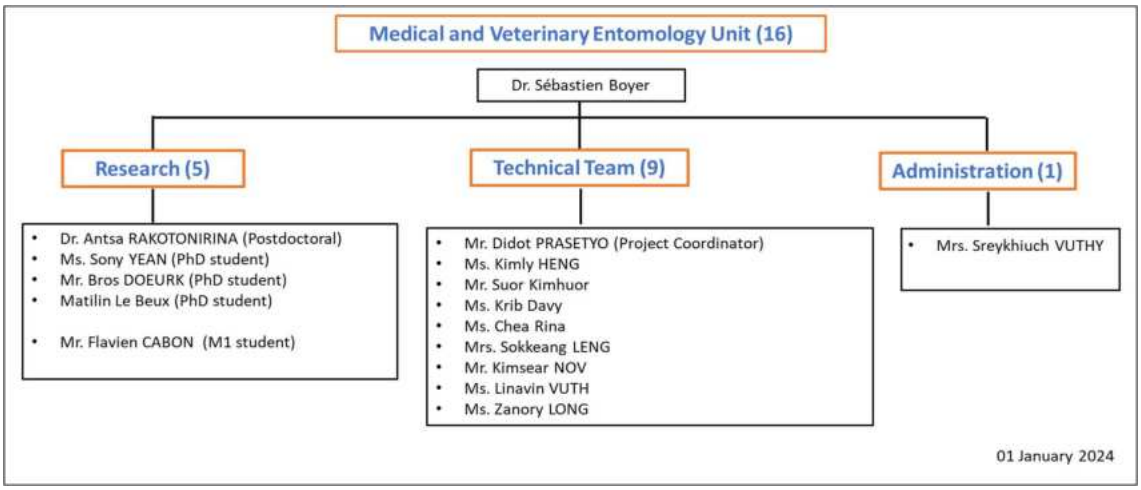


Figure 27: Organizational chart of the Medical and Veterinary Entomology Unit

4.5.2 Major Achievements in 2023

Field Missions in 2023

During the year 2023, the Medical and Veterinary Entomology Unit undertook **58 field missions** in Cambodia (compared to 39 in 2022 and 35 in 2021), for a total of **539 mission days** in the field (compared to 399 in 2022 and 393 in 2021).

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

The PICREID project aims 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, the understanding of endemic RNA virus transmission, the determination of factors influencing RNA adaptations to new hosts, and on the 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, and to further analyze and model the spatial distribution and the land-use effects on the dynamics. The other objective will be to model the risk of dengue by linking the number of dengue vectors and the number of dengue cases in humans. Finally, we also coupled the pathogen discovery objective with an entomological-based genomic surveillance, in collaboration with Institut Pasteur in Paris.

In 2023, 17 field missions were accomplished. Twelve missions covered the dynamics of relative mosquito densities in Kampong Thom provinces, in relation to different types of land use. Two

missions were dedicated to larva collection for the identification of breeding sites. Two other missions were carried out to determine the biting patterns of mosquito species in the field. Finally, one mission's purpose was to sample ticks that will be sent to Institut Pasteur in Paris.

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

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

The project will analyze the mechanisms underlying the impact of the 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. If deemed feasible, surveillance and pathogen detection strategies will be implemented.

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

The project's strategies will be implemented in Europe and in three tropical biodiversity hotspots, in Southeast Asia, West Africa and the Caribbean. BCOMING 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 ability to detect emerging epidemics and stop them before they turn into pandemics. The project's consortium is a strong multi-actor group of partners with a history of successful cooperation, including academics from the 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 of the proposal's expected outcomes. The embedding of BCOMING in the PREZODE Initiative will help to scale up the project's innovations as well as disseminate cutting-edge socio-economic and environmental strategies.

Field missions begun in 2023. In 2023, the Unit participated to four missions in the Stung Treng Province. During these missions, bats were captured at dusk on three karst mountains using mist nets: Chhgnauk, Ka Ngoark and Chab Pleurng (longitudinal follow-up). Ectoparasites, mainly Nycteribiidae, Streblidae, Arachnida (mites, ticks) and bat-bugs, were collected with forceps directly from the bats' bodies, before being placed in Eppendorf tubes with alcohol. A total of over 1,500 ectoparasites were captured during the four campaigns carried out in 2023. In addition to bats' ectoparasites, CDC light traps were also installed to capture mosquitoes and phlebotomines (sand flies). All the specimens obtained during the missions were then analyzed in the unit's laboratory, and for the most part identified to a specific level. In September 2023, Flavien CABON, a master's student in biology at Paul Sabatier University (Toulouse, France) and taxonomic entomologist, joined the Unit to work on bat ectoparasites, mainly Nycteribiidae and Streblidae. His research focuses on the taxonomy of these insects and on the host-parasite specialization between these batflies and bats in Cambodia.

Collaborations	CIRAD (Julien Cappelle) Virology Unit Ministry of Environment, Cambodia Institut de Technologie du Cambodge (ITC), Cambodia Royal University of Phnom Penh, Cambodia
Funding	French National Research Agency (ANR - Agence Nationale de la Recherche) 2023-2026

Proof of Concept: Detection of Dengue and Chikungunya Viruses in Mosquitoes using MALDI-TOF MS 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 the performance of the MALDI-TOF MS to analyze mosquito spectra.

In this study, we 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 were infected by dengue and chikungunya viruses in a biosafety security level P3 (Pathogen level 3) laboratory, through their exposition to an infectious blood meal. In parallel, other *Ae. aegypti* and *Ae. albopictus* were fed with uninfected blood. At day ten post blood feeding, mosquito saliva were collected. All mosquitoes were subsequently killed and dissected. Mosquito abdomens were used for RNA extraction and RT-PCR while the protein extraction and MALDI-TOF MS analysis were carried out using saliva, legs and thorax individually.

The experiment was repeated three times (three batches) independently. In the first experiment, 29 *Ae. aegypti* and 47 *Ae. albopictus* were used. In the second experiment, 61 *Ae. aegypti* and 89 *Ae. albopictus* were used and in the third experiment 92 *Ae. aegypti* and 39 *Ae. albopictus* were used.

The preliminary data on the first batch indicated that RT-qPCR and MALDI-TOF MS were both able to detect arboviruses in infected mosquitoes. The distinction between infected and uninfected *Ae. aegypti* and *Ae. albopictus* was made using mosquito saliva, legs, and thorax. Importantly, these results seem to allow the identification of potential biomarkers of DENV and CHIKV infection in the two mosquito species. Even through this preliminary data remains to be confirmed, it seems that MALDI-TOF MS is able to recognize CHIKV and DENV-infected mosquitoes according to their spectral patterns.

Matilin Le Beux (from the University of Sud Bretagne in France) begin working on these data during his Master 2 internship. He is currently a PhD student and continues the analysis. His main work on this part is to find the optimal model allowing to distinguish infected form uninfected mosquitoes and to test the reproducibility of the results using the second and the third batches. The results could provide interesting perspectives on the use of this technique as an inexpensive tool for exploring the immune response of arbovirus-infected mosquitoes.

Collaborations	Virology Unit, IPC Institut Pasteur of Korea
Funding	IPC, Medical and Veterinary Entomology Unit

Ecological characteristics and temporal dynamics of mosquito species in Mondulkiri province, Cambodia

Based on the important work accomplished last year in taxonomy, in 2023 a second-year master's degree student, Mr. Bros Doeurk worked on the characterization of the main factors driving the

distribution and the dynamics of mosquito species in Mondulkiri's forests. The mosquitoes were collected monthly, over 11 months in 2020, from ten collection sites in villages and forests. Among the ten sites, nine used human odor-baited, double net traps only, and one site used both human and cow odor-baited double net traps.

A total of 54,680 individual mosquitoes belonging to 16 genera and 119 species were collected. Regarding the nine sites with a human odor-baited net only, the three dominant species, *Culex vishnui.g, Aedes albopictus*, and *Anopheles dirus* represented 35.4% of the collected mosquitoes. They are known for their public health importance, being known vectors of the Japanese encephalitis virus, the dengue virus and the malaria parasite, respectively. Meteorological factors impacting the relative density of these species, as well as that of the other species present, were analyzed. The biting patterns of the three dominant species were significantly different over the 24-hour collection period. Interestingly, they all proved to be active during both day and night, which means that they can infect humans in villages and forests at any time. Our study showed a lower diversity of mosquitoes in villages compared to the forests. Many mosquito species displayed a zoo-anthropophilic behavior and occurred in a similar proportion in both human odor and cattle odor-baited traps. Occurring in both villages and forests, they could be potential bridge vectors.

Following its works, we planned to publish two research articles in 2024. The first one focuses on "***Bionomics of Anopheles mosquitoes in Cambodia*".** This study aims to investigate the investigate the abundance, distribution, seasonal patterns, biting behavior of Anopheles mosquitoes, and prevalence of Plasmodium, in Mondulkiri province, Northeastern Cambodia. The manuscript will be submitted to the Malaria journal at the end of March 2024. The second articles will be about "**Biting patterns and seasonality of 30 mosquito species in Cambodia topical forest**". This study aims to determine for the first-time biodiversity and activity pattern of the mosquito species in the forests of Mondulkiri in Cambodia. This manuscript is currently under development and planned for submission in the middle of 2024.

Two articles related to the subject "Anopheles Mosquitoes in Mondulkiri Forest, Cambodia: Abundance, Distribution, Seasonal Patterns and Plasmodium prevalence" and "Bionomics of 30 species in the forests" will be written in 2024.

Collaborations	Malaria Research Unit, IPC CNM, Ministry of Health, Cambodia
Funding	5% Initiative Project, France IPC, Medical and Veterinary Entomology Unit

Southeast Asia Tick Identification Key (SEA TICKEY)

This project began in April 2022 and end in March 2023.

The project aims to develop an identification key for tick species for all the countries in Southeast Asia. This project will initiate and develop veterinary entomology activities in Cambodia. Our main partners are the Royal University of Agriculture (RUA), and the Ministry of Agriculture, Forestry and Fisheries (MAFF). This project was funded by a FSPI regional dedicated One Health projects in Southeast Asia.

We created and developed a complete and updated key for tick species identification in Southeast Asia that will be freely distributed to all public and private stakeholders. Over the medium term, the identification key created through the project in its current form will be used: (1) to draw up an inventory of tick species present in Cambodia and in the Lao People's Democratic Republic (PDR) and explore their diversity and distribution, (2) to share knowledge with local veterinary and public health services and universities, and (3) to build capacity for the monitoring of 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 with a One Health approach.
Alongside the creation of the tick identification key and its dissemination throughout Southeast Asia, we will also initiate the implementation of MALDI-TOF MS databases using ticks collected in the field in Cambodia and the Lao PDR (with the involvement of veterinary and agricultural stakeholders). A 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 MS method will be further used for pathogen discovery.

Collaborations	Ambassade de France au Cambodge, Cambodia Royal University of Agriculture (RUA), Cambodia Ministry of Agriculture, Forestry and Fisheries (MAFF) Cambodia IRD (Ecoland, GeoHealth) University of Hokkaido, Japan Institut Pasteur, Paris
Funding	Regional FSPI - *Fonds de Solidarité pour les Projets Innovants*, Paris, France 2022-2023

Veterinary Entomology in Cambodia (VECAM)

During the last few decades, we have seen the emergence of several pandemics, the expansion or re-emergence of historical pathogens, and changes in the ranges of major vectors, raising questions about the possible emergence of new vectors and new epidemics, often at the human-animal interface*, i.e.* zoonosis. For several years, Cambodia has been suffering from epizootic diseases that have heavily affected its cattle, sheep and poultry production sectors. These diseases are mainly transmitted by ticks, fleas or mosquitoes and yet, apart from the latter, the country has no basic information on these vector species, their distribution on the national territory, and even less on how to control them. The country needs to establish baseline data to monitor and control emerging epidemics. It is of vital importance to build the capacity of the Cambodian technical, academic and research sectors involved in arthropod vector management, for the benefit of public and veterinary health.
The overall objective of this project is to develop an expertise that does not exist in Cambodia: veterinary entomology. The staff who will directly benefit from the project are from the four partner organizations, namely the Institut Pasteur du Cambodge (IPC), the Royal University of Agriculture (RUA) and the two institutions attached to the Ministry of Agriculture, Forestry and Fisheries (GDAHP and NAHPRI).
This will enable the development of new medium and long-term surveillance and control methods as well as diagnostic tools. It will also increase the awareness of livestock farmers about arthropod vectors in Cambodia.
Through the formation of a unique working group bringing together stakeholders from three different ministries, this FSPI project will create a sustainable development think tank to address the issue of zoonotic diseases in relation to the environment in socio-ecological systems. In other words, this FSPI project will allow the One Health approach to be strengthened in Cambodia, through a reinforced Franco-Cambodian cooperation on these broader health issues.

Collaborations	Ambassade de France au Cambodge, Cambodia Royal University of Agriculture (RUA), Cambodia Ministry of Agriculture, Forestry and Fisheries (MAFF) Cambodia IRD (Ecoland, GeoHealth) University of Hokkaido, Japan Institut Pasteur, Paris
Funding	Fonds de Solidarité pour les Projets Innovants, Paris, France 2022-2024

Taxonomy

After intensive taxonomy work in 2021 on all mosquitoes from the Malaria Unit (20,237 mosquitoes), and after the publication of the mosquito checklist, our Unit has encountered new challenges, and has met them with new efforts and objectives in 2022.

In 2022, several different taxonomy projects were initiated and continued in 2023:

- Creation of a MALDI-TOF MS database for mosquito species.
- Since 2020, we have made efforts to develop a method using 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.
- At the end of 2022, 211 individual mosquitoes and 3,424 spectra were implemented.
- Barcoding of mosquito species of Cambodia.
- Barcoding of COI genes is in progress. Up until now, 111 mosquitoes belonging to 24 species have been barcoded, or sequenced.
- We have sequenced the DNA of *Aedes unalom,* a new species of the Scutellaris Group recently recorded in Cambodia. Their DNA sequences were analyzed alongside those of other Scutellaris Group species from Asia and the Pacific islands. The results suggest that the speciation of *Aedes* species within the Scutellaris Group may be driven by diversity in mammalian hosts, climate and environmental changes, or geological dynamics rather than human migration.
- Identification key for tick species.
- As previously mentioned in the SEA TICKEY project, the identification key for tick species (around 97 species) in Southeast Asia is in progress. The difficulty in obtaining tick specimens limits the key's accuracy for species identification. Our objective is to send a preliminary identification key to partners in all Southeast Asian countries to test the key and obtain their feedback.
- A MALDI-TOF MS database for tick species was also launched. It comprises 133 spectra of 16 species. More species will be added in the future. The barcoding using COI is also in progress.
- Identification key for mosquito species.
- At the same time, we also wanted to develop an identification key for the 290 mosquito species described until now in Cambodia. However, completing this identification key would take a lot of time, especially if done concurrently with the key for tick species. Work on this project began in 2022. We aim to obtain a final key by 2024.
- Streblidae and Nycteribiidae (bat flies).
- Since last year, and with the upcoming BCOMING project, we have been collecting bat ectoparasites. Our objective is also to develop a key for these species, and to determine the

number of species, the exchanges between these species, and the pathogens they carry. We have also developed COI and MALDI-TOF database for those specimens.

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

Collaborations	Virology Unit, IPC, Cambodia
Funding	FSPI for SEATICKEY (2022-2023) ZooCoV (2020-2022) and BCOMING (2023-2026) for ectoparasites samples Medical and Veterinary Entomology Unit for MALDI-TOF MS and Barcoding

4.5.3 Research Programs - Outlook for 2024

Written and deposal of projects in 2023.

In 2023, the following scientific projects were written and deposit to be funded:

1. 5% Initiatives — Urban Anopheles with Myanmar
2. DEKLIK IRD — Plasmodium knowlesii
3. MIE-PEPR — SEA-ROADS : Surveillance Dengue
4. Sweden — JEV vectors and reservoirs
5. WTDiscovery — RACMSEY – National serosurvey
6. JSPS Hokkaido Univ — Ticks and vector competence
7. Japan Initiatives — Drones in Kampong Thom
8. SATREPS — Tticks (viro-entomo) with Japan
9. MIE-PREZODE — Rats and ticks as sentinels
10. PRFI (ANSES) — Dengue vectors in 5 cities
11. PRFI (ANSES) — Japanese encephalitis in Cambodia

Amongst all these projects, the projects 1 to 5 were not selected to be granted, the project 6 and 7 have been granted and the projects 8 to 11 are still under evaluation. This represents an important year of investment time in the writing of scientific projects.

Expected projects for 2024

Preventing Zoonotic Diseases Emergence (PREZODE)

Through the PREZODE Initiative, Cambodia is the only Asian country involved in the AfriCam project (2022-2024), along with four African countries (Cameroon, Guinea, Madagascar, and Senegal). The AfriCam project will aim, 1) to study how the risks of emergence of zoonotic diseases are impacted by hydrological dynamics, by the climate, and by the environment in various ecosystems representing key animal/human/environment interfaces, and 2) to implement measures limiting the risks of the emergence of zoonotic risks and strengthening, in coordination with local and national partners, existing surveillance systems to enable the future implementation of a One Health integrated surveillance system.

The overall project has three main components: 1) a risk assessment for zoonotic disease emergence, 2) determining the environmental and climatic influences on them, and (3) establishing preventive strategies that can reduce the risk of zoonotic disease emergence, reinforcing surveillance to work towards an integrated One Health system.

ECOMORE 3

Briefly, for the entomological work package of the Ecomore 3 projects, the main objective in Cambodia should be the study of the transmission of vector-borne diseases as a function of entomological indices and land use in order to assist national health authorities. The specific objectives will be the

identification and characterization of mosquito vector species related to the impact of land-use on mosquito species, including vector species. Regionally, the main objective will be the study of the impact of human activities, land use change and climate change on the distribution of the main vectors in South-East Asia in order to model the transmission dynamics of vector-borne diseases and support the development of a regional health surveillance system. Regionally, the specific objectives will be to study the impact of the climate on the different species, the impact of the climate change and land-use change on the mosquito species, and to standardize a regional capture method.

4.5.4 Support to National Authorities

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

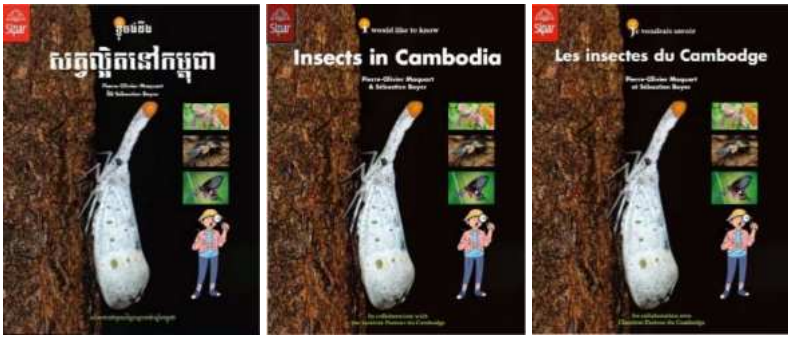
At the request of the Ministry of Agriculture, Forestry and Fisheries, and with the help of Royal University of Agriculture, two projects were written and submitted to establish and expand veterinary entomology capabilities in Cambodia.

Project VECAM has for sole purpose to support national institutions. In 2022, several training courses were organized for the personnel of the Royal University of Agriculture and the Ministry of Agriculture. Capacity building is one of VECAM's objectives (including the purchase of material for these two institutions). This program is funded by the French Embassy. The second project (mentioned before) has the objective to develop a determination key for tick species.

Support to National knowledge: Publication of a book in Khmer, French and English on "Insects in Cambodia"

Our Unit published a book to the destination of the children and confirms its commitment to civil society. We realized an educational scientific project, for everyone, especially aimed at young people. As a result, the captivating book entitled "Insects of Cambodia" was published during Christmas 2023. This book, published in three languages (Khmer, French, English), is primarily intended for young minds aged 8 to 12.

The lack of scientific books in the Khmer language is often criticized. Our Unit, in collaboration with SIPAR editions, has successfully met this challenge through this publication. We led this project, carefully selecting the most iconic species in Cambodia to be grouped in this comprehensive and didactic work. A cocoon of general information has also been woven to explain the life cycles and peculiarities of these fascinating species.



The covers of books on Insects in Cambodia

4.5.5 Teaching and Training

Master's Students

- Mr. Doeurk Bros (Master of Science in Biodiversity Conservation, RUPP, Cambodia), January 2022-December 2022.
  M2 thesis title: "Ecological Characteristics and Temporal Dynamics of Mosquito Species in Mondulkiri Province, Cambodia"
- Mr. Antoine Bellois (Master of Data science and statistical modeling, University of South Britany, France), 01 February 2023 – 30 June 2023.
  M2 thesis title: Studies of the impact of landscape fragmentation on the distribution of mosquito species
- Mr. Antoine Pelras (Master of Data science and statistical modeling, University of South Britany, France), 30 January 2023 – 30 June 2023.
  M2 thesis title: Modelling the impact of longitudinal and spatial factors on the distribution of mosquito species in Cambodia
- Mr. Matilin Le Beux (Master of Interactive application of digital data, University of South Britany, France), 01 February 2023 – 28 July 2023.
  M2 thesis title: Development of artificial intelligence technique for the recognition of mass spectra of mosquito vectors of Japanese Encephalitis virus.
- Mr. Flavien Cabon (Master of Biodiversity, Ecology and Evolution, Paul Sabatier University), September 2023 – August 2024
  M2 thesis title: Bat's ectoparasites of Cambodia (six months) / Study of disease vectors along a longitudinal gradient from the large city of Battambang to the floating and flooded cities of the south of the country (six months).

PhD Students

- Ms. Yean Sony, October 2022-September 2025, Doctoral school SDSV of Paris-Saclay (SDSV = Structure et dynamique des systèmes vivants)
  Thesis title: "Tick Species and Tick-Borne Diseases in Cambodia"
- Mr. Doeurk Bros, October 2023-September 2026, Doctoral school SDSV of Paris-Saclay (SDSV = Structure et dynamique des systèmes vivants)
  Thesis title: "Bionomic of dengue vector mosquitoes in Cambodia"

Teaching

Since 2020, Sebastien BOYER has been responsible for the "Vector Borne Diseases and Vector Transmission" module within year two of the International Joint Master's in Infectiology: 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 each. Our team also conducted the examinations and grading.

Training

In 2022, all of the newly recruited staff has taken the online "Aptitude training course on laboratory security and good laboratory practices".
In addition, **9 trainings** were provided this year.

15-17 March 2023	**Tick taxonomy training** Trainer: Dr. Mackenzie L. Kwak, Hokkaido University, Sapporo, Japan Trainees: 4 staff from IPC, 4 from MAFF and 4 from RUA
15 May 2023	**Training on how to use regional tick taxonomic key** Trainers: Mr. Didot Prasetyo, Mr. Kimhuor Sour & Ms. Saoya Sen Trainees: 4 staff from IPC, 4 from the MAFF and 4 from RUA
22-26 May 2023	**2nd Training on qGIS Mapping and Spatial Analysis for One Health Studies** Trainers: GeoHealth, IRD Trainees: 4 staff from IPC, 4 from the MAFF and 4 from RUA
29 May – 09 June 2023	**Training on fleas taxonomy & slide mounting technique** Trainer : Mr. Tojo Ramihangihajason, Institut Pasteur du Madagascar (IPM) Trainees: Medical & Veterinary Entomology staff
7-10 August 2023	**PCR for Tick Taxonomy** Trainer: Dr. Antsa Rakotonirina, Institut Pasteur du Cambodge (IPC) Trainees: 4 staff from IPC, 4 from MAFF and 4 from RUA
14-16 August 2023	**MALDI-TOF MS for Tick Taxonomy** Trainer: Dr. Antsa Rakotonirina, Institut Pasteur du Cambodge (IPC) Trainees: 4 staff from IPC, 4 from the MAFF and 4 from RUA
2-12 October 2023	**Masterclass Macro Photography of Insects** Trainers: Mr. Nathanaël Maury and Ms. Somchit Sudavanh Trainees: 4 staff from IPC
30 October 2023	**ELISA for pathogen screening** Trainer: Dr. Antsa Rakotonirina, Institut Pasteur du Cambodge (IPC) Trainees: 4 staff from IPC, 3 from MAFF and 4 from RUA
4-6 December 2023	**3rd qGIS Mapping and Spatial Analysis for One Health Studies** Trainers: GeoHealth, IRD Trainees: 4 staff from IPC, 4 from MAFF and 4 from RUA

4.5.6 Outlook

Within 3 years

- Thesis defense of a PhD student in Medical and Veterinary Entomology
- New insectarium and new space (laboratory and office)
- Mobility of the head of unit towards another establishment of the Pasteur Network might be considered
- Develop mechanistic research on transmission
- Ecomore 3

Within 5 years

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

4.5.7 Scientific Publications 2023

1. **Description of a new species of Toxorhynchites (Diptera: Culicidae) from Nepenthes pitchers in Cambodia**
   Pierre-Olivier Maquart, Nil Rahola, Kalyan Chhuoy, Kimly Heng, Moeun Chhum, Kimhuor Suor, Sébastien Boyer
   Journal of Asia-Pacific Entomology 26 (2023) 102064 DOI : 10.1016/j.aspen.2023.102064

2. **Longitudinal Study of Viral Diversity Associated with Mosquito Species Circulating in Cambodia**
   Souand Mohamed Ali, Antsa Rakotonirina, Kimly Heng, Elise Jacquemet, Stevenn Volant, Sarah Temmam, Sebastien Boyer and Marc Eloit
   Viruses 2023, 15, 1831. https://doi.org/10.3390/v15091831

3. **Mosquito diversity (Diptera: Culicidae) and medical importance in four Cambodian forests**
   Antsa Rakotonirina, Pierre-Olivier Maquart, Claude Flamand, Chea Sokha & Sébastien Boyer
   Parasites & Vectors 2023-03-21 DOI : 10.1186/s13071-023-05729-w

4. **Phylogeny and spatial distribution of Japanese encephalitis virus vector species in Cambodia**
   Dusadeepong R, Maquart PO, Hide M, Boyer S.
   Medical and Veterinary Entomology 2023 Jul 5. doi: **10.1111/mve.12678**.

5. **pWCP is a widely distributed and highly conserved Wolbachia plasmid in Culex pipiens and Culex quinquefasciatus mosquitoes worldwide**
   Amani Ghousein, Jordan Tutagata, Hans Schrieke, Manuel Etienne, Victor Chaumeau, Sebastien Boyer, Nonito Pages, David Roiz, A Murat Eren, Guillaume Cambray, Julie Reveillaud
   ISME Communications 2023 Apr 28;3(1):40. **doi: 10.1038/s43705-023-00248-2**

6. **Ribosomal RNA (rRNA) sequences from 33 globally distributed mosquito species for improved metagenomics and species identification**
   Cassandra Koh, Lionel Frangeul, Hervé Blanc, Carine Ngoagouni, Sébastien Boyer , Philippe Dussart, Nina Grau, Romain Girod, Jean-Bernard Duchemin, Maria-Carla Saleh
   Elife. 2023 Jan 23;12:e82762. doi: 10.7554/eLife.82762.

7. **Stag beetle fauna of Cambodia (Coleoptera: Lucanidae)**
   Maquart PO, Yamamoto S, Sopha S, Chhorn S, Phak S, Sinovas P, Phauk S, Boyer S.
   Journal of Asia Pacific Entomology 26(2023) 102008. **Doi: 10.1016/j.aspen.2022.102008**

4.6 Bacterial Phylogenomics group (G2+2)

The Bacterial Phylogenomics Group was founded in 2021, thanks to the backing of a collaborative research fund aimed at establishing a research focus on AMR genomics at the IPC. Initially conceived as a two-year commitment, the potential of the project led to an extension of an additional two years. The initiative is supported by an international collaboration involving the University of Melbourne (Australia), Institut Pasteur (France), and Institut Pasteur du Cambodge, highlighting a global commitment to advancing AMR genomic research. 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. 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, the Bacterial Phylogenomics group 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 have been organizing retrospective snapshot surveys of a number of high-priority AMR Gram-negative pathogens causing bloodstream infections in Cambodia. High-throughput WGS methods have facilitated the sequencing of large, geographically representative collections of disease isolates. Our retrospective genomic snapshot surveys are one of the first to comprehensively characterize the AMR gene repertoire, the resistance mechanisms, and the relatedness of high-priority AMR pathogens in Cambodia. Genomic characterization has made it possible to define AMR with much greater precision compared to phenotypic categorization. As a result, our work provides insights that can help contain AMR and protect Cambodian public health.

4.6.1 Organizational Structure

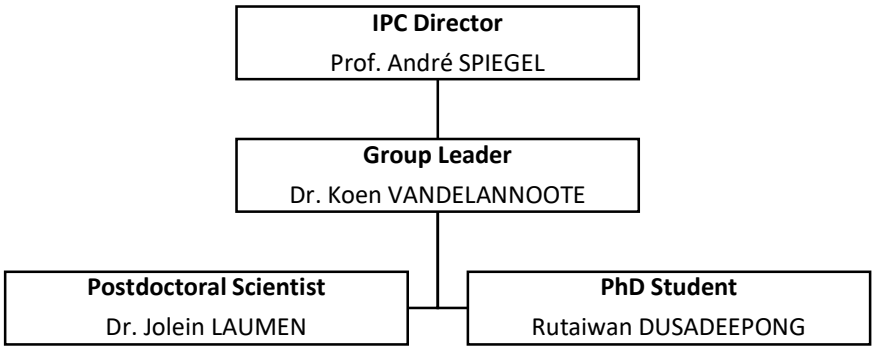


Figure 28: Bacterial Phylogenomics group organizational chart

4.6.2 Research Programs – Major Achievements in 2023

Axis 1: Phylogenomic investigation of an outbreak of fluoroquinolone-resistant Salmonella enterica subsp. enterica serovar Paratyphi A in Phnom Penh, Cambodia.

In early 2020, the MBL of the IPC isolated an unusually high number of fluoroquinolone-resistant *Salmonella enterica* subspecies *enterica* serovar Paratyphi A strains during its routine bacteriological surveillance activities in Phnom Penh, Cambodia. A public-health investigation was supported by genome sequencing of these Paratyphi A strains to gain insights into the genetic diversity and population structure of a potential outbreak of fluoroquinolone-resistant paratyphoid fever. Comparative genomic and phylodynamic analyses revealed the 2020 strains were descended from a previously described 2013-2015 outbreak of Paratyphi A infections. Our analysis showed sub-lineage

2.3.1 had remained largely susceptible to fluoroquinolone drugs until 2015, but acquired chromosomal resistance to these drugs during six separate events between late 2012 and 2015. The emergence of fluoroquinolone resistance was rapidly followed by the replacement of the original susceptible Paratyphi A population, which led to a dramatic increase of fluoroquinolone-resistant blood-culture-confirmed cases in subsequent years (2016-2020). The rapid acquisition of resistance-conferring mutations in the Paratyphi A population over a 3-year period is suggestive of a strong selective pressure on that population, likely linked with fluoroquinolone use. In turn, emergence of fluoroquinolone resistance has led to increased use of extended-spectrum cephalosporins like ceftriaxone that are becoming the drug of choice for empirical treatment of paratyphoid fever in Cambodia.

Funding	IPC internal project G2+2
Project duration	2021-2023
External Collaborator	Sihanouk Hospital Center of HOPE, Phnom Penh, Cambodia Institute of Tropical Medicine, Antwerp, Belgium

Axis 2: Introduction of Fluroquinolone-Resistant Salmonella enterica subsp. enterica serovar Typhi Strains into Cambodia: A Genomic Analysis

Sihanouk Hospital Center of HOPE, a non-governmental referral hospital located in Phnom Penh, has been conducting surveillance for bloodstream infections among adult patients across its primary facility and affiliated clinics since 2007. In January 2017, two ciprofloxacin resistant strains of *Salmonella Typhi* were identified with a particularly high MIC (MIC ≥ 24 µg/ml). This finding represented an interesting result, since full ciprofloxacin *Salmonella Typhi* resistance has never been described in Cambodia. Whole Genome Sequencing was used to characterize the two isolates. It was found that their genomes were identical. Limited patient data revealed the two isolates were from two Indian brothers. Analysis of the sequenced strain also revealed the strains carried three QRDR mutations which are known to raise ciprofloxacin MIC to 8–32 mg/L. These triple mutants were found to belong to *Salmonella Typhi* genotype 4.3.1.2.1, which emerged in India in the mid-1990s and has since been introduced into Pakistan, Nepal, Bangladesh, and Chile. The nationality of the brothers is suggestive of an introduction of the 4.3.1.2.1 lineage from India where it emerged.

Funding	IPC internal project G2+2
Project duration	2021-2023
External Collaborator	Sihanouk Hospital Center of HOPE, Phnom Penh, Cambodia Institute of Tropical Medicine, Antwerp, Belgium

Axis 3: Phylogenomic analysis reveals pervasive nosocomial spread of Carbapenem-Resistant *Acinetobacter baumannii* in Cambodia.

Acinetobacter baumannii emerged as an important opportunistic nosocomial pathogen in intensive care units worldwide causing ventilator-associated pneumonia, central line-associated bloodstream infections, catheter-associated urinary tract infections, and surgical site infections. To date, *A. baumannii* has become resistant to almost all currently available antimicrobial agents. One of the main concerns has been the acquisition of carbapenem resistance, depriving physicians of one of the most well-tolerated and active antimicrobials to treat *A. baumannii* infection. Genomic sequencing enables a comprehensive analysis of the entire pathogen genome, providing unparalleled precision in distinguishing closely related bacterial lineages and transforming outbreak investigations in hospitals. We sequenced the genomes of a panel of 97 carbapenem-resistant *A. baumannii* (CRAB) strains

isolated by IPC and ICER between 2017-2022 from patients who presented at 8 different public and private hospitals/clinics in Phnom Penh, Kampong Cham, Takeo, Siem Reap, and Battambang. Comparative genomic and phylodynamic analyses revealed the predominance of Global Clone 2 CRAB strains circulating in Cambodian hospitals and indicated Cambodian CRAB isolates are related to those from neighbouring countries Thailand and Vietnam. While our ability to track fine grained transmission events was limited due to the lack of available patient movement data, we were still able to discern pervasive nosocomial spread of CRAB in two large Phnom Penh hospitals. Notably, each of the two hospitals experienced the spread of a distinct clone, which was shared among 11 patients over the course of a year. We also detected particular CRAB clones of interest that were identified in multiple hospitals. Furthermore, comprehensive drug susceptibility testing showed that colistin, a last-resort antibiotic, continues to be effective in treating CRAB infections in Cambodia. However, concerns about its serious side effects, limited availability, and high treatment costs persist.

Funding	IPC internal project G2+2, NIH (NIAID - International Center of Excellence in Research ICER), USA
Project duration	2021-2023
External Collaborator	NIH (NIAID - International Center of Excellence in Research ICER), USA

Axis 4: Identification of suspected Bacillus anthracis strains grown from water samples in Cambodia.

In 2022, the Cambodian Department of Fisheries sent bacterial strains, which were isolated from water samples collected at a fish hatchery, to IPC for identification using MALDI-TOF MS. This particular hatchery produces stripe catfish (*Pangasianodon hypophthalmus*) which is heavily cultivated in Cambodia. Alarmingly, five of the bacterial strains identified by MALDI-TOF were suspected *Bacillus anthracis*, an obligate pathogen responsible for the zoonotic disease anthrax, affecting humans as well as domestic and wild animals. Despite fish being unlikely anthrax hosts due to their low body temperatures, they can still transmit the disease through contaminated water or as carriers of *B. anthracis* on their surfaces or in their diet. As *B. anthracis* can be misidentified with other closely related species in the *Bacillus cereus* group - including the foodborne pathogen *B. cereus* - we assembled the complete genome sequences of the five strains and carried out a characterization. The genetic similarity of the five suspected isolates to *B. anthracis* was established by comparing their genomes with those of known *B. anthracis* strains available in public databases. While our five isolates were closely related to *Bacillus anthracis*, genomic analysis in this study did not taxonomically classify any of them as belonging to the anthracis subspecies. Furthermore, *B. anthracis* plasmids pXO1 and pXO2 that carry the main virulence factors responsible for anthrax were not detected in any of the 5 strains indicating they represent attenuated bacteria with reduced virulence and ability to cause illness in humans or animals.

Funding	IPC internal project G2+2
Project duration	2022-2023
External Collaborator	

4.6.3 Research Programs – Outlook 2024

Axis 1: Exploring the phylogenomic landscape of paediatric *Burkholderia pseudomallei* in Cambodia: A comprehensive analysis within southeast Asia.

Burkholderia pseudomallei, the causative agent of melioidosis, presents a significant health threat with a high mortality rate, especially in regions with limited resources. This study's primary goal is to unravel the molecular epidemiology, evolutionary history, and antimicrobial resistance of the *B.*

pseudomallei population in Cambodia, contextualized within the wider Southeast Asian framework. We aim to identify the predominant sequence types, clonal complexes, and the overall population structure of this bacterium in Cambodia. Our research is guided by two main hypotheses: first, that the pathogenic strains of *B. pseudomallei* implicated in infections among Cambodian children evolve autonomously within local populations, marked by numerous mutations and recombination events; second, that the evolutionary trajectory and spatial distribution of *B. pseudomallei* in Cambodia are characterized by significant overlap, likely due to repeated reintroductions of the bacterium from neighbouring Southeast Asian nations. Thailand, a known epicentre for melioidosis, is particularly suspected of being a source of these reintroductions. A total of 104 clinical *B. pseudomallei* isolates of were systematically collected from paediatric patients aged from birth to 13 years old at Kantha Bopha Hospital Children's Hospitals in Siem Reap and Phnom Penh over a one-year period.

Funding	IPC internal project G2+2, University of Melbourne
Project duration	2022-2024
External Collaborator	Kantha Bopha Hospital Network, Cambodia University of Melbourne, Australia

Axis 2: Defining the genomic characteristics, diversity, and population structure of Cambodian *Neisseria gonorrhoeae*

Gonorrhoea is a sexually transmitted infection caused by the bacterium *Neisseria gonorrhoeae*. For the past 80 to 90 years, gonorrhoea has been treated successfully by antimicrobials. However, since the introduction of antimicrobial treatment against gonorrhoea, bacterial resistance has rapidly emerged to sequentially introduced classes of antibiotics used for treatment. It is clear that *N. gonorrhoeae* is evolving into a superbug and that untreatable gonorrhoea represents a critical global health threat. *N. gonorrhoeae* is presently excluded from Cambodia's national AMR surveillance system, which is solely centred on blood cultures. High quality, up-to-date, and representative gonococcal AMR data is imperative to monitor AMR trends, identify emerging AMR, and inform refinements of international and national clinical management guidelines and public health policies. Currently, a lack of AMR data in Cambodia places serious limitations on the country's ability to control the spread and minimize the impact of drug resistant *N. gonorrhoeae*. This is why in this study we wish to define the AMR, genomic characteristics, diversity and population structure of Cambodian *N. gonorrhoeae*.

Funding	IPC internal project G2+2
Project duration	2022-2024
External Collaborator	

4.6.4 Teaching and Training

PhD Students

- Rutaiwan Dusadeepong, University of Melbourne, Australia (2023-2025): "Molecular epidemiology and evolution of *Mycobacterium tuberculosis*, *Burkholderia pseudomallei* and carbapenemase-producing *Enterobacterales* in Cambodia."

Internship/Master students

- Nabi Nge, University Paris-Saclay, France (2023): "Complete characterization of five suspected *Bacillus anthracis* isolates using whole genome sequencing."

4.6.5 Outlook

Our research strategy for the upcoming years is dedicated to designing and implementing research programs that are closely aligned with the priorities identified in Cambodia. Our objective is to generate actionable insights that can directly inform and enhance targeted public health strategies. This approach ensures that our efforts are both relevant and impactful, contributing to the improvement of public health outcomes in the region. The Bacterial Phylogenomics group's prowess in infectious disease research is anchored in the dedication and expertise of our team, the robustness of our resources, and our dynamic collaborations. Our success stems from enduring partnerships with other IPC units and a global network of distinguished researchers. We are committed to fostering a culture of collaboration within a diverse and inclusive environment. Our goal is to cultivate a rich educational setting for students, PhD candidates, and postdoctoral fellows. Looking ahead to 2024, are launching several innovative grant proposals. These are designed not only to reinforce our existing research endeavors but also to broaden our scope by exploring new topics and forging partnerships with new collaborators. Such strategic initiatives are set to shape the direction of our research programs over the next five years, promising an era of expanded discovery and impact.

4.6.6 Scientific Publications 2023

1. **High performance Legionella pneumophila source attribution using genomics-based machine learning classification.**
   Andrew H Buultjens, Koen Vandelannoote, Karolina Mercoulia, Susan Ballard, Clare Sloggett, Benjamin P Howden, Torsten Seemann, Timothy P Stinear.
   Appl Environ Microbiol. 2024 Jan 30:e0129223. doi: 10.1128/aem.01292-23. Online ahead of print.

2. **Phylogenomic investigation of an outbreak of fluoroquinolone-resistant Salmonella enterica subsp. enterica serovar Paratyphi A in Phnom Penh, Cambodia.**
   Rutaiwan Dusadeepong, Gauthier Delvallez, Sokleaph Cheng, Soda Meng, Navin Sreng, Joanne Letchford, Kimcheng Choun, Syna Teav, Liselotte Hardy, Jan Jacobs, Tuyet Hoang, Torsten Seemann, Benjamin P Howden, Philippe Glaser, Timothy P Stinear, Koen Vandelannoote.
   Microbial Genomics 2023 Mar;9(3). doi: 10.1099/mgen.0.000972.

3. **Statistical modeling based on structured surveys of Australian native possum excreta harboring Mycobacterium ulcerans predicts Buruli ulcer occurrence in humans.**
   Koen Vandelannoote, Andrew H Buultjens, Jessica L Porter, Anita Velink, John R Wallace, Kim R Blasdell, Michael Dunn, Victoria Boyd, Janet A M Fyfe, Ee Laine Tay, Paul D R Johnson, Saras M Windecker, Nick Golding, Timothy P Stinear.
   Elife. 2023 Apr 14;12:e84983. doi: 10.7554/eLife.84983.

4. **Unraveling the intricacies of host-pathogen interaction through single-cell genomics.**
   Emanuele Gioacchino, Koen Vandelannoote, Anthony A Ruberto, Jean Popovici, Tineke Cantaert.
   Microbes Infect. 2024 Feb 16:105313. doi: 10.1016/j.micinf.2024.105313. Online ahead of print.

4.7 Medical Biology Laboratory

4.7.1 Functional Structure

The Medical Biology Laboratory (MBL) provides a comprehensive platform for biological analyses, offering approximately 150 tests to public/private hospitals and clinics, non-governmental organizations (NGOs), and walk-in patients. The MBL's team comprises 37 staff members across seven divisions: Reception, Sampling, Microbiology, Mycobacteriology, Blood biology, Molecular biology, and the Bacteriology Research Laboratory (LMI DRISA / International Joint Laboratory – Drug Resistance in Southeast Asia).

In 2018, the MBL achieved accreditation according to ISO 15189 for biochemistry, hematology, and microbiology. In 2022, the renewal of this accreditation by the French Committee for Accreditation (COFRAC) has been granted for another 5 years period.

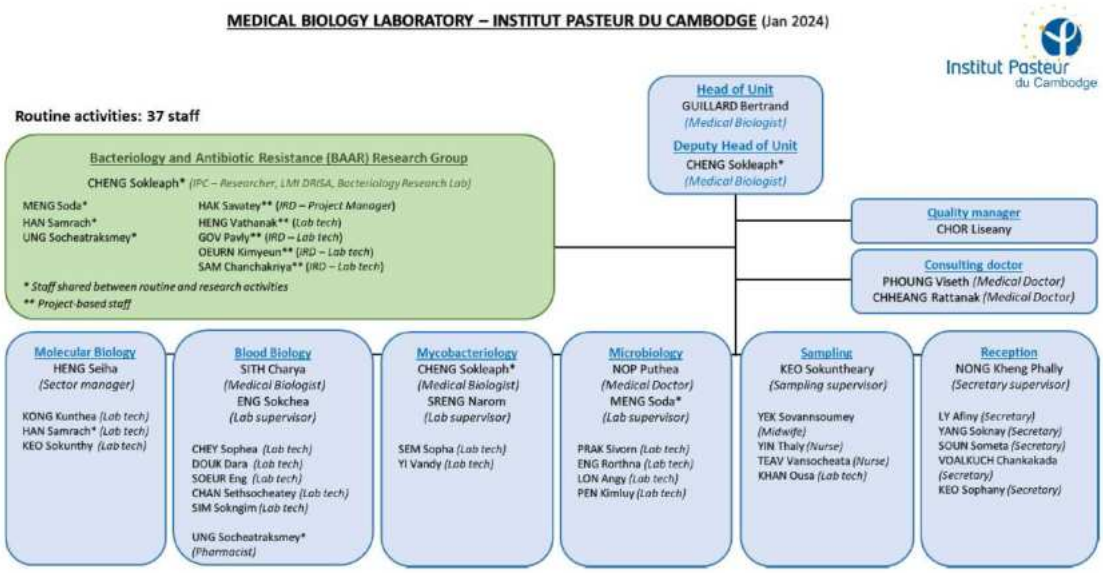


Figure 29: Organigram - Medical Biology Laboratory (January 2024)

4.7.2 Service Activities in 2023

To evaluate the Laboratory's activity levels, analyses are expressed in "key-letter B" units, according to the French nomenclature of medical laboratory procedures (*Nomenclature des Actes de Biologie Médicale: NABM*).

In 2023, the MBL conducted 177 745 analyses (+10% compared to 2022), resulting in 6.2 million B, representing a decrease of 25 % compared to 2022.

The year 2023 marked a resurgence of diverse activities: COVID-19 testing accounted for 4.6% of MBL's total activity in 2023 (compared to 53.6% in 2022 and 56.5% in 2021).

Activity in 2023, in comparison to that of 2019 (prior to the emergence of COVID-19 infection) showed a 59% increase. Figure 30 illustrates the trend of our activities over recent years.

The MBL remains a reference laboratory in Cambodia, despite an evolving medical biology sector in Cambodia, characterized by the emergence of new private laboratories in Phnom Penh and health service providers now establishing internal laboratories. Nonetheless, MBL is frequently approached for its microbiology and molecular biology services and to verify certain pathological blood results obtained from other laboratories.

MBL forwards specialized medical analyses to Laboratoire Cerba in France for specialized analyses or infrequently requested tests: 241 files were sent in 2023 compared to 116 in 2022.
In December 2023, MBL transitioned to a new laboratory information system (LIS) despite significant implementation challenges (a 6-month delay in start-up) associated with the LIS company (DATAMED, Switzerland).

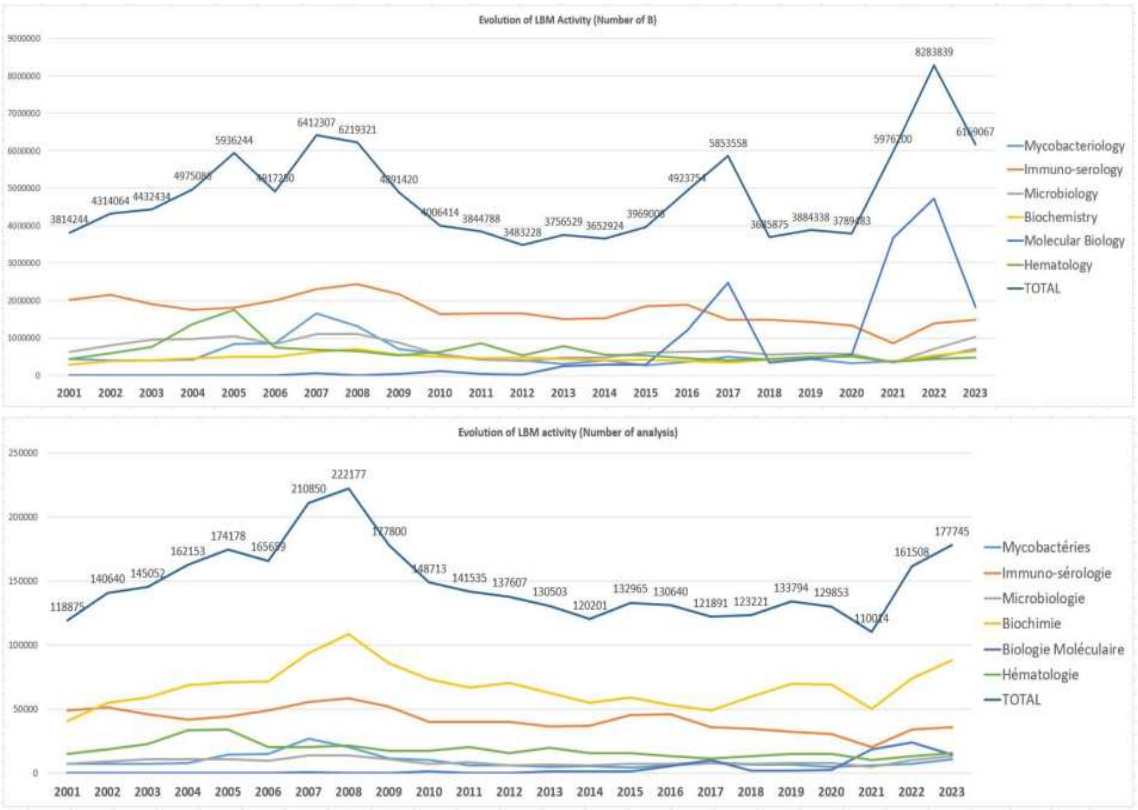


Figure 30: Trends in MBL activities, values expressed in key-letter B (adapted for the MBL using the French Nomenclature for Medical Biology Acts, or NABM)

4.7.2.1. HIV

Voluntary Confidential Counselling and Testing for HIV (VCCT)

In 2023, 373 patients consulted the VCCT and benefited from a free HIV consultation and screening (see Figure 30). Among these consultation, HIV-positive patients accounted for 20.6 %. This high rate of positivity is due to the fact that most patients were referred by other health centres for a free confirmation of a positive HIV antibody rapid test administered elsewhere. Of the 373 patients, 56 were referred by NGOs (such as Men's Health Cambodia, Reproductive Health Association of Cambodia, Chouk Sor Clinic) working with the most at-risk populations, including entertainment workers, men who have sex with men and transgender individuals. In addition, 66 patients were referred by National Hospitals (Calmette Hospital, Ang Duong Hospital, Preah Kossamak Hospital, Khmer-Soviet Friendship Hospital, etc.) and private clinics. In recent years, we have noted a decrease in VCCT activities, triggered by the opening of additional HIV testing centres focused on the most at-risk populations. On the other hand, the MBL's VCCT activities remain the reference for these centres to confirm their results.

HIV Nominative Serology (MBL routine patients)

In 2023, we observed a 9.5 % increase in the number of HIV serology screenings compared to 2022 (1952 vs 1665).

The seropositivity rate among MBL patients rose to 7.5 % (see Figure 31). This prevalence exceeds that of the general population, partly due to samples sent by other laboratories to confirm their positive results.

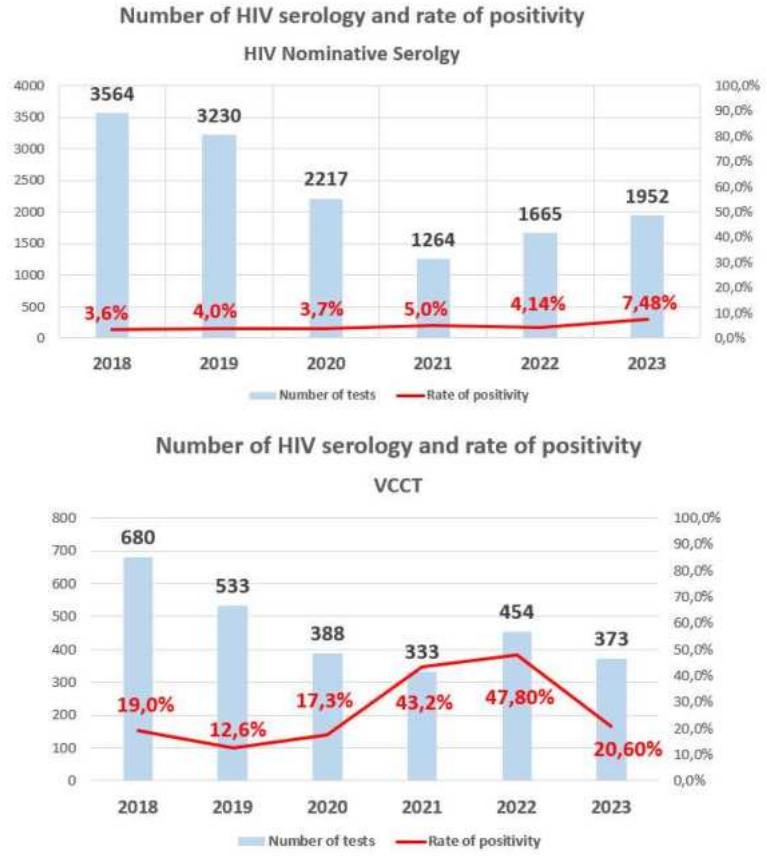


Figure 31: HIV serology and rate of positivity at VCCT and MBL

4.7.2.2. Bacteriology

In 2023, the number of bacterial cultures increased by 41%. We observed a 6% decrease in the rate of extended-spectrum-beta-lactamase (ESBL) *Enterobacteriaceae* (28%) compared to 2022, while Carbapenemase-producing *Enterobacteriaceae* (CPE) decreased by -3.3%. The Methicillin-resistant rate of *Staphylococcus aureus* (MRSA) remains stable at 35% and Carbapenem-resistant *Acinetobacter baumannii* (CRAB) decreased significantly by 11%. Finally, 18 strains of *Burkholderia pseudomallei* were isolated (Table 5).

Bacteria of particular public health importance	2020	2021	2022	2023
Samples for bacteriological culture	4114	2508	5023	7101
Positive cultures with AST	1466 (35.6%)	967 (38.6%)	1548 (30.8%)	2623 (36.93%)
Extended spectrum beta-lactamase (ESBL)				
Enterobacteriaceae	200/701 (28.5%)	143/503 (28.4%)	290/851 (34.1%)	405/1466 (27.62%)
Escherichia coli	155/366 (42.3%)	107/254 (42.1%)	240/487 (49.3%)	347/893 (38.86%)
Klebsiella pneumoniae	31/206 (15.0%)	25/127 (19.7%)	34/242 (14.0%)	43/341 (12.61%)
Others	14/129 (10.9%)	11/122 (9.0%)	16/122 (13.1%)	15/232 (6.46%)
Carbapenemase-producing Enterobacteriaceae (CPE)				
Enterobacteriaceae	26/701 (3.7%)	30/503 (6.0%)	40/851 (4.7%)	20/1466 (1.36%)
Escherichia coli	12/366 (3.3%)	21/254 (8.3%)	20/487 (4.1%)	17/893 (1.90)
Klebsiella pneumoniae	9/206 (4.4%)	7/127 (5.5%)	17/242 (7.0%)	2/341 (0.59)
Others	5/129 (3.9%)	2/122 (1.6%)	3/122 (2.5%)	1/232 (0.43%)
Carbapenemase type	*NDM and OXA-48*	*NDM, OXA-48 and KPC*	*NDM, OXA-48 and KPC*	*NDM and OXA-48*
Staphylococcus aureus				
Methicillin-resistant *S.aureus* (MRSA)	93/238 (39.1%)	43/139 (30.9%)	71/204 (34.8%)	116/333 (34.83%)
Acinetobacter baumanii				
Carbapenem-resistant *A.baumannii* (CRAB)	9/20 (45.0%)	9/17 (52.9%)	8/21 (38.1%)	21/78 (26.92%)
Enterococcus faecium				
Vancomycin-Resistant Enterococci (VRE)	0/12 (0%)	0/6 (0%)	0/25 (0%)	0/15 (0%)
Neisseria gonorrhoeae				
Resistance to 3GC	0/11 (0%)	3/6 (50.0%)	1/4 (25%)	1/1 (100%)
Salmonella paratyphi A (Blood culture)				
Resistance to 3GC	0/37 (0%)	0/10 (0%)	0/2	0/1 (0%)
Resistance to nalidixic acid	37/37 (100%)	10/10 (100%)	1/2 (50%)	1/1 (100%)
Burkholderia pseudomallei	14	22	15	18

Table 5: Bacterial cultures

In 2023, as an alternative to the classic bacterial culture for the diagnosis of sexually transmitted infections (STIs), the MBL conducted 489 *Chlamydia trachomatis*/*Neisseria gonorrhoeae* PCRs on vaginal, urine, urethral, throat, and anal samples. Among these, 9.6 % (n = 47) tested positive for *Chlamydia trachomatis*, and 6.5 % (n = 32) for *Neisseria gonorrhoeae*, while we identified 7 co-infections (1.4 %).

4.7.2.3. Tuberculosis

The mycobacteriology activities experienced a continuous surge. In 2023, 10519 analyses were conducted for service activities, marking a notable increase of 48.5% from 2022's 7082 analyses. The increase was observed in all types of analyses, including a 48% increase for smear examination, 55% for mycobacteria culture, and 29.3% for Xpert MTB/RIF Ultra assay. In 2023, 2229 Xpert MTB/RIF Ultra tests were performed for rapid TB diagnosis, with 21% yielding *Mycobacterium tuberculosis* complex (MTBC) positive results, and 1.1% of positive samples showing resistance to Rifampicin. Figure 32 illustrates the detection of *M.tb* and Rifampicin resistance by the Xpert MTB/RIF Ultra assay over the past five years.

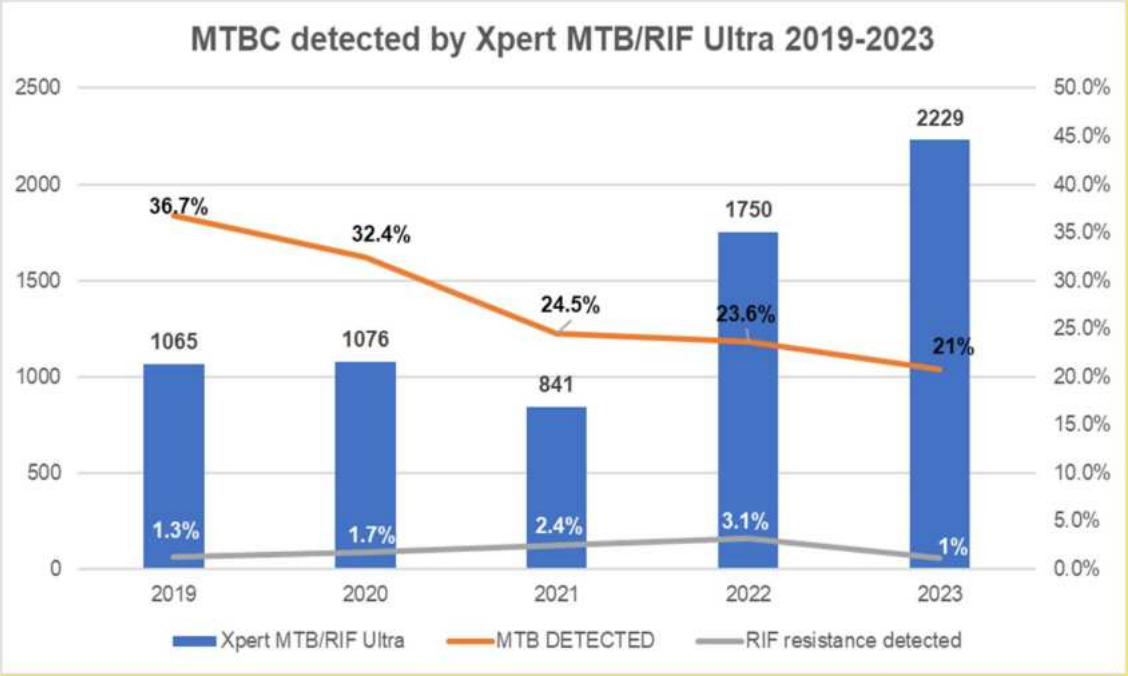


Figure 32 : Xpert MTB/RIF Ultra Activity 2019-2023

In addition to the service activities, the Mycobacteriology laboratory also participated in the 3rd National TB prevalence survey as a culture-performing laboratory for the survey, which also contribute to the escalation in activities. The survey began in May 2023, with 476 sputum samples received in 2023 for smear microscopy and culture. The laboratory was also involved in the rapid molecular testing for first- and second-line anti-TB drugs using line probe assay for multi-drug-resistant TB patients identified by the National TB Control Program.

4.7.3 Research Programs - Major Achievements in 2023

Circus – Pilot phase in Cambodia

This multicenter study aims to investigate the circulation of Antimicrobial Resistance (AMR), particularly focusing on Multi-Drug-Resistant Enterobacteriaceae (MDR-E) and mobile genetic elements across humans, animals, and the environment in Burkina Faso, Cambodia, Ivory Coast, and Madagascar. The project received partial funding in 2022 to initiate its pilot phase in the four countries. The year 2022 focuses on project preparation, including securing funding, selecting study sites, obtaining support from partners and local authorities, developing study protocols, obtaining ethical clearance, and staff recruitment and training. Takeo Provincial Referral Hospital was selected as a study recruitment site. In 2023, CircUs organized a kickoff meeting in April 2023 and commenced hospital data collection alongside patient household visits. Current progress includes the inclusion of 38 patients in the Hospital work package (WP1) and subsequent visits to 16 patient households for the Patient's household and environmental work package (WP2). Of the 38 patients recruited, 57.9% present infection with MDR-E, including 47.4% (n=18) with Extended Spectrum Beta-Lactamase producing Enterobacteriaceae (ESBL-PE) and 10.5% (n=4) with Carbapenem-Resistant Enterobacteriaceae (CRE). In WP2, 185 samples (59 humans, 29 animals, 48 environments, 11 rodents, and 38 food samples) have been collected, with 89.2% of the collected samples presenting MDR-E. Patient recruitment and household sampling are ongoing until June 2024, with sample processing and

analysis expected to be completed by August 2024. The genomic DNA will be extracted from selected isolates for Next-generation sequencing at the Institut Pasteur in Paris (WP3) by the end of 2024.

Collaborations	PIs: N. Guessend (IP in Ivory Coast), A. Salam, E. Cardinal (Cirad), A.L. Banuls (IRD) Team Leader for Cambodia: S. Cheng (MBL) MBL: B. Guillard, V. Heng LMI-DRISA$_{OH}$: P. Gao, S. Hak Takeo Provincial Referral Hospital: S. Seang General Directorate of Animal Health and Production (GDAPH), MAFF: S. San
Funding	AVIESAN AMR-SUD ANR "Antibiorésistance : comprendre, innover, agir (AMR)"

Implementation of Flow-cytometric Immunophenotyping

The collaboration between the MBL, the Immunology Unit of IPC and Kantha Bopha Hospital aimed to improve the diagnosis of hematological malignancies in Cambodia. This project was initiated at the request of Kantha Bopha Hospital and initially financed by an internal IPC budget of $15,000. After a test phase, 30 immunophenotypings were carried out in routine before the end of this collaboration in December 2023, initiated by Kantha Bopha Hospital.

ACIP ORACAN "Geographic origin of the parasite Angiostrongylus cantonensis and role of the mollusc Achatina fulica in its dispersion, in Central and West Africa and in the French departments of America

Nerve angiostrongylosis is an anthropozoonosis caused by *Angiostrongylus cantonensis*, a parasitic nematode of the genus *Angiostrongylus*. The parasite cycle of *A.cantonensis* requires the presence of definitive hosts (mainly rats) and intermediate hosts (snails, notably *Achatina fulica*) for its complete maturation. Humans are incidental hosts and infection can occur through the consumption of raw or undercooked intermediate or paratenic hosts. Neurologic manifestations include eosinophilic meningitis, encephalitis/encephalo-myelitis. Today, human cases of nerve angiostrongylosis are primarily described in Southeast Asia (Thailand, Malaysia, China), the Pacific Islands, Indian Ocean islands, the Greater Antilles (Cuba, Puerto Rico, Jamaica), and more recently Brazil. The ORACAN project, funded by the Institut Pasteur through Actions Concertées InterPasteuriennes - ACIP 2022, aims to determine the origin of the *A.cantonensis* and introduction pathways (rats or snails) in the French Department of America and Africa where no data exists. This involves assessing prevalence and generating phylogenetic data of *A.cantonensis* and its major intermediate host, *A.fulica* in all regions of the world. In 2023, Samrach Han, a technician in the Medical Biology Laboratory, attended the ORACAN Workshop (22-26/05/2023) at the Institut Pasteur de Guinée in Conakry, Guinea, alongside participants from other institutes. The workshop aimed to provide onsite opportunities for participants to exchange ideas and implement techniques suitable for all institutions involved. Throughout the workshop, participants received training in various techniques, including snail dissection, microscopic examination, extraction methods using Chloroform and Qiagen, Nanodrop analysis, PCR, gel electrophoresis, and trapping rats. On the final day, a discussion was held to summarize the ideas, workflows, and techniques, ensuring all participants were aligned. Subsequently, an online meeting was organized to finalize the testing protocol. In 2024, the planned activities include the collection of snails in the first trimester, collection of rodents tissue sample from the IPC Biobank, and laboratory testing.

Collaborations	PI: Ferdinand, Séverine (IP in Guadeloupe) MBL of IPC: B. Guillard, S. Cheng, S. Heng, S. Han Institut Pasteur in Bangui Institut Pasteur in Cambodia Institut Pasteur in Cote d'Ivoire Institut Pasteur in French Guiana Institut Pasteur in Guadeloupe Institut Pasteur in Guinea Institut Pasteur in Madagascar Institut Pasteur in New Caledonia
Funding	Actions Concertées Inter-Pasteuriennes - ACIP 2022

Households and close contact investigations of persons with tuberculosis in Cambodia

Cambodia, having recently transitioned out of the global list of high TB-burden countries, still faces challenges in identifying and treating TB cases effectively. Current national guidelines and World Health Organization (WHO) recommendations for TB contact investigations focus on bacteriologically confirmed cases, excluding individuals with smear-negative TB who could still transmit the infection. Additionally, a significant proportion of TB cases in Cambodia are detected without the typical symptoms of coughing. Despite this, the prevalent strategy relies heavily on symptom screening, potentially missing a substantial number of TB cases. Notably, testing for TB infection (TBI) using tuberculin skin tests (TST) or interferon-gamma release assays (IGRA) is not routinely implemented in Cambodia. The project aims to fill this gap by utilizing IGRA to assess the feasibility of screening latent TB infection rates among household and close community contacts with bacteriologically and non-bacteriologically confirmed pulmonary TB (regardless of symptoms) and identify factors associated with TB infection among eligible household contacts. The MBL collaboration in the project ensures the proper performance of the IGRA test as per protocol and provides timely results to decide whether to initiate preventive therapy. The study protocol was submitted and approved by the ethical committee, followed by IGRA laboratory training and a kick-off meeting workshop in August 2023. Patient recruitment and testing started in October 2023 and will continue until May 2024.

Collaborations	PIs: C. Hout (National TB Control Program) MBL: B. Guillard, S. Cheng, S. Ung University of Sydney, Australia: AKJ. TEO KHANA, Cambodia: S. Tuot
Funding	University of Sydney Qiagen supplies the QuantiFERON-TB Gold Plus (QFT-Plus) for the study and the training required to perform the Qantiferon test. Dynamic Pharma Co., Ltd provides the equipment to be used to perform the ELISA test for the duration of the study.

Effectiveness analysis and economic evaluation of a modified program for cervical cancer screening in Cambodia assisted by a cloud-based digital system

Cervical cancer screening is a global and Cambodian public health priority, with emerging methods indicating superiority over existing VIA-based screening. The WHO recommends primary HPV screening as the preferred strategy wherever affordable. Important country-specific implementation issues include the choice of the most effective triage test for HPV-positive women and the most accepted mode of HPV test collection. The study aims to compare VIA and HPV as primary screening methods, determine the most effective triage tests for HPV-positive women, establish the most

desired HPV collection method, and conduct an economic evaluation alongside the implementation of different screening strategies. A cloud-based digital platform is utilized for comprehensive data collection, storage, and analysis. Within the collaboration framework, the MBL coordinates laboratory activities for the project, including High-risk HPV testing using the Cobas4800® platform (Roche), High-risk HPV genotyping, liquid-based cytology (LBC) slide preparation for dual-stain cytology (which will be conducted in a specialized lab in Germany), and histology analysis on biopsies from HPV-positive participants. The LBC slide preparation and histology were performed in the Pathology laboratory of Calmette Hospital. In 2023, 10,075 samples were received and tested for High-risk HPV PCR testing. Additionally, 2,777 ThinPrep samples and 139 biopsy samples were sent to Calmette for LBC slide preparation and histology analysis, respectively. The analysis of the data is ongoing.

Collaborations	PIs: H. Bussmann (Heidelberg University) and Dr S. Vong (Ministry of Health) MBL: S. Cheng, S. Heng, B. Guillard
Funding	Kreditanstalt für Wiederaufbau (KfW)

Prezode AFRICAM Cambodia

As part of the fight against zoonotic diseases and the international PREZODE (Preventing Zoonotic Disease Emergence) initiative, the AfriCam Cambodia project, funded by the French Development Agency (AFD), was launched on June 1st, 2023, in Phnom Penh. The aim of AfriCam Cambodia is to study zoonotic risks associated with hydrological dynamics and climatic and environmental changes at the human-animal-environment interface. The project will implement activities aimed at reducing the emergence of zoonotic risks and strengthening surveillance systems towards "One Health" surveillance. At a time of environmental, climatic, and social upheaval, the project aims to prevent the emergence of new epidemics, coordinated with local and national partners. The MBL is involved in several activities across the three components (C1: risk assessment, C2: Prevention strategies for risk reduction, and C3: Reinforcement of surveillance toward "One Health") of the AfriCam project. The MBL will be involved in activities such as (1) assessing the circulation/exposure of zoonotic pathogens (bacteria and parasites) in humans, animals, and the environment, (2) investigating the etiology of unexplained syndromes (bacterial infections), and (3) implementing tools for the early detection of infectious risks (e-DNA). In 2023, following the Kick-off meeting in June, the project activities mainly involved organizational meetings focused on developing the project activities and protocols.

Collaborations	PI: A.L. Banuls (IRD) IPC (MBL, Virology, Epidemiology and Public Health Unit, Medical Entomology) IRD (MIVEGEC, Espace-Dev), 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), WCS (Wildlife Conservation Society)
Funding	AFD

LMI-DRSAOH “Laboratoire Mixte International - Drug Resistance in Southesat Asia: a one Health approach to tackle AMR spread”

This is a continuing project from LMI-DRISA that was established in 2016 as part of a regional effort involving Vietnam, Cambodia, and Lao PDR. The main objective of the LMI is to share competencies and best practices among different academic and non-academic institutions in the three mentioned

countries and France to study the mechanisms and factors influencing the emergence and 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 (2021-2025), named 'LMI-DRISA$_{OH}$,' aims to integrate a 'One Health' approach to understand antimicrobial resistance emergence and spread at the interface of humans, animals, and the environment. In 2023, the LMI-DRISA$_{OH}$ activities in Cambodia focused on supporting ongoing project activities, data analysis, valorization, and capacity building. These activities included:

- Colistin resistance in multidrug-resistant Enterobacteriaceae in Cambodia: Manuscript submission to the International Journal of Antimicrobial Agents
- FSPI ARCAHE project: Genomic/Metagenomic data analysis is ongoing using Baargin workflow
- CircUs Pilot phase: ongoing data collection
- FSPI Wat-Health: data analysis and manuscript preparation
- Supporting two Cambodian participants to attend the 4th Antimicrobial Resistance Course (AMR): a "One Health" challenge organized by the Fondation Mérieux

Collaborations	Team Leader: S. Cheng (MBL), T.K.O. Nguyen (USTH), Q.H. Nguyen (USTH) and A.L. Banuls (IRD) MIVEGEC Unit, IRD (M. Hide) Medical Biology Laboratory, IPC (B. Guillard) 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)

4.7.4 Research Programs - Outlook for 2024

Nontuberculous mycobacterial (NTM) infections associated with climate change and major weather events: enhancing surveillance and mitigation strategies

Nontuberculous mycobacteria (NTM), known to cause chronic infections, are environmental pathogens commonly found in water and soil. The incidence of NTM infections is increasing dramatically in Australia, Japan, the United States, and other global regions. In many Asian countries, however, epidemiological data on NTM are lacking as public health prioritizes tuberculosis cases. Therefore, this multi-party collaboration will evaluate the epidemiology of NTM infection in Australia, the US, Japan, Thailand, and Cambodia. We will document the impact of the dispersal of environmental NTM that occurs following major weather events through geospatial analysis of infection incidence, in parallel with climate and weather data, and use modeling techniques to forecast future disease patterns. The specific aims of this study are: (1) To identify climate change variables and major weather events that predict patterns of NTM incidence in Australia, the US, Japan, Thailand, and Cambodia; and (2) To evaluate novel disinfection methods that can be applied to drinking water distribution systems to mitigate the impact of NTM dissemination caused by major weather events and climate change (Australia only).

Collaborations	PIs: R. Thomson (The University of Queensland, Australia), D.R. Prevots (National Institutes of Health, USA), K. Morimoto (Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association, Japan), S. Mahasirimongkol (Ministry of Public Health, Thailand), S. Cheng (Institut Pasteur du Cambodge, Cambodia)
Funding	e-ASIA Joint Research Program (e-ASIA JRP) NIAID funding to support activities in Cambodia

Prevalence of invasive fungal infections – Histoplasma capsulatum, Talaromyces marneffei, Cryptococcus spp – in severe immunocompromised HIV-infected patients in Cambodia

Histoplasmosis, talaromycosis, and cryptococcosis are serious invasive fungal infections (IFI) in patients with advanced HIV disease. Even though these pathogens are probably endemic in Cambodia, little or no data are available, especially for histoplasmosis and talaromycosis. The lack of awareness of these infections and the insufficient availability of reliable diagnostic methods lead to delayed diagnosis, resulting in a late introduction of specific treatment and an excessive burden. In recent years, antigenic and molecular techniques have emerged as promising tools for the rapid diagnosis of IFI. Our main objective is to assess, using these new methods, the prevalence of these IFI in Cambodia in patients living with HIV (PLHIV) at an advanced stage (CD4+ T lymphocytes <100/mm3) with no previous antiretroviral therapy (ART) and having participated in the STATIS study (ANRS_12290), a study on the management of tuberculosis associated with HIV. Our secondary objective is to raise awareness and train local actors on these infections and new and simple diagnostic tools. The project has been accepted for funding in 2023 - ANRS MIE. Project activities in 2023 focus on the preparation of the study protocol, which will be submitted to Cambodia's National Ethical Committee for Health Research in Feb 2024.

Collaborations	PIs: S. Cheng (Institut Pasteur du Cambodge, Cambodia) and A. Sturny Leclere (Institut Pasteur de Paris, France) E. Mosnier, University of Health Sciences, Cambodia A. Adenis, Cayenne Hospital, French Guiana, France N. De Rekeneire, Institut Pasteur du Cambodge, Cambodia
Funding	Inserm – ANRS MIE Sponsor: IPC

4.7.5 Support to National Authorities

- In partnership with the National Centre for HIV/AIDS, Dermatology and Sexually Transmitted Diseases (NCHADS) of Cambodia, follow-up of HIV seropositivity
- Participation in CENAT's Technical Working Group on multi-drug resistant tuberculosis
- Participation in the technical working group on AMR with the Ministry of Health
- Participation in the development of the protocol for the 3rd National TB Prevalence survey (2022-2023)

4.7.6 Teaching and Training

Continuing Professional Training and Development for the MBL's Staff in 2023

Training	MBL Staff trained
Chemical risk and biological risk (online, Kaptitude)	- Chan Sithsocheatey - Ung Sochearaksmey - Chor Liseany - Bertrand Guillard
Fire Fighting Training (onsite, IPC)	- Sem Sopha
Workshop for the project ORACAN in Institut Pasteur de Guinée	- Han Samrach
ISO 15189:2012 Awareness training (online)	- Ung Sochearaksmey - Chor Liseany
ISO 15189:2012 Certified Auditor (online)	- Chor Liseany
MOOC Tuberculosis (online)	- Heng Seiha
ISO 17025: 2017 Certified Auditor (online)	- Chor Liseany
Awareness training of ISO 15189:2022 (online)	- All the personnel of MBL
Method validation on ISO 15189 2022	- Bertrand Guillard - Chor Liseany - Heng Seiha - Sreng Narom - Eng Sokchea - Cheng Sokleaph - Sith Charya
Internal auditor ISO 15189:2022 (online)	- Chor Liseany - Heng Seiha - Sreng Narom - Eng Sokchea
ISO 17025: 2017 Awareness training (online)	- Chor Liseany
English training (Australian Center for Education, Phnom Penh, onsite/online)	- Chor Liseany - Sith Charya - Nop Puthea - Eng Sokchea - Chan Sethsocheatey - Sayulim Sereyrith - Khan Ousa - Sem Sopha - Han Samrach - Sreng Narom - Yi Vandy
Cerba Live sessions (Webinars) Topics: Helicobacter pylori, Thrombophilie biologique, Tuberculose multirésistante, Best of congrès européen de microbiologie…	- Bertrand Guillard - Cheng Sokleaph - Sith Charya - Nop Puthea - Heng Seiha
Antimicrobial Resistance Course (AMR) – A One Health Challenge	- Cheng Sokleaph

Teaching

- Cheng Sokleaph: Master 2 Infectiology (UHS, Phnom Penh) - Antibiotics and Antibiotic resistance

Internships

One of the missions of Institut Pasteur du Cambodge is training. In 2023 we welcomed trainees with various backgrounds:

- Master's Degree, Medical Biology, UHS: ..... **2 students**
- Master's Degree, Medical Biology, University of Puthisaastra: ..... **4 students**
- Master's Degree of Infectiology, UHS and Paris-Saclay University..... **1 student**
- BA, Pharmacy, UHS: .....**20 students**
- Associate's Degree, Medical Laboratory Technician, University of Puthisaastra: ..... **6 students**
- Associate's Degree, Medical Laboratory Technician, UHS: .....**18 students**
- High school, Lycée Français René Descartes : ..... **1 student**

4.7.7 Outlook for 2024

Quality

The ISO 15189 accreditation of the IPC's Medical Biology Laboratory demonstrates the quality and reliability of our services. Our laboratory will continue to be regularly reassessed to ensure that we are maintain our standard in terms of technical expertise. In April 2024, there will be a surveillance audit coupled with a transition audit (transition to the new ISO 15189:2022) conducted by the *French Committee for Accreditation* (COFRAC).

Laboratory information system (LIS) follow-up

In 2024, our goal is to stabilize the situation with the implementation of updates by Datamed, which will allow us to improve the service provided to customers and enhance their satisfaction.

Development plan

The MBL of IPC must adapt to the evolving landscape of medical biology in Cambodia. In 2024, a development plan for the laboratory will be implemented. This plan involves collaboration between MBL and the IPC's management:

- Opening the laboratory on Saturday afternoons
- Recruitment of an additional biologist (or biologist in training courses)
- Reviewing prices for analyses in the MBL catalogue
- Implementing new tests and acquiring new equipment
- Considering the establishment of an off-campus sampling site

Research activities

- Enhancing Research Capacity: The establishment of the BAAR group in 2022 was a significant step forward. However, the group currently consists of 5 staffs with only one researcher, who can allocate only 60% of their time to research activities. Therefore, it is imperative to strengthen the team's research capacity by recruiting PhD students, post-docs, or visiting scientists through research collaborations.
- Strengthening expertise in statistical data analysis, modern clinical microbiology, genomic, and bioinformatics.
- Boosting scientific publication output: It is crucial for the team to publish the data generated by various projects, which has been delayed due to routine activities and limited human resources.

4.7.8 Scientific Publications 2023

1. **Colistin resistance in multidrug-resistant enterobacteriaceae in Cambodia.**
   Hide M, Meng S, Cheng S, Bañuls Al, Ky S, Chantana Y, Laurent D, Delvallez G.
   The manuscript was submitted to the International Journal of Antimicrobial Agents in November 2023.

2. **Comparative Performance of Anyplex II HPV28 and Cobas 4800 Human Papillomavirus (HPV) Assays for High-Risk HPV Detection in Self-collected Anal Samples.**
   Delvallez G, Cheng S, Marot S,Malonga GA, Cocherie T, Wignall S, Calvez V, Phal S, Vichet K,Marcelin AG,Jary A.
   Open Forum Infectious Diseases 2023 Oct 31;10(11):ofad540. doi: 10.1093/ofid/ofad540. eCollection 2023 Nov.

3. **Genomic insights into anthropozoonotic tuberculosis in captive sun bears (Helarctos malayanus) and an Asiatic black bear (Ursus thibetanus) in Cambodia.**
   Kirsty Officer, Timothy M. Walker, Sokleaph Cheng, Seiha Heng, Mallorie Hidé, Anne-Laure Bañuls, Jonathan Cracknell, Nev Broadis, Nhim Thy, Sam Abraham, Kris Warren, Bethany Jackson.
   The manuscript was submitted to the Scientific Reports – Nature in August 2023.

4. **Phylogenomic investigation of an outbreak of fluoroquinolone-resistant Salmonella enterica subsp. enterica serovar Paratyphi A in Phnom Penh, Cambodia**
   Rutaiwan Dusadeepong, Gauthier Delvallez, Sokleaph Cheng, Soda Meng, Navin Sreng, Joanne Letchford, Kimcheng Choun, Syna Teav, Liselotte Hardy, Jan Jacobs, Tuyet Hoang, Torsten Seemann, Benjamin P Howden, Philippe Glaser, Timothy P Stinear, Koen Vandelannoote
   Microbial Genomics 2023 Mar;9(3). doi: 10.1099/mgen.0.000972.

5. **Prevalence and risk factors of anal Human Papillomavirus infections among men having sex with men and transgender women in Phnom Penh, Cambodia**
   Aude Jary, Stéphane Marot, Gervillien Arnold Malonga, Théophile Cocherie, Vincent Calvez, Anne-Geneviève Marcelin, Sokleaph Cheng, Gauthier Delvallez, Steve Wignall, Sophat Phal, Kem Vichet
   The Journal of Infection 2023 Feb 28;S0163-4453(23)00124-X. DOI: 10.1016/j.jinf.2023.02.038

Abstracts and Presentations

1. **Investigating the circulation of AMR between animals, humans and their environment in Cambodia – ARCAHE**
   Juliette Hayer, Ella Marcy, Mallorie Hidé, Savatey Hak, Sivhour Chiek, Navin Sreng, Meymey Lem, Rina Dork, Chiva Ma, Gauthier Delvallez, Patrice Piola, Véronique Chevalier, Anne-Laure Bañuls, Sokleaph Cheng
   Oral presentation at the 9th Symposium on Antimicrobial Resistance in Animals and the Environment, July 3-5, 2023, Palais des Congrès, Tours, France.

2. **Understanding the circulation and factors associated with extended-spectrum β-lactamase-producing enterobacteria in Cambodia – FSPI ARCAHE study**
   Sokleaph Cheng at 2nd RAPID SYMPOSIUM, South Korea.

4.8 Laboratory of Environment and Food Safety

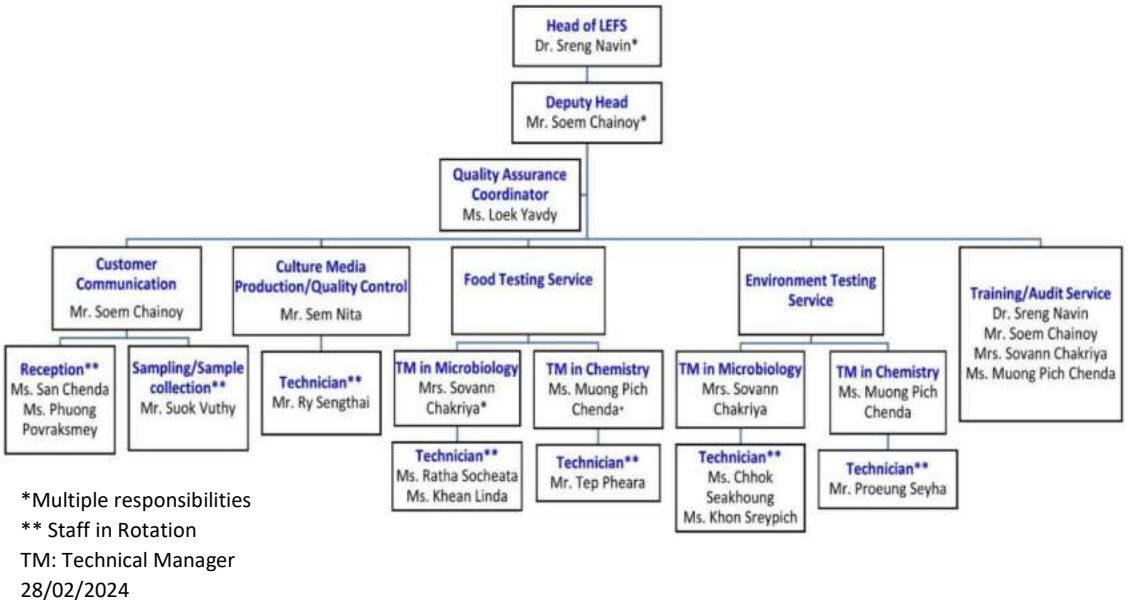
The Laboratory of Environment and Food Safety (LEFS) was created in 1995. Its activities mainly involve microbiological and chemical analyses of food and water. In more detail, the LEFS aims to:

- Identify and quantify public health issues related to food and water consumption and caused by the presence of pathogens (*Clostridium perfringens*, Coagulase positive Staphylococci, *Salmonella*…) and parasites;
- Promote hygiene practices in restaurants and food industries (training, consulting, auditing).

The laboratory provides the following analysis services, in compliance with international protocol standards:

- Microbiology of food, water and surface swab samples;
- Physical chemistry assays, quality of water samples;
- Identification of Legionella in tap water, cooling towers, pools and spa water.

4.8.1 Functional Structure



*Multiple responsibilities
** Staff in Rotation
TM: Technical Manager
28/02/2024

Figure 33: Laboratory of Environment and Food Safety organigram

Changes occurred to the team's composition in 2022: five new technician was recruited which among them, one replaces a staff member who had left. The new Technical Manager in chemistry was also recruited to replace the one who resigned in 2022. A new quality assurance coordinator was recruited for replacement.

4.8.2 Service Activities in 2023

During 2023, our laboratory tested 7,822 samples, comprising 3,404 food samples, 2,572 water samples for microbiology testing and 18 food samples and 1,828 water samples for chemical testing. In 2023, we have signed a partnership with b.Consulting for organizing training on Good Hygiene Practice and HACCP for public. Thank to this partnership, we could provide 2 trainings 2023.

Compared to 2022, the total number of samples tested decrease slightly.

The analytical activities (sample numbers and test numbers) over the last five years are shown in the figure below:

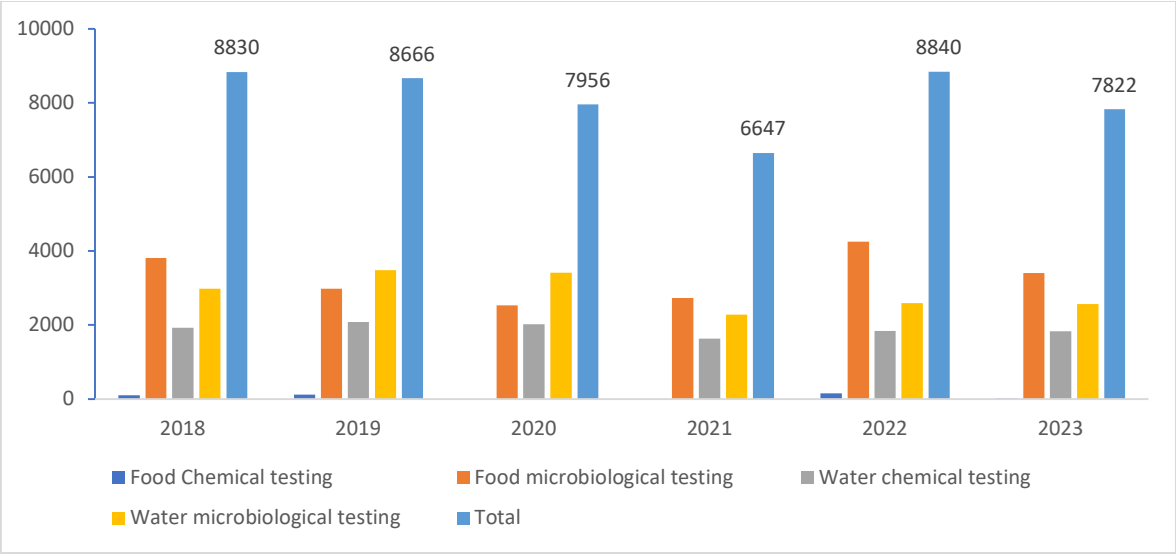


Figure 34: Evolution of the number of samples by year (2018-2023)

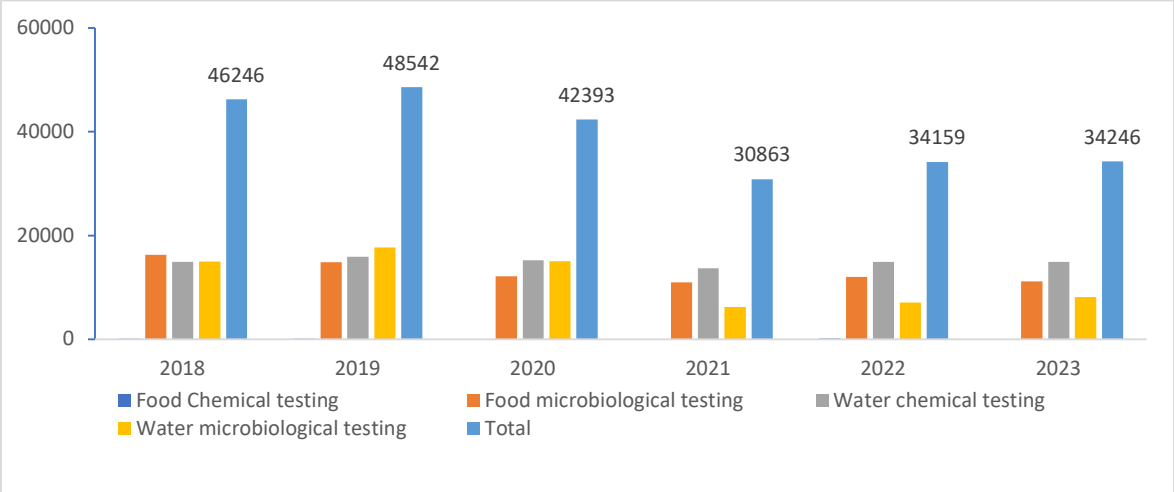


Figure 35: Evolution of the number of analysis by year (2018-2023)

If we look more closely at the data collected for each sample category in terms of quality, we note that:

Food sample

- 42 % of food samples (1,441/3,404) were requested to compare the results with reference standards or customer requirements, and 4 % (53/1,441) were found to be unsatisfactory. The unsatisfactory results were due to *Salmonella* contamination (6%), and to high levels of hygiene risk indicators such as coliform bacteria (17%), E. coli (13%), Bacillus cereus (6%), and Total plate count (38%). 30% of these unsatisfactory results were due to two or three different bacteria contaminations.
- 54 % of Salmonella-positive food samples were raw meat (19/35) followed by fish and fishery products at 43 % (15/35), and feed products at 3 % (1/35).

Water

- 30 % of water samples sent to microbiology testing (769/2,572) were requested to compare the results with reference standards or customer requirements, and 15 % (112/769) were unsatisfactory due to the contamination of Coliforms bacteria (83%) and Legionella (7%).
- 15 % of ice cube samples (56/382) from restaurants and bars were found to be contaminated by faecal bacteria, such as coliforms and E. coli.

Quality Management System

The LEFS was accredited for food microbiology analysis by the International Accreditation Service (IAS) on 6 September 2022, under the accreditation number TL-1056, according to the ISO/IEC 17025:2017 standard.

Scope of accreditation: food microbiology

1. Culturable microorganisms at 30˚C – NF EN ISO 4833-1
2. Enterobacteriaceae – NF EN ISO 21528-2
3. Coliform bacteria – NF ISO 4832
4. *Escherichia coli* – NF ISO 16649-2
5. *Salmonella* – NF EN ISO 6579-1

In order to maintain the ISO 17025 accreditation, various quality control documents have been created or revised in 2023. Below is the total number of such documents/procedures/forms created and revised in 2023.

Description	New creation		Revision		Total
	Completed	In process	Completed	In process	
Procedure	0	20	29	14	**63**
Form	14	0	44	3	**61**

Table 6: Quality Management Documents in LEFS

4.8.3 Research Programs - Major Achievements in 2023

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** of 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 as follow.

1. ***Identification of critical control points:***
   - Identify two prominent bacterial pathogens associated with vegetable-borne disease(s);
   - Conduct a longitudinal study to map and characterize microbial pathogen contamination points, persistence, and transmission in vegetable supply chains;
   - 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:***
   - Identify and/or design interventions to reduce microbial contamination;
   - Assess food safety awareness and willingness to apply potential interventions for specific control points;
   - Establish and strengthen food safety networks and public–private partnerships to promote relevant safety measures identify early adopters, and help in positioning interventions.
3. ***Delivery of data-driven engagement programs:***
   - Create and deliver engagement programs to foster greater adoption of food safety measures by farmers, distributors, vendors, and market management groups;
   - Deliver engagement programs that improve food safety awareness among consumers;
   - Measure the impacts and efficacy of all engagement programs and refine outreach activities to produce greater reductions in foodborne disease risks associated with vegetable consumption.

The LEFS is mainly involved in part 1 of the project, which involved collecting clinical data from children under the age of 15 suffering from diarrheal diseases over the last 24 months in Phnom Penh, Battambang and Siem Reap hospitals, and requires analysing the collected samples. Based on literature review and on clinical data collected from children under 15 suffering from diarrheal diseases over the past 24 months at the Medical Biology Laboratory of *Institut Pasteur du Cambodge*, Battambang's Provincial Hospital and the Angkor Hospital for Children in Siem Reap, *E. coli* and *Salmonella* were determined to be the two main bacterial pathogens associated with vegetable-borne diseases. Sample collection was done monthly, starting in April 2022 and ending in November 2022. Battambang and Siem Reap Provinces were the two selected locations for sample collection. The LEFS was in charge of analysing the samples from Siem Reap province, whereas ITC did the analyses for Battambang Province.

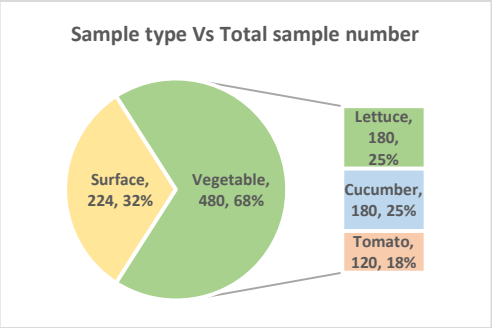
30 farms, 30 distribution centre vendors (DC) and 30 market vendors were included in this project as sampling sites for each province. Cucumbers, lettuce, and tomatoes were defined as high-risk vegetables (if eaten raw) and thus selected for this project. Besides vegetables, environmental samples such as direct food contact surface (FCS) and non-direct food contact surface (NFCS) samples were also collected from each site."

Sample analysis was done with two different protocols. *E. coli* screening began with an enrichment broth, followed by DNA extraction and completed by a conventional PCR and gel electrophoresis. The *Salmonella* screening protocol starts with a selective enrichment step, followed by isolation on selective agar. Typical colonies were first detected with latex agglutination, and then confirmed with Real-Time PCR.

The preliminary data from Siem Reap Province's samples is shown below:

Location	Sample Number
Farm	224
Distribution centre vendors	240
Market vendors	240

Table 7: Sample number according to location



A total of 704 samples were collected between April and November 2022, comprising 480 vegetable samples and 224 environmental samples. These samples were collected across the entire vegetable value chain, including farms, DC vendors, and market vendors

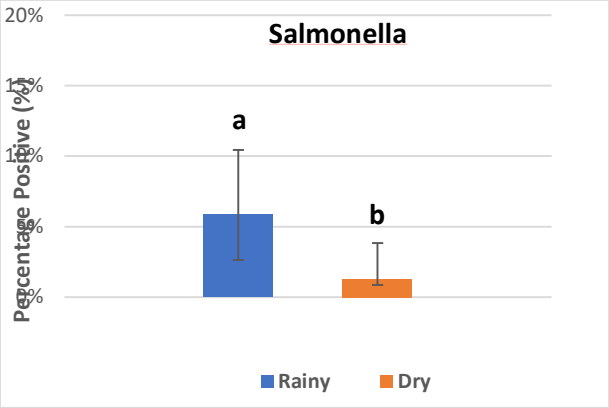


Figure 36: Estimated Salmonella detection rates by season, averaged across sample types and sites along the value chain. Whiskers depict 95% confidence intervals.

The prevalence of *Salmonella* was found to be higher during the rainy season compared to the dry season (P<0.05). Interestingly, no significant evidence of a difference in *Salmonella* prevalence was observed along the various stages of the value chain.

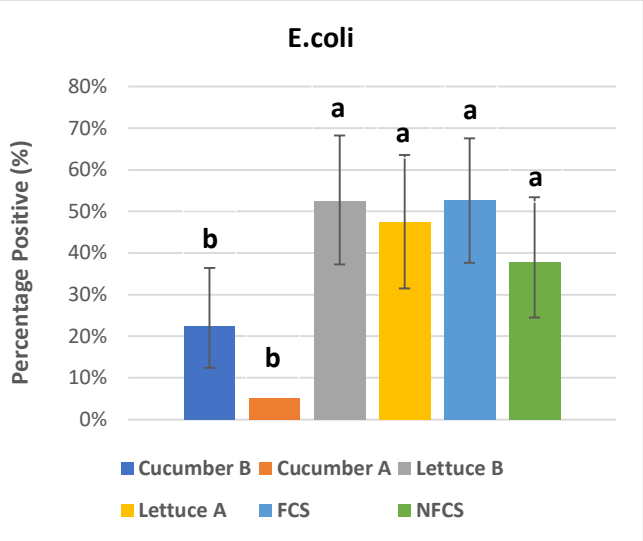


Figure 37: Estimated E. coli detection rates for each of sample type, regardless of season or location.
Whiskers depict 95% confidence intervals. a, b P<0.05.
B: before at farm (sampled at farm), DC and market (sampled when DC or market vendor just start their business).
A: after at farm (sampled after packaging at farm), DC and market (sampled when DC or market vendor finish their business).
FCS: direct food contact surface
NFCS: non-direct food contact surface"

The prevalence of *E. coli* was notably high at rate of 43%, surpassing that of *Salmonella* (5%). *E. coli* prevalence in lettuce and on FC surfaces was significantly higher than in other sample types (P<0.05). Though, seasonal variations did not show a significant impact on *E. coli* detection rates.

In-depth data analysis is in progress, and all of the isolated strains have already been shipped to Penn State University for whole genome sequencing (WGS).

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)

4.8.4 Support to National Authorities

For several years, the LEFS has supported different national authorities in Cambodia, including the Food and Drug Department of the Ministry of Health, and the National Animal Health and Production Research Institute, attached to the Ministry of Agriculture, Forestry and Fisheries.
In 2023, as part of a national monitoring program, the Ministry of Health sent 814 samples to LEFS. These samples were obtained through product hygiene campaigns of industrial foods imported and exported.
Also, as part of a national monitoring program, the Ministry of Agriculture, Forestry and Fisheries sent 134 sample to LEFS.

4.8.5 Teaching and Training

The Laboratory supervised eleven trainees from various universities in Cambodia for internships lasting between one and three months. Details are provided below.

University	Number of students	Program Year	Period (Month)	Date
University Paris Cite	1	Year 3	2	01/05/2023–05/07/2023
University of Health Sciences (UHS)	8	Year 4	2	26/12/2022–10/02/2023 13/02/2023–28/03/2023 15/05/2023–27/06/2023 03/07/2023–18/08/2023
Institute of Technology of Cambodia	1	Year 4	1	07/08/2023–31/08/2023
	1	Year 3	2	07/08/2023–30/09/2023
	1	Year 3	1	11/09/2023–15/10/2023

Table 8: Internship Students at the LEFS in 2023

4.8.6 Outlook for the Upcoming 3-5 Years

The LEFS's outlook for the upcoming three to five years comprises the following objectives:

- Maintaining and improving the quality of service;
- Increasing laboratory visibility/recognition with the public;
- Extension of our accredited services for food, water microbiological testing and food, water chemical testing;
- Set up testing equipment and procedures for chemical analysis in water, using GF-AAS
- Set up pesticide, heavy metals, and antibiotic residue analysis capabilities;
- Develop increasing cooperation with internal and external partners for research projects.

4.9 Vaccination Service

The Vaccination Service at the Institut Pasteur du Cambodge is composed of the International Vaccination Center (IVC) in Phnom Penh and three Rabies Prevention Centers (RPC), in Phnom Penh, Battambang, and Kampong Cham.

4.9.1 Functional Structure

The unit has 23 team members based in Phnom Penh and two provinces: nine medical doctors (including the head of the Vaccination Service and four medical supervisors), eleven nurses, two administration staff, and one person in charge of hygiene.

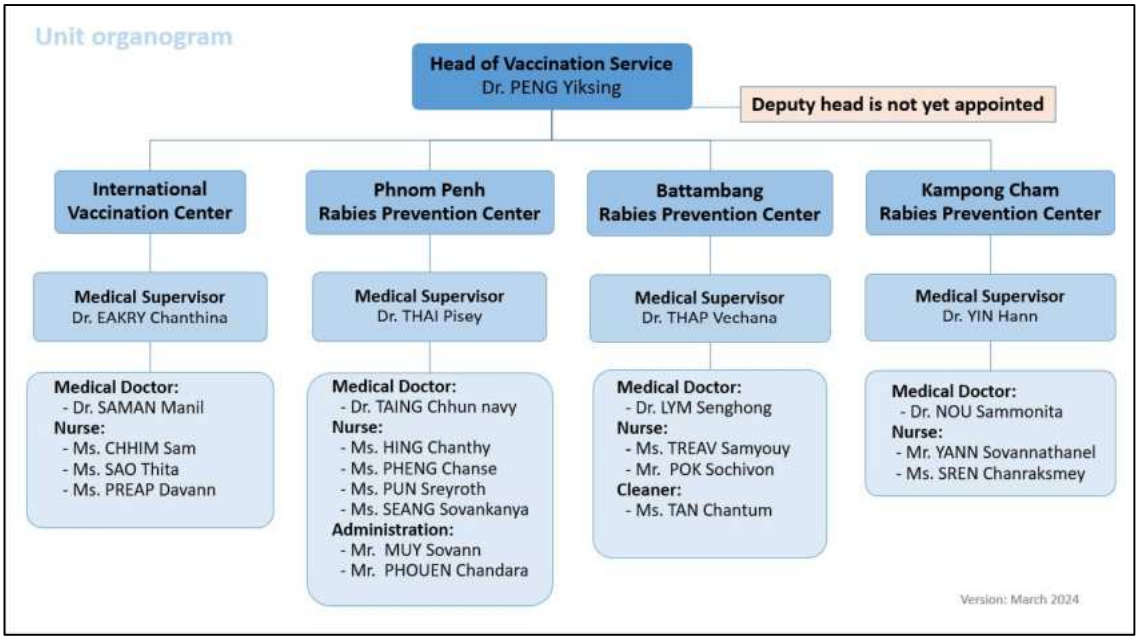


Figure 38: Vaccination Service Organigram

4.9.2 Rabies Prevention Centers

The three rabies prevention centers employ 17 full-time staff under the leadership of the Head of the Vaccination Service. 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 and following the 2018 WHO recommendations, IPC has offered a full rabies PEP intradermal protocol, which consists of three sessions of 2-site ID injection using 0.1 mL vaccine per site (IPC protocol). This is proposed 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 2023:

- Provided rabies post-exposure prophylaxis to 60,476 patients.
    - 36,099 patients received rabies PEP at the Rabies Prevention Center in Phnom Penh
    - 15,432 patients received rabies PEP at the Rabies Prevention Center in Battambang
    - 8,945 patients received rabies PEP at the Rabies Prevention Center in Kampong Cham
- Despite efforts from MoH-CDC and GDAPH to eliminate human rabies through dog mass vaccination campaigns in various areas in 2023 (PP and Battambang), the number of patients who received PEP at IPC remained stable compared to 2022.
- A total of 220 animal heads were tested by immunofluorescence for rabies virus at the Virology Unit. 162 samples (74%) were positive for rabies:
    - 77.6% of all dog samples (159/205) were positive for rabies,
    - 20% of all cat samples (3/15) were positive for rabies.
- The animal rabies diagnosis information is regularly communicated to MoH-CDC, WHO, and GDAPH.

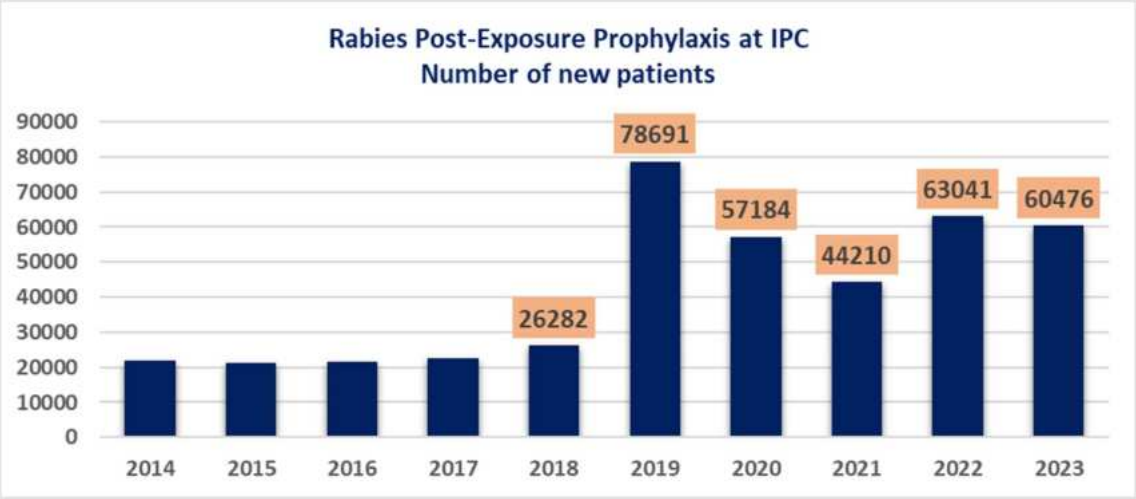
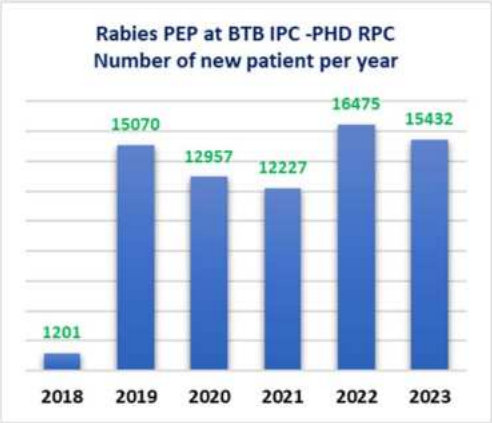
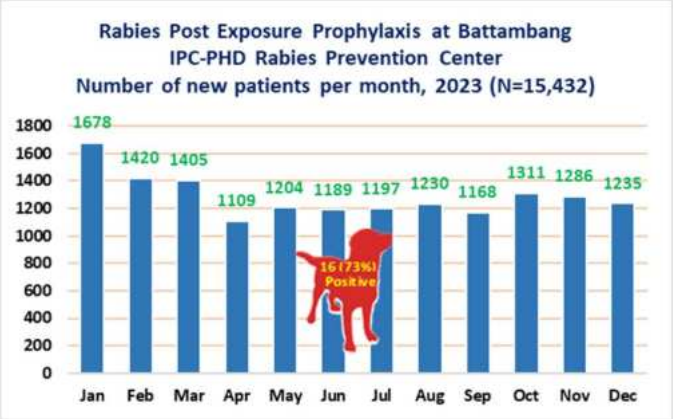


Figure 39: Rabies Post-Exposure Prophylaxis at IPC, number of new patients

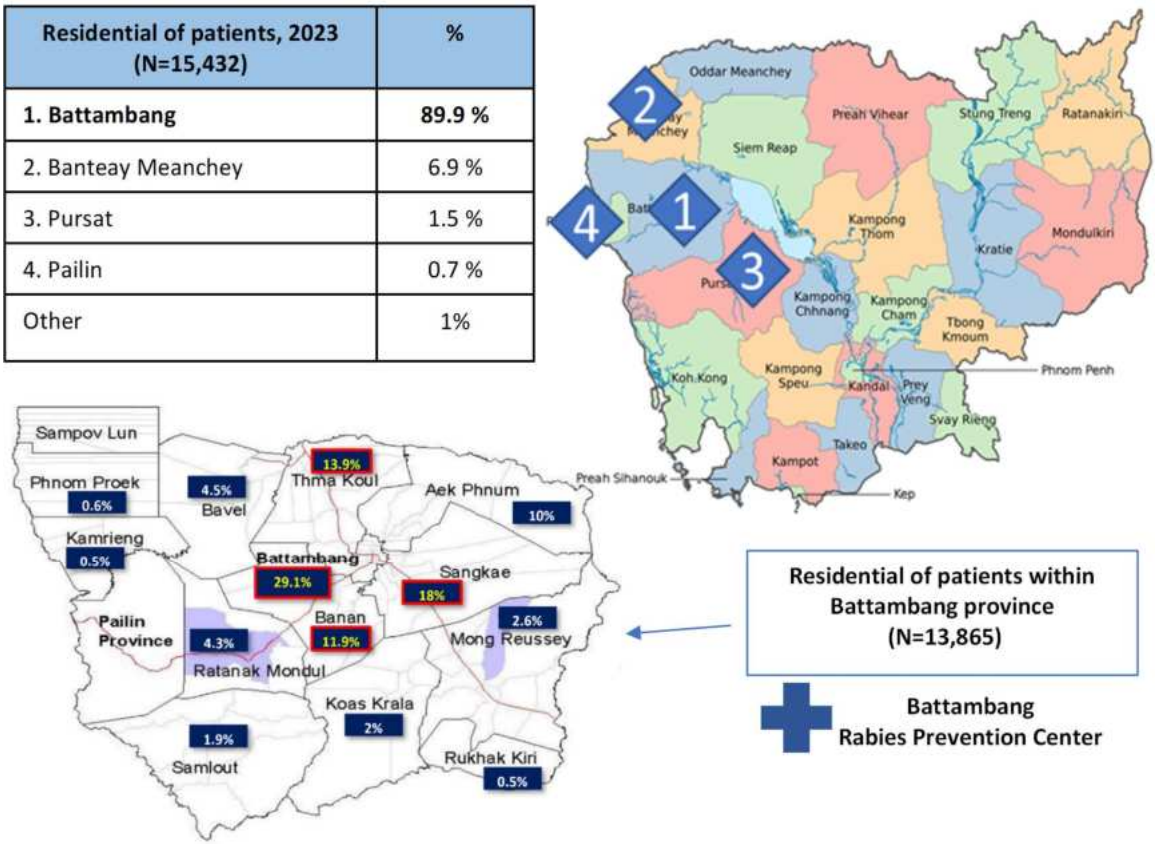
4.9.2.1 Battambang Rabies Prevention Center

The Center located within the provincial hospital was opened in July 2018, following a memorandum of understanding signed on 25 December 2017 between the Battambang Provincial Health Department (PHD) and the Director of Institut Pasteur du Cambodge. In this collaboration, the PHD contributes the building and utility services. The official inauguration ceremony was held on 28 September 2018. This center is expected to cover Battambang and 5 other neighboring provinces.



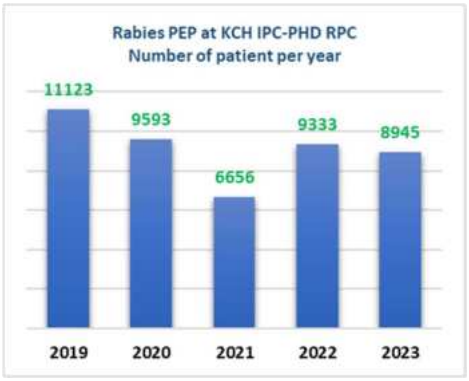


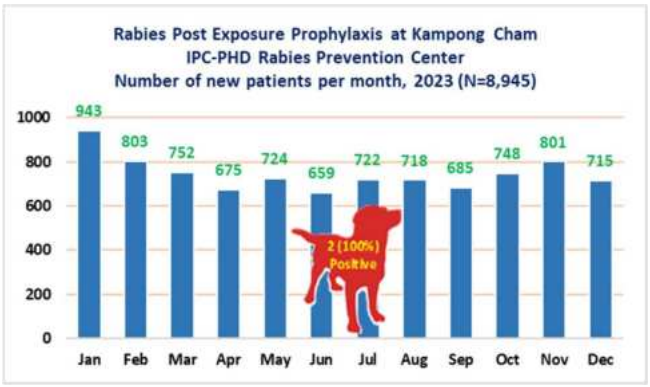
Residential of patients, 2023 (N=15,432)	%
1. Battambang	**89.9 %**
2. Banteay Meanchey	6.9 %
3. Pursat	1.5 %
4. Pailin	0.7 %
Other	1%



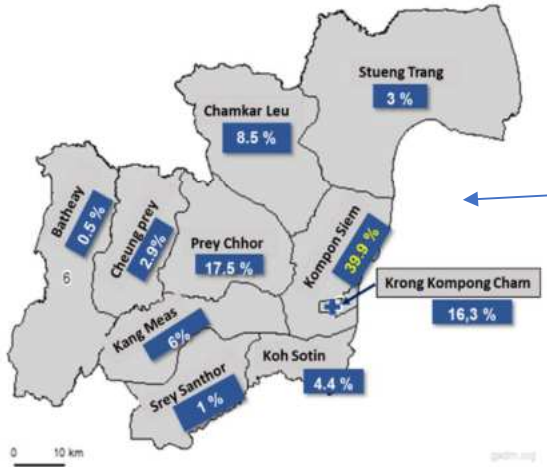
4.9.2.2 Kampong Cham Rabies Prevention Center

This IPC-PHD Rabies Prevention Center was opened on 7 March 2019 in Kampong Cham Province as part of the response to a sudden surge of patients seeking rabies prevention following wounds caused by dogs or cats. The center is located within Kampong Cham Provincial Hospital and uses a temporary building provided by the hospital. The coverage may be extended to six other provinces of Northeast Cambodia. The agreement between IPC and KC-PHD for the construction of a permanent Rabies Prevention Center in Kampong Cham was signed on 26th April 2023. The construction of the center was started in Dec 2023, and it is expected to be finished in April 2024.





Residential of patients, 2023 (N=8,945)	%
1. Kampong Cham	**77.1 %**
2. Tboung Khmom	20.1 %
3. Kampong Thom	1 %
4. Prey Veng	0.9 %
Other	0.9 %



Residential of patients within Kampong Cham Province (N=6,895)

Kampong Cham Rabies Prevention Center

4.9.3 International Vaccination Center

The International Vaccination Center at the Institut Pasteur du Cambodge has a medical team of 5 full-time staff under the responsibility of the head of the Vaccination Service. One additional nurse was recruited in 2023 to support the team in response to the significant increase in the number of clients. Furthermore, our cold room capacity was also extended (one more cold room has been installed), this improvement provides further possibility for us to secure vaccine safety stock at IPC for both local supply and international purchase products.

A wide range of vaccines (including those that are part of the national immunization program) and immunoglobulins are available at the International Vaccination Center. We maintain international

standards with certified products, proper cold chain management, a high level of quality control, and professionalism.

In 2023, a total of 44,374 injections (including the immunoglobulins) were done as part of 25,953 vaccine protocols. There is a 24% massive increase in overall activity in 2023 compared to 2022. For 2023, our activity was led by HPV, Flu, and Hepatitis B among others. Notably, the service activity of the International Vaccination Center in 2023 was strongly contributed by optional vaccines that significantly increased following the adaptation of our strategic plan to address vaccine shortage issues.

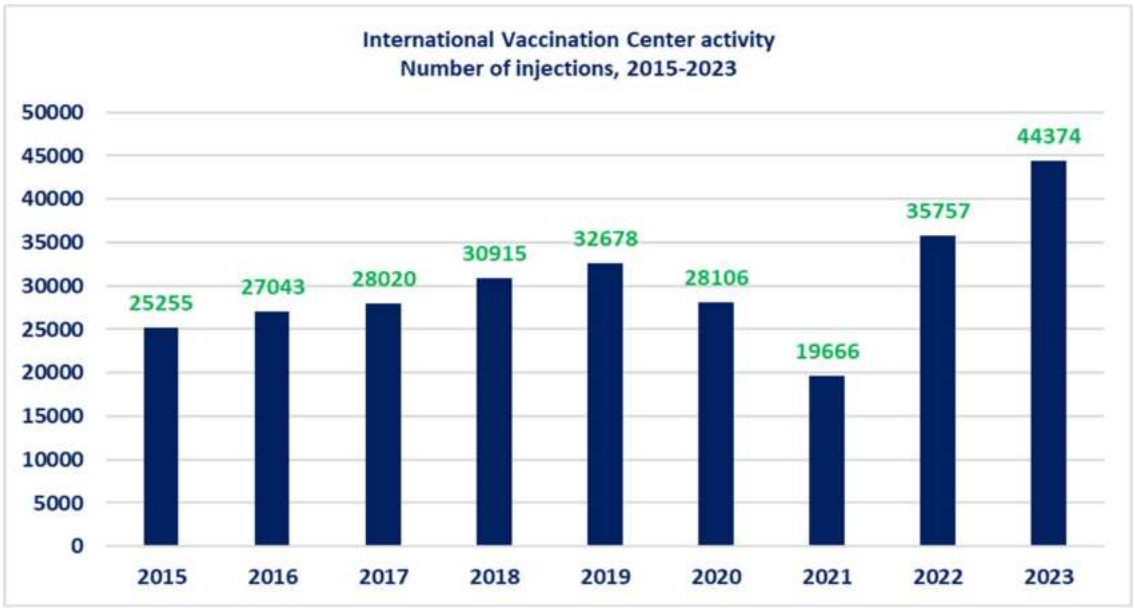


Figure 40: International Vaccination Center activity, number of injections, 2015-2023

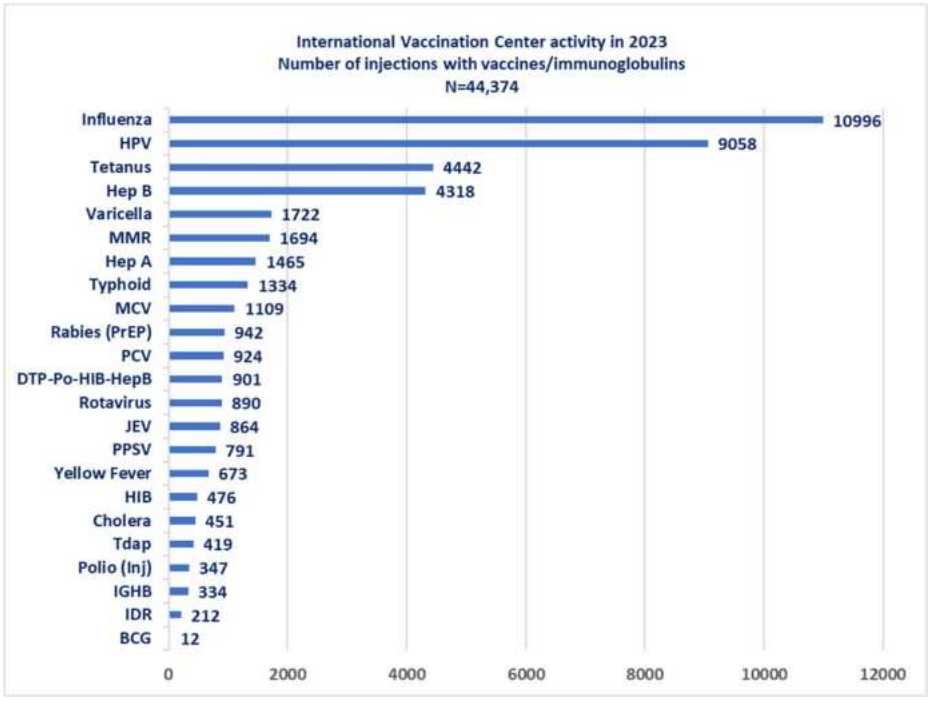


Figure 41: International Vaccination Center activity in 2023, number of injections with vaccines/immunoglobulins

4.9.4 Support to National Authorities

Contributions to the fight against rabies in Cambodia

The Vaccination Service of IPC supports the Ministry of Health in Cambodia in the fight against rabies through various key activities: it contributes to the development of the National Rabies Elimination Strategy, participates in EIC (education, information, and communication) activities, provides rabies PEP to the public at an affordable price, and is a national reference training center for rabies PEP.

The Vaccination Service cooperates with several national authorities and international agencies (MoH, US-CDC, Institut Pasteur in Paris, WHO, GDAPH, FAO, CIRAD, GIZ, and others) to contribute to rabies elimination programs and research studies, especially on rabies.

4.9.5 The Vaccination Service's Vision for Next 2-5 Years

Contributing 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:

- Increasing accessibility to rabies PEP by improving the visibility of our centers.
- Raising awareness about rabies through education, information, and communication activities, IPC is ready to support the MoH in the rabies elimination mission, including by being a teaching center for rabies PEP.

Supporting and promoting additional research

- We aim to enhance staff career development in research studies, focusing on the young talents within the Cambodian staff.
- We will continue to promote the Vaccination Service's research activities in close collaboration with other units of IPC.

Enhancing the quality of our service

- We aim to maintain the quality of our vaccination services by upholding high-quality standards and professionalism, for the benefit of the public.

4.9.6 Scientific Publications 2023

1. **Side-by-side comparative study of the immunogenicity of the intramuscular and intradermal rabies post-exposure prophylaxis regimens in a cohort of suspected RABV exposed individuals**
   Heidi Auerswald, Alvino Maestri, Sothy Touch, Saraden In, Nisa Ya, Borita Heng, Valérie Bosch-Castells, Christele Augard, Céline Petit, Philippe Dussart, Yiksing Peng, Tineke Cantaert, Sowath Ly. Clinical Infectious Diseases 2023 Jun 20;ciad304. doi: 10.1093/cid/ciad304

4.10 Technical platforms

4.10.1 Biobank

4.10.1.1 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 group of local developers called 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 the 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 it has been put in use since July 2022.

4.10.1.2 Functional Structure

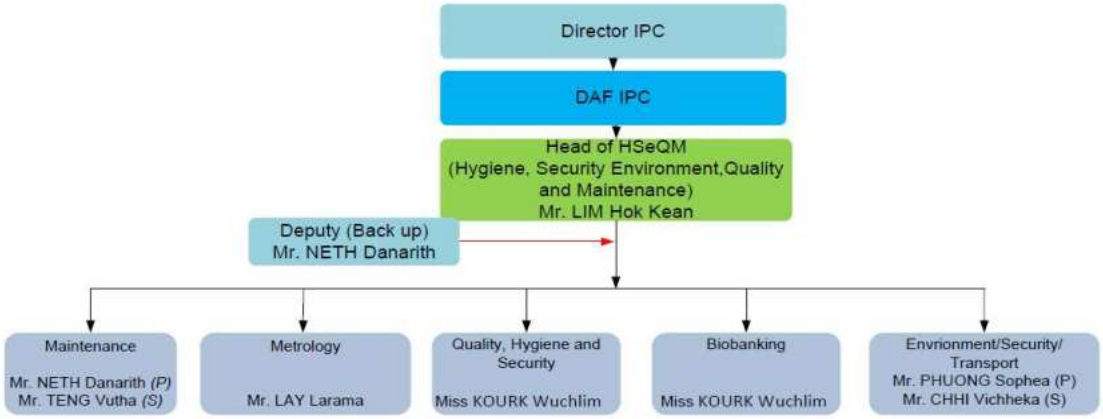


Figure 42: Biobank Organigram

The biobank section is under the Hygiene, Security, Environment, Quality and Maintenance (HSeQM) service/Administration unit. It has a cross-cutting role to support research and testing laboratories at IPC with different materials including documents such as standard operating procedures (SOPs) and software to monitor the samples. Current key responsibilities and functions in biobanking at IPC are as described below.

- Biobank software: managing the biobank software, working with developers, handling complaints from users, and solving problems/errors reported by users relating to the software;
- Sample labelling: printing labels for users (biobank label template + QR code);
- Material transfer agreement (MTA): reviewing MTA and providing reference numbers

The current users of the biobank are the Virology Unit and the Epidemiology and Public Health Unit.
All samples are stored in -80°C freezers. In addition, we now have 47 units of such freezers and in the future, some samples will be stored in liquid Nitrogen (N_2). IPC also has its own liquid nitrogen generators for daily production of around 100 liters per day which meets the demand of use for all the laboratories at IPC.
All of the freezers are installed in a purpose-designed room equipped with air conditioners working permanently. This room is only accessed by a group of authorized personnel, which means that the entry of the biobank rooms is strictly controlled. The freezers are constantly monitored through the use of monitoring sensors (the Oceasoft system).

4.10.1.3 Biobank Database

At present, there are 13 research projects registered in the biobank software with the total number of 56150 samples as shown in more detail in the Table below.

Collection/Project	No. of samples	Nature of samples	Storage
LEFS			
Food Safety Innovation Lab	674	*E. coli* *Salmonella*	-80°C freezer (HSM-04) Freezer code: I/01599
LBM			
Aspergilloma in Cambodia	3051	Serum Aspergillus	-80°C freezer (HSM-04) Freezer code: 439
VIROLOGY			
ECOMORE 2	6421	Serum Saliva	-80°C freezer (HSM-04) Freezer code:
Immuno PEP follow up 2019	234	Serum	-80°C freezer (VIR-02) Freezer code: 1324
RAB00056 IM/ID (Sanofi study)	26	Serum	-80°C freezer (VIR-02) Freezer code: 1324
Rabies surveillance	14050	Ammon's horn Spinal bulb	-80°C freezer (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	1938	Kidneys, Swab, Urine, Heart, Lung, Liver, Ectoparasite, Blood clot, blood serum, Pool organ	NA
Epidemiology and Public Health			
HEPEDIAC- Pilot therapeutic study of DAA treatment for children and adolescents with active HCV infection in Cambodia	105	Whole blood and plasma	-80°C freezer (HSM 04) Freezer code: 01609
Lowering Interleukin-1 Receptor Antagonist Concentrations after TB Treatment Onset: A proof of concept study in Cambodia and Ivory	303	Plasma Buffy coat	-80°C freezer (HSM-04) Freezer code: LILAC-TB
TB-Speed Output 2 - Severe pneumonia	1794	Plasma Stool Whole blood	-80°C freezer (HSM-04) Freezer code: 1551
Tenofovir As Prevention Of Hepatitis b Mother-to-child transmission	27509	Plasma Blood Red blood cell Buffy coat	-80°C freezer (HSM-04) Freezer code: 1475
Zika Sentinel Surveillance in prenatal care visit and maternity ward in Calmette Hospital (Phnom Penh, Cambodia)	16	Serum Urine	-80°C freezer (HSM-04) Freezer code: 439
Total samples	**56121**		

4.10.1.4 Action Plan

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

The person responsible for Biobanking is currently pursuing a Master Degree in MSc Biobanks and Complex Data Management for two years (October 2023 – September 2025) at the Université Côte d'Azur in Nice, France. The objective of this master is to focus on both technical and practical knowledge in Biobanking. This will serve an opportunity to develop biobanking in IPC as well as the development of biobank networking in the future.

4.10.2 Sequencing Mini-platform

4.10.2.1 Functional structure

Established in April 2021, the Sequencing Platform is a technical facility whose mission is to strengthen - in transversal within IPC – research activities and public health responses related to genomic sequencing. At its core, the platform is equipped with cutting-edge infrastructure including a high-throughput next generation sequencer (Illumina MiSeq System), alongside an array of other instruments integral to conduct next generation sequencing. As of April 2023, the platform has evolved into an autonomous entity, boasting a dynamic composition comprising two scientists (Dr. Janin NOUHIN, Ph.D., Senior Scientist, 30% of his working time and Dr. Nimol KHIM, Ph.D., Deputy Head of Malaria Research Unit, 20% of her working time) in charge of general coordination and one skilled research engineer (Mr. Vireak HEANG). Our activities comprise nucleic acid library preparation and next generation sequencing (NGS) using the state-of-the-art Illumina MiSeq System.

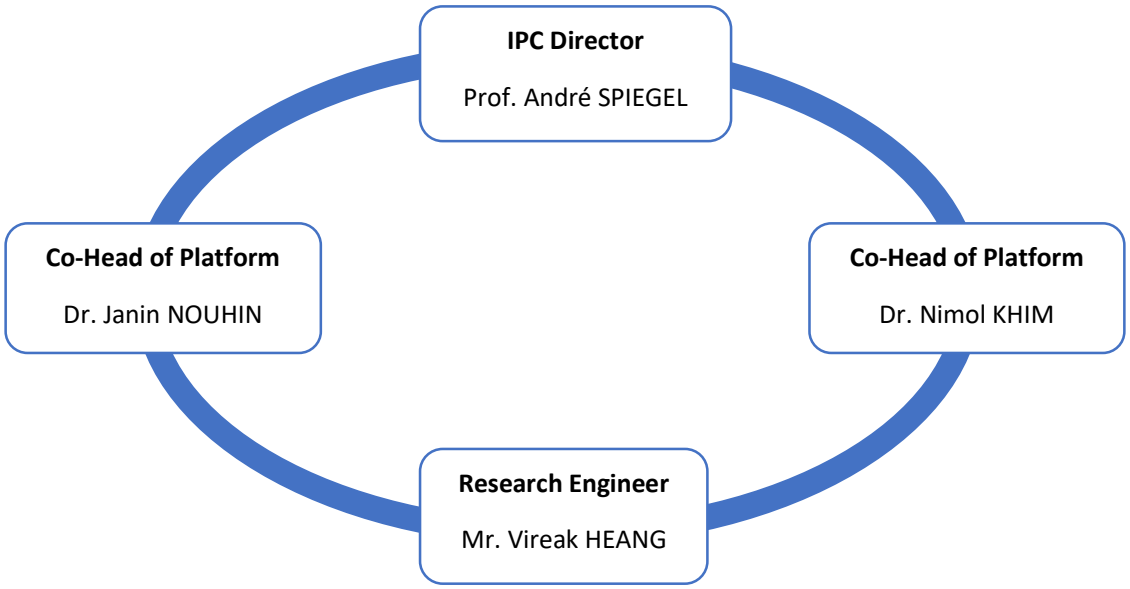
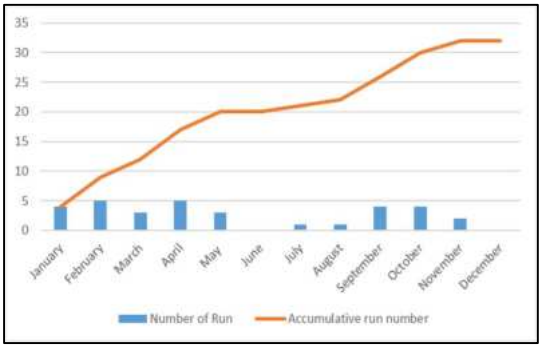


Figure 43: Sequencing Mini-Platform organigram, 2023

4.10.2.2 Research Programs – Major Achievements in 2023

In 2023, the platform delivered 32 runs of NGS service for two types of research programs: Collaborative projects (n=20 runs) and fee-based user services (n=12 runs). The distribution of these runs over monthly period is depicted in Figure 44.A. The primary users of the platform were from Virology Unit, accounting for 97% of platform's activities. Among these, 63% of runs were conducted as part of collaborative projects between Sequencing Mini-Platform and Virology Unit, while 34% of runs were carried-out for fee-based user services (see Figure 44.B). The secondary users were from Malaria Research Unit, requested fee-based service, accounting for 3% of platform's activities.

A



B

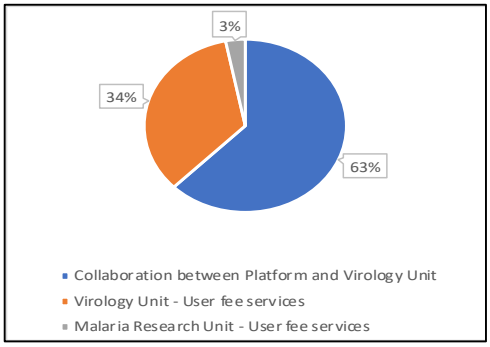


Figure 44: Distribution of Next-Generation Sequencing runs in 2023. (A) Distribution of runs over monthly period in 2023. Monthly totals are indicated by blue bars. Cumulative total is indicated by orange line. (B) Distribution of runs by research unit. Sequencing run performed in the framework of collaborative projects between the Sequencing Mini-Platform and the Virology Unit are in indicated in blue. Sequencing runs delivered to the Virology Unit as user fee services are indicated in orange. Sequencing runs provided to the Malaria Research Unit are indicated in gray.

4.10.2.3 Collaborative Projects

Project 1: In the Air Tonight: Metagenomic Pathogen Discovery as Tools in Pathogen Surveillance.

This is a collaborative project of Sequencing Mini-Platform and Virology Unit, with coordination led by Mr. Vireak Heang (Sequencing Mini-Platform) and Dr. Jurre Y. Siegers (Virology Unit) serving as Co-Principal Investigators. In 2023, a total of 17 NGS runs were carried-out.

Quickly identifying zoonotic viruses at high-risk human-animal interfaces is paramount for effective early warning systems and initiation of preventative actions. Current surveillance systems rely on sampling individual animals – a costly and time-consuming practice that prevents widespread surveillance. Incorporating environmental sampling into pathogen surveillance programs has the potential to vastly increase coverage at high-risk interfaces, while reducing the cost and biosecurity risks associated with direct animal sampling.

In this study, we collected both individual animal and environmental samples from three high-risk human-animal interfaces in Cambodia: live bird markets, pig slaughterhouses, and bat caves or roosts. Samples were tested for locally-relevant viral pathogens by PCR (e.g., coronaviruses, orthomyxoviruses, paramyxoviruses), and subjected to targeted metagenomic sequencing using the Twist Comprehensive Viral Research Panel. To assess the feasibility of using metagenomics for routine pathogen surveillance in a resource-limited setting, we compared the results generated by several commercially available metagenomic pipelines (One Codex, Genome Detective, and Chan Zuckerberg ID) to the results from the PCR assays.

Overall, we found an agreement between the viruses identified from environmental samples and those found in individual animal samples at all interfaces, with some variability. Notably, avian influenza virus (AIV) detection from environmental samples in live bird markets strongly correlated with AIV diversity revealed through animal sampling. However, the use of enriched metagenomics significantly improved the sensitivity of AIV detection, while also returning full and partial AIV genome sequences. At all interfaces, the use of metagenomics revealed the presence of additional animal and human viruses that were not included in routine pathogen surveillance activities in these locations, but may warrant future consideration. In sum, we demonstrate that the inclusion of environmental sampling and targeted metagenomics can substantially improve the efficiency and safety of pathogen surveillance programs, without sacrificing our ability to rapidly detect and respond to outbreaks.

Collaboration	Institut Pasteur du Cambodge: Sequencing Mini-Platform (Vireak Heang), Virology Unit (Jurre Y. Siegers and Erik A. Karlsson), EcoHealth Alliance (Cadhla Firth)
Funding	NIH CREID-Network (award number 1U01AI151378), $147,675, May 01, 2022 – Jul. 31, 2023

Project 2: Human papillomavirus E6 and E7 coding gene variations and their possible association with the occurrence of cervical intraepithelial neoplasia.

The study has been carried-out in collaboration with the Virology Unit with Dr. Janin Nouhin as the Principal Investigator. In 2023, a total of 3 NGS runs were carried-out for the present study.
The main objective of the study is to describe and characterize human papillomavirus (HPV) diversity circulating in HIV-infected women in Cambodia using NGS approach. Secondarily, we will assess the associations between variants in HPV E6 and E7 coding genes and cervical intraepithelial neoplasia (CIN) status. The Sequencing Platform has been involved in performing HPV whole genome sequencing using rolling-circle amplification metagenomics approach and Illumina MiSeq technology. A total of 60 women co-infected with HIV and HPV were included in the study. The sequencing activities were completed for all included samples (20 samples in 2022 and 40 samples in 2023). Data analysis is ongoing and a publication is expected by end of 2024.

Collaboration	Institut Pasteur du Cambodge: Sequencing Mini-Platform (J. Nouhin, N. Khim and V. Heang), Virology Unit (J. Nouhin, N. Boukli, L. Khun, J. Guillebaud and G. Gonnella). Calmette Hospital, Phnom Penh, (S. Limsreng, A. Korn). University of Health Sciences, Phnom Penh (S. Kim, S. Moeung). ANRS (O. Segeral). Institut de Recherche pour le Développement (P. De Beaudrap).
Funding	IPC Internal Funding, 2022 - 2023

4.10.2.4 User Fee Services

The Sequencing Mini-Platform provided fee-based user services for four research projects led by scientists from Virology Unit and Malaria Research Unit.

- **Screening of respiratory viruses in the context of Severe Acute Respiratory Infection (SARI) (Virology Unit):** The study was conducted with coordination led by Dr. Erik A. Karlsson, Ph.D., Deputy Head of the Virology Unit. The aim of the study was to screen the sentinel specimens collected through the Kuntha Bopha SARI surveillance system for other endemic and emerging pathogens using family level PCR and metagenomics. The platform performed eight runs of metagenomics sequencing on 100 SARI specimens collected at Kantha Bopha hospital from 2014 to 2020, using Twist Comprehensive Viral Research Panel kits on Illumina MiSeq System.
- **Characterization of bat coronaviruses in the framework of Ms. Tey Putita Ou's PhD project (Virology Unit):** The Platform carried out two runs of sequencing on bat specimen using Illumina Miseq System.
- **Current knowledge of ticks and tick-borne pathogens in Cambodia (Virology Unit):** The study has been conducted with coordination led by Dr. Janin NOUHIN Ph.D. One of the main study aims was to assess diversity of tick-borne pathogens in a rural community of Cambodia. The platform assisted the study with one run of metagenomics sequencing on a subset of tick samples using Twist Comprehensive Viral Research Panel kits on Illumina MiSeq System.
- **Molecular markers of antimalaria drug efficacy in *Plasmodium falciparum* infection (Malaria Research Unit):** The project aims to investigate the utility and benefits of amplicon deep sequencing as a tool for genotyping of antimalaria drug efficacy in *Plasmodium falciparum* infection. The experiments involved 87 samples and targeted five specific markers of interest (*ama1-D3, cpp, csp, cpmp, and msp7*). All wet-lab procedures were carried-out within the

Malaria Research Unit. Following the completion of the library preparation, the platform provided support to the study by conducting one run of NGS using the Illumina MiSeq system.

4.10.2.5 Outlook for 2024

Research Programs

Environmental Surveillance of Zoonotic Pathogens in Live Animal Markets.

The study will be conducted with coordination led by Dr. Erik A. Karlsson, Ph.D., Deputy Head of the Virology Unit. The objective of the research is to evaluate environmental surveillance methods for detecting infectious disease emergences or outbreaks in live animal markets in Cambodia. The Platform has played a key role in supervising and conducting sequencing using Oxford Nanopore and Illumina technologies. In total, we anticipate performing metagenomics sequencing for 480 samples collected from live bird market. As of February, 2024, metagenomics sequencing runs have been conducted on 20 samples using Oxford Nanopore Technology (4 runs) and Illumina Miseq System (2 runs).

User fee services

The platform continues to provide supports related to NGS across IPC and external customers. Several activities have been planned as following:

- Antimalarial resistance markers (Pfk13, Pfcrt, Pfmdr1, Pfcytb) in Plasmodium falciparum (Malaria Research Unit): The study will emphasize how crucial it is to maintain genetic surveillance in order to monitor the spread of recognized molecular markers associated to antimalarial drug resistance in the Grand Mekong Sub region. The platform will be involved in performing target deep amplicon sequencing of antimalarial resistance markers (Pfk13, Pfcrt, Pfmdr1, and Pfcytb) of Plasmodium falciparum.
- Fee-based service for validation of nucleic acid library using TapeStation instrument: This service will be open for internal and external users.

4.10.2.6 Outlook (3 – 5 years)

The Sequencing Mini-Platform envisions an important role as a cornerstone technical facility supporting a wide spectrum of research projects across IPC. Currently, the platform is underutilized by our internal researchers, primarily due to competitive pricing offered by commercial facility in the region. However, a significant challenge confronting the platform is the elevated cost of annual preventive maintenance of Illumina MiSeq system, accounting for approximately 29% of the instrument cost. Over the next three to five years, it is imperative to address this challenge to optimize the platform's functionality, ensuring its cost-effectiveness for researchers within our institution. This necessitates robust support from IPC leadership through financial contribution for the annual preventive maintenance cost. Additionally, forging stronger ties with various units within IPC is crucial to encourage internal scientists to utilize our platform. Strengthening collaborations and communication channels will promote awareness of the platform's capabilities and benefits, fostering increased utilization among our research community. Furthermore, the platform plans to recruit additional personnel to ensure sustain human resources capacity. These strategic approaches align with our goal of making the Platform a central hub for genomic sequencing within our institution.

4.10.2.7 Meeting and Workshop

- Mr. Vireak Heang attended 1) the 2023 CREID Network Annual Meeting, Rockville, MD USA, June 13 – 15, 2023 and 2) Grant Concept and Proposal Development Workshop, Rockville, MD USA, June 16, 2023
- Dr. Janin Nouhin attended the Nanopore Community Meeting 2023 in Singapore, September 27, 2023.

4.10.2.8 Publication List in 2023

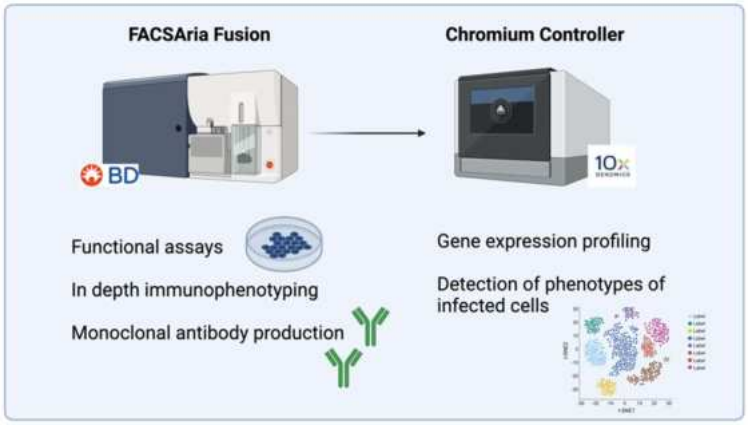
1. **Construction and characterization of a new hepatitis C virus genotype 6a subgenomic replicon that is prone to render the sofosbuvir resistance.**
   Liu C, Guo M, Han L, Lu J, Xiang X, Xie Q, Nouhin J, Duong V, Tong Y, Zhong J.
   J Med Virol. 2023 Sep;95(9):e29103. doi: 10.1002/jmv.29103.

4.10.3 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 have purchased and implemented a new 4 laser, 18-color single cell sorter (FACSAria Fusion III). Funding was obtained from Wellcome Trust (Multi User Equipment grant, PI: Cantaert Tineke, Co-investigators: Jean Popovici, Benoit Witkowski and Erik Karlsson) and GIZ. Moreover, we purchased and implemented a 10x Genomics Chromium Controller for single cell RNA sequencing, funded by the NIH PI-CREID grant (country PI: Cantaert Tineke). Both pieces of equipment are placed in a BSLII+ biosafety environment.

In terms of functioning, the platform is open to researchers both inside 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 the experiment.



4.10.3.1 Functional structure of the platform

The platform is integrated within the Immunology Unit.

4.10.3.2 Research Programs – Major Achievements in 2023

In 2023, the platform was used for the following research projects:

- Understanding the role of regulatory T cells in dengue virus infection
- Evaluation of B cell receptor (BCR) and T cell receptor (TCR) genes in the context of dengue virus infection
- Production of monoclonal antibodies directed to dengue virus

- Understanding of immune responses to chikungunya virus infection leading to chronic symptoms
- Uncovering of the mechanisms governing IgG glycosylation
- Assessment of skin immune responses to mosquito saliva and arbovirus infections
- Identification of mechanisms leading to clinical protection from Plasmodium vivax infection
- Understanding the mechanisms of plasmodium vivax receptor-ligand interactions involved in reticulocyte invasion

4.10.3.3 Research Programs – Prospects 2024

In 2024, a continuation of the above mentioned research projects is envisioned. In addition, our pipelines for the production of monoclonal antibodies will be extended to other viruses of major concern in South-East Asia.

4.10.3.4 Perspectives

The platform can be utilized to study interactions between host and pathogens of major health importance in Cambodia. The platform can help in the design, planning and execution of the experiments. In 2023, we hired a bioinformatician that will establish with the single cell RNA-sequencing data analysis and is involved in various collaborative projects. In 2024, the bioinformatician will continue to establish analysis pipelines and train Cambodian staff. Within Cambodia, we aim to setup novel collaborations with universities and other research entities. Indeed, we have an ongoing collaboration with the NIH-funded ICER (International Center for Excellence in Research) which is located at the National Center for Parasitology, Entomology and Malaria Control (CNM), Ministry of Health. Moreover, the platform aims to support research programs in Cambodia and in the region through workshops and training. A course in flow cytometry and cell sorting was held in September 2023 open to Cambodian students and other students from low-middle income countries.

4.10.4 BSL3 Laboratory

4.10.4.1 2023 at IPC's BSL3 Laboratory: A Year of Strategic Innovation and Collaboration

As Cambodia's sole BSL3 facility, IPC has been pivotal in handling pathogens like avian influenza, Mycobacterium tuberculosis, and SARS-CoV-2, contributing significantly to both diagnostic and research capabilities. This role has significantly bolstered both diagnostic and research capabilities within the region. In 2023, the IPC's BSL3 laboratory remained a beacon of excellence in infectious disease research. As IPC's frontline in handling high-risk pathogens, the BSL3 lab has been instrumental in advancing public health safety and scientific understanding of infectious diseases.

The BSL3 laboratory's governance is defined by a clear organizational structure emphasizing specialized roles and collaborative decision-making (Figure 45). The facility management is a cooperative effort spearheaded by the General Scientific Manager and the Technical Manager. The Scientific Manager is tasked with the rigorous oversight of biosafety protocols, ensuring that the laboratory's handling of pathogens and execution of procedures are in strict adherence to safety standards. This is carried out in close collaboration with the scientific supervisors of each module, enabling a tailored approach to biosafety that aligns with the specific requirements and risks associated with the various research activities and pathogens handled in the lab. Key to the laboratory's operation is the Technical Manager, who ensures the technical proficiency and operational integrity of the laboratory's functions. This role involves developing and implementing stringent biosecurity protocols to prevent unauthorized access to pathogens, along with overseeing

the regular maintenance, calibration, and validation of laboratory equipment and infrastructure to guarantee that they meet the necessary standards and regulations for safe and effective operation. These pivotal management roles are supported by specialized teams that manage waste, oversee quality, and include an occupational health doctor. Each BSL3 module is managed by two dedicated supervisors, responsible for overseeing pathogen-specific protocols, and maintaining biosafety standards, ensuring that all laboratory activities are executed with the highest safety and efficiency. Decision-making within the BSL3 team is likely collaborative, informed by expertise from each section, ensuring that all decisions are well-informed, comprehensive, and conducive to maintaining the highest quality of laboratory operations.

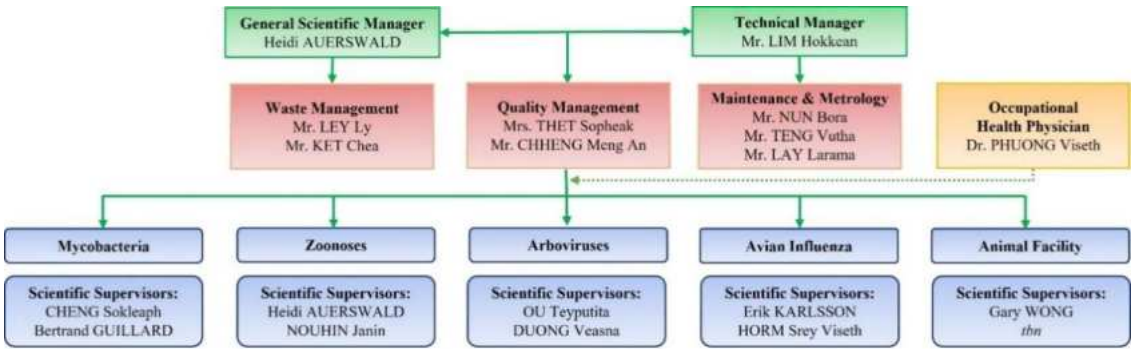


Figure 45: Organigram of the BSL3 Management

This year, IPC showcased its commitment to continuous improvement by enhancing waste management within the BSL3 facility. The introduction of a new integrated system that includes preliminary shredding and steam sterilization (at 135°C for 10 minutes) of biohazardous waste (STERIPLUS 80) marks a significant step in ensuring effective and safe disposal of hazardous materials. This machine is capable of handling the daily waste production from all laboratories, approximately 100 kg per day. It is crucial that biohazardous wastes are shredded and decontaminated, transforming them into non-infectious wastes, which aligns with the main goal of IPC's waste management strategy to prevent contamination spread to the public. The BSL3 underwent renovations to enhance its ventilation with stainless steel and upgraded its decontamination system to a more efficient H_2O_2-based system, reducing the time required for decontamination. Additionally, a generator was installed, dedicated to the cooling system (Chiller) of BSL3. In 2023, IPC focused heavily on specific staff training, ensuring that both new and existing employees were well-versed in biosafety and biosecurity. Specialized training was provided on BSC Class III, and maintenance staff received certification training for BSC Classes II and III. This initiative was extended to personnel from NIPH and NAHPRI, reflecting IPC's collaborative spirit. Preparation for ISO 35001:2019 certification was a key focus for the second half of the year, with preparation of all documentation now completed, setting the stage for further reviews and staff training in ISO 35001:2019 awareness in 2024.

4.10.4.2 Support for National Authorities: IPC's BSL3's Commitment to Biosafety and Biosecurity Standards

In 2023, IPC's BSL3 laboratory continued to play a crucial role in supporting national authorities, particularly in fulfilling international standards on biosafety and biosecurity. IPC's commitment to these standards is evident in its ongoing support for government actions related to the construction of other BSL3 facilities and general laboratory enhancements. This support is exemplified through joint training initiatives, such as those conducted under the EU Project 81, which focused on enhancing regional biosecurity capabilities. The EU Project 81, also known as the BIOSEC project, aims to enhance

biosecurity in the ASEAN region by strengthening national capabilities to prevent, detect, and respond to biological threats. Key objectives include improving border control for biological threats, enhancing regulatory controls over high-risk biological materials, and building capacity for best practices in biological materials storage and transport. The project focuses on collaborative efforts, including training workshops and the development of a network laboratory testing capability tool. Institute Pasteur Cambodia (IPC) actively supports the objectives of the BIOSEC project. IPC's involvement includes participation in multiple workshops organized by the Ministry of Defense's CBRN department, contributing to the project's goals of improving laboratory capacity and biosecurity measures. These efforts align with IPC's commitment to enhancing regional biosafety and biosecurity standards. Furthermore, IPC has extended its support to the Ministry of Defense, particularly in implementing a National Inventory for High-Risk Biological Agents. This collaboration underscores IPC's role as a leader in biosafety and biosecurity, contributing significantly to national and regional efforts in managing biological risks.

4.10.4.3 Teaching and Training: Educational Initiatives and Skill Development at IPC's BSL3

In 2023, IPC hosted a notable Biological Safety Cabinet (BSC) training, funded by EU Project 81 and organized by experts from the UK National Health Agency. This specialized training focused on enhancing skills related to the operation and maintenance of BSC Class III units, critical for ensuring biosafety in high-risk pathogen handling. Participants included staff from IPC, NIPH, and NAHPRI, reflecting a collaborative effort in building regional biosafety capacity. The training covered theoretical and practical aspects, ranging from understanding BSC functionalities to hands-on maintenance techniques. This initiative not only improved the technical capabilities of the participants but also reinforced IPC's commitment to fostering a culture of safety and excellence in biosecurity practices. The success of this program highlighted the importance of international partnerships in enhancing biosafety standards across institutions.

4.10.4.3 Outlook for 2024: Enhancing Operations and Ensuring Compliance

A significant focus for 2024 will be on achieving ISO 35001:2019 certification. The implementation of the Quality Management System according to ISO 35001 standards will be carried out across all processes in the laboratory. The lab has completed the necessary documentation preparation in 2023 and is now moving towards the application and certification process. This step is crucial for demonstrating IPC's commitment to global standards and requirement of ASEAN in biorisk management.

A major upgrade for the BSL3 lab in 2024 will be the replacement of the autoclave. This upgrade is pivotal for enhancing the sterilization process, ensuring that all biohazardous waste and equipment are decontaminated effectively, thereby maintaining a safe working environment.

Aligned with the 18-month maintenance schedule, the BSL3 lab is set for its regular maintenance in May 2024. This routine maintenance is critical for ensuring the BSL3's operational integrity and safety compliance, and this applies equally to its equipment

The planned closure of the BSL3 facility offers a unique opportunity to conduct comprehensive emergency drills. These drills are essential for testing and improving emergency response protocols, ensuring preparedness for any biosecurity incidents. Utilizing the BSL3 lab's closure period for emergency drills reflects IPC's dedication to continuous improvement and staff preparedness. These drills not only enhance safety protocols but also serve as practical training sessions for the staff, reinforcing their skills in emergency management. The plans for 2024 reflect IPC's commitment to

maintaining the highest standards of biosafety and operational excellence, while also preparing for future challenges and advancements in the field.

4.10.4.4 Vision for the Future: IPC's BSL3 Laboratory's Blueprint for Advancement and Innovation

Looking ahead, IPC's BSL3 lab is aligned with ambitious objectives as detailed in its strategic. The next three years will focus on enhancing biosafety protocols, particularly by updating guidelines to meet top international standards and acquiring the ISO35001:2019 certification. Concurrently, IPC will invest in advanced diagnostic technologies and initiate plans for a new BSL3 construction.

Extending this vision to the next five years, IPC aims to evolve its BSL3 lab into a hub for capacity building and training. This will involve organizing specialized programs for staff and collaborators, further embedding IPC as a leader in infectious disease management. The planning and eventual completion of the new BSL3 construction are key components of this vision, expanding IPC's capabilities in disease research and diagnostics.

Throughout this journey, IPC is committed to maintaining the highest standards in biosafety, developing cutting-edge diagnostic methods, enhancing disease surveillance, and conducting innovative research. This comprehensive strategy not only underlines IPC's dedication to advancing global health and biosecurity but also its commitment to scientific research, thereby contributing to a safer and healthier future.

5 Scientific Publications in 2023

NOTE *The name of authors from the Institut Pasteur du Cambodge are underlined*

1. **A qualitative study of the experience of COVID-19 patients in Burkina Faso.**
   Konaté B, Médah R, Traoré I, Ouedraogo S, Kaboré NF, Mamguem AK, Billa O, Kania D, Badolo H, Ouédraogo E, de Rekeneire N, Poda A, Diendéré AE, Ouédraogo B, Tinto H, Dabakuyo-Yonli TS.
   American Journal of Tropical Medicine and Hygiene 2023 Dec 18;110(1):170-178. doi: 10.4269/ajtmh.22-0351.

2. **Acceptability of decentralizing childhood tuberculosis diagnosis in low-income countries with high tuberculosis incidence: experiences and perceptions from health care workers in sub-Saharan Africa and South-East Asia**
   Basant Joshi, Yara Voss De Lima, Douglas Mbang Massom, Sanary Kaing, Marie-France Banga, Egerton Tamba Kamara, Sheriff Sesay, Laurence Borand, Jean-Voisin Taguebue, Raoul Moh, Celso Khosa, Guillaume Breton, Juliet Mwanga-Amumpaire, Maryline Bonnet, Eric Wobudeya, Olivier Marcy, Joanna Orne-Gliemann, the TB-Speed Decentralization study group
   PLOS Global Public Health Published: October 11, 2023 doi.org/10.1371/journal.pgph.0001525

3. **Antimalarial drug efficacy and resistance in malaria-endemic countries in HANMAT-PIAM_net countries of the Eastern Mediterranean Region 2016-2020: Clinical and genetic studies.**
   Adam M, Nahzat S, Kakar Q, Assada M, Witkowski B, Tag Eldin Elshafie A, Abuobaida D, Safi N, Khan MA, Nagi M, Mustafa SA, Kohestani K, Muhammad J, Khim N, Al-Hadi M, Elfaki TM, Habib MN, Khairy AKA, Hamid H, Uddin Z, Amer Y, Hassan AH, Elhag MS, Sediqi AW, Kakar I, Abdul-Ghani R, Amran JGH, Abdallrahim TA, Tamim MS, Aljasari A, Rasmussen C, Azkoul L, Warsame M.
   Trop Med Int Health. 2023;28(10):817-29. doi: 10.1111/tmi.13929

4. **Arbovirus researchers unite: expanding genomic surveillance for an urgent global need**
   Gabriel Luz Wallau Author links open overlay panelNgu Njei Abanda, Adriano Abbud, Saro Abdella, Adugna Abera, Steve Ahuka-Mundeke, Francesca Falconi-Agapito, Kalichamy Alagarasu, Kevin K Ariën, Constancia Flávia Junqueira Ayres, Luisa Barzon, Joseph Humphrey Kofi Bonney, Sanaba Boumbaly, Philippe Buchy, Van-Mai Cao-Lormeau, Yu Kie Chem, Paul A Cardenas, Andres E Castillo, Adriana Delfraro, Gregor Devine, Veasna Duong, Myrielle Dupont-Rouzeyrol, Artem V Fadeev, Alvaro Fajardo, Luis Adrián Diaz, Lara Ferrero Gómez, Eduardo Samo Gudo, Gladys Gutierrez-Bugallo, Hapuarachchige Chanditha Hapuarachchi, Jean-Michel Heraud, Martin L Hibberd, Osvaldo Frederico Inlamea, Nik Jasmin, Kalysbek Kydyshov, Maria Ezekiely Kelly, Salim Khan, Andrey B Komissarov, Pornsawan Leaungwutiwong, Mariana Leguia, Yaniv Lustig, Rafael Maciel-de-Freitas, Gathsaurie Neelika Malavige, Alexander A Martinez, Maria L Mendoza, Luong T Mo, Brechla Moreno, Lydia Mwasi, Felipe Gomes Naveca, Lee Ching NG, Richard Njouom, Mauricio Lacerda Nogueira, Francine Ntoumi, Nehemie Nzoyikorera, Barbara A Parra, Mauricio Vázquez Pichardo, Kristine Joy Ragual Privaldos, Ricardo Rivero, Alejandra María Rojas, Richard Steiner Salvato, R Tedjo Sasmono, Jonas Schmidt-Chanasit, Etienne Simon-Loriere, Ava Kristy Dy Sy, Michael Talledo-Albujar, Daniel Thakuma Tizhe, Usenbaev Nurbolot Toloshovich, Vi Thuy Tran, Cécile Troupin, John Timothy Kayiwa, Andrew van den Hurk, Nikolaos Vasilakis, Atsbeha Gebreegziabxier Weldemariam, Sophie Yacoub, Zainun Zaini, Gabriel Luz Wallau
   The Lancet Global Health 2023-08-01 DOI : 10.1016/S2214-109X(23)00325-X

5. **Characterization of soluble TLR2 and CD14 levels during acute dengue virus infection.**
   Upasani V, Ter Ellen BM, Sann S, Lay S, Heng S, Laurent D, Ly S, Duong V, Dussart P, Smit JM, Cantaert T, Rodenhuis-Zybert IA.
   Heliyon 2023 Jun 21;9(6):e17265. doi: 10.1016/j.heliyon.2023.e17265. eCollection 2023 Jun.

6. **Comparative Performance of Anyplex II HPV28 and Cobas 4800 Human Papillomavirus (HPV) Assays for High-Risk HPV Detection in Self-collected Anal Samples.**
   Delvallez G, Cheng S, Marot S, Malonga GA, Cocherie T, Wignall S, Calvez V, Phal S, Vichet K, Marcelin AG, Jary A.
   Open Forum Infectious Diseases 2023 Oct 31;10(11):ofad540. doi: 10.1093/ofid/ofad540. eCollection 2023 Nov.

7. **Construction and characterization of a new hepatitis C virus genotype 6a subgenomic replicon that is prone to render the sofosbuvir resistance.**
   Chaolun Liu, Mingzhe Guo, Lin Han, Jie Lu, Xiaogang Xiang, Qing Xie, Janin Nouhin, Veasna Duong, Yimin Tong, Jin Zhong
   Journal of Medical Virology 2023 Sep;95(9):e29103. doi: 10.1002/jmv.29103.

8. **Description of a new species of Toxorhynchites (Diptera: Culicidae) from Nepenthes pitchers in Cambodia**
   Pierre-Olivier Maquart, Nil Rahola, Kalyan Chhuoy, Kimly Heng, Moeun Chhum, Kimhuor Suor, Sébastien Boyer
   Journal of Asia-Pacific Entomology 26 (2023) 102064 DOI : 10.1016/j.aspen.2023.102064

9. **Detection and Phylogenetic Analysis of Contemporary H14N2 Avian Influenza A Virus in Domestic Ducks in Southeast Asia (Cambodia)**
   Jurre Y. Siegers, Michelle Wille, Sokhoun Yann, Songha Tok, Sarath Sin, Sokha Chea, Alice Porco, Sreyem Sours, Vutha Chim, Samban Chea, Kimtuo Chhel, Sothyra Tum, Sorn San, Peter Thielen, Vijaykrishna Dhanasekaran, Erik A. Karlsson
   Emerging Microbes & Infections DOI: 10.1080/22221751.2023.2297552

10. **Detection and Phylogenetic Analysis of Highly Pathogenic A/H5N1 Avian Influenza Clade 2.3.4.4b Virus in Chile, 2022**
    Pedro Jimenez-Bluhm, Jurre Y. Siegers, Shaoyuan Tan, Bridgett Sharp, Pamela Freiden, Magdalena Johow, Katherinne Orozco, Soledad Ruiz, Cecilia Baumberger, Pablo Galdames, Maria Antonieta Gonzalez, Camila Rojas, Erik A. Karlsson, Christopher Hamilton-West, Stacey Schultz-Cherry
    Emerging Microbes & Infections **https://doi.org/10.1080/22221751.2023.2220569**

11. **Detection of Clade 2.3.4.4b Avian Influenza A(H5N8) Virus in Cambodia, 2021**
    Kimberly M Edwards, Jurre Y Siegers, Xiaoman Wei, Ammar Aziz, Yi-Mo Deng, Sokhoun Yann, Chan Bun, Seng Bunnary, Leonard Izzard, Makara Hak, Peter Thielen, Sothyra Tum, Frank Wong, Nicola S Lewis, Joe James, Filip Claes, Ian G Barr, Vijaykrishna Dhanasekaran, Erik A Karlsson
    Emerg Infect Dis. 2023 Jan;29(1):170-174. doi: 10.3201/eid2901.220934.

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    Eva Legendre, Florian Girond, Vincent Herbreteau, Sokeang Hoeun, Stanislas Rebaudet, Aung Myint Thu, Jade Dean Rae, Laurent Lehot, Sokhna Dieng, Gilles Delmas, François Nosten, Jean Gaudart & Jordi Landier
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13. **Hemisynthetic alkaloids derived from trilobine are antimalarials with sustained activity in multidrug-resistant Plasmodium falciparum.**
    Nardella F, Dobrescu I, Hassan H, Rodrigues F, Thiberge S, Mancio-Silva L, Tafit A, Jallet C, Cadet-Daniel V, Goussin S, Lorthiois A, Menon Y, Molinier N, Pechalrieu D, Long C, Sautel F, Matondo M, Duchateau M, Médard G, Witkowski B, Scherf A, Halby L, Arimondo PB.
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Maryline Bonnet, Delphine Gabillard, Serge Domoua, Conrad Muzoora, Eugène Messou, Samreth Sovannarith, Duc Bang Nguyen, Anani Badje, Sylvain Juchet, Dim Bunnet, Laurence Borand, Naome Natukunda, Thị Hong Tran, Xavier Anglaret, Didier Laureillard, François-Xavier Blanc;
Clinical Infectious Diseases 2023 Mar 8;ciad125. doi: 10.1093/cid/ciad125.

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Yamin R, Kao KS, MacDonald MR, Cantaert T, Rice CM, Ravetch JV, Bournazos S.
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Janin Nouhin, Philip Lei Tzou, Soo-Yon Rhee, Malaya K. Sahoo, Benjamin A. Pinsky, Miri Krupkin, Joseph D. Puglisi, Elisabetta V. Puglisi, Robert W. Shafer
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Quentin D. Watson, Lenore L. Carias, Alyssa Malachin, Karli R. Redinger, Jürgen Bosch, Martino Bardelli, Lea Baldor, Lionel Brice Feufack-Donfack, Jean Popovici, Robert W. Moon, Simon J. Draper, Peter A. Zimmerman & Christopher L. King
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Francisco J Martinez, Micheline Guillotte-Blisnick, Christèle Huon, Patrick England, Jean Popovici, Hélène Laude, Laurence Arowas, Marie-Noëlle Ungeheuer, Jenny M Reimer, Darrick Carter, Steve Reed, Paushali Mukherjee, Virander S Chauhan, Chetan E Chitnis
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19. **Inappropriate antibiotic prescribing and its determinants among outpatient children in 3 low- and middle-income countries: A multicentric community-based cohort study**
Antoine Ardillon, Lison Ramblière, Elsa Kermorvant-Duchemin, Touch Sok, Andrianirina Zafitsara Zo, Jean-Baptiste Diouf, Pring Long, Siyin Lach, Fatoumata Diene Sarr, Laurence Borand, Felix Cheysson, Jean-Marc Collard, Perlinot Herindrainy, Agathe de Lauzanne, Muriel Vray, Elisabeth Delarocque-Astagneau, Didier Guillemot, Bich-Tram Huynh; BIRDY study group
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Benjamin L Sievers, Robert E Sievers, Eric L Sievers
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Nilandone Senvanpan, Vilayouth Phimolsarnnousith, Sayaphet Rattanavong, Mayfong Mayxay, Daniel Reinharz, Amanda E Fine, Paul F Horwood, Philippe Dussart, Stuart D Blacksell, Mathieu Pruvot, Paul N Newton, Matthew T Robinson
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Souand Mohamed Ali, Antsa Rakotonirina, Kimly Heng, Elise Jacquemet, Stevenn Volant, Sarah Temmam, Sebastien Boyer and Marc Eloit
Viruses 2023, 15, 1831. https://doi.org/10.3390/v15091831

23. **Malaria temporal dynamic clustering for surveillance and intervention planning**
Eva Legendre, Laurent Lehot, Sokhna Dieng , Stanislas Rebaudet, Aung Myint Thu, Jade D Rae, Gilles Delmas, Florian Girond, Vincent Herbreteau, François Nosten, Jordi Landier, Jean Gaudart
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Bernard C, Font H, Ziadeh S, Tine JM, Diaw A, Ndiaye I, Samba O, Bottai T, Jacquesy L, Verdeli H, Ngom NF, Dabis F, Seydi M, de Rekeneire N; IeDEA West Africa Cohort Collaboration.
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Antsa Rakotonirina, Pierre-Olivier Maquart, Claude Flamand, Chea Sokha & Sébastien Boyer
Parasites & Vectors 2023-03-21 DOI : 10.1186/s13071-023-05729-w

26. **National dengue surveillance, Cambodia 2002-2020**
Christina Yek, Yimei Li, Andrea R Pacheco, Chanthap Lon, Veasna Duong, Philippe Dussart, Katherine I Young, Sophana Chea, Sreyngim Lay, Somnang Man, Souv Kimsan, Chea Huch, Rithea Leang, Rekol Huy, Cara E Brook, Jessica E Manning
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27. **Natural Killer Repertoire Restoration in TB/HIV Co-Infected Individuals Experienced an Immune Reconstitution Syndrome (CAMELIA Trial, ANRS 12153)**
Pean P, Madec Y, Nerrienet E, Borand L, Laureillard D, Fernandez M, Marcy O, Scott-Algara D; CAMELIA Study Team.
Pathogens. 2023 Oct 13;12(10):1241. doi: 10.3390/pathogens12101241.

28. **Naturally acquired antibodies to gametocyte antigens are associated with reduced transmission of Plasmodium vivax gametocytes to Anopheles arabiensis mosquitoes**
Surafel K Tebeje, Wakweya Chali, Elifaged Hailemeskel, Jordache Ramjith, Abrham Gashaw, Temesgen Ashine, Desalegn Nebret, Endashaw Esayas, Tadele Emiru, Tizita Tsegaye, Karina Teelen, Kjerstin Lanke, Eizo Takashima, Takafumi Tsuboi, Nichole D Salinas, Niraj H Tolia, David Narum, Chris Drakeley, Benoit Witkowski, Amelie Vantaux, Matthijs M Jore, William J R Stone, Ivo S Hansen, Fitsum G Tadesse, Teun Bousema
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Volle R, Luo L, Razafindratsimandresy R, Sadeuh-Mba SA, Gouandjika-Valisache I, Horwood P, Duong V, Buchy P, Joffret ML, Huang Z, Duizer E, Martin J, Chakrabarti LA, Dussart P, Jouvenet N, Delpeyroux F, Bessaud M.
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31. **One assay to test them all: Multiplex assays for expansion of respiratory virus surveillance**
Narjis Boukli, Claude Flamand, Kim Lay Chea, Leangyi Heng, Seangmai Keo, Kimhoung Sour, Sophea In, Panha Chhim, Bunthea Chhor, Lomor Kruy, Jelena D. M. Feenstra, Manoj Gandhi, Obiageli Okafor, Camilla Ulekleiv, Heidi Auerswald, Viseth Srey Horm and Erik A. Karlsson
Frontiers of Medicine, 24 April 2023 doi.org/10.3389/fmed.2023.1161268

32. **Phylogenomic investigation of an outbreak of fluoroquinolone-resistant Salmonella enterica subsp. enterica serovar Paratyphi A in Phnom Penh, Cambodia**
Rutaiwan Dusadeepong, Gauthier Delvallez, Sokleaph Cheng, Soda Meng, Navin Sreng, Joanne Letchford, Kimcheng Choun, Syna Teav, Liselotte Hardy, Jan Jacobs, Tuyet Hoang, Torsten Seemann, Benjamin P Howden, Philippe Glaser, Timothy P Stinear, Koen Vandelannoote
Microbial Genomics 2023 Mar;9(3). doi: 10.1099/mgen.0.000972.

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Dusadeepong R, Maquart PO, Hide M, Boyer S.
Medical and Veterinary Entomology 2023 Jul 5. doi: 10.1111/mve.12678. Online ahead of print.

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Welmoed van Loon, Rafael Oliveira , Clara Bergmann, Felix Habarugira, Costanza Tacoli, Julia Jäger, Darius Savelsberg, Djibril Mbarushimana, Jules M Ndoli, Augustin Sendegeya, Claude Bayingana, Frank P Mockenhaupt
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35. **Plugging the leaks: antibiotic resistance at human–animal interfaces in low-resource settings**
Maya L Nadimpalli, Marc Stegger, Roberto Viau, Vuthy Yith, Agathe de Lauzanne, Nita Sem, Laurence Borand, Bich-tram Huynh, Sylvain Brisse, Virginie Passet, Søren Overballe-Petersen, Maliha Aziz, Malika Gouali, Jan Jacobs, Thong Phe, Bruce A Hungate, Victor O Leshyk, Amy J Pickering, François Gravey, Cindy M Liu, Timothy J Johnson, Simon Le Hello, Lance B Price
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36. **Prevalence and characterization of piperaquine, mefloquine and artemisinin derivatives triple-resistant Plasmodium falciparum in Cambodia.**
Mairet-Khedim M, Roesch C, Khim N, Srun S, Bouillon A, Kim S, Ke S, Kauy C, Kloeung N, Eam R, Khean C, Kul C, Chy S, Leang R, Ringwald P, Barale JC, Witkowski B.
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Jary A, Cheng S, Marot S, Malonga GA, Cocherie T, Wignall S, Calvez V, Phal S, Vichet K, Marcelin AG, Delvallez G.
The Journal of Infection 2023 Feb 28;S0163-4453(23)00124-X. DOI: 10.1016/j.jinf.2023.02.038

38. **pWCP is a widely distributed and highly conserved Wolbachia plasmid in Culex pipiens and Culex quinquefasciatus mosquitoes worldwide**
Amani Ghousein, Jordan Tutagata, Hans Schrieke, Manuel Etienne, Victor Chaumeau, Sebastien Boyer, Nonito Pages, David Roiz, A Murat Eren, Guillaume Cambray, Julie Reveillaud
ISME Communications 2023 Apr 28;3(1):40. doi: 10.1038/s43705-023-00248-2

39. **Rabies surveillance in Madagascar from 2011 to 2021: can we reach the target?**
Andriamandimby SF, Volasoa MH, Razafindraibe NP, Ranoaritiana DB, Razafindramparany MH, Rafisandratantsoa T, Nomenjanahary LA, Rakotondrabe N, Andriamananjara MA, Guis H, Lacoste V, Dreyfus A.
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Cassandra Koh, Lionel Frangeul, Hervé Blanc, Carine Ngoagouni, Sébastien Boyer , Philippe Dussart, Nina Grau, Romain Girod, Jean-Bernard Duchemin, Maria-Carla Saleh
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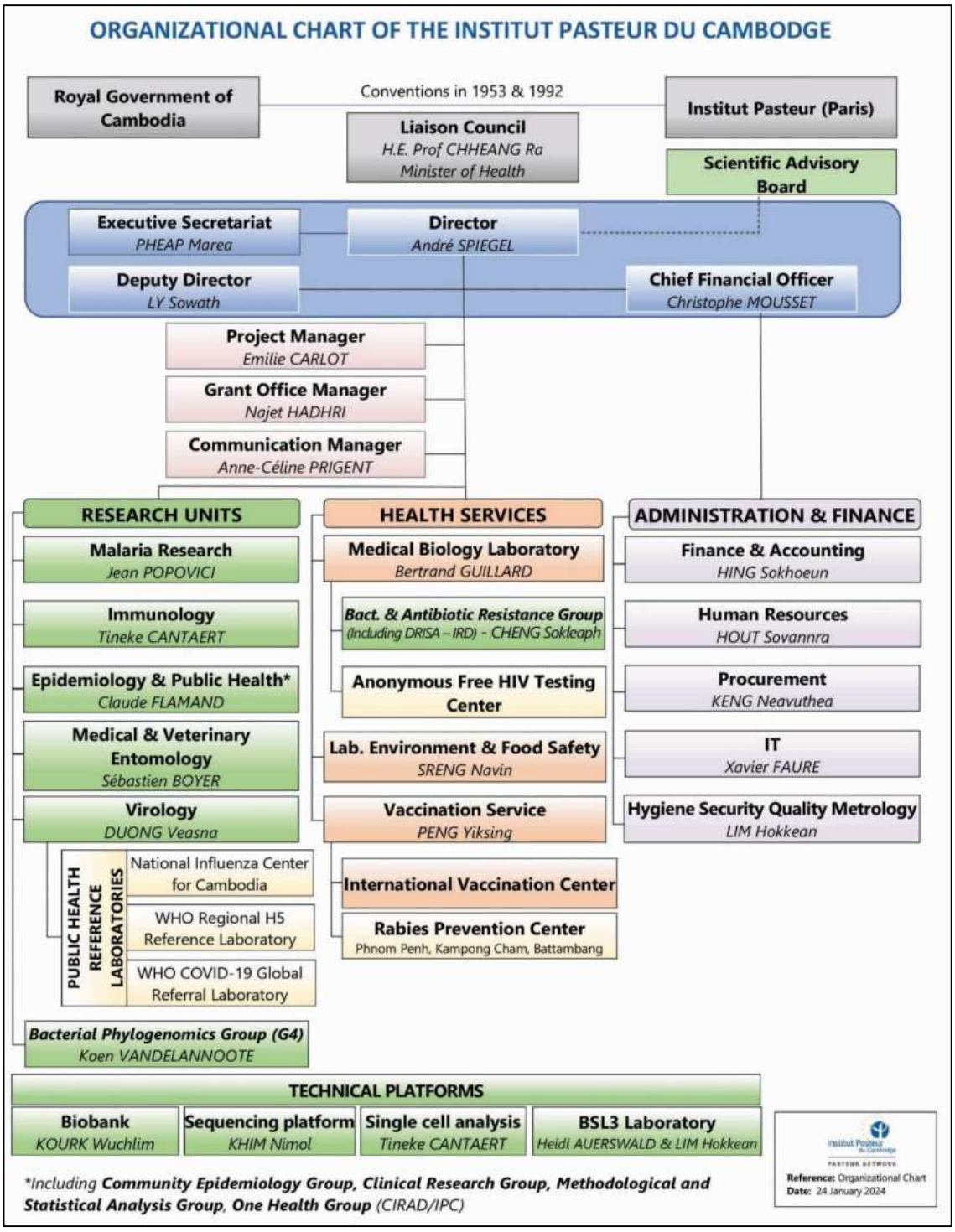
6 Annex


ORGANIZATIONAL CHART OF THE INSTITUT PASTEUR DU CAMBODGE
Royal Government of Cambodia
Conventions in 1953 & 1992
Institut Pasteur (Paris)
Liaison Council
H.E. Prof CHHEANG Ra
Minister of Health
Scientific Advisory Board
Executive Secretariat
PHEAP Marea
Director
André SPIEGEL
Deputy Director
LY Sowath
Chief Financial Officer
Christophe MOUSSET
Project Manager
Emilie CARLOT
Grant Office Manager
Najet HADHRI
Communication Manager
Anne-Céline PRIGENT
RESEARCH UNITS
Malaria Research
Jean POPOVICI
Immunology
Tineke CANTAERT
Epidemiology & Public Health*
Claude FLAMAND
Medical & Veterinary Entomology
Sébastien BOYER
Virology
DUONG Veasna
PUBLIC HEALTH REFERENCE LABORATORIES
National Influenza Center for Cambodia
WHO Regional H5 Reference Laboratory
WHO COVID-19 Global Referral Laboratory
Bacterial Phylogenomics Group (G4)
Koen VANDELANNOOTE
HEALTH SERVICES
Medical Biology Laboratory
Bertrand GUILLARD
Bact. & Antibiotic Resistance Group
(Including DRISA – IRD) - CHENG Sokleaph
Anonymous Free HIV Testing Center
Lab. Environment & Food Safety
SRENG Navin
Vaccination Service
PENG Yiksing
International Vaccination Center
Rabies Prevention Center
Phnom Penh, Kampong Cham, Battambang
ADMINISTRATION & FINANCE
Finance & Accounting
HING Sokhoeun
Human Resources
HOUT Sovannra
Procurement
KENG Neavuthea
IT
Xavier FAURE
Hygiene Security Quality Metrology
LIM Hokkean
TECHNICAL PLATFORMS
Biobank
KOURK Wuchlim
Sequencing platform
KHIM Nimol
Single cell analysis
Tineke CANTAERT
BSL3 Laboratory
Heidi AUERSWALD & LIM Hokkean
*Including Community Epidemiology Group, Clinical Research Group, Methodological and Statistical Analysis Group, One Health Group (CIRAD/IPC)
Reference: Organizational Chart
Date: 24 January 2024